CHAPTER ONE

INTRODUCTION

1.1 Background and Problem of Study

African trypanosomosis is a devastating disease of man and animals which is enzootic in Sub-Saharan Africa. The causative protozoa of genus *Trypanosoma* include species like *Trypanosoma brucei*, *T. congolense*, *T. vivax* whose infection of subjects is marked by varying conditions usually involving fluctuating parasitaema, intermittent fever, anaemia, weight loss, other production losses and deaths. The disease poses a threat to food security in the endemic region with over 100 million goats at risk of infection across Africa (Offor 2000, Abenga, *et. al*., 2005, Eze and Ochike, 2007).

Comparative studies in species and breeds of animals affected by trypanosomosis have been advocated (Ikede, 1991). Elsewhere (in the Sudan), it was reported that where nomadic tribes kept cattle, carmels and goats, simultaneously, an experimental infection of goats lead to chronic form of trypanosomosis with animals recovering spontaneously from strains which caused acute fatal disease in cattle (Mahmoud and Elmalik, 1977).

It is well known that certain breeds of livestock show resistance to trypanosomosis (Murray, et. al., 1984, Radostits, *et. al*., 2000) which has been termed trypanotolerance (Seifert, 1992). But this resistance is not absolute and less well defined in small ruminants like goats compared to cattle (Mare, 1998).

There exist a dearth of information on comparative studies in Nigerian small ruminants bothering on tolerance or susceptibility to trypanosomosis such as immune response, packed cell volume, haemoglobin concentration etc. (Kumshe, *et. al*., 2010). In attempt to fill these gaps, experimental infection of Nigerian West African Dwarf (WAD) and Red Sokoto goats (RSG) was undertaken in Makurdi, Benue State using strains of two trypanosome species namely *Trypanosoma brucei* (Federe Strain) and *Trypanosoma congolense* (Karu strain) previously isolated from cattle at Federe in Plateau State and Karu in Nassarawa State. The location of the experimental site and the sites where the isolates were obtained are all within the same agro-ecological zone in Nigeria. This raises

the possibility that such trypanosome strains could even be circulating naturally in the goat population of the area.

Available information on the natural infection of Nigerian goats with trypanosomosis has not been very consistent on prevalence levels. Report show that prevalence of trypanosomosis in Nigerian goats was 4.5% countrywide (TTIQ, 2001). It was 33.9% in Konshisha area of Benue State (Omotainse, *et. al.*, 2000), 1.1% in Abraka area of Delta State (Odoya, *et. al.*, 2003), 57.39% in Red Sokoto goats at Ibadan (Fayemi, 2006), 1.2% in Abia (Ohaeri, 2010). These reports indicated occurrence of wide variations in the prevalence from one area to another and probably changes over time period which might be associated with variation in occurrence of vectors. At time of design of this study, no such information on prevalence status was found for Makurdi area where the experimental infection was sited. The investigation therefore included a survey on trypanosome occurrence in goats around the study site in Makurdi area.

It is common knowledge that obtaining evidence of an infection in a population or flock of animals may depend on the diagnostic technique used (Audu and Pila, 2008). For trypanosomes, common parasitological detection methods used include wet film, thick and thin smear or microhaematocrit centrifuge techniques (Audu and Abifarin, 2001). The trypanosomes used in experimental infection of goats were usually obtained from Nigerian Institute for Trypanosomiasis and Onchocerchiasis Research (NITOR) at Kaduna or Vom. However, such isolates were occasionally lost due to strategic reasons and reacquired from field scientists who previously got them from the institute but usually handled multiple stocks in their investigations. The situation therefore created need for confirmatory molecular identification of isolates used in experimental infections. This investigation included application of a molecular method to confirm species of the trypanosome isolates used to infect the goats.

1.2 Aim and Objective

1.2.1 Aim

To determine comparative tolerance or susceptibility of West African Dwarf goats and Red Sokoto goats to African Animal trypanosomosis.

1.2.2 Specific Objectives

1. To evaluate natural occurence of trypanosomes (*T. brucei and T. congolense)* in the West African Dwarf and Red Sokoto Goats retrospectively from clinic treatment records.
2. To determine the occurence of *T. brucei* and *T. congolense* in goats sampled around the experimental site in Makurdi, Benue State, Nigeria.
3. To experimentally infect West African Dwarf and Red Sokoto Goat with *T. congolense, T. brucei* and mixed inoculum of both species and to assess the post-infection response of West African Dwarf and Red Sokoto Goats to experimental infection.
4. To confirm the genotype of Trypanosomes used in experimental infection using Polymerase Chain Reaction (PCR).

1.3 Justification

Experimental infection was conducted with aim of gauging response of the breeds to the trypanosome inoculums used. In African Animal Trypanosomosis, severity of the clinical response is dependent on the species and breed of animal affected, the dose and virulence of infecting trypanosome (Mare, 1998). Generally, when given good feed and management, some animals like those of trypanoresistant breeds recover from transient infections especially after exposure to infection with low numbers of trypanosomes or strains of low virulence (Seifert, 1992).

Since natural variation exists within and across animal breeds, the absolute performance or phenotype of an individual host is determined by genetic make up (genotype), the environment (such as management systems, climate, nutritional factors etc.), plus any interaction between the genotype and the environment (Drinkwater and Hetzel, 1991). In trypanosomosis, the physiologic and metabolic disturbance in affected animal include anaemia, loss of body weight, poor immune response, intolerance to stress and poor reproductive performance (Abebe, 1991). Exhibition of these systemic variables is usually graded and measurable in infections especially where different breeds are involved which could respond differently to a given stimulus. Some animals are more

susceptible, while some are more tolerant. However, the real cause which lead to death of trypanosome affected animals is not fully understood (Seifert, 1992).

Report of previous surveys elsewhere on trypanosomosis in Nigerian goats apparently revealed variations in prevalence based on locality of survey, therefore there is need to probe into status of trypanosomosis in goats at Makurdi area. Moreover, tsetse distribution which majorly influences the prevalence of trypanosomosis is both time and area specific, making generalization over large areas vague (Agyemang, 2005).

Diagnostic technique used is an important factor in unveiling trypanosome infections. The three main criteria on which detection of trypanosomosis is usually based are clinical signs and symptoms of disease, detection of parasites in body fluids and detection of parasite products in body fluids (Luckins, 2007). Microscopy techniques are commonly performed to detect infections. But these microscopic methods which work best for detecting trypanosomes are not as well suited for distinguishing species where molecular methods are better. But these are often not enough even in the primary detection of trypanosome infection, not to talk of specific identification of species involved. For instance, in detecting infection with megatrypanuum species like *T. theileri* which is a stercorarian with common cryptic infections in domestic animals, blood culture methods are required (Hussain, et. al., 1985). To distinguish accurately trypanosome species involved in infection, more sensitive molecular biological methods such as polymerase chain reaction which are based on parasite DNA sequences need to be done on blood samples from infected animals and tsetse (ILRAD, 1988). Such specific tests become most crucial where possibility of mistaken identity exist as in working with a mixture of species.

1.4 Research Questions

1. Was *T.brucei* or *T.congolense* present in goats around the study site in Makurdi?
2 Was *T.brucei* or *T.congolense* present in the inoculums administered to experimentally infected West African Dwarf and Red Sokoto Goats?
3. What was the comparative susceptibility of the West African Dwarf and Red Sokoto goats to the Was *T.brucei* (Federe Strain) and *T.congolense* (Karu Strain) with which they were experimentally infected?

1.5 Definition of Terms

1.5.1 Goats

In the order Artiodactyla, the family Bovidae has genus capra that comprise 5-6 wild species (ibex, bezoar, aegagrus markhor etc) from one or more of which the domesticated goat portraying more than 200 breeds could have originated (Mowlem, 1992).

The genus capra shares the tribe caprini to which it belongs with another genus Hemitragus (Tahrs) which are wild goats found in Arabia, the Himalayas and South India having only 48 chromosomes in their cells and do not cross breed with the Capra that has 60 chromosomes (Steel, 1996).

1.5.2 Specie

This is defined among higher organisms as a group of organisms that can naturally interbreed to produce fertile offspring. However, in lower organisms with no classical sexual processes like the protozoa, the definition of specie is more arbitrary and may depend on what is recognized by a good taxonomist (FAO, 2005).

1.5.3 Breed

Breeds are groups of animals of the same species which belong together because they have the same origin, share certain physical and physiological characteristics, and are of a commercial value but are not constant entities in view of their biological behaviour (Brem, *et. al.,* 1989). Breeds have genes common to the specie but which as a result of selection, have segregated into groups with restricted ranges of variability. In goats, breeds may differ widely or may overlap in functional abilities and efficiencies (French, 1970). A breed of goat as a group distinguishable from others has characteristics that are genetically controlled (Steele, 1996)

1.5.4 Infection

This is defined by Blood and Studdert (1999) as invasion and multiplication of organisms in body tissues especially that causing local cellular injury due to competitive metabolism, toxins, intracellular replication or antigen-antibody response. The infectivity

of trypanosomes defined as their ability to invade and multiply in a host, is variable depending on the specie and strain of trypanosomes (Losos, 1986).

1.5.5 Trypanosoma

The genus Trypanosoma was coined by Gruby in 1843 for the organism *Trypanosoma sanguinis.* The first of several trypanosomes described from frogs (Vickerman, 1997). The Genus Leishmania is closely related to it.

1.5.6 Trypanosomosis (Trypanosomiasis)

The disease produced by Pathogenic trypanosomes is termed trypanosomiasis (Smyth, 1996). It has been decided by the World Association for the advancement of Veterinary Parasitology (WAAVP) that the terms trypanosomiasis and Leishmaniasis should now be called Trypanosomosis and Leishmaniosis respectively (Hide *et. al.*, 1997).

1.5.7 Trypanotolerance/Trypanoresistance

Trypanotolerance is a term that was coined in the late nineteen fourties (Seifert, 1992). It has been defined as the relative capacity of an animal to control the development of the parasites and to limit other pathological effects, the most prominent of which is anaemia (d'Ieteren, *et. al.,* 1998). This resistance to infection with Trypanosomes is inherent in some breeds of cattle like N'dama, Lagune and others (Blood and Studdert, 1999). Trypanotolerance also occurs in some indigenous breeds of small ruminants notably the West African Dwarf sheep and goats as well as the East African goats (Radostits *et. al.* 2000).

The term Trypanoresistance is however preferred since trypanotolerance misrepresents immune tolerance which is a situation where specific immune response to an antigen is absent while the animal reacts to other antigens (Seifert, 1992).

1.5.8 Resilience

Based on the fact that goats were unable to maintain their packed cell volume (PCV) during infection, (Goossens, *et. al.,* 2001) concluded that the mechanism of

trypanotolerance is different in small ruminants compared to cattle and should be called Resilience.

1.5.9 Anaemia

Anaemia is a decrease in the level of haemoglobin in the blood (haemoglobin concentration) below a set reference range (Everything, 2006). Typical values for normal Haemoglobin concentrations range from about 11 to 13g/100ml in domestic animals (Frandson, et. al., 2003).

1.5.10 Packed Cell Volume (PCV) or (Haematocrit)

Haematocrit means to separate blood and when this is done by centrifugation, the compartment of erythrocytes at the bottom is called packed cell volume (Schalm, 1971).

1.5.11 Parasitaemia

This refers to presence of parasites in the circulating blood (FAO, 2005). Trypanosomes multiply at the site where infection is introduced into host animal, leaving the location to enter the blood and lymphatics to become disseminated round the body by circulating blood. Different levels of parasitaemia are found with different species and strains of trypanosomes (Losos, 1986).

1.5.12 Ecotype

This is variety of a species that has special inherited characteristics which allow it to thrieve in a particular habitat (Luck, 1998).

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Trypanosoma, meaning tendril-like body and referring to how the undulating membrane wraps around the organism, was given as generic name in 1843 by Hungarian doctor Gruby to *Trypanosoma rotatorium*, the organism seen in a frog (Itard, 1989). The genus of kintoplastid parasites is known to cause African Animal Trypanosomosis, African human Trypanosomosis (African human sleeping sickness) and American Trypanosomosis also known as chagas disease.

Sub-genera having species involved in domestic ruminant animal infection were identified about a century ago, as *Trypanosoma (Trypanozoon) brucei* by Plimmer and Bradford in 1899, *Trypanosoma* (*Nannomonas) congolense* by Broden in 1904, and *Trypanosoma* (*Duttonella) vivax* by Ziemann in 1905 (Itard, 1989; Stevens and Brisse, 2004). Other species related to these are known which can be involved in infection of man, domestic and wildlife. However, some species in Africa cannot be identified as any currently recognised species (CFSPH, 2005).

The great majority of Trypanosomes are non-pathogenic and live at peace with their hosts, some of which carry the infection for years (Itard, 1989; Symth, 1996; Luckins 1998). Non pathogenic trypanosome of ruminants include *T. melophagium* in sheep (Georgi and Georgi, 1990), *T. theileri* and *T. ingens* in cattle (FAO, 2005).

However, pathogenic African Trypanosomes wreck havoc in endemic areas where trypanosomosis associated with them is a debilitating and commonly fatal disease of domestic cattle and small ruminants (ILRAD, 1993). All the pathogenic trypanosomes in Africa are in section salivaria with subgenera Duttonella, Nannomonas, Trypanozoon and Pycnomonas. But section stercoraria with subgenera megatrypanum, Herpetosoma and schizotrypanum comprise slightly or non pathogenic species although some highly pathogenic stercorarian species occur outside Africa (Itard, 1989). Upon introduction of parasite into the host through natural or experimental infections, physical and metabolic

relationships and interactions between the parasite and host occur such that parasite could adapt to host which is susceptible or fail to adapt where host is insusceptible (Symth, 1996).

Protein related factors play critical roles in infection. For instance, proteins such as proteases act as factors to enhance invasion of tissues while variant surface glycoproteins of trypanosome act as factors to confound host immunity (Dorelson, 1988).

In general, complex and changing host and vector environment confront the trypanosome (ILRAD, 1988). It uses complicated developmental cycle in the two different hosts; the mammalian species and arthropod vectors where it undergoes serial changes in form. These include slender or broad trypanosome forms in the blood which were thought to reflect the complex relationship between parasite and host tissues in *T. cruzi*. In African human Trypanosomosis, host-parasite relationship marked by a poor adjustment between man and *T. brucei rhodesience* that quickly kills host and is considered less efficient than *T. brucei gambiense* which is slower in destroying the host so its relationship is adjudged much older in evolutionary terms (Jordan 1986).

Trypanosomes are highly reactive parasites not known to be idle in the host animal. Indeed, phenomena of fundamental biological significance first discovered in the trypanosome include antigenic variation, glycosphospho-inositol anchors, eukaryotic polycistronic transcription, trans-RNA splicing, Mitochondrial RNA editing and distinctive organelles such as the kinetoplast and the glycosome (EL-sayed and Donelson, 1997).

Biological adaptations of trypanosomes are therefore fascinating. But equally fascinating is the fact that among animal hosts, trypanotolerant animals control parasitaemia better, have less severe anaemia and organ damage (Radostits *et. al* 2000). Trypanotolerant breeds are therefore able to survive and remain productive under the trypanosomosis risk (Agyemang, 2005).

Since there are different degrees of trypanotolerance, one must not only identify the phenomenon but also measure it both between animal breeds and within breeds (Verhulst and Pandey, 1998). Apart from the varying susceptibility shown by different breeds of a host species, there is also marked variation in the virulence of various strains of each trypanosome (Anosa, 1991). Indeed, African trypanosomes are among parasites

with an incredible potential for variation (Majiwa, 1998). The behaviour of one trypanosome specie may differ among its different strains within the system of a given host animal specie and among its different breeds. The infection in different animals could therefore be expected to show variation in responses.

Among animals, the ranking of genotypes can vary across environment such as single or mixed infections (Drinkwater and Hetzel, 1991) which may involve haematic species like *T. congolense* or *T. vivax* and humoral species like *T. brucei* (Mutayobu *et.al.*, 1989, Jeffeoate and Holmes 1997, Abubakar *et.al.*, 1999). This variable performance of genotype has given rise to the situation whereby in cattle, there is a slow progressive trend towards more cross breeding in choice of breed under trypanosomosis risk (Zakpa *et.al.* 2001).

Between the two main groups of goat the brevipes (short legged) which gave rise to the West Africa Dwarf breed in Fouta Djallon region of Guinea were the first to enter Africa about 5000BC and lived in humid tsetse infested forest environment to which they adapted unlike the Longipes (long legged) found in the more arid Sahel and Savanna areas (Leak *et. al*., 2002). Keeping of these goats has been a valued traditional and modern occupation. Indeed, goats along with poultry are by far the most important animals kept by households and small holder farmers in the humid tsetse infested areas of Nigeria (ILCA, 2005). Three important breeds of goats found in Nigeria are the Sahel goat which is confined to arid area around Maiduguri, the more widely distributed West African Dwarf and Red Sokoto goat (Okonkwo *et. al*., 2011).

Fig: 2.1 Distribution of Red Sokoto goats in Nigeria

Source : Blench (1999).

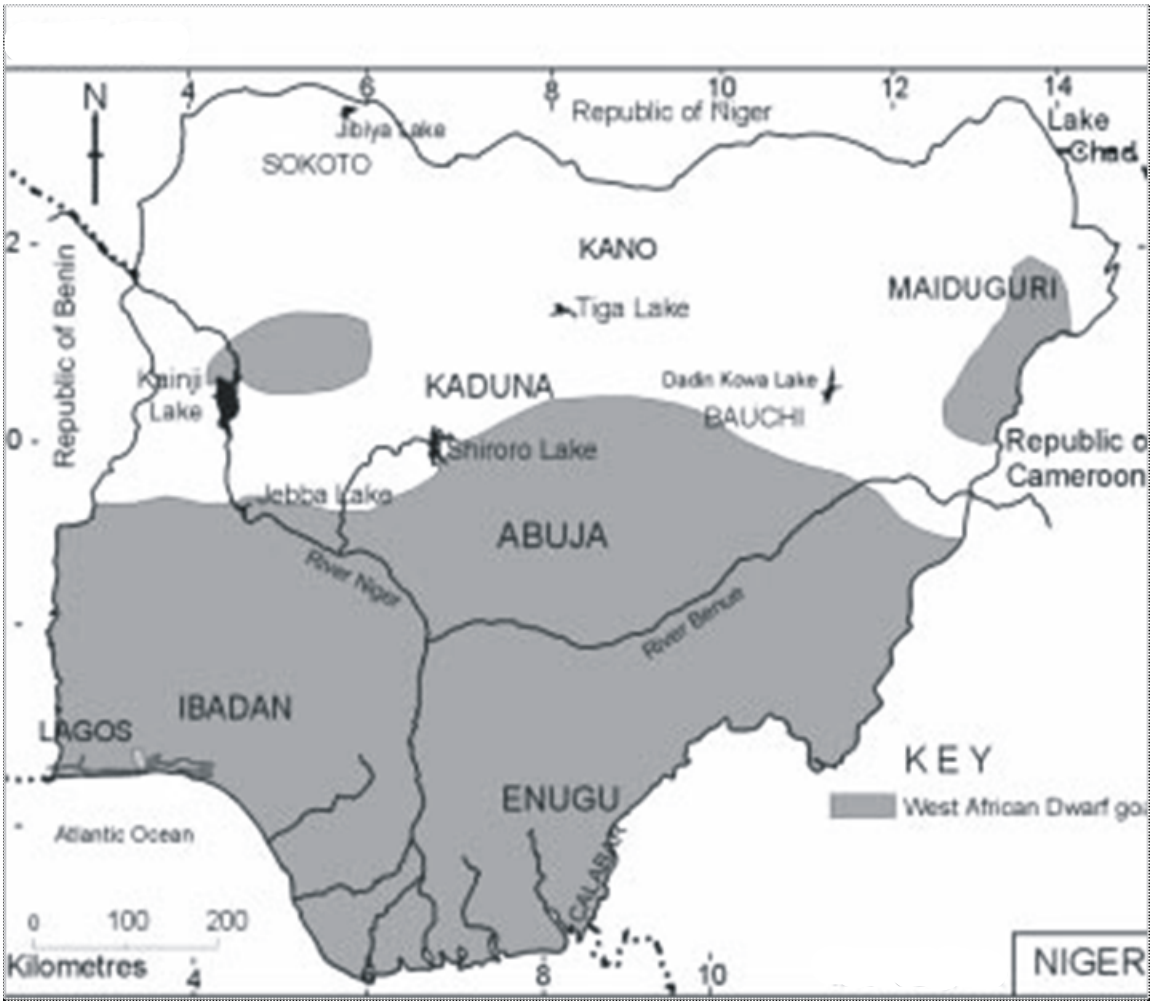


Fig. 2.2 Distribution of WAD goats in Nigeria

Source: Blench (1999).

The two more widely distributed West African Dwarf and Red Sokoto breeds constitute the major types of goat found in Nigeria (Steele, 1996). Distribution of these two breeds of goat overlap in the Guinea Savanna vegetation zone (Blench, 1999). The zone envelops Nigeria's middle belt states. That includes Benue State where Makurdi which serves as experimental site for this investigation is located. It equally has Nassarawa and Plateau State which respectively contain Karu and Federe being the sites where trypanosome isolates used in the work were obtained.

These middle belt states of Nigeria are known to be endemic to trypanosomosis due to presence of thick vegetation in the area (Lamorde, 1991). A cursory observation would reveal that while Makurdi the experimental site is located within this trypanosomosis endemic zone and is at interphase of distribution of WAD and RSG breeds, both types of goat survive and reproduce in the area. In this area as elsewhere in Nigeria, goats are kept by several households as source of income and insurance against crop failures (Uza *et. al.,* 2005). However, genetic resources of Livestock are threatened by erosion as local disease resistant and adopted breeds become replaced by others (Akinyemi and Salako, 2012). Accordingly, it has been reported (Leak *et.al.,* 2002) that West African Dwarf goats are found to change phenotypically and probably genetically in North-South transects across West African countries, which might be due to intromission of genes of trypanosusceptible breeds into their population with consequent mortalities during experimental trypanosome infections. Red Sokoto goat is a more homogenous breed since an attempt was made to standardise the breed in the old Sokoto state so as to maintain the quality of morocco leather produced by this goat (Blench, 1999).

2.2 African Animal Trypanosomosis

Pathogenic trypanosomes are responsible for some pathological entities that are given vernacular names like Nagana (African trypanosomosis), Sura and Dourine (Taylor and Authie 2004).

The group of diseases due to typical African trypanosomes namely *T. vivax, T. uniforme, T. congolense, T. simiae, T. brucei* and *T. suis* which are cyclically transmitted by tse-tse flies (Glossina species) is termed Nagana (Itard, 1989, EL-Sayed and

Donelson, 1997; Mare, 1998; Encyclo, 2011).The term Nagana is unscientific and was derived from a Zulu term meaning 'to be in low or depressed spirits' (Infonet, 2011).The Trypanosomes involved cause disease either as single species or simultaneous infection with one or more species (Mare, 1998). The most important Trypanosome species in economic terms are those which infect cattle, sheep and goats in Africa namely *T. congolense, T. vivax* and *T. brucei* (ILRAD, 1988). This is especially because Africa has 11% of the world cattle population, and 26% of the world small ruminant population making the ruminant group the most important on the continent as it comprises 82% of total livestock biomass (d'Ieteren *et.al.,* 1998).

2.3 Pathogenic Trypanosome Species Involved in Single and Mixed Infections of Goats

The major tsetse transmitted trypanosome species of cattle, sheep and goats are *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei* (Olatoye and Ogundipe, 2003). These cause debilitating and commonly fatal disease of domestic Ruminants (ILRAD, 1993A). Trypanosoma vivax is the specie mostly encountered in Livestock infections in West Africa and is the most important specie while *T. brucei* is the least common, detectable mostly in sheep and goats (Kalu and Lawani, 1996, Omotainse *et. al*., 2000). However, in East Africa *T. vivax* is less pathogenic than *T. congolense* which is the single most important cause of African animal Trypanosomiasis in that region (Mare, 1998).

Among the African Trypanosomes occurring outside the tsetse belt of Africa, non-cyclically transmitted *T. evansi* and *T. vivax* are the most extensively distributed trypanosomes responsible for diseases in livestock (Uzcanga *et. al.*, 2002). In the case of *T. vivax,* it was transported from Africa to West Indies, Central and South America inside Senegalese zebu cattle imported into French Guiana and the West Indies in 1830 (Itard, 1989). The spread of *T. evansi* from Africa to Asia and South America was facilitated by its suitability for mechanical mode of transmission by blood sucking insects rather than cyclical transmission by vector insects since it lacks KDNA maxi circle (Ventura *et. al.,* 1997; Williamson *et. al.,* 1996).

2.3.1 *Trypanosoma (Trypanozoon) brucei* infection

Trypanosoma (Trypanozoon) brucei, is the only kinetoplastid known to undergo genetic exchange (Gibson, 2001), being a parasite that is intermediary on a scale of sexuality/clonality (Tibayrenc, 1995; Tibayrenc, 1997A).

The parasite occurs in three blood stream forms, the slender form, the stumpy form and the intermediate form, a phenomenon described as polymorphism or pleomorphism (Smyth, 1996). The specie has three sub-species possessing similar blood stream forms. These are *T. brucei brucei* which affects all domestic and wild animals but not man, *T. brucei gambience* which causes chronic sleeping sickness of man in West Africa and *T. brucei rhodesiense* the cause of acute sleeping sickness of man in East Africa (Itard, 1989). Cattle are the main reservoir for *T. brucei rhodesiense*, by contrast with *T. brucei gambiense* in West Africa where there appears to be no epidemiologically significant animal reservoir (Hutchinson *et. al*., 2003). Apart from cattle, other domestic animals kept in *T. brucei rhodesiense* endemic area such as sheep, goats and pigs also acquire the trypanosome and are important in the transmission cycle (Ng'ayo *et al*, 2005).

2.3.2 *Trypanosoma (Nannomonas) congolense* infection

Trypanosoma (Nannomonas) congolense comprises organisms that are morphologically identical but genotypically heterogenous being identified as *T. congolense* Savannah type, T.congolense West African Forest/riverine type, *T. congolense* Kilifi type and T.congolense Tsavo type (Majiwa *et. al.*, 1993). T. *congolense* is probably a clonal species (Tibayrenc 1997). Different genetically distinct *T. congolense* differ in their pathogenicity (Bengaly et al. 2002). Though morphologically similar, such genotypically heterogenous *T. congolensce* express different phenotypes in terms of vector and host specificities and disease symptoms (Majiwa *et al*., 1993). A regional variation explained by heterogeneity was seen in reaction of the small East African goat following *T. congolense* infection, which reflected in the levels of Parasitaemia, severity of anaemia, weight loss and mortality (Mutayoba *et. al.*, 1989). While *T. congolense* infected Sahelian goats showed severe drop in PCV followed by death (N'doutamia *et. al.*, 2002). West African dwarf goats were not severely affected,

where it was reported that all infected Red Sokoto goats by 11 days post infection (Adah *et.al*., 1993).

2.4 Structure of Trypanosomes

Trypanosomatid cellular forms include Amastigote, promastigote, epimastigote and trypomastigote, the names being derived from Greek word 'mastig' meaning whip, which refers to the trypanosome's whip-like flagellum (Wikipaedia, 2012).

2.4.1 Morphology of Species

The major tsetse transmitted trypanosomes species of cattle, sheep and goats are *Trypanosoma congolense, Trypanosoma, brucei* and *Trypanosoma vivax* (Olatoye and Ogundipe, 2003; Mare, 1998).

2.4.1.1 *Trypanosoma (Trypanozoon) brucei*: Plimmer and Bradford, 1899

This has the subspecies *T.T. brucei brueci*: Plimmer and Bradford 1899, T.*T. brucei gambiense*: Dutton, 1902 and *T.T brucei rhodesiense*: Stephens and Farthan, 1910 among others in the subgenus (Itard, 1989) which bears *brucei* in the binomial name as a tribute to David Bruce who discovered the parasite as agent of Nagana in Zululand (Vickerman, 1997). *Trypanosoma brucei* (Sensu lato) comprises sub species with Long slender (23 – 30µm), short stumpy (17 – 22µm) and intermediate forms of varying lengths (FAO, 2005). A very well developed undulating membrane, pointed posterior end and sub- terminal kinetoplast (Uquhart *et. al*., 1998, FAO, 2005)

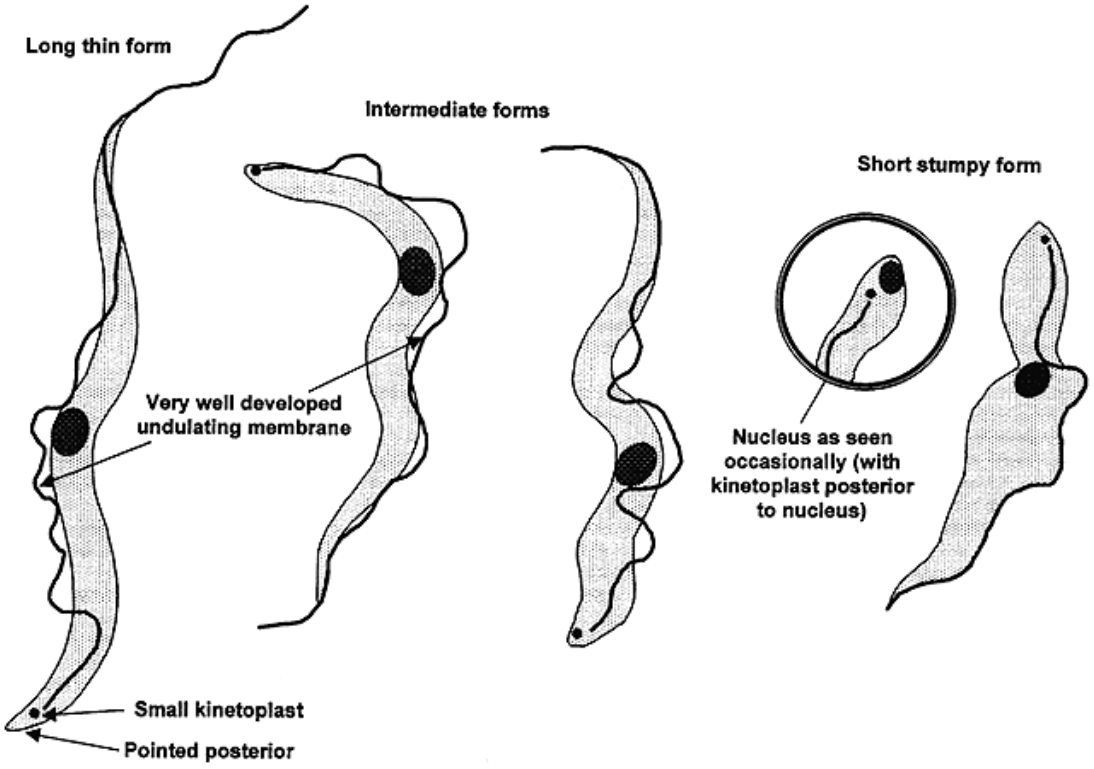


Fig: 2.3 ***Trypanosoma brucei***

Source: FAO (2005)

2.4.1.2 *Trypanosoma congolense*

T. congolense is a monomorphic parasite with inconspicuous undulating membrane, no free flagellum, and marginal kinetoplast having variable length (18µm) that reaches 25µm in the longer variants so called dimorphic strains. (FAO), 2005). From repetitive DNA sequence variation, different molecular types namely, *T.congolense* savanna type, *T. congolense* Tsavo type, *T. congolense* West African Forest/riverine type and *T. congolense* Kilifi type have been identified as those, whose DNA probes could not hybridise with each other and other trypanosomes (Majiwa *et. al*., 1993).

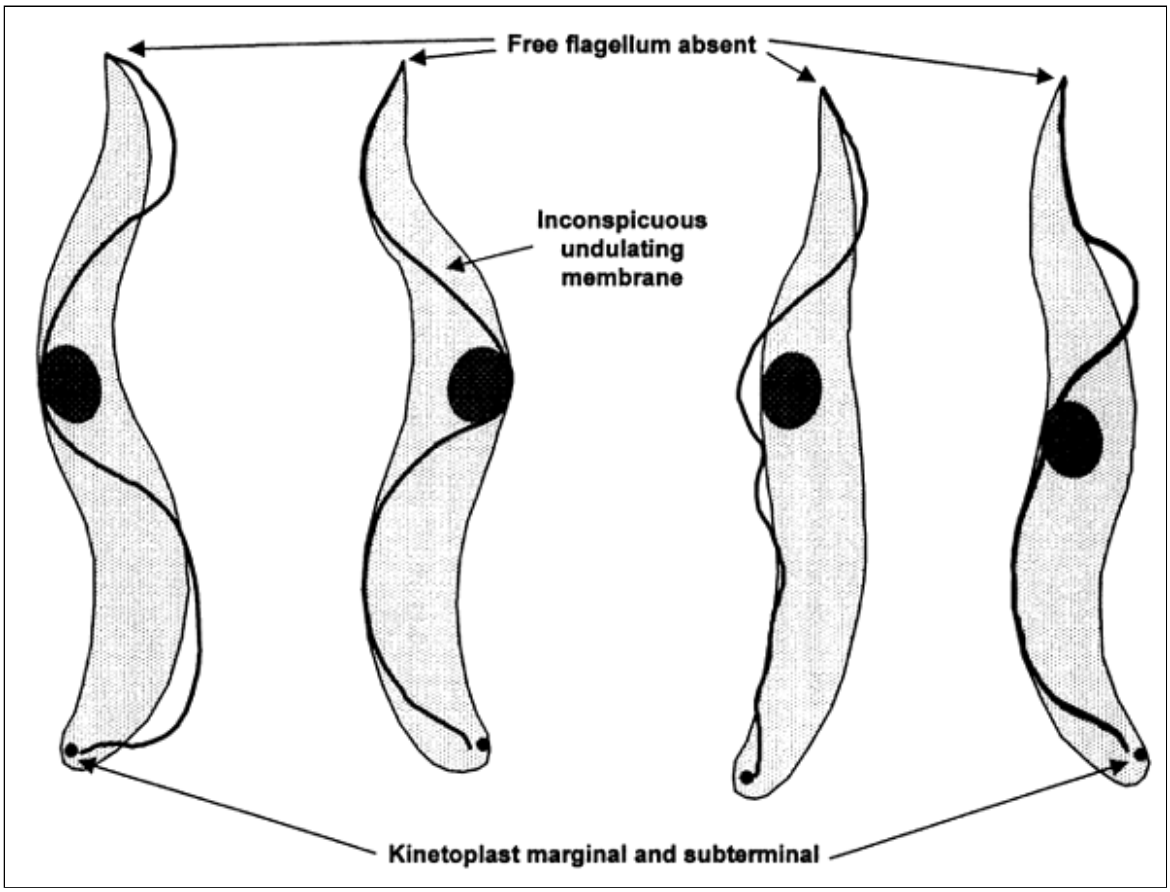


Fig: 2.4 ***Trypanosoma congolense***

Source: FAO (2005)

2.4.1.3 *Trypanosoma vivax*

These are monomorphic trypanosomes whose blood stream forms have expanded or club shaped posterior end tapering towards anterior end with large terminal kinetoplast and rapid movement in wet blood films (Stevens and Brisse, 2004). There is a free flagellum and its length including the flagellum varies from 18 - 26µm while the closely related *T. unforme* is smaller, reaching only 12 – 20µm (FAO, 2005). Laboratory rodents are usually not susceptible to *T. vivax* and *T. uniforme* but recent finding of strains that infect rodents reveal longer more granular blood stream forms with sub terminal kionetoplast (Stevens and Brisse, 2004).

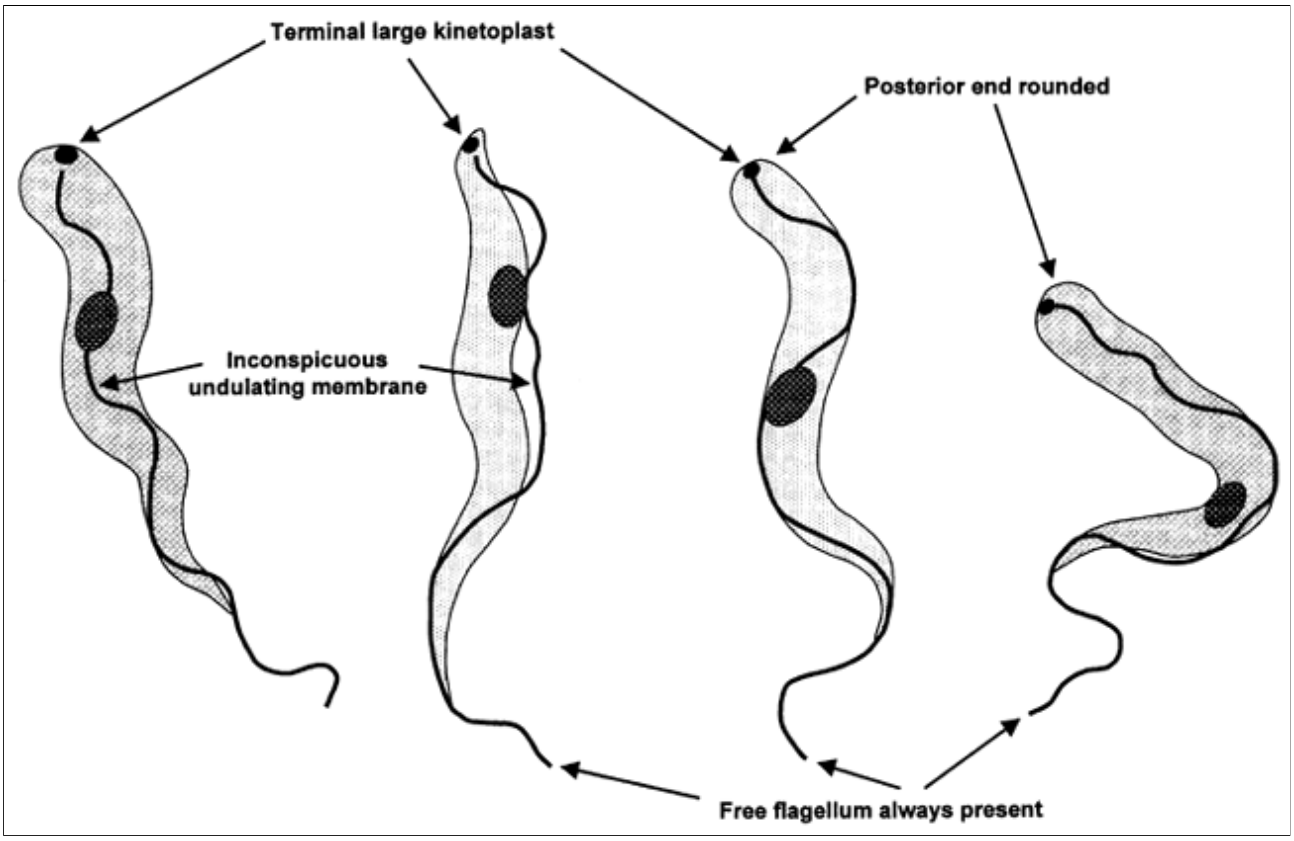


Fig: 2.5 ***Trypanosoma vivax***

Source: FAO (2005)

2.4.2 Trypanosome Genetics/Genome

The genome is the nucleic acids representing entire genetic information of an organism e.g. the 46 chromosomes of humans (Otokunefor and Otokunefor, 2009). In some prokaryotic micro-organisms, a single strand of DNA seem to be the store for the genetic information and average gene in *E.coli* has about 1200 nucliotide pairs while the entire DNA molecule of bacteriophage has over 5000 nucleotide pairs (Dhanotiya, 2004). In the case of mammals, the total genetic constitution carries about 50,000 to 100,000 genes in the cell with about 3 billion base pairs of information (ILRAD, 1991).

The nuclear organization of trypanosomes differs from that of higher eukaryotes since there is lack of chromatin (chromosome) condensation during metaphase and nuclear membrane persists during cell division. Their nucleus has at least 120 chromosomal associated DNA molecules grouped by size into two classes with about 20 rehular DNA molecules ranging from 208 to 5700 kb and 100 minichromosomal DNA molecules of 50 to150 kb (EL – Sayed and Donelson, 1997). The 20 chromosomes were described as 'regular' chromosomes by Cross (1996) who gave their number as approximately 12 pairs of DNA molecules having 150 to 5,700 kb which add to the 100 mini chromosomes having 25 to 100 kb as observed from *T, brucei* on pulsed – field gel electrophoryesis. The mini chromosomal DNAs which contain a highly repetitive 177 base pair sequence as well as unexpressed telomere – linked VSG genes, may serve primarily as repositories for spare VSGgenes (EL – sayed and Donelson, 1997). Trypanosome genome contains repeated DNA sequences whose functions are unknown while some of these sequences have been cloned and used as probes for molecular identification of the organisms at the level of subgenus or species, their repetitive nature offering high signal and hence high sensitivity (Diallo, 1991).

2.4.2.1 Mitochondrial Genome

Within their mitochondrion, trypanosomes and Leishmania posses a unique structure called kinetoplast (kinetonucleus), which used to be also called parabasal body (Smyth, 1996). In addition to mitochondrial, DNA as is found in most nucleated cells, the kinetoplast contains a large network of small circular DNA molecules known as minicircles. The function associated with kinetoplast DNA is to help "edit"

mitochondrial RNA to produce genetic codes that will be transcribed into functional mitochondrial proteins, that is, mitochondrial RNA editing (ILRAD, 1993a).

2.4.2.2 Genetic Control of cell Division and RNA Editing

The central gene regulating cell division in the trypanosome, Cdc 2 differs significantly from the corresponding host gene. Trypanosomes also differ from their hosts in having a single mitochondrion which necessitates the coordination of parasite processes involved in mitochondrial and nuclear division whereas other organisms having multiple mitochondria do not need to coordinate these events (ILRAD 1993b).

Eukaryotic pre MRNAs are spliced to form mature MRNA, alternative splicing sites occurring across introns or exons to produce different MRNA isoforms. RNA editing and trans – splicing are some of the molecular mechanisms regulating alternative splicing (*Zhang et.al.,* 2004). RNA editing encompass a variety of alterations of RNA primary squence that arise from base modifications, nucleotide insertions or deletions and replacements, so that the definition of RNA editing has evolved as new systems are described (Koslowsky 2004).

Current evidence suggests that post – transcriptional control of nuclear gene expression (control exerted after the generation of mRNA transcript) is important in trypanosomes and requires direct interaction between proteins and MRNA transcripts. Parts of kinetoplast DNA minicircles are transcribed into 'guide' RNA molecules, which participate in the editing process (ILRAD 1993).

2.4.2.3 Genome Replication and Transcription

The important difference between replication and transcription is that during replication the entire chromosome is copied yielding daughter DNA identical to parent DNA but in transcription not the entire cell DNA is necessarily transcribed, usually only individual genes are transcribed. Therefore, transcription of DNA is selective being turned on by regulating sequences, which indicate the beginning and end of DNA segment to be transcribed (Dhanotiya, 2004).

The understanding that there was a polycistronic length of DNA coding for several proteins (peptides) or genes (Coombs, 1992) in eukaryotic transcription was first

associated with trypanosomes (EL – sayed and Donelson 1997) where a long polycistronic operon known as an expression site (ES) is found in blood stream forms (Cross 1996).

2.4.2.4 Blood Stream Expression Sites (BESs)

The genome of *T. brucei* contains about 1000 VSG genes, many of which may be incomplete or interrupted by termination codons rather than non coding *(trypanosome genes contain no introns)* yet only one VSG is expressed at a time (Cross, 1996), The variant surface glycoproteins are expressed individually presenting a complex gene control problem that is solved by transcription being possible only at specific telomeric loci called Blood Stream expression Sites (BES) which are polycistronic transcription units under the control of a strong promoter. There are multiple BES in the genome but only one is active at a time (Robinson *et.al*., 1999).

Within the *T. brucei* polycistronic transcription units of VSG gene are linked homologous expression site associated genes (ESAG) such as ESAG 7/6 pair of gene encoding transferin receptor (which receptor is usually hidden in flagellar pocket) and another gene family ESAG 4 encoding cell surface adenylate cyclase as member of the large gene encoding the cyclase isoforms. This shares the same receptor – like structures which may be found as well on flagella surface (Pays *et. al*., 1997). Usually, among the thousands of genes carried in each mammalian or parasite cell, only a fraction at any one time is expressed, that is, transcribed into MRNA and then translated into proteins (ILRAD, 1992). All the expression sites (ES) appear to be structurally and functionally similar but the transcription unit appear to be quite accommodating. ES sizes vary, ESAG duplications occur in some ES_S and alien genes can be inserted at will into silent and active ES_S (Cross, 1996). Lateral transfer of genes as distinct from reproducing (from one generation to next) has been achieved in trypanosomes through vectors, transfecting being achieved by process of electroporation (ILRAD, 1992).

There have been studies in attempts to understand how switching variation occurs within trypanosomes to achieve antigenic variation. The genetic rearrangements underlying the phenomenon of antigenic variation occurring at the ESs have attracted a complicity of published mechanisms some of which probably are artefactual or

insignificant in real infection (Barry, 1997). Robinson et. al. (1999) explained that the best approach to identify the most active switch mechanism is by the analysis of single relapses in which an infection with a single variant relapses to a second peak from which clones are isolated and their activation mechanisms are identified. They listed six possible of such mechanisms for activation of blood stream expression sites (BES) towards achieving antigenic variation. These mechanisms have been grouped into three categories as:

(a) In situ mechanism operating by transcriptional switching between BES, which effects a switch between VSGs occupying such sites.

(b) Homologous recombination's such as duplication of a silent gene into BES and concurrent deletion of VSG resident at that site. There is either (1) duplicative transposition (if silent gene is located within a chromosome or (2) telomere conversion (if silent gene is located at telomere). (3) Reciprocal recombination involving exchange of VSG sequences between chromosomes. (4) Mosaic gene formation involving the splicing together of segments of VSGs or pseudogenes.

(c) Point Mutagenesis.

2.5 Life cycle, Transmission and Infection

Trypanosomes multiply in the mid gut or proboscis of biological vector (ILRI, 2009) but do not multiply in mechanical vector such as non-tsetse biting flies (Urquhart *et. al.*, 1998). The bite of infected vector introduces trypanosome into mammalian host where the organism first multiplies at local site which may swell, known as Chancre before spreading to other tissues and blood stream (Jordan, 1986)

2.5.1 Transmission and Infection

The cycle of *T. congolense* involves tse tse fly mid-gut and probosics lasting two weeks while that of *T. brucei* is more complex and lasts three weeks or more, involving mid-gut and Salivary glands (Radostits *et. al.*, 2000). These kinetoplastids attach to epithelium of salivary gland or the cuticle of probosics through the enlarged flagellum whose membranes are anchored by plaques resembling hemi-desmosomes (Bastin et. al., 1996). Natural transmission by the tsetse vectors occurs over one third of Africa

occupying some of the best watered lands where susceptible ruminants have to be kept under close veterinary supervision, given frequent and costly treatment (ILRAD, 1989).

In natural infection, the animals acquire the trypanosomes on exposure to the vectors in the field. Barriers exist in the vector which must be overcome by the trypanosome. Such a barrier, Trypanolysin present in tse tse mid gut kills procyclic and bloodstream trypanosomes (Osir *et. al.*, 2001). So only a low proportion, of tse tse flies are infected and could transmit trypanosomiasis given as 0.1 – 0.4% (Seifert, 1992), 1 – 20% (Unquhert *et al.*, 2008) Mechanical vectors like tabanid flies can increase infection rate of animals (Rahman 2002).

Trypanosome infections have been experimentally achieved by natural (cyclical) and non-cyclical (artificial) methods (Whitelaw *et. al*., 2006). In the natural experimental method, Naessens *et.al.*, (2003) shaved sites on the left flank of each animal to be infected, introducing tse tse flies to the sites in 7 cm x 3 cm tubes with netting at one end, which was attached to the animal. These flies were allowed to feed until becoming engorged. The infective status of the flies was ascertained after inducing them to salivate onto warmed glass slide. Artificial syringe transferred infections have been more common. The Inoculation of trypanosomes by transfer of infected blood from diseased to healthy animal was known and mentioned by David Bruce at the time he discovered N'agana (Vickerman, 1997). Different routes have been used. Biryomumaisho *et.al.*, (2003) employed sub-cutaneous inoculation of goats using 2ml of blood containing approximately 101 trypanosomes per ml. Rabo and Oyejide (1996) inoculated animals intravenously using $1x10^6$ trypanosomes. Intradermal inoculation of T. vivax was as effective as the bite of infected tsetse in transmitting the disease (ILRAD, 1984).

2.5.2 Monomorphic and Pleomorphic Trypanosome Lines

Trypanosome lines called Monomorphic lines which are adapted by rapid syringe passaging to continual growth under laboratory conditions routinely yield only the proliferative blood stream form but do not develop through life cycle stages like the unadapted pleomorphic lines (Robinson *et al*., 1999). The non-dividing stumpy forms transmitting infection to tsetse flies are lacking (Barry, 1997). Spontaneous Variant Surface Glycoprotein (VSG) switches allow the trypanosome to achieve its potential to

express hundreds of different VSGs although only one is actually expressed at a time before being changed eventually to evade antibody responses (Rudenko *et al.*, 1998).

Different VSGs have entirely distinct N-terminal sequences while each VSG is encoded by a distinct gene or in pieces of different genes, some interstitial in loci within chromosomes others sub-tolemeric at end of chromosome but all kept silent until activated (Barry and Carrington, 2004; Borst, 2002). Non-adapted or pleomorphic trypanosomes have an active VSG switch mechanism involving gene duplication that is depressed or from which a component is absent in monomorphic lines resulting to VSG switch rates about 4 or 5 orders of magnitude lower than those of non adapted lines (Robinson *et al.*, 1999).

2.5.3 Vector of Trypanosomes

The well known biological vector of African Trypanosomes: Glossina species are commonly called 'Tsetse', the word of Tswana tribe of Botswana for this family of flies (Jordan, 1986).

A number of species of tsetse fly occur in Africa, given as 22 species (Omoogun, 1991; Omotainse *et. al.*, 2004), 30 species (Minter, 1989; Urquhart *et. al.*, 1998). Eleven species occur in Nigeria, shown below by grouping into sub-species:-

Table 2.1: *Glossina* Species occurring in Nigeria

Morsitans group
Glossina morstans Submorsitans. Newstead,1910
Glossina longipalpis. Wiedemann,1830.
Palpalis group
Glossina palpalis palpalis. Robineau-Desvoidy, 1830
Glossina tachinoides. Westwood, 1830
Glosinna pallicera pallicera. Bigot, 1891
Glossina calliginea.Austen, 1911.
Fusca group
Glossina tabaniformis.Westwood, 1850.
*Glossina nigrofusca nigrofusca.*Newstead, 1910.
Glossina medicorum. Austen, 1911
Glossina fusca congolensis.Newstead and Evans, 1921.
Glossina haningtoni.Newstead and Evans 1922.

Source: Omoogun(1991).

Out of these species, four are important as transmitters of trypanosomosis in Nigeria namely, *Glossina submorsitans, Glossina tachinoides, Glossina palpalis and Glossina longipalpis* (Omotainse, *et. al.*, 2004).

Trypanosomes have interactions with tsetse fly vectors. Following challenge by Trypanosomes, tsetse flies also mounted an immune response by elaborating humoral antimicrobial peptides (AMPs) which were identified by Boulanger *et al.*, (2002) to include Cercopin, attacin and defensin.

Among the four species transmitting trypanosomes in Nigeria, *Glossina morsitans submorsitans* and *Glossina longipalpis* are on decline while *Glossina palpalis* and *Glossina tachinoides* are expanding from riverine to peridomestic areas (Omotainse *et.al.,* 2004). It has been noted by Agyemang (2005) that tsetse distribution relates to changes in climate, changing over time so its population density and distribution are area and time specific making regional or national generalizations misleading. Thus highlands like Jos, Mambila and Obudu Plateau previously considered tsetse and trypanosomosis free are now infested (Omotainse *et. al.,* 2004).

Mechanical vectors of trypanosomes comprise biting flies like Tabanidae, Stomoxyinae and Hippoboscidae which could transmit the parasite in their mouth parts if they feed on more than one host within a short interval (Radostits *et. al.,* 2000; Itard, 1989). This mode of transmission has proved to be sufficiently effective to maintain *T. Vivax* and *T.evansi* outside Africa in South and Central America as well as Asia in case of *T. evansi* (FAO, 2005)

2.6 Pathology

Distinct pathological changes may be caused by the different livestock – infective trypanosome species and pathology in tissues is associated with the relative ability of the parasite to invade extravascular spaces and tissues (Taylor and Authie, 2004). Gross changes observed in trypanosomosis are not pathognomonic, including emaciation, splenomegaly and hepatomegaly, enlargement of lymph nodes, atrophy of body fat, testicular atrophy and corneal opacity (Anosa, 1991).

The cardinal lesion of trypanosomosis is anaemia, which is initially proportional to parasitaemia, that eventually becomes low while anaemia either persist or resolve, being haemolytic in expanded mononuclear phagocyte system (Urquhart *et. al.,* 1998).

Anosa (1991) grouped lesions in trypanosome infection into three:-

Firstly, those associated with response to stimulation of host by trypanosome antigens, found in lymphoid organs and characterized by proliferation of plasma cells, macrophages and lymphoid hyperplasia.

Secondly, Lesions associated with increased destruction of blood cells in haemopoietic organs (spleen, liver, bone marrow, haemolymph nodes) such as erythrophagocytosis, haemosiderosis, phagocytosis of leucocytes and thrombocytes.

Thirdly, Lesions associated with extra vascular localization of parasites in tissues with tissue destruction and mononuclear cell infiltration. In the cause of infection, the immune response is vigorous and immune complexes course inflammation, which contributes to signs and lesions of the disease (Kahn and Scott, 2010).

2.6.1 Pathology in *T. vivax* Infection

T. vivax multiplies rapidly in blood and is dispersed throughout the cardiovascular system (Radostits *et. al*., 2000). Variation in its virulence occurs with infective dose, immune status of host (Taylor and Authie, 2004). Microscopic features reported in *T. vivax* infection were active follicles with enlarged germinal centers in lymph nodes and spleen, hyperplastic lymphocyte, haemosiderin laden macrophages in spleen, lymph node and liver necrosis with erythrophagocytosis by Kupffer cells (Ibrahim *et. al*., 2005).

2.6.2 Pathology in *T. brucei* Infection

T. brucei escapes from the capillaries to invade interstitial tissues, serous cavities and cerebrospinal fluid where its multiplication continues (Radostits *et. al.,* 2000). Despite being likely to infect other tissues including central nervous system, *T. brucei* is less pathogenic that *T. vivax* and *T. congolense* in cattle (Taylor and Authie, 2004). Lesions found in association with invading parasites occur in the heart, kidney, testes, muscles, skin, adrenal glands, pituitary glands and brain where perivascular mononuclear cell infiltration is characteristically found in *T. brucei* infection (Anosa 1991).

2.6.3 Pathology in *T. congolense* Infection

T. congolense aggregates on small blood vessels and capillaries of the heart, brain skeletal muscles and rarely causes heavy parasitaemia (Radostits *et.al.,* 2000). Parasitaemia in both *T. congolense* and *T. brucei* is generally lower than *T. vivax* and in chronic stages remains below threshold detection using standard techniques (Taylor and Authie 2004). *T. congolense* is the commonest cause of infection in small ruminants (Jordan, 1986; Samdi *et. al.*, 2010).

Like *T. vivax*, lesions due to *T. congolense* are due to anaemia, which is an important factor in the pathology of these infections (ILRAD, 1989).

2.7 Influence of Trypanosome Infection on Productivity

Direct impact of the disease on livestock productivity (mortality, fertility, milk yield, ability to work as traction animals) and expenditure incurred on control activities adds to indirect impact which include the way presence of disease limits production opportunities as well as influence choice on livestock keeping and migration (Shaw 2004). About 37 percent of Africa's land mass or 11 million km^2 in 37 countries is infested with tsetse where as 65 percent of this area (7 million km^2) could be used for livestock or mixed agriculture development without stress to the environment if trypanosomosis was controlled (Agyemang, 2005). Presence of the disease therefore negatively influences productivity of Livestock and mixed farming on some of the best lands in Africa.

2.8.0 Immunity in Trypanosomosis

Genetic variation in disease resistance of farm animals can be observed at all levels of defence against infectious agents. Disease resistance is achieved by vaccination and or genetic improvement through breeding strategies. Presently these strategies include modifications of genome such as improvement in candidate genes like MHC genes, T cell receptor genes, immunoglobulin genes, genes encoding lymphokines and specific resistance genes (Brem and Muller, 1991). Generally, there is a failure of the immune system to eliminate a trypanosome infection because of the adaptive responses of the parasite to the immune mechanism of the host (Cross, 1996).

2.8.1 Immune Response

Innate immune responds to a limited number of molecules restricted to major groups of pathogens. On the other hand antigen sensitive Lymphocytes (T-helper, cytotoxic T cells and B- lymphocytes) of acquired immune system recognize and respond to a large number of diverse foreign antigens which may happen to bind specifically to one of their randomly generated antigen receptors (Tizard, 2004). Adaptive immunity detects non-self through recognition of peptide antigens using antigen receptors expressed on the surface of B and T. cells (Takeda *et. al.*, 2003). This immune response begins when helper T cells (which act as regulators) recognize antigenic components of invading organisms, become activated, proliferate and produce soluble factors which enable other effector cells of immune system to mount a protective response. For an example, cytotoxic T-lymphocytes kill cells containing the organism or B-lymphopcytes produce antibodies that mark invaders for destruction (ILRAD, 1987; Otokunefor and Otokunefor, 2009.).

The T helper cells are only capable of recognizing foreign peptides when they are associated with MHC glycoproteins. These glycoproteins are known to occur in types I, II, III etc.

The immune system includes five major systems, (a) the CD4 helper/inducer T cells (b) The CD8 (suppressor/cytotoxic) T cells, (c) The B cells, (d) The NK cells and (e) macrophages/monocytes (Targan and Shanahan, 1990). The ratio of CD4+ to CD8+ cells in blood is used to estimate lymphocyte function, elevated CD4+ imply lymphocyte reactivity but Elevated CD8 count imply depressed lymphoctye reactivity. However, CD4 is not restricted to T helper cells but is also on monocytes, macrophages, neutrophils and eosinophils in some species of animals while in humans T lymphocytes that are CD 4+ CD8- comprise 65%, CD4-CD8+ 30% and 5% are neither CD4+ CD8+ but double negative that is CD4- CD8- (Tizard 2004).

2.8.2 Innate Immune Response

Host defense relies both on innate and adaptive immunity of which the innate immunity is an evolutionary, ancient defense mechanism.It’s similarities are found between Drosophilia and mammals in its pathogen recognition, signaling pathways and

effector mechanisms. This point to a common ancestry (Hoffman *et.al.,* 1999). It distinguishes self from non-self, has links with adaptive immunity and acute inflammation is its central feature. It recognizes invading organism by pattern – recognition receptors that bind and recognize conserved molecules on surfaces of the organism. The most important of these many receptors being Toll – like receptors (TLR), a family of at least 10 different receptors found on the surfaces or in the cytoplasm of cells such as macrophages, intestinal epithelia and mast cells (khan and Scott, 2010).

2.8.3 Adaptive Immune Response

Adaptive immunity detects non-self through recognition of peptide antigens using antigen receptors expressed on the surface of B and T cells (Takeda *et.al.*, 2003).

2.8.4 MHC and Immune Response Genes

Whether or not and to what extent an individual responds to the antigens associated with a particular disease is influenced by the extremely polymorphic MHC genes which gives glycoprotein products that may be able or fail to form association with the antigen (ILRAD, 1987).

In order to respond to a wide range of potential antigens, B and T cells rearrange their immunoglobulin and T cell receptor genes to generate over 10^{11} different species of antigen receptors (Takeda *et.al.*, 2003). Several associations have been identified in humans and laboratory animals between MHC type and disease resistance (ILRAD, 1988). The magnitude of immune response, is controlled by immune response (ir) genes and it is believed that class II genes function as ir genes (Vegad 1995)

In mice, previous studies on survival to trypanosome infection have localized three genomic regions that regulate the trait and these three quantitative trait Loci (QTL) have been called Tir 1, Tir 2 Tir 3 (for Trypanosoma infection response 1- 3) (Goodhead *et. al.,* 2010).

2.8.5 MHC Products

MHC consist of numerous genes located next to each other which together with associated genetic material could constitute up to 1/1000 of total genome Their products

are glycoproteins which occur as integral components of cell membranes, The class 1 glycoproteins on surfaces of most mammalian cells except R.B.C. and the class 11 glycoproteins occur on antigen presenting cells (ILRAD, 1987). The MHC molecules on cell surfaces may normally be partially occupied by peptides that are, self derived or formed by enzymatic cleavage.

Antigen presentation in conjunction with MHC class 11 molecules has been important in understanding how T-cell receptor 'sees' each antigen while interaction between antigen and MHC class 11 occurs at slow association rate but also at low dissociation rate (Targan and Shanahan, 1990). Cytotoxic T lymphocytes, which recognize foreign antigen on the surface of a variety of cell types, generally do so in association with antigenic components of the glycoproteins encoded by class 1 MHC genes. By contrast, the helper T lymphocytes, which 'see' foreign antigens on antigen – presenting cells, tend to recognize antigenic components of the glycoproteins encoded by class 11 MHC genes (ILRAD, 1987).

2.8.6 Lymphocyte Mitogens

T – independent antigens like polyclonal B cell activators (mitogens) can occur on organism, for instance bacterial cell wall lipopolysaccharide (Otokunefor and Otokunefor 2009). Proteins, especially lectins bind to cell surface glycoproteins and so trigger lymphocyte division as mitogens, the different mitogens having variable effects on T and B cells include plant lectins, neutral proteases and FC fragment of immunoglobulins (Tizard, 2004).

2.8.7 Signalling in Trypanosomosis

In cell there occur binding of extracellular signaling molecules (chemicals like amino acids, lipid derivatives, acetyl chloline, Peptides and Proteins) or ligands to surface receptors and even intracellular receptors (in case of some hydrophobic molecules like steroids, retinoids, thyroxine) being regarded as first messengers (Lodish *et. al.*, 2004; Berridge 1985). This causes conformational changes that induce specific cytosolic activation responses. These transduce into nucleus to regulate activity of transcription factors, the events controlling metabolic processes including growth and differentiation,

synthesis and secretion of proteins, composition of intracellular and extracellular fluids (Lodish *et. al.*, 2004)

Signal molecules operate either through autocrine, paracrine or endocrine stimulation (Snyder, 1985) to bind as ligands to receptors (e.g. growth factor receptor which can be trans-membrane glycoprotein whose intracellular domain interact with elements of the cytoskeleton to signal locomotion or differentiation). When signal molecule binds to the erstwhile receptor, quiescent cell is stimulated to induce or inhibit growth, differentiation or activate a gene depending on signaling molecule and the pathway that was stimulated (Vegad 1995). Ligand molecules from host that bind to parasite receptors or conversely, such ligands from parasite that bind to receptors on host cells can generate one type of response or the other in the cause of a parasitism.

Activation of all surface receptors lead directly or indirectly to changes in protein phosphorylation through the action of protein kinases or the opposing protein phosphatases such that the activity of a protein in a cell is a complex function of the activities of multiple kinases and phosphatases acting on it (Lodish *et. al.,* 2004). Equally, what fraction of the full genetic potential, a cell displays depend on which of its genes are turned on and which are turned off, since each cell is characterized by a specific pattern of active and inactive genes that undergo sequential changes as development proceeds (Gethring, 1985).

Major barrier to flow of information is the cell's plasma membrane where signal transduction (transfer of messages across plasma membrane) mechanism translate external signals into internal signals (whose pathways in cells are remarkably universal) that are carried by a small number of second messengers including cyclic adenosine monophosphate (cyclic AMP), calcium ions, inositol triphosphate (IP3), diaglycerol (DAG), guanosine triphosphatae (GTPases) activating protein (GARP) that activate transcription factors (Berridge, 1985; Vegad, 1995). This leads to activation or inhibition of cell growth while the changes second messengers induce in the concentration of cyclins initiate DNA synthesis and cell division (Vegad, 1995).

Among pathogens, one class of pattern recognition receptors described as Toll receptor like (TRL) are involved in detection of conserved molecules termed pathogen associated molecular patterns such as lipopolysaccharide, CPG DNA and flagellin (Harris

et. al., 2007). This TRL family induces inflammatory response and antigen specific adaptive immunity through common signaling pathways mediated by the nuclear adaptor molecule MyD88, although other pathways may also mediate TRL ligand specific biological responses (Takeda *et. al.*, 2003).

Host to trypanosome and trypanosome to host signaling events permitting interactions between trypanosome cell surface and host components occur. In this way, trypanosomes themselves direct host mediated regulation of parasitaemia as they release factors triggering production of two opposing cytokines. These are interferon gamma (IFN-ɤ) from CD8+ lymphocytes which induces their proliferation and tumor necrosis factor alpha (TNF-α) from macrophages which lyses them, thus maintaining equilibrium to permit chronic infection (Pays *et. al*., 1997)

2.8.8 Factors in Immune Response to Trypanosomes

The mammalian immune system is regulated by cell-to-cell contact and by messenger molecules secreted by cells of the immune system (ILRAD, 1993). Disturbance in homeostasis by invading parasites usually induce hosts immune system to righten it by response mechanisms whose nature is determined by a given set of cytokines which parasite- reactive naïve T cells secret. Either type I cytokines (interferon gamma IFN -, interleukin 2 (IL-2) etc.) or type 2 cytokines (IL – 4, IL-10, IL-13 etc) are expressed by the T cells in affected tissues where failure to control parasite is linked with development and expression of inappropriate rather than insufficient immune response since type I response leads to cure and stable immunity while type 2 response provides optimal environment for parasite expansion, progression of lesion and absence of immunity to re-infection in leishmaniasis (Milon, 1994).

Recent identification of toll-like receptors in mammals as a family characterized by presence of leucine rich repeat (LRR) domain in their extra cellular domain and a trypanosome infection response - TIR which resides in (Toll/IL -1 receptor) domain in their intracellular domain created awareness that innate immunity plays an important role in detection of invading pathogens as these receptors recognized specific patterns of components on pathogens, regulating activation of both innate, and adaptive immunity (Takeda *et al.*, 2003).

Relative resistance to African trypanosomes is based on the development of a type I cytokine response which is partly dependent on innate immune responses generated through MyD88 and Toll – like receptors 9 whose agonist CPG oligodeoxynucleotide (ODN) treatment resulted to enhanced protection against trypanosomes (Harris *et. al*., 2007)

2.8.9 Antigens

The term antigen is used in two senses, the first, to describe a molecule, which generates an immune response (also called an immunogen), and the second, a molecule which reacts with antibodies or primed T cells irrespective of its ability to generate them (Dhanotiya, 2004). Mole cules with high molecular weights like those ≥600,000 Daltons that are usually complex are good antigens especially the proteins and carbohydrates (Wilkinson, 1975, Dhanotiya, 2004, Otokunefor and Otokunefor, 2009).

Antigen presenting cells (APCs) like Macrophages or Dendritic cells (Tizard, 2004) could present easily degradable molecules especially proteins with few copies of different epitopes as T- dependent antigens requiring help of T- lymphocytes for their recognition by immunoglobulin (Ig) secreting B- lymphocytes. But those that are T-independent, usually also resistant to degradation which stimulate B- lymphocytes directly commonly induce IgM production and are weaker antigens either as polyclonal activators (mitogen) activating the B-lymphocytes regardless of antigenic specificity e.g. lipopolysaccharides or having many copies of similar epitopes which are non-polyclonal (non-mitogen) activators requiring participation of cytokines released by T- helper lymphocyte but not direct contact with the T- helper Lymphocyte e.g. highly repetitive proteins like flagellin. (Otokunefor and Otokunefor, 2009).

In most protozoal parasites as in mammalian cells, antigens may be water-soluble cytosolic components or membrane- bound molecules with a hydrophobicity, which requires detergents to solubilise (Maizels *et.al*., 1991). As all membrane proteins reside in a liquid bilayer, they can diffuse side ways just as the lipid molecules do. How fast they diffuse is determined in part by how liquid the membrane phospholipid matrix is. Unless they are constrained from doing so, membrane proteins in most eukaryotic cells

can therefore diffuse from one end of the cell to the other in a few minutes (Bretscher, 1985).

Many trypanosome membranes are accessible to components of the extra cellular milieu and their proteins represent potential target molecules as antigens (Burleigh, 1993). Within a specie of trypanosomes, stocks from different geographical areas occur and in any such stock different variant antigenic types, (VATs), may be found having antigenically distinct populations where by a population with same repertoire is called a serodeme (ILRAD, 1988, Smyth 1996).

2.8.9.1 Variant Antigens:- Variant Surface Glycoprotein (VSG)

Among many other immune evasion mechanisms, pathogens and parasites have evolved two major molecular strategies by which they vary the protein antigens presented to their hosts namely antigenic polymorhism and antigenic variation (Crampton *et, al.*, 1998)

During their time in the blood stream, each trypanosome is covered by about 10^7 copies of a single surface protein, the variant surface glycoprotein (VSG), which is periodically switched from one type to synthesis of another type effectively evading the immune response (Donelson, 1988; Cross, 1996; Barry, 1997). In polymorphism, parasites present diverse range of different alleles encoding the offending immunogen. Therefore, in a population of hosts where individuals display restricted immune response, different clones are capable of surviving in different host so that survival of diverse range of parasite genotypes in host population is assured as in MSPI antigen of *P.falciparum*, while Trypanosome VSG are involved in antigen variation (Crampton *et.al*.,1998).

The VSG appears to do nothing more for the trypanosome than form a densely packed replaceable physical barrier to macromolecular penetration, protecting the underlying plasma membrane from immune attack (Cross, 1996). During infection, each peak of parasitaemia usually consists of a mixture of variable antigen types, while the repertoire is expressed in a hierarchical fashion. This means that, some variant antigenic types (VATs) have tendency to appear early in infection; some during late infection and the rest in between (Robinson *et. al.*, 1999). A single trypanosome population causing infection in a single susceptible host animal will change its antigenic coat every few days.

Not only can a single parasite population display a great number of VATs, but also an animal may be infected with any of several species or subspecies of trypanosomes, each with its own set of VATs (ILRAD,1989).

2.8.9.2 Invariant Antigens

Divergence occured some 200 – 300 million years ago between the VSG bearing antigenically variant salivarians and the non-salivarians whose surfaces are coated with antigenically non – variant molecules comprising carbohdrate – rich mucin –like glycoproteins (Overath *et.al.*, 2001). It was reported that when Salivarian African trypanosomes, were collected from infected trypano-tolerant N'dama and trypano-susceptible Boran cattle, stored frozen but viable in liquid nitrogen from which soluble non-VSG proteins, purified VSG and whole trypanosomes were used as antibody targets, invariant antigens were found which consisted of all the molecules of the parasite other than surface glycoproteins. Through western blotting, antigens were obtained such as a 69 kDa protein found in all life cycle stages and which also resembled heat shock proteins and could act as molecular chaperon to assist correct folding of proteins such as newly synthesized VSG protein. Both the N'dama and Boran cattle reacted to it producing high IgM but only N'dama produced detectable IgG, just as only N'dama reacted to another invariant antigen, a 33 kDa protein found to be a cysteine protease (ILRAD, 1991). Report of another similar study, showed the 35 kDa and 65 kDa protein were found as invariant integral membrane glycoproteins on the trypanosome (Burleigh 1993).

2.8.10 Antibodies to Trypanosomosis

Trypanosomes like other protozoans living in the vasculature are challenged and killed by antibodies since their variant surface glycoprotein coat is a prominent immunogen eliciting high titres of lytic antibodies, but through antigenic variation rare individuals change to another coat to survive and produce a new wave of growth (Barry and Carrington, 2004). A variant may be eliminated by antibody specific for the surface exposed conformational epitopes of the VSG but antibody responses are also elicited to buried VSG antigenic determinants (Taylor *et. al.*, 1996).

Every one of the many different strains of trypanosomes displays a particular set of variant surface glycoproteins (VSGs) while the VSGs are reshuffled in the switching of variant antigenic types so that trypanosomes present a confounding variety of antigens in the course of an infection. This property of salivarian trypanosomes to counter vertebrate host's immune system by producing a large number of antigenically different populations through out the infection makes protection by conventional vaccination impossible (Jones, 1997). Signals from the infecting parasite prevent the host from mounting an appropriate immune response to combat establishment of the infection (Murphy and Pelle, 1997).

Host antibody against trypanosomes are produced by B lymphocytes which are mostly stimulated not by multiplying trypanosomes but by dead trypanosomes especially the stumpy forms that eventually die (ILRAD, 1985). It was reported (ILRAD, 1991) that when subjected to panel of antigens, both susceptible Boran and tolerant N'dama cattle first mounted a defence against exposed VSG molecules, elaborating IgM. Subsequent death of parasites and exposure of hidden epitopes induced continued production of IgM in susceptible Boran cattle, but tolerant N'dama switched to IgG sub class indicating matured immune response. In another comparism between the two breeds of cattle, Taylor *et. al*., (1996) measured B-cell activation and the quantity and isotype of antibody produced at the cellular level. Their report indicated that trypanotolerant N'dama had more circulating B cells that were activated and more VSG – specific IgG1 while susceptible Boran cattle had higher level of splenic cells secreting IgM specific for surface VSG. They argue that much of the IgM would bind to circulating dead trypanosomal antigens in immune complexes destined for elimination but still part of IgM produced by susceptible animal was polyspecific, aimed against non-trypanosomal antigens so susceptible animal produced antibody but much of it was aimed at unnecessary target such as the polyspecific antibodies that include auto-antibodies which rather contribute to pathology of host and not just the defence (Taylor, 1998). Thus, while high and sustained levels of trypanosome specific IgGI isotype were found in trypanotolerant N'dama, their level was low and transient in trypansusceptible Boran cattle (d'Ieteren *et al* 1998). The immunoglobulins produced in trypanosusceptible animal seems therefore directed to unnecessary target unlike the case of the trypanotolerant animal.

Host antibodies bound to parasite VSG mediate killing of parasite by other elements of the immune system such as Macrophages and Neutrophils. However unidirectional clearance of VSG-bound antibodies into the flagellar pocket occurs in trypanosomes assisting parasite in the process of antigenic switching such that antibody to a particular VSG may not kill but speed up the process of coat substitution to advantage of the parasite (Russo, 1992).

2.9 Diagnostic Methods of Trypanosomosis

Ikede (1991) classified the diagnostic techniques into clinical, parasitological and serological methods. The more recent molecular techniques have broadened the spectrum of diseases that can be diagnosed by detection of genetic material which need neither viable pathogens as do isolation assays nor high amounts in specimen as required for microscopic observation.

2.9.1 Clinical Diagnosis

Ruminants affected by trypanosomosis show certain features which are however not pathognomonic such as intermittent fever and loss of appetite, dullness, weakness, swollen lymph glands, pale mucous membrances, stunting of the young, emmaciation, abortion, irregular oestrus cycle, infertility, ocular discharge (Ikede, 1991; Rabo and Oyejide, 1996; Urquhart *et.al.*, 1998). The disease typically runs a chronic course over months in which cases of death may occur associated with congestive heart failure due to anaemia and myocarditis. But, some strains of *T.vivax* induce acute death where anaemia and widespread haemorrhages are found (Urquhart *et. al.*, 1998; ILRAD, 1984).

2.9.2 Parasitological Methods

Included in this group are the blood (wet, thick and thin) films, parasite concentration methods (Dark ground/phase contrast buffy coat technique, haemotocrit centrifugation technique that are more common). Others are capillary concentration technique especially for *T.congolense*, as well as the animal inoculation method (Ikede, 1991).

2.9.2.1 Wet Blood Film

When the parasitaemia is massive it is possible to detect motile trypanosomes in fresh film of blood (Urquhart *et. al.*, 1998). A drop of blood is placed on to a glass slide and covered with a cover slip. A number of random fields are examined using 10 x eyepieces and 40x objective or lower to identify trypanosomes present, on basis of size and motility and estimate level of parasieaemia as low, medium or heavy (Ikede, 1991). With the wet film, a drop of blood can be examined next to the animal provided that a microscope is available (Eisler *et. al*., 2004).

2.9.2.2 Thick and Thin Smear

A thick blood smear is made by placing one or two drops of blood on a clean slide and drawn by edge of another slide centrifugally over an area of about 2 cm dried and lysed in distilled water and stained for detection of trypanosomes. Thin blood smear where the drop of blood is spread by holding spreader slide at an angle of about 30^0 and drawn quickly to smear thinly over a wider area that is fixed in alcohol before staining assist in species identification. (Ikede, 1991; Cheesbrough, 1998 and Mare, 1998). Stained films are components of standard detection method that also include wet film and animal inoculation (Kalu *et.al.*, 1985). It was in a carbolfuchsin stained smear that Bruce first observed trypanosome as a curiously shaped object lying among the corpuscles before he later found living wriggling ones in fresh blood. (Vickerman 1997), However, these techniques are not sensitive enough to detect the low parasite levels characteristic of the disease in large animals (Eisler *et. al.*, 2004).

2.9.2.3 Haematocrit Centrifugation Techniques (HCT) Dark Ground Buffy Coat Technique (BCT)

To concentrate few trypanosomes and detect them as motile organisms , the haematocrit method is done by filling anticoagulated blood in a capillary tube sealing one end with cristaseal and spinning for 3 –5 minutes in the microhaematocrit centrifuge at 12, 000g when PCV can also be read off a haematocrit reader (Cheesbrough, 1998; Abenga *et. al.*, 2005). The capillary tubes can be cut with a glass saw or diamond tipped pencil at a point 1mm below the buffy coat to include the top layer of red cells in content

that is smeared on the slide and covered with cover slip to be viewed under x40 objective (Rabo and Oyejide 1996).

The technique is named after Woo who in 1970 first reported it in the form the capillary tube was mounted in DPX for visualization of centrifuged blood preferably with a 20x or 25x achromatic objective that has a long working distance of about 6.7 mm in order to focus through the depth of the capillary tube (Ikede 1991, Cheesbrough 1998.). Cutting the capillary tube to express and smear buffy coat in the buffy coat technique (BCT) it is viewed under dark ground or phase contrast illumination. If a special condenser provides the illumination it eliminates requirements of special objective and increase diagnostic sensitivity to the least detectable numbers of $2.5x10^2$, $5x10^2$, and $5x10^3$ for *T. congolense, T.vivax* and *T. brucei* respectively in estimation of parasitaemia scoring system (Eisler *et. al.*, 2004). Kalu *et. al.*, (2002) recommended a combination of HCT and BCM for routine diagnosis of caprine trypanosome infections.

2.9.2.4 Animal Inoculation

Trypanosomes vary in their ability to grow in laboratory rodents especially mice that are commonly injected intraperitoneally with 0.2 – 0.3ml of blood, lymph aspirate or cerebrospinal fluid followed by examination of wet films three times weekly through four weeks to detect presence of trypanosomes (Ikede, 1991). Rats, mice, guinea pigs, rabbits are used for *T. brucei* and *T. congolense* while monkeys are also used for *T.simiae* but rodents are usually not susceptible to *T.vivax, T. uniforme, T.theileri* and *T. godfrayi* (FAO, 2005). Laboratory animal experiments are however, expensive and time consuming (Diallo, 1991).

2.9.3 Sero-immunological Diagnosis

These methods detect antibodies to the organism or antigen in serum or other body secretions. Detection of antibodies is not sufficient to conclude the diagnosis while detection of antigen is considered more specific for diagnosis, but each test has its advantages and disadvantages, none being fool proof. (Chauhan and Agarwal, 2006).

2.9.3.1 Enzyme Linked Immunosorbent Assay (ELISA) Technique

As a primary binding test, the Elisa developed by Engvail and Perimann in 1972 measures binding of antigen to antibody through the amount of enzyme label in the immune complex and is sensitive to the level of detecting less than 1 mg/L (Thrusfield, 1991; Babu, 2003). Other advantages of the technique like safety, reduced cost and extreme stability of reagents as well as use of computerization have been listed (Anderson and Mckay, 1991; Chauhan and Agarwal, 2006).

From a diagnostic point of view, limitation of parasitological tests to detect animals infected with trypanosomes promoted development of indirect methods such as ELISAs for detection of either trypanosomal antigens or antibodies. (Mutugi, 2006). There appears to be a variation in the ability of ELISA to detect infection according to the stage of infection. ELISA was more effective in detecting chronic infection where as parasitological techniques were more likely to detect infection in acute stage of trypanosomosis (Luckins, 1998). There is sceptism on use of immunological methods in diagnosis where the tests are based on detection of antibodies in sera or other body fluids which may not necessarily be indicative of current infection since the test would be positive in animal that had long recovered (Ikede, 1991).

Through the use of trypanosome antigen detection ELISA employing monoclonal antibodies, Olatoye and Ogundipe (2003) reported 16.45% prevalence of trypanosomes in sheep, compared to 4.33% prevalence in same animals obtained by blood smear technique. There is a dogma that antigen ELISAs have more potential than antibody for distinguishing between the infected and non infected state, although too little has been done in critically comparing these tests for it to be accepted unconditionally (Lukins 1998). Mutugi *et. al.,* (2006) conducted both detection of trypanosomal antigens through sandwich ELISA and the antibody detection ELISA on samples from the same animals. They reported low sensitivity for the antigen ELISA attributing it to instability of conjugate where as antibody ELISA was more promising but affected by quality of water used which they recommended should be freshly distilled and deionised in order to produce fresh buffers.

In developing countries, water used in buffers to make reagent dilutions especially conjugate solution may contain impurities that inhibit enzyme activity or impair the

enzyme's substrate (Jacobson and Crowther, 1998.). Luckins, (1998) observed that with antibody ELISAs, little attention has been given to the use of specie specific diagnostic assays and most work has been done with ill-defined trypanosome antigens. Basically, the soluble antigens from ultra sonicated parasites may be incapable of discriminating between species in a multispecie infection, unless it was fractioned by gel chromatography to enable production of antigen with specie specific characteristics. Olatoye and Ogundipe (2003) concluded that ELISA technique could successfully overcome difficulty of distinction between pleomorphic forms of *T. brucei* and *T. congolense* encountered in smear techniques but clear serum was required since false positive cases could be recorded with haemolysed serum due to action of peroxidase enzyme of the reagent.

2.9.3.2 Indirect Fluorescent Antibody Test (IFAT)

Immunoflurescence is a process in which dyes called fluorochromes are exposed to UV, violet or blue light to make them fluoresce or emit visible light. Such fluorescing dyes as fluorescein or fluorescein isothisocynate (FITC) and Rhodamine or tetramethyl rhodamine isothiocynate (TRITC) can be coupled to antibody molecules without changing the antibodies capacity to bind to a specific antigen so that they are used to identify and localize antigens in cells and on cell surfaces in fluorescence immunoassay. (Babu, 2003).

Deposits of immune complexes (i.e. antigen – antibody – complement) in immunologically mediated diseases are commonly found in kidney and skin when examined by immnofluorescent stain. For direct fluorescent test, the specimen is stained with species – specific anti – IgG conjugated with the fluorescent dye (FITC OR TRITC) such that by incubation the FITC- anti- IgG antibodies attach to deposit in tissue giving a fluorescence under the fluorescent microscope after unbound reagent is washed off and examined (Barta and Barta, 1984). In the indirect immunofluorescence antibody test (IFAT) a known antigen is fixed onto a slide and test serum (antiserum) is added so that if the specific antibody is present, it reacts with antigen to form a complex. When the fluorescence – labeled anti specie IgG is added, it reacts with the fixed antibody and after incubation followed by washing unbound reagent and examination under fluorescence

microscope, it fluoresces if antibody specific to the antigen is present in serum (Babu, 2003). The indirect immunoflurescence antibody test (IFAT) is used to detect trypanosomosis where trypanosomes obtained from artificially infected laboratory animals are fixed to the slide as the antigen to test suspect serum for presence of antitrypanosomal antibodies. If serum was from trypanosome infected animal, presence of antibodies would cause fluorescence, but absence of fluorescence indicates trypanosome – free animal. (ILRAD, 1989).

2.9.3.3 CATT (Card Agglutination Trypanosomiasis Test)

Agglutination is the clumping of particulate antigens in the presence of an immune serum, the particles involved have size of 200 – 250 nm to produce visible reaction when the antigen – antibody complexes are formed (Chauhan and Agarwal, 2006). In human African trypanosomiasis (HAT), parasitological tests have low sensitivity and are hampered by fluctuating parasitaemias so the identification of seropositive individuals on whom to focus parasitological examination is done by means of Card Agglutination Trypanosomiasis Test (Garcia *et.al.,* 2000). While suitable antigens are still being sought for *T. congolense* and *T. vivax*, available antigens for the test originate from particular variable antigenic types (VATS) of *T. gambiense* that are highly conserved across the range of this specie such that majority of infected persons develop antibodies which cause agglutination when blood or serum is mixed with the antigen on the card (Eisler *et. al.*, 2004).

2.9.4 Molecular Methods

The useful objectives of accurate diagnosis of animal trypanosomiasis and definitive identification of causative trypanosome species is elusive by parasitological and immunological technologies but molecular methods which detect and amplify nucleic acids (DNA and RNA) have potential to achieve them (Eisler *et al*. 2004). A number of organizations including ILRI, CIRDES and TseTse Research laboratotry (TRL), which supply the probes, have been developing the molecular biology techniques. These are still not fully controlled, prohibitive in cost and can not be used

on large scale studies but are very useful for small-scale targeted field and laboratory investigations (Reifenberg *et al*., 1997).

It is supposed that detection and identification of trypanosomes by molecular means should be based upon stable parasite specific genetic characteristics that can withstand environmental influences exerted by either the host or the vector (Eisler *et al*., 2004).

In designing molecular tools for identification, Tibayrenc 1997(b) expected problem for *T. brucei* and *T. congolense. T. brucei* viewed as monophylectic was considered a good specie phylogenetically that could have common molecular tools to identify its isolates even though doubts existed on its genetic stability on account of possible genetic exchange occurring during its transmission. But *T. congolense* having Savannah, Forest and Kilifi types was viewed as likely artificial specie which if confirmed to be polyphylectic, designing molecular tools of identification specific for its whole range of organisms might be impossible.

Despite such initial fears, specie-specific probes now exist for both DNA hybridization and primers for PCR to be used to characterize specific trypanosome species infections in hosts and vectors. Unique regions of highly repetitive multicopy DNA sequences found in genome of trypanosome species as well as kDNA minicircles (though lacking in some, varying in sequence and proportion among trypanosomes) provide target sequences for identification probes (Eisler *et al*., 2004).

2.9.4.1 Nucleic Acid Hybridization Assays

Nucleic acid hybridization assays are used to identify individual recombinant bacteria harbouring parasite sequences of interest, analyse given organism and expression of cloned genes as well as screen vectors or clinical samples (Maizels *et. al.*, 1991). The tests are able to show presence of infecting organism through the genome instead of its products like the proteins (Diallo, 1991). The test indicates the degree of relatedness of two genes or detects RNA and DNA using a specific nucleic acid probe (Ignacimuthu, 1995).

The probes are purified, characterized sequences specific to a given species which are either radiolabelled (^{32}P, ^{125}I, or ^{3}H) detectable by radiography or non-

radioactive biotin labeled to be detected by avidin – peroxidase conjugate and using enzyme substrate like diamino benzidine tetrachloride (Chauhan and Agarwal, 2006). Detection of infectious agents by nucleic acid hybridization using radiolabelled probes is hazardous; the probes have short half-life and their handling require special equipment (Diallo, 1991).

Four major components of the hybridization assay were listed by Walker and Rapley (2005) who also briefly explained the process as follows; (a) the target nucleic acid in the sample being sought out for by (b) probe (a single stranded nucleic acid of known origin that hybridizes to it, (c) reporter or detection system and (d) hybridization format adopted. DNA or RNA from target organism is denatured by alkali treatment or heating above its melting point so as to separate strands. Through baking or use of UV light, the DNA or RNA strands are attached to a solid support such as nitrocellulose or nylon membrane so as to prevent re-annealing. A probe labeled with a reporter group(radioactive or non-radioactive) is allowed to react with target nucleic acid for few hours before removing unreacted probe by washing in buffer solution.

Target DNA attached to complementary sequences of probe in stable hybrids remains on support membrane for probe's reporter group to be detected either enzymatically, flurometrically or by autoradiography.

Majiwa (1998) listed different recombinant plasmids containing repetitive DNA sequences useful as hybridization probes for detecting African trypanosomes in mammalian blood, buffy coat, vector gut and proboscis as follows:

Table 2.2 Probes for Hybridization Assays

Parasite Specificity	Recombinant Plasmid
Trypanozoon	PgDRI
Trypanozoon	177 Repeat
T. evansi, type A	PKT 420
T. evansi, type B	PKT 700
T. congolense, Savanna type	Pg NRE – 372
T. congolense, Kilifi type	Pg NIK – 450
T. congolense, Tsavo type	Pg Ngulia – 11
T. congolense, WA forest/Riverine type	TSW 103
T.simiae	Pg NS – 600
T. vivax	Ig Dil – 10k
T. vivax `	TV 47
T. vivax	PK DIL 900
T. vivax	PK DIL 900 WA
T. vivax	Pg DSIL 800/3

Source: Majiwa (1998)

The probes were ordinarily used in radioactive format; however, with the availability of systems of non-radioactive labeling and detection of DNA, these are converted to this format (Majiwa, 1998).

Hybridization assays are limited in large scale field studies partly due to requirement for sources of radioactive nucleotides and inavailability of equipment in developing countries (Hide and Trait, 2004). Consequently, derivatives of the probes with dinitrophenol were introduced such that positive hybridization using repetitive sequence DNA probes can be revealed by an enzyme labelled anti – dinitrophenol antibody (ILRI, 2009).

Among the three main formats in which hybridization reaction is done, that is, on solid support, in solution and in situ, hybridization in solution is more efficient than the more common solid support format but the insitu format has the unique advantage that probe penetrates tissue in their natural configuration on slide so that localization of an organism can be known (Diallo, 1991).

2.9.4.2 Polymerase Chain Reaction (PCR)

The polymerase Chain Reaction (PCR) is for amplifying nucleic acid so that when only a small amount is present in clinical samples it can still be detected (Chauhan and Agarwal, 2006). PCR based approaches having advantages of high specificity and sensitivity have superceded hybridization assays (Hide and Trait, 2004)

If the sequence at the end of a DNA is known, the intervening fragments can be amplified by PCR (Lodish *et. al.,* 2004). The general format of the method which was invented by Karry Mullis in 1984 has been explained by Dhanotiya (2004). After extraction of target DNA, two oligonucleotides used as primers are synthesized, each being complementary to a short sequence of the desired (target) DNA to be replicated invitro. The primers are positioned just beyond the end of the sequence to be amplified.

In the first cycle, the DNA is heated (to 95^{o}C) briefly to denature into single strands before being cooled to (55^{o}C) permitting annealing to primer in the presence of a large excess of the synthetic oligo-nucleotide primers. Heat stable DNA polymerase (Taq polymerase) and the four dinucleoside triphosphate (as substrates) are then added and temperature is raised (to 72^{o}C) for optimal polymerization in the selective replication

of the primed DNA segment. These stages comprise a PCR cycle and several such cycles are repeated so that 20 cycles yield amplification of 10^6 while 30 cycles yield 10^9 times the DNA segment.

2.9.4.2.1 Benefits of PCR Techniques

PCR has high sensitivity and advantage of speed over some other detection systems employed in the detection and diagnosis of infectious diseases (Gould et. al., 1991). Other areas the PCR techniques finds application were given as DNA cloning for sequencing, DNA based phylogeny or functional analysis of genes, diagnosis of hereditary diseases and identification of genetic finger prints employed in forensic sciences and paternity testing (Mesotheliomalawyers, 2011).

2.9.4.2.2 Development of PCR Technique in Diagnosis of Trypanosomosis

Specific objective underlying use of PCR technique in trypanosomosis was given as development of a 'Pan trypanosome test' that will amplify the DNA of all pathogenic trypanosomes of mammals. Such a test could be applied in specific geographical regions to assess trypanosomosis prevalence and assist decision makers in focusing and implementing appropriate disease control measures. As a more sensitive technique, it could identify animal reservoirs of human trypanosomosis, improve monitoring of control and eradication programmes, since larger number of animals infected would be identified, treated and those no longer infected also correctly identified .(Diallo and Radwanska, 2010). Technical research contracts were given by joint FAO /IAEA research coordination meeting (RCM) to institutions such that preparation of standardized protocol for trypanosomal PCR, was tackled by Center for Ttropical Veterinary Medicine (CTM), development of pan-pathogenic trypanosome primer was contracted to CIRAD – EMVT and IRD while details of the pcr technique, in creation of reference blood bank for collection of trypanosome DNA for PCR test was based at FAO / IAEA Agriculture and Biotechnology Laboratory at Seibersdort Vienna Austria (Diallo and Radwanska, 2010)

Table 2.3 List of possible DNA to be sent to Seibersdorf Laboratory for reference on Trypanosome PCR is given below:

Trypanosome Protocol	Donor Institute	Laboratory Code	Gene Target
Trypanozoon	ITG	ESAG 6/7	
Pantryp	ITG	185	
T.b. gangiense	ITG	TS 19	
T.b. Rhodesiense	ITG	SRA	
Trypanozoon	FUB	TBR ½	Satellite Repeats
T. Congolense (Sav)	FUB	TCN ½	Satellite Repeats
T. Congolense (For)	FUB	TCF ½	Satellite Repeats
T. Vivax	FUB	T.V 80.24	Plasma membrane gene
T. evansi	KETRI	____	kDNA mini circles
T. congolense (*Tsavo)*	KETRI	____	Satellite Repeats
T.godfreyi	KETRI	____	Satellite Repeats
T.vivax (west Africa)	KETRI	____	Satellite Repeats
T. cogolense (Kilifi)	KETRI	____	Satellite Repeats
ITS 1 – 2	Fiocruz	____	rDNA ITS
ITS 1	Fiocruz	____	rDNA ITS
ITS 2	Fiocruz	____	rDNA ITS
ITS 3 – 4	Fiocruz	____	rDNA ITS
ITS 3	Fiocruz	____	rDNA ITS
ITS 4	Fiocruz	____	rDNA ITS
T. vivax	Fiocruz	____	____
T. gambiense	Cote d' Ivoire	____	____
T.brucei (Trypanozoon)	ILR1	____	INGI Repeat

Key: Fiocruz (Brazil)
ITG (Belgium)
FuB (Berlin)
KETRI (Kenya)

Source: Diallo and Radwanska (2010)

2.9.4.2.3 Components and Reagents in the basic PCR set up

The required components include DNA template with region to be amplified (target). Two primers complementary to 3 ends of the sense and anti-sense strand of DNA target. Recently, Kin 1 and Kin 2 primers were used in the molecular characterization of trypanosomes in the Kachia grazing reserve, north-west Nigeria. Use of this primer was on the basis that it is designed to amplify the internal transcribed spacer (ITS 1) of ribosomal deoxyribonucleic acid (rDNA) and serve as a *universal diagnostic* test for all *pathogenic trypanosomes* since *spacer length* is *variable* between *species* but *constant* within *a species*. Their reports showed PCR products of 147 bp, for *T.vivax*, 710 bp for *T.congolense* and 480 bp for *T.brucei* (Enwezor *et.al.*, 2008)

Thermostable taq polymerase which has its optimal temperature at the elevated level of 72^{o}c (Gould and Mccoll, 1991), deoxynucleoside triphosphates (dNTPs) as building blocks for new DNA strand, buffer solutions for optimum activity, divalent cations (generally Mg^{2t} but also Mn^{2t}), monovalent cations (K^{t}), reaction volume of 10-200ml in small reaction tubes (0.2 – 0.5µl) the thermal cycler to heat and cool reaction.

Thermal cycler may be new version with heating lid to prevent condensation on top or old types without heating lid which therefore requires overlay of a layer of mineral oil on top of reaction mixture (Mesotheliomalawyers, 2011).

2.9.4.2.4 Variation in PCR Technique

A variety of PCR types exist obtained from varying the basic technique

2.9.4.2.5 Nested PCR

Nested PCR means that two pairs of PCR primers were used for a single locus. The first pair (A set) generates an amplicon within the locus as seen in any PCR experiment, the second pair of primers (B set, nested primers) bind within the first amplicon and produce a second PCR product that will be shorter than the first one ,so if wrong locus was amplified mistakenly, the probability is low that it will also be amplified a second time by the second pair of primers (Griffin, 2011).

Trypanosoma brucei and *T. evansi* are both of subgenus Trypanozoon. On a PCR investigation on *Trypanosoma evansi*, a pair of outer primers TE 1 and TE 2 amplified

821 bp primary PCR product, the second amplification using nested (internal) pair of primers TE 3 and TE 4 yielded a 270 bp PCR product from *T. evansi* (Aradaib and Majid, 2006).

2.10 Control of Trypanosomosis

This involve vector control, chemoprophylaxis and keeping trypanotolerant animals (Seifert, 1992; Ogunsanmi and Taiwo, 2004; d'Ieteren *et. al.*, 1998)

Jordan (1986) gave a breakdown of methods directed against parasite to include use of trypanotolerant breeds, avoiding contact by moving animals and men as well as movement out of disease area, Trypanocidal drugs and probable immunization.

2.10.1 Treatment with Trypanocidal Drugs

The requirement for treatment of exposed animal is one of the criteria used to assess resistance (Murray *et. al.*, 2004). Only three compounds, isometamidium chloride, homidium (bromide and chloride) and diminazene aceturate are available, having been in the market for over 40 years (Holmes *et. al*., 2004). Resistance to these trypanocides has been reported in many countries in individual farms and on regional basis (d'Ieteren *et. al*., 1998). This has led to trials of treatment strategies using these trypanocides that aim at extending their usefulness such as employing biodegradable sustained release devices (SRD) in form of implants (Geerts *et. al.* 1997).

2.10.2 Control of Vector

Measures directed against the vector include clearing vegetation, application of persistent and non-persistent insecticides, traps and insecticide impregnated targets, genetic control, biological control (Jordan 1986, Itard 1989, Allsopp and Hursey 2004, Vale and Torr, 2004).

Tsetse control became widespread with the arrival of synthetic insecticides when in many countries including Nigeria, Helicopters were also employed in spraying agents like dieldrin and endosulfur (Offor, 2000, FAO, 2005).

Sterile male technique or sterile insect technique (SIT) where laboratory reared males are sterilised through irradiation with gamma rays has been employed, it is costly

and was only successful on the isolated unguja island of Zanzibar in Tanzania (FAO 2005).

Different kinds of traps are used against anthropod vectors of disease. Nitse and Biconical traps were employed by Ahmed and other workers. (2000) at the Kainji Lake National Wild life Park, who reported trapping hundreds of tsetse flies belonging to G. *tachinoides, G. morsitans submorsitans* and *G. Palpalis* species.

2.10.3 Trypanotolerance and its Mechanism

Several genetic, sero-immunological and cellular factors are probably involved in trypanotolerance while ecological and physio-pathological factors may help its maintenance but it breaks down through poor nutrition, excessive fatigue and intestinal parasitism (Itard, 1989).

A comparison between trypanotolerant and trypanosusceptible cattle shows differences in antibody response, complement level and cytokine response (Naessens *et. al.*, 2002). The work of Naessens *et. al.*, (2003) exploited bone marrow chimerism in trypanotolerant N'dama/trypanosusceptible Boran twins and singletons, infecting the animals with *T. congolense* to study control of parasitaemia and anaemia. They reported that the trypanotolerance trait in N'dama cattle comprised at least two distinct mechanisms. The one limiting anaemia and loss of white blood cells depend on haemopoietic tissue genotype. The second mechanism involves the host's capacity to control parasitaemia which was innate and not dependent on haemopoietic tissue genotype.

Further reports point to the fact that constitutive factors play important role in the trypanotolerant associated with different species and breeds of animals since those lacking them are trypanosusceptible.Trypanotolerant cape buffalo survives in areas of high trypanosomosis challenge because it contains Xanthine oxidase which is a constitutive serum protein that kills all species of African trypanosomes (Black *et. al.*, 1997). Human resistance to animal trypanosomes is associated with trypanosome lytic factor which is a non-immune serum trypanosome-killing factor which lysis *T. brucei brucei* in-vitro and in-vivo (Encyclopaedic Reference, 1997).

Magez *et. al.*, (2006) reported that immunoglobulin G plays a role in control of trypanosome infection in mice. The non-immunological mechanisms play a primary role

in trypanotolerance which is subsequently supported by a secondary defense mechanism mounted by the immune system (Verhulst and Pandey, 1998)

2.10.3.1 Factors Influencing Trypanotolerance

Factors such as helminth infection, seasonal nutritional constraints and management are important as they can interfere with trypanotolerance which is not absolute (Goossens *et. al.,* 2004).

Nutrition modulates the severity of trypanosomal infection in animals (Otesile *et. al.,* 1991). Faye *et. al.,* (2002) reported that parasitaemia level was significantly influenced by diet with the group under high supplementation having a higher mean parasitaemia than the group under low supplementation. Nutritional changes have a major impact on PCV which is used to estimate anaemia (ILRAD, 1989).

Oral and parenteral administrations of some nutritional agents were reported to reduce severity of trypanosome infections (Ibrahim et al, 2005, Egbe – Nwiyi *et. al*, 2005).

Stress (overwork, parturition, suckling, disease) is an important factor influencing the infection (ILRAD, 1989) Concurrent worm infection has effect on trypanosomosis (Faye *et. al.,* 2002) and even trypanotolerant West African Dwarf goat concurrently infected with worms can have outbreak with the infection (Chiejina *el. al.,* 2005). The affected animal is intolerant to stress (Abebe, 1991).

2.10.3.2 Indicators of Trypanotolerance

Practical reliable markers of resistance or susceptibility to trypanosomosis may not be found among animals but results from works done mainly with cattle have yielded a number of indicators, the key ones being parasitaemia, anaemia, and weight gain (Naessens *et. al.*, 2003). Others are production and Reproduction parameters (Verhulst and Pandey 1998). Verhulst and Pandey, (1998) suggested a list of five groups of criteria that could be used in attempt to measure trypanotolerance which were more or less similar to those stated later on by Murray *et. al*., (2004). These are: direct pathological criteria such as parasitaemia, anaemia, survival time duration, prepatent period.Indirect production criteria are body weight, meat and milk production as well as reproductive performance.

2.10.3.2.1 Anaemia and Trypanotolerance

Trypanosomes are haemotropic parasites (Akinboade, 2001) and anaemia is a principal pathological feature of trypanosomosis (Ibrahim *et. al.*, 2005). Measurement of the anaemia gives a reliable indication of the disease status and productive performance of trypanosome infected animal (Abubakar *et. al*, 1999). The anaemia results if either the number of functional erythrocytes or the quantity of haemoglobin per unit of blood is below normal (Frandson *et, al.* 2003). This can arise from decreased rate of red blood cell production or their increased loss or destruction by haemorrhages or haemolysis (Harper, 1975). Control of anaemia development is the criteria of trypanotolerance most linked to over-all productivity but interpretation of PCV variation is only meaningful if other factors affecting it are identified, quatified and controlled (Murray *et, al.*, 2004).

Deficient blood formation can occur in diseases of bone marrow and kidney where inadequate production of erythropoietin occurs as well as in dietary deficiencies of iron, copper, vitamins or amino acids (Frandson et. al., 2003). Many anaemic goats are also hypo-proteinaemic, their condition is precipitated by trauma, feeding of materials like brassicas and onions, parasitisms involving external parasites such as lice, as well as helminths like *Fasciola* specie and protozoa like Eperythrozoon (Matthews, 1999). In assessing the anaemia by packed cell volume (PCV) it could arise from other causes such as haemo-concentration. Concurrent estimation of plasma or serum proteins would rule out such factors (Schalm, 1971).

Among the trypanosomoses, anaemia in infection of *T. vivax* may assume an acute or hyperacute haemorrhagic form especially in cattle and even animals resisting infections with *T. congolense* and *T. brucei* are susceptible to it (ILRAD, 1984). A rapid progressive anaemia was reported in Zambian goats infected intravenously with pathogenic *T. congolense* (Witola and Lovelace, 2001). In an experimental infection of both *T. congolense* and helminth affecting West African dwarf goats and their Fl crosses with Sahelian breed, Faye *et. al.*, (2002) reported severe drop in PCV which was not significantly affected by breed. Dhollander *et al*, (2005) reported that following infection of *T. congolense* to West African dwarf goats and their crooses with Saanen goats neither were able to control drop in PCV while course of infection was similar. A chronic

anaemia was reported with a significantly lower PCV, red cell count and haemoglobin in trypanotolerant Djallonke ewes and West African dwarf Does (Goossens *et. al.,* 1998). However, anaemia is of less importance in *T. brucei* which affects organs deeper in the body and causes inflammatory lesions accompanied by degeneration and necrosis (Itard, 1989).

2.10.3.2.2 Pathogenesis/Mechanism of Anaemia in Trypanosomosis

Anaemia in trypanosomiasis is haemolytic in nature and the haemolysis occurs principally extravascularly in expanded mononuclear phagocytic system (Ibrahim *et. al.*, 2005). Investigation concerning erythrophagocytosis as a cause of anaemia was done by Witola and Lovelace (2001), who incurbated mixtures of 51 Cr-labelled red blood cells and self mononuclear cells (MNCs) from trypanosome infected and uninfected goats. Their reports showed that Giemsa stained smears from infected goat, had 50% higher incorporation of the radioactive Cr in mononuclear cells than uninfected controls. It was proved that infected red cells were destroyed by the mononuclear cells beyond the level of physiological turnover in normal animals for erythrocytes reaching the end of their finite life span (Reagen *et. al.*, 1998).

Through binding to erythrocytes by trypanosomal proteins or components of immune system or auto-antibodies, immunological mechanisms play a role in anaemia of trypanosomosis (ILRAD, 1984). Such immune-mediated processes result in formation of spherocytes, agglutination and ghost cells in the immune mediated haemolytic anaemia (Reagan *et. al.*, 1998). There could be direct damage to red blood cells by biologically active substances acting as haemolysins from the trypansome, for instance their phospholipases (ILRAD, 1984).

Infection induced peroxidative injury to erythrocytes have been reported by Ogunsanmi and Taiwo (2001). Higher serum free fatty acid concentration in *Trypanosoma brucei* and *Trypanosoma congolense* infections reported by Biryomumaisho *et al*. (2003) were also thought to be cytotoxic and haemolytic while enabling multiplication of the parasites.

2.10.3.3 Parasitaemia and Susceptibility

Parasitaemia occurs through out the course of infection but its level varies with different species and strains of trypanosome and with various species of host (Losos, 1986). The first wave of parasitaemia from a given parasite is similar in different host animals but subsequent waves vary, each having some parasites which express a different surface coat not recognized by lytic host antibody (ILRAD, 1985).

Differences in level of parasitaemia occur between species, so the extravascular *T. brucei* usually has lower level of parasitaemia than vascular *T. congolense* whose sub-populations also cause different levels of parasitaemia (Losos, 1986). The overall level of parasitaemia is important as it is a major determinant of the virulence of infection. In both *T, brucei* and *T. congolense* infections, higher parasitaemia leads to greater degree of pathogenesis (Turner *el. al.,* 1995).

Faye *et al,* (2002) reported that parasitaemia in *T. congolense* infection tended to be higher in crosses with sahelian breed, though not significantly different from West African dwarf. But nutritional supplementation had a significant influence on level of parasitaemia. Regional differences in the small East African breed of goast were reported by Mutayoba *et. al.,* (1989) to cause differences in levels of parasitaemia in *T. congolense* infection. This was attributed to underlying heterogeneity.

In the case of *Trypanosoma brucei* it has a dependence on purines which is interconverts into cellular nucleotides for replication of blood stream stages and would cease to replicate if its purine supply becomes growth-limiting (Black *et. al.,* 1997). At high density *T. brucei* replicating slender blood stream forms associated with parasitaemia become limited to a sub-lethal level by differentiation into the intermediate form and then to the non-replicative stumpy form (Tyler *et. al.,* 2001). The transition from slender to stumpy forms limits parasite population expansion found in *T. brucei* while other parasite populations do not yield stumpy forms or do so slowly. As a result, low parasitaemia is associated with *T. brucei* while other species like *T. congolense* have high parasitaemia (ILRAD, 1985).

However, unlike the pleomorphic field isolates, laboratory-adapted syringe-passaged trypanosome lines have lost the ability to differentiate so their growth rate equals their replication rate (Turner, 1995).

2.10.3.3.1 Control of Parasitaemia

The ability of the trypanosome infected animal to control parasitaemia and anaemia is directly related to the positive clinical outcome of the disease in terms of productivity and survival (Taylor *et al*., 1996).

Parasitaemia is higher in more severe syndromes and level of parasitaemia in infected animals is initially usually high, fluctuating between peaks and low values in about every 6 days (Losos, 1986). Resistant animals manage to control level of parasitaemia after initial wave (ILRAD, 1985). Precisely how they cope with antigenic variation in controlling and eventually eliminating their parasitaemias is unknown, even as a rapid, effective antibody response and other possible factors are suggested (Unquhart *et. al*., 1998). Cytokines, anti-trypanosome antibodies like Immunoglobulin M and G2 and other factors have influence on parasitaemia control (Magez *et. al.*, 2002).

Trypanotolerant animals survive by influencing parasitaemia so that parasite numbers are controlled to a state of host - parasite equilibrium. This is maintained through signals between host and parasite population and among the parasites, but trypanosusceptible animals having defects or lacking these signaling events die of the infection (ILRAD, 1993(b)). Infecting trypanosomes rapidly differentiate to actively-dividing forms in response to a temperature change and signaling factors from the host. (Murphy and Pelle, 1997).The nature of these signals has not been elucidated. With their capacity to rid themselves of trypanosome parasites and maintain low parasitaemia, trypanotolerant animals indirectly reduce the trypanosome parasite load associated with any given location (Agyemang, 2005).

2.10.3.4 Serum Factors

A number of serum factors have been increasingly assessed in the trypanosome affected animal especially complement in trypanotolerance of cattle.The complement system comprises proteins found in plasma in inactive forms that play a role both in inflammation and in immunity notably by opsonisation of target organism, prior to lysis by phagocytosis, which are activated either by rapid classical pathway when antigen –

antibody complex binds to Cl component or by slow alternative pathway through non immunological stimuli (Vegad, 1995).

Several blood biochemical parameters including total serum proteins, albumin and cholesterol were determined in small East African goats infected with *Trypanosome brucei* and *T. congolense* by Biryomumaisho et al (2003) using a ceiba corning 560 blood chemistry autoanalyser while cholesterol was analysed by calorimetric enzymatic end point method. They reported lower albumin in both *T. congolense* and *T. brucei.* While total proteins increased in *T. brucei*, there was decrease in *T. congolense.* Also, decrease in cholesterol was reported in their work.

Serum proteins were assayed by Anosa and Isoun (1976) who reported that total serum proteins and gamma globulins increased while serum albumin decreased in *T. vivax* infected sheep and goats. Abubakar *et. al.,* (1999) measured serum proteins in *T. brucei* infected rabbits by using the folin phenol reagent method. Their reports showed increased total serum protein associated with elevated globulins, but a decrease in albumin which was suggested to arise from either plasma expansion, proteinuria or hepatocellular damage.

In trypanosome infection especially *T. brucei,* injurious mechanisms occur in target connective tissues of solid organs inducing their malfunctioning (Abenga *et. al.*, 2005). Destruction affecting the organs include myositis and myocarditis of the heart (Sarror, 1980) necrosis of hepatocytes and erythrophagia by Kupffer cells in Liver while adhesion of parietal layer of glomeruli to bownman's capsule and necrosis of renal tubules with mononuclear cell infiltration occurred in the kidney (Ibrahim et al, 2005). Lymphoid organ enlargement and splenomegaly associated with plasma cell hyperplasia and hyperganmaglobulinaemia occur concurrent with suppression of immune response to other antigens (Urquhart *et. al.*, 1998).

Creatinine, a nitrogenous waste product of skeletal muscle metabolism having excreted quantities closely related to body muscle mass and metabolic rate of animal (Egan 1976) is an important test of kidney function. It has elevated levels in impaired glomerular filtration rate while muscle wasting reduces the blood level but the levels are less influenced by dietary proteins, unlike urea. As a test of kidney function, creatinine is

therefore preferred to urea, being also cheaper with more colorimetric manual test kit availability (Cheesbrough, 1998).

Reports of Adah *et. al*., (1993) on levels of transaminases in West African dwarf and Red Sokoto goats infected with *T. congolense* show that these enzymes increased in both breeds but remained within the normal reference range while treatment using berenil lowered the elevated levels. Blood and Studdert (1999) explained two of these aminotransferases (transaminases – colloquial term) as Alanine aminotransferase (ALT) formerly known as serum glutamate pyruvate transaminase (SGPT) an enzyme present in hepatocytes which has high serum levels after acute damage to liver cells. The second one Aspartate aminotransferase (AST) formerly called serum glutamate oxalo acetate transaminase (SGOT) is an enzyme with high serum levels after skeletal muscle damage or acute damage to liver cells. Large amounts of it are also found in kidneys so there is lack of organ specificity.

Measurement of these indicators of tissue damage in assessment of trypanoresistance may be necessary as the animal's capability to limit and bear the damage may have bearing on the trypanoresistance. These would fall among direct pathological criteria used in attempt to measure trypanotolerance (Verhulst and Pandey 1998).

2.10.3.5 Productivity Losses

Trypanoresistant animals also suffer loss of productivity due to chronic trypanosome infection (Barrett, 1997). However specie and breed susceptibility matters. Whereas in tsetse infected areas, trypanosomosis is a very obvious problem in susceptible livestock, it may remain practically in apparent where trypanotolerant breeds are concerned although even these breeds may not be very productive when challenge is high (FAO, 2005). Among trypanotolerant, cattle Muturu has shown superior ability to maintain itself in good condition while harbouring trypanosomes (Yanan *et. al.*, 2003).

The invasion of trypanosomes and leukocytes into the brain parenchyma is thought to trigger loss of weight and death of the affected animal (Agbo *et. al.*, 2002). The cytokine tumor necrosis factor – alpha (TNF-α) is involved in physiological and metabolic abnormalities found in Cachectic states (from abnormalities of its production)

just as it can play key role in obesity (from abnormalities of its action) thus exerting a role in control of body weight (Argiles *et. al.*, 1997). Nitric oxide that is synthesized from L – arginine via endothelial nitric oxide synthase (eNOS) is involved in control of vascular tone and permeability affecting trypanosone distribution in surrounding tissues and central nervous system (viswambharan et al, 2003). Also enzymes such as proteases (trypanopains and oligopeptidase), cell surface associated acid phosphatases, may cause tissue degradation to ease passage of invading trypanosomes (Lonsdale – Eccless and Grab, 2002). These factors enabling spread of trypsnosomes after infection is established in the host while they could vary between individuals and breeds of animals affecting outcome of infection.

Control of parasite proliferation and limitation of pathological effects are important components of the mechanism underlying trypanoresistance while productivity traits like oestrus cyclicity and body weight are part of the criteria used in resistance studies (Murray *et. al.*, 2004).

CHAPTER THREE

RETROSPECTIVE AND PROSPECTIVE SURVEY FOR TRYPANOSOMES IN GOATS IN MAKURDI AREA

3.1 Introduction

Investigations on vectors and trypanosomes should be carried out together, but this is rarely done (ILRAD, 1989). The current set of global priority goals for tsetse and trypanosomosis research seeks that research and intervention must be supported by farmers, for poverty reduction and increased food security (Ilemobade, 2002)

Field situation study involving retrospective and prospective survey for trypanosome status of goats in the area was done with the two fold objectives:

(a) Periscope the practical field situation of trypanosomosis of goats in the area.

(b) Forging link between the goat owner, treatment centre and the research being conducted for the purpose of creating increased awareness on the disease and future flow of benefits from the research towards poverty reduction and increased food security.

3.2.0 Materials and Methods

3.2.1 The study Area

Markurdi the study location is the capital of Benue State, North central Nigeria. It is located within the Guinea Savannah vegetation zone between longtitude 8^0 00^1 and 9^0 00^1 being traversed by longitude 8^0 30^1 within latitude 8^0 00^1 and 7^0 $30^{1.}$ See figure 3.1 Active agricultural activities are undertaken in Markurdi including research. The town host two universities, the University of Agriculture and Benue state University.

3.2.2 Retrospective Survey (April, 2005 – March, 2009)

The aim of this study was to establish the prevalence of trypanosome infection from previous diagnoses of caprine cases presented at veterinary clinics in Makurdi.

Diagnoses of ruminant cases presented at government veterinary clinics in Markurdi were reviewed. Four government veterinary clinics were involved. These include Markurdi local government veterinary clinic Wadata, and state veterinary clinic near fire service on JS Tarka road, both south of the River Benue. The others are; State veterinary Clinic North Bank and the University of Agriculture Veterinary Teaching hostpital annex, both are in the north of the River Benue. See figure 3.2

The caprine cases were extracted and compiled through adoption of the diagnoses found in the treatment files. Cases recorded as trypanosomosis were noted.

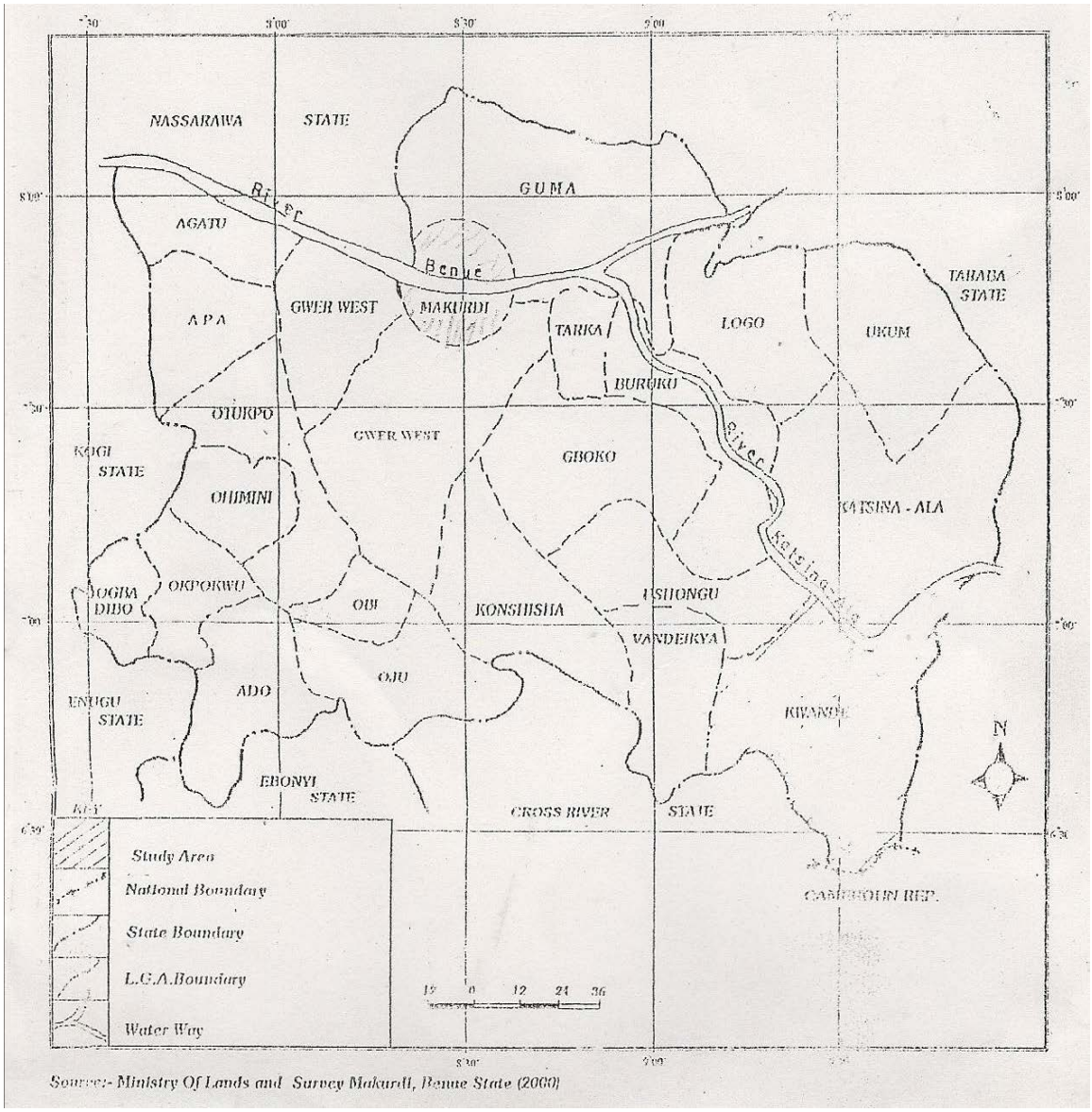


Fig. 3.1 **Benue state map showing Makurdi local government area**

Source: Ministry of Lands and Survey Makurdi.

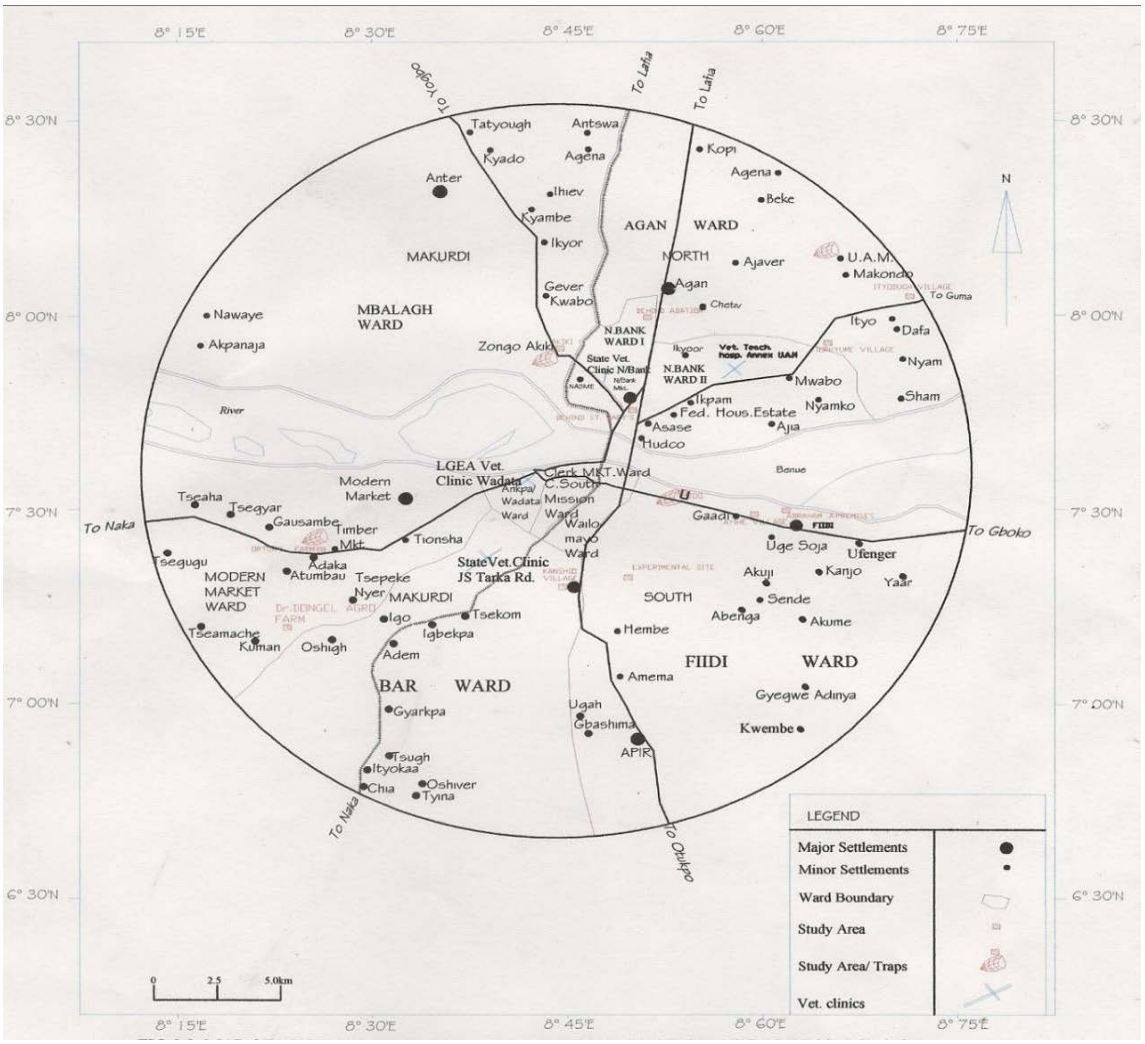


Fig. 3.2 **Map of Makurdi showing Vet Clinics, sites of prospective survey for Trypanosomes in goats and traps for vectors.**

Source: Ministry of Lands and Survey Makurdi/Project Data.

3.2.3.0 Blood Sampling Survey for Occurence of Trypanosomosis in Goats in Makurdi Area

Out of the 11 wards of Makurdi local government area, 7 wards were purposely selected to sample for trypanosomes among goats. The selection was done in favour of less urbanised perimetric wards where goats production was common. Words at the centre of the town were ministries were housed were not favoured since goats production was not likely to occure in those areas. Calculation of required sample size was determined using Win Episcope software (Ortega *et. al.*, 1996), taking expected prevalence at 50% (number recommended when disease frequency is not known), a confidence interval of 95% and an error of 5% with an estimated population of 3000 goats.

Survey sites are indicated on map of Markurdi showing study sites in figure 3.2. Jugular blood samples were collected from sampled animals to examine for trypanosomes.

3.2.3.1 Blood Collection

7.5ml blood was collected from animal's jugular vein and placed in vacutainer containing EDTA. Blood in vacutainer was immediately placed on ice pack or in a refrigerator at $4^{o}C$.

3.2.3.2 Examination for Parasite

Detection of parasite was carried out using the Buffy Coat Method (BCM) as described (Woo, 1970). Giemsa or Leishman stained thin blood smear was used to confirm the identity of parasite.

3.3.0 Results

Table 3.1 shows result of the retrospective study. A prevalence of 1.7% was found from the retrospective caprine cases diagnosed at the clinics surveyed. Table 3.2 shows occurrence of trypanosomes in survey animals using blood sampling. Jugular blood samples showed prevalence of 1.3% with absence of *T. brucei* but presence of *T. congolense* detected among the positive smears. As shown in table 3.2, the *T. congolense* positive samples occurred only in Agan council ward on northern bank of the river Benue.

Table 3.1 Clinically Diagnosed (Annual) Cases of Trypanosomosis at Makurdi between 2005 and 2009

Year	Cumulative no. of Caprine Cases presented at Clinics	No. of Cases Diagnosed as Trypanosomosis	% Positive
2005	30	-	
2006	33	1	
2007	33	-	
2008	66	1	
2009	79	2	1.7%

Table 3.2 Clinically Diagnosed (monthly) cases of Trypanosomosis at Makurdi between 2005 and 2009

Month	Cumulative no of caprine cases presented in the month	No of cases Diagnosed as Trypanosomosis
January	24	
February	17	
March	14	
April	12	
May	34	2
June	18	
July	15	
August	20	1
September	26	
October	16	
November	18	1
December	27	

Table 3.3 Occurence of trypanosomes in survey animals

Total no. of cases	Stratum/ward		Trypanosome species diagnosed (no. of cases)	Total no. of positive cases	Prevalence
		(A) Agan	(A) + *T congolense* (3)		
	North Bank	(B) Mbalagh	(B) -	3	1.3%
		(C)North Bank W_1	(C) -		
254		(D)North Bank W_2	(D) -		
		(E)Modern Market Ward	(E) -		
	South Bank				
		(F) Bar Ward	(F) -		
		(G) Fiidi Ward	(G) -		

3.4 Discussion

The finding of 1.7% trypanosomosis in retrospective survey and 1.3% from blood sampling survey of goats was similar to a prevalence of 1.7% reported in female WAD goats at Ibadan by Leigh and Fayemi (2011).It was however lower than 5% trypanosomosis prevalence reported by Daniel *et.al.* (1994) from goats in Bauchi State. It was also lower than 33.2% found in Gboko Local government area by Kalu *et. al.* (1991) and 33.9% prevalence in peridomestic goats reported by Omotainse et.al. (2000) in Konshisha, both of which are also local government areas in Benue State.

The finding of *Trypanosoma congolense* in all positive blood samples from survey in Makurdi was similar to report of Ameen et. al. (2008) in Ogbomosho area where this specie accounted for all infections found in ruminants (cattle, sheep and goats) surveyed.

Apart from factors like presence of vector, method used to detect trypanosomes in prevalence surveys vary in their sensitivity. Daniel *et.al.* (1994) reported superiority of concentration techniques such as buffy coat method and haemotocrit centrifugation over standard trypanosome detection methods like wet and thin films. The lower prevalence found in this work may be due to influence of factor that change over space and time which have potential to affect vectors. Such factors include: recent increased flooding along the Benue trough, expansion in Fadama farming of rice with increasing use of pesticides, changes in livestock demographic patterns with escalating Fulani nomad versus native conflicts in the area.

CHAPTER FOUR

EXPERIMENTAL INFECTION OF WEST AFRICAN DWARF AND RED SOKOTO GOATS WITH *T.BRUCEI* AND *T. CONGOLENSE*

4.1 Introduction

Trypanosomosis remains an important constraint to optimal productivity of animals. In particular when the disease co-exist with other diseases or in poor nutritional state and poor husbandry practices, there is a clear effect on productivity (Goossens et. al., 2009). Stress such as multiple infections usually prevalent under natural infections may prevent recovery of affected animal (Kahn and Scott, 2010). But where such stresses are removed under experimental infection, it becomes difficult to precipitate clinical disease (Mare, 1998). Therefore the degree of devastation induced by experimental infection could differ from that caused by natural infection.

Parasite factors such as when proteases degrade tissues to enable their penetration while control of their proliferation and limitation of pathological effects by host are important features of resistance that can affect responses to infection (Murray et. al, 2004). Even when the same breed of small East African goats were experimentally infected with the same *Trypanosoma congolense* species, regional differences were seen in the response that could be explained by underlying heterogeneity (Mutayoba *et.al.*, 1989). It has been reported that phenotypic differences probably underlined by difference in genotype were noticeable within WAD population when moving across a South to North transect in many West African countries (Leak *et. al.*, 2002). Such observation raised suspicion about genetic dilution of WAD with other breeds and possible loss of trypanotolerance.

Report based on recent studies alluded to the intromission of genes of trypanosusceptible breeds into WAD goat population with consequent loss of trypanotolerance in these animals (Geerts, *et. al.*, 2009). Simmilary, it has been argued that even with purebred indigeneous Livestock, resistance to trypanosomosis is not always complete. So a portion of animals often suffered some degree of production loss as a result of the disease (Agyemang, 2005). In the light of these observations, probing

the susceptibility to experimental trypanosome infection by WAD and Red sokoto goats around zone of overlap in the distribution of these breeds such as in Makurdi should yield clues on current level of susceptibility to trypanosomosis by their comparative populations.

4.2.0 Materials and Methods

4.2.1 Experimental Animals

Albino rats and mice were obtained from the experimental animal unit of college of Health sciences Benue State University, Markurdi. The mice were fed a commercial diet of grower mash (Vital Feeds Ltd. Jos)

Trypanosoma brucei and Trypanosoma congolense were obtained from the National Institute for Trypanosomiasis and onchocerchiasis Research (NITR / NITOR) Vom. Federe strain of *T. brucei* was originally obtained from a muturu-N'dama cattle in Federe, Plateau state in 1995.

Karu strain of *T. congolense* was originally obtained from white Fulani cow in Karu, Nassarwa state in 1995. Each parasite was transported to the experimental site through inoculation into both a donor goat and a pair of mice. Water was provided ad libitum. These animals on arrival were monitored daily for parasitaemia by wet mount and buffy coat technique. At peak (massive) parasitaemia, the animals were bled for sub-inoculation of more mice preparatory to infection of the goats.

Thirty-two goats comprising 16 WAD and 16 RSG were used for the experimental infection. The goats were obtained from farms and markets in and around Makurdi, Benue State, Nigeria.

4.2.2 Management of Experimental Goats

Goats were housed in insect proof pen that was separated into wooden stalls (cubicles) for individual housing of the animals on a concrete floor. Exit doors were double phased comprising the outer wooden panel door and the inner netted door. Each exit door located on western side opened into an aisle between separating rows of stalls and measuring 110 cm which ran through pen to terminate on eastern wall where a

conduit pipe drained at the back. Stalls measured 106 cm (L) X 82 cm (W) with partially netted doors on one row facing those of the other row across dividing aisle.

Feeding of goats was done using a mixture of freshly cut grasses and legumes. Supplemental feed was given based on the formulation of Dried Brewers Grain (DBG) 20%; maize offal 64.5%, Soya beans 12.0%, Bone ash 2.5% and salt 1.0%. Water and salt lick were provided adlibitum.

The animals were covered prophylactically with the following:

(1) Broad-spectrum antibiotic Terramycin long acting (LA) preparation 200 mg/ml at a dose of 20mg / 10kg body weight, equivalent to 1 ml of the prepation (Tridox ® _ Farvet, Bladel Holland) was given.

Parenteral Sulphonamide Sulfavet ® injection (Kepro B.V. Deventer _ Holland) at a starting dose of 3ml per 10kg body weight maintained by 1.5 ml per 10 kg for the next 3 days.

The anti-coccidial Amprolium 250 WSP (Kepro B.V. Daventer _ Holland) was administered also. This 250 mg per gram of powder preparation was given as 750 mg / 20 kg body weight in drinking water for 5 days

(2) An Ectoparasiticide Diazintol containing Diazinon Viantylate 152mg/ml as 16.2% W/V was given at recommended dilution of 100ml /100 – 200 litres of water for a 0.5 – 1.0% wash solution containing 10g / ml. While still in Quarantine, the animals were dusted three weeks later with pyrethrin preparation piffpaff (® Gongoni, Kano)

(3) Deworming was done with two drugs. Ivermectin (Kepromec ® _ Deventer, Holland) was given at rate of 1mg/5kg equivalent to 1ml/50kg of the preparation to cover both internal and external parasites. Second deworming (repeat) using Albendazole (Albendabolus 600 ® _ Eagle Chemical co. Ltd. Chungchongnam _ do, Korea).

(4) Vaccine administration against endemic peste des petits Ruminants (PPR) using PPR Vaccine (NVRI, VOM) at recommended administration of 1ml / animal subcutaneously was given.

Plate 4.1: Inner view of Pen with Goats in Cages

4.2.3 Experimental Design

An initial number of fifty male goats were acquired made up of twenty five each of the West African Dwarf and Red Sokoto breed which were estimated by their dentition to be about one year old. These were quarantined for the experiment and allowed to stabilize through two months. The goats were blocked by weight as sixteen each of the two breeds weighing 15kg $\pm$2kg were recruited and assigned randomly into four groups.

Animals were tagged with wooden tags which were in turn tied to a rope running loosely around the neck for permanent identification. The number on the tag was also painted by the side as additional identification for weak animals becoming recumbent. The number carried prefix denoting breed as either WD (for West Africa Dwarf or RS for Red Sokoto) followed by the number of group being 1, 2, 3, or 4 and the specific replicate within the group being A, B, C, or D. Pooled blood from mice infected with same specie of parasite showing similar parasitaemia count wet mount was obtained from bleeding through retro orbital plexus into specimen bottle.

Group 1 of each breed of goat received single *T. congolense* infection as 1ml of 5.2×10^5 trypanosomes intravenously through jugular vein.

Group 2 of each breed of goat received single *T.brucei* infection as 1ml of 5.2×10^5 parasites intravenously.

Group 3 of each breed of goat were given mixed infection having 0.5ml of 5.2×10^5 *T.congolense* and 0.5ml of 5.2×10^5 *T. brucei*.

Group 4 of each breed served as control

4.2.4.0 Determination of Parameters

Clinical parameters (Temperature, body weight, scrotal circumference, and parasitaemia), Haematological parameters (RBC and WBC counts, Haematocrit, RBC indices etc), Serum biochemical parameters (Creatinine, Alanine Aminotransferase, Aspartate Aminotransferase, Total protein etc) and pathological assessments (Mortality, Histopathology etc) were determined.

4.2.4.1 Determination of Rectal Temperature

Between 6 – 7 am daily temperature was taken. The bulb of digital thermometer was inserted into the rectum and left to make a beeping noise. Temperature was read and bulb cleaned in alcohol for use on another animal.

4.2.4.2 Determination of Weight

A meat type weighing scale was strapped onto a metal trolley having an articulated cage to hold the animal. With the aid of metal trolley, scale was pushed on concrete floor to individual cubicle, then animal entered the cage. Its weight was read from scale. Weighing was done weekly.

Plate 4.2 The use of weighing Scale

4.2.4.3 Determination of Scrotal Circumference

Scrotal tape was used to measure size of testicles at their greatest diameter.

4.2.4.4 Determination of Log_{10} parasitaemia

Mean number of Parasites counted in microscopic fields on the blood film were calculated and transformed using Log_{10} parasitaemia estimation method of Walker, (1969).

4.2.4.5 Determination of Haematological Parameters

4.2.4.5.1 Principle of Automation in Haematology by Use of Autoanalyzers

Two principles are utilized in haematology autoanalysis. The first is Voltage Pulse Counting. This is based on the fact that cells passing through an aperture in a conductive fluid cause changes in electrical resistance (impedence) resulting in voltage pulses that are proportional to the volume of the cell. The instrument directs these pulses through a threshold circuit where only those pulses above a certain threshold are captured and counted, so that as magnitude of pulse is proportional to cell size it can give data on cell size such as mean corpuscular volume (mcv), during the counting and sizing of cells in appropriately heated sample. For white blood cell counts, 1 in 500 dilution of sample is made using drops of diluents which lyses stroma of cells thus eliminating RBC but leaving WBC nuclei intact.

For RBC counts 1 in 50,000 dilution is made by which procedure the WBC being only little in number will not significantly affect RBC count. The second principle employed by autoanalyzers is the electro optical system where the counting is done by making use of optical flow cell. Cells passing through create deflection, scattering beam of light beyond a dark field disc where subsequent collection of the scattered rays by photo multiplier tube converts them – to electrical pulses that are counted to denote the cells since absence of cells cause no scattering of rays. An alternative third principle of digital image analysis utilizes stained blood film in computerized method where a modified microscope captures the illumination of the blood film and transforms it into digital data (Baker *et. al.*, 2001, Davidson and Henry, 1974).

Platelet counts are however, difficult on multipurpose automated impedence counters because it is necessary to use centrifugal and double threshold procedures to discriminate between them and other blood cells since the small microcytic RBC may be included with platelets or the large platelets may be excluded so that counts can be with error (Baker *et. al.*, 2001). Counting of platelets employs phase contrast microscopy as method of choice (Davidsohn and Henry 2974).

4.2.4.5.2 Procedure of Haematology Autoanalysis

About 7.5ml blood was collected from animals' jugular using vacutainer containing EDTA while 2.5ml was left in syringe for separation of serum.Blood in vacutainer was immediately placed on ice pack or in a refrigerator at 4^0C. Micro haematocrit capillary tube was used to aspirate sample for microscopy by buffy coat method while from same a little quantity was taken in 1ml syringe for other test. The blood sample for haematology autoanalysis was mixed properly by vortexing, then its sample number entered on sysmex KX 21 N haematology analyzer machine and stopper opened. Tube was brought to the sample aspirator nozzle and start knob switch opened When buzzer sound two times 'beep' 'beep' and LCD screen displayed analysing, the tube was removed as sample had been aspirated for units to execute automatic analysis and display result on screen. The unit then turned to ready status becoming ready for analysis of the next sample.

4.2.4.6 Determination of Serum Biochemical Parameters

4.2.4.6.1 Principle of Clinical Chemistry Autoanalysis

Methods employed in clinical chemistry analysis were listed to include manual techniques which may employ test kits using ready made reagents or rapid solid phase (dry reagent) strip-tests and the use of read-out analyzers employing ready made liquid reagents (Cheesbrough, 1998). The auto analyzer is an automated analyzer using a special liquid flow technique named continuous flow analysis (CFA) where continuously flowing stream of reagent is separated by air bubbles. It eliminated slow, clumsy and error prone manual methods of analysis (Wikipedia, 2011). Common errors eliminated

by auto analyzers include incorrect pipetting and dispensing, inadequate mixing of sample with reagents, use of an automatic pipettor that has been set to measure incorrect amount, etc. all of which give rise to imprecision or inaccuracy (Cheesbrough, 1998).

In 1974, Ruzicka and Hansen introduced a competitive technique termed Flow Injection Analysis (FIA) where continuous flow was replaced with computer controlled programmable flow. On the basis of operating principle, there are continuous flow analyzers or Flow Injection Analyzers (Wikipedia, 2011).

4.2.4.6.2 Procedure of Serum Biochemistry Autoanalysis

All analytes except electrolytes were analysed using Hitachi 902 automatic analyzer. Up to 35 serum samples were arranged on the sample disk. The sample probe picked and dropped the required microvolume of serum into the reaction cell/cuvette on the cuvette disk. The disk automatically rotated according to the programmed order and positioned the reaction cell/cuvette at a point where the reagent probe added the required volume of reagent according to the analyte's procedure on the written program. The cuvettes/reaction cell is then incubated by the system in a water reservoir at 37^{o}C for the required time frame while other analysis are in progress. At expiration of incubation period the reaction cell/cuvettes rotates on its axis and is positioned against a spectrophotometric component which quantifies the analytes by comparing its reaction color intensiity with that of standard solution. The system then prints out a calculated result and the process continued.

4.2.4.7 Assessment of Pathology

4.2.4.7.1 Determination of Mortality

Daily observations for cases of mortality and post-mortem examination on the animals were carried out during the experiment.

4.2.4.7.2 Post-Morterm Examination

Animals were observed daily after infection for changes in appearance and behaviour as well as signs exhibited. Post mortem examination was conducted on dead carcases using sharp knife to expose internal organs.

4.2.4.7.3 Histopathological Sections, Principles and Procedure

4.2.4.7.3.1 Principle of Histopathology Sections

Although often overlooked in veterinary sciences, histopathological diagnosis is useful in establishing pathogenesis and pathology of diseases caused by bacteria, parasites etc. As other tissues may be destroyed on transit from remote locations, formalin fixed tissues may be the only ones left on which to establish a diagnosis (Chauhan and Agarwal, 2006). These tissues should not be > 1cm thick but should be kept in 10% preservative $\geq$10 times their volume (Kahn and Scott, 2010).

4.2.4.7.3.2 Procedure for Histopathological Sections

Tissue sections of liver, spleen, lung, heart and kidney were fixed in 10% formaldehyde for over 48 hours. It was dehydrated by automated tissue processor Ser. No. 2337 (Shandom Elliot Ltd., Runcorn, Made in England) in ethanol of increasing strength (70%, 95%, 100% and 100%) each for 2 hours. It was transferred into three changes of xylene each lasting 2 hours in order to remove alcohol that had bathed the tissues. Infiltration of tissue with molten wax done in a wash oven (Baird & Tatlock, Laminton Limited, channel Health Essex, Made in England) at a temperature of 60^{o}c for 6 hours. This was followed with embedding in Mould that was left for solidification of wax. Little blocks of tissues were cut, held in paraffin wax. Using hot spatula, these were attached to pieces of wood to serve for clamping, positioning and trimming preparatory for sectioning. Using the microtone and microtone knife, tissues were sectioned at 4µm.

Tissue sections serially produced were floated out in floating water bath with 1% alcohol dropped on the water to enable floating of tissues which were picked on the side of a microscope slide that was pre coated with glycerine – egg albumin. It was labeled

with diamond pen oven dried for 30 minutes in readiness for staining with H & E. Briefly, slides were dewaxed in xylene by 3 dippings of 3 minutes each.They are hydrated for 3 minutes each in decreasing strengths of ethanol (100%, 95%, 70%) and water. Staining in Haematoxylin was done for 15minutes followed by washing excess stain, differentiation in 1% acid alcohol for 3 –5 seconds, washing off acid in water. This was followed by placing in ammonia water for blueing for 10 minutes, and then dehydrated in increasing strength of ethanol (50%, 70%, 95% and 100%) before clearing in three changes of xylene. It remained in the last until ready for mounting of cover slide using D.P.X. mountant.

Slides were examined microscopically at x 40 objective and histopathological findings recorded.

4.3.0 RESULTS

4.3.1 Clinical Parameters

The findings on Clinical parameters namely; temperature, body weight, scrotal circumference and parasitaemia are presented below:

4.3.1.1 Temperature

Table 4.1 shows the rectal temperature of control and infected goats. Mean $\pm$ (SD) of rectal temperatures were higher in infected animals than control with the highest values occurring in the *T.brucei* infected goats (RSG 41.28^{o}C and WAD 41.37 ^{o}C). The temperatures of mixed infected WAD were higher than those of WAD with *T.congolense* infection but conversely RSG with *T.congolense* infection had higher temperature than RSG with mixed infection.

Table 4.1 Temperature (ºC) of West African Dwarf and Red Sokoto goats infected with T. *brucei* and *T. congolense*

Inoculum	WAD	RSG
Control	38.05±0.03^{d}	38.07±0.03^{d}
T.brucei	41.37±0.50^{a}	41.28±0.48^{a}
T.congolense	41.12±0.04^{cd}	41.21±0.05^{ab}
Mixed Infection	41.17±0.04^{abc}	41.19±0.05^{ab}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test.

4.3.1.2 Body Weight

Table 4.2 shows values of weights(kg) of infected and controlled goats. No significant ($P > 0.05$) increase or decrease in weights of all treatment groups occurred across the duration of the experiment. Infected animals of both breeds experienced general depression in weight in first 3 weeks Pi (WADtb) RSGtbc, WADtbc or 4 weeks Pi (WADtc, RSGtc, RSGtb, RSGtbc, WADtbc). This however, dropped again in week 5 Pi (WADtb) and week 6 Pi (WADtc, RSGtb, WADtbc) but was sustained through week 6 Pi (RSGtc) and week 7 Pi (RSGtbc). Fluctuating rises and falls occurred in weights of control goats.

Table 4.2: Infection Weights(Kg) of West African Dwarf and Red Sokoto goats inoculated with *T. brucei* and *T. congolense*

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mixed infection	WAD_Mixed infection	RSG_control	WAD_control
Pre-infection	14.20±0.67^{a}	14.05±0.73^{a}	13.53±0.19^{a}	13.90±0.61^{a}	13.53±0.53^{a}	14.15±0.81^{a}	14.73±1.00^{a}	13.43±0.27^{a}
1	14.13±0.60^{a}	13.80±0.50^{a}	13.53±0.24^{a}	14.10±0.69^{a}	13.38±0.28^{a}	14.10±0.97^{a}	15.05±2.26^{a}	13.20±0.08^{a}
2	14.03±0.48^{a}	13.45±0.26^{a}	13.43±0.28^{a}	14.18±0.71^{a}	13.48±0.62^{a}	14.03±0.86^{a}	15.15±0.83^{a}	13.35±0.17^{a}
3	13.40±0.30^{a}	13.33±0.26^{a}	13.35±0.29^{a}	13.60±0.29^{a}	13.40±0.61^{a}	13.90±1.73^{a}	14.58±0.91^{a}	13.15±0.10^{a}
4	13.38±0.31^{a}	13.10±0.06^{a}	13.35±0.29^{a}	13.82±0.25^{a}	13.35±0.25^{a}	13.93±0.86^{a}	14.53±0.88^{a}	13.13±0.09^{a}
5	14.17±1.02^{a}	14.55±0.67^{a}	13.38±0.28^{a}	14.23±0.80^{a}	13.50±0.44^{a}	14.20±0.95^{a}	15.80±1.55^{a}	13.00±0.00^{a}
6	14.60±0.74^{a}	14.33±0.38^{a}	13.23±0.19^{a}	14.00±0.73^{a}	14.13±0.91^{a}	14.05±1.05^{a}	15.58±1.49^{a}	13.18±0.12^{a}
7	14.10±0.95^{a}	14.08±0.35^{a}	13.23±0.19^{a}	13.65±0.52^{a}	14.75±1.05^{a}	13.83±0.83^{a}	14.83±1.06^{a}	13.13±0.8^{a}
Total	13.97±0.20^{b}	13.83±0.16^{bc}	13.38±0.80^{bc}	13.93±0.19^{b}	13.62±0.17^{bc}	14.02±0.28^{b}	15.03±0.36^{a}	13.19±0.05^{c}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.1.3 Scrotal Circumference

Table 4.3 shows the scrotal circumference of infected and control goats. Mean scrotal circumference was similar during pre-infection across all the groups. It varied across the groups following infection. There was fluctuation in control groups both of Red Sokoto and West African dwarf breed. This fluctuation in control through the weeks. However, it decreased progressively in Red Sokoto goats with mixed infections. In West African dwarf goats with single *T.brucei and T. congolense* infections, there was a decrease from week 2 post infection (pi).

Table 4.3 Scrotal/Testicular Circumference (cm)

	Treatments (Mean±SE)							
Week	***RSG_T. congolense***	**WAD_*T.* *congolense***	**RSG_*T.* *brucei***	**WAD_*T.* *brucei***	**RSG_mixed infection**	**WAD_Mixed infection**	**RSG_control**	**WAD_control**
Pre-infection	8.20±1.19^{a}	17.7±0.36^{a}	18.38±0.18^{a}	18.05±1.85^{a}	19.23±1.69^{a}	16.90±0.65^{a}	19.75±0.32^{a}	17.13±0.24^{a}
1	18.80±1.43^{ab}	18.00±0.41^{ab}	17.95±0.79^{ab}	17.25±2.10^{ab}	17.93±0.78^{ab}	17.00±0.71^{b}	20.75±0.78^{a}	17.38±0.55^{ab}
3	18.500±1.04^{ab}	17.50±0.50^{b}	17.63±0.49^{b}	14.83±1.69^{c}	20.70±0.64^{a}	16.25±0.92^{bc}	18.50±1.22^{ab}	16.35±0.4^{bc}
5	18.33±1.42^{ab}	17.65±0.91^{ab}	18.20±0.36^{ab}	14.00±2.08^{c}	19.75±1.19^{a}	16.38±0.69^{bc}	19.20±0.69^{ab}	17.13±0.43^{ab}
7	15.97±1.79^{ab}	15.80±1.13^{ab}	16.83±0.43^{ab}	13.77±1.71^{b}	18.00±1.50^{a}	15.68±0.93^{ab}	19.00±0.58^{a}	16.45±0.25^{ab}
Total	18.02±0.59^{bc}	17.33±0.34^{cd}	17.80±0.23^{c}	15.82±0.87^{e}	19.24±0.49^{ab}	16.44±0.33^{de}	19.46±0.30^{a}	16.89±0.17^{cde}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.1.4 Parasitaemia

Figure 4.1 shows the parasitaemia. Parasitaemia fluctuated in all infected groups. Low log10 Parasitaemia was found in mixed infected WAD in week 3 Pi and RSG. Among WAD, the highest was in WAD with *T. brucei* and mixed infection in week 3 and 6 Pi respectively. Among RSG, lowest log10 Parasitaemia was in *T. brucei* infection in week 7 Pi while highest was in *T. congolense* infection in weeks 3 and 4 Pi. Consistently, higher log10 Parasitaemia in infected RSG than WAD with same infection.

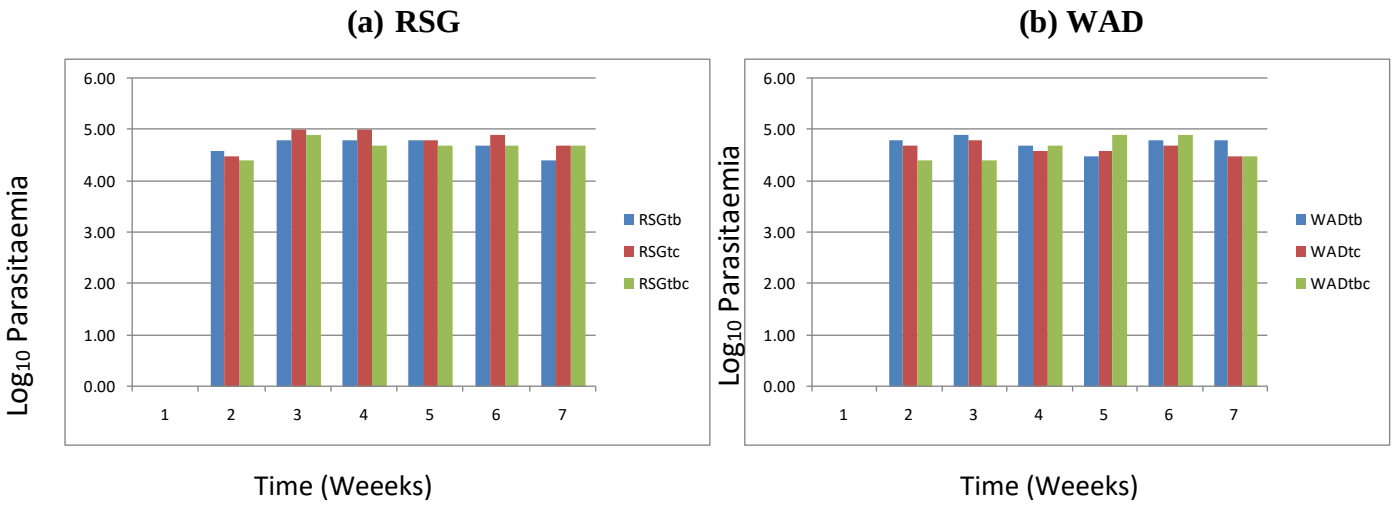


Fig. 4.1 Log_{10} Parasitaemia of all infected Goats

4.3.2 Haematological parameters of infected and non-infected goats

4.3.2.1 Total Red Blood Count

Figure 4.2 shows Total Red Blood cell Count of control and infected goats. RBC values fluctuated in both control and infected animals of the two breeds but in each breed, values of control group were higher than values of infected groups. Generally, RBC was higher in WAD than RSG of equivalent treatment.

RSG_{tb} had significantly lowest RBC of all the treatment groups. Among the WAD goats, mixed infected group (WAD_{tbc}) had the least mean RBC values.

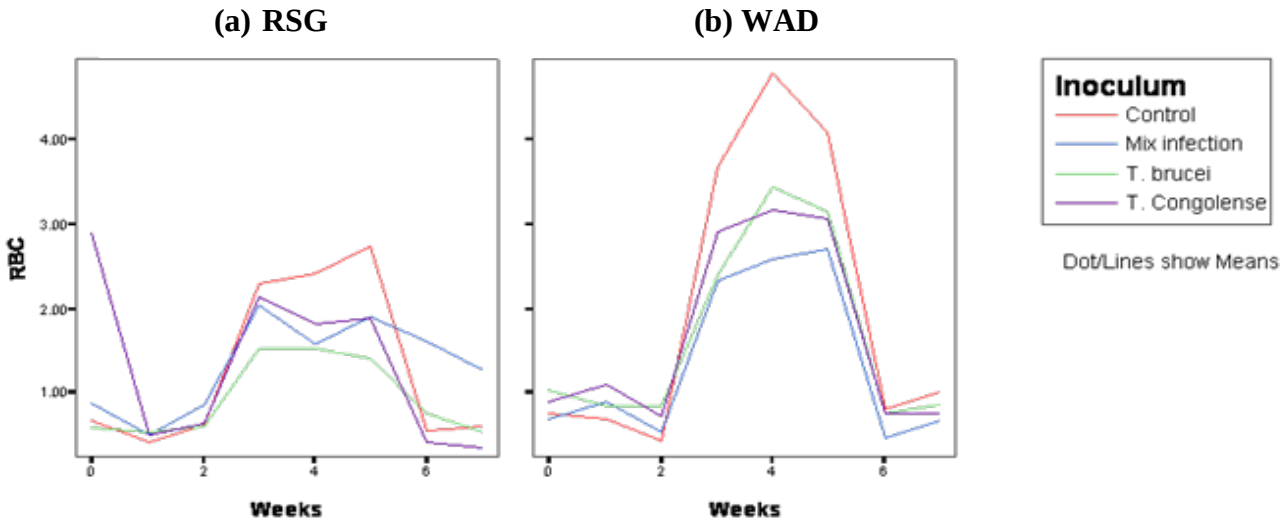


Fig 4.2 Total Red blood(x 10^6/µL) count of West African dwarf and Red Sokoto goats infected with *T.brucei* and *T.congolense*

4.3.2.2 Haematocrit

Table 4.4 shows the PCV (haematocrit) of both control and infected goats. Slight rise and fall occurred in control animals in week 1 Pi (Red Sokoto) and week 2 Pi (West African dwarf) but their levels remained high and in tune with pre infection values. Significantly low values were also associated with Red Sokoto goats having mixed infection (RSG_{tbc}) in week 6 Pi.

In each inoculum, haematocrit values of WAD were higher than those of RSG.

Table 4.4 Levels of Haematocrit (HCT) in West African dwarf and Red Sokoto goats infected with *T.brucei and T.congolense*

	Treatments (Mean±SE)							
Week	**RSG_*T. congolense***	**WAD_*T. congolense***	**RSG_*T. brucei***	**WAD_*T. brucei***	**RSG _mixed infection**	**WAD_Mixed infection**	**RSG _control**	**WAD _control**
Pre-infection	20.25±4.07^{a}	32.25±6.32a	27.50±5.78^{a}	37.00±4.49^{a}	31.00±7.52^{a}	24.00±5.08^{a}	23.25±3.15^{a}	25.75±7.03^{a}
1	17.75±3.20^{d}	39.50±7.50a	19.50±1.55^{cd}	29.75±1.31^{abc}	21.50±4.44^{bcd}	31.67±2.19^{ab}	16.00±1.78^{d}	24.00±5.12^{bcd}
2	23.00±4.60^{a}	26.75±3.42a	22.75±8.44^{a}	29.75±4.64^{a}	30.5±3.30^{a}	18.75±4.97^{a}	29.50±7.40^{a}	17.25±2.46^{a}
3	12.95±2.65^{c}	34.25±8.10ab	21.87±5.23^{bc}	29.33±5.33^{abc}	20.25±3.49^{bc}	29.360±2.56^{abc}	29.97±4.60^{abc}	43.75±3.08^{a}
4	14.87±3.25^{d}	30.58±3.85ab	17.43±2.83^{cd}	24.08±4.20^{bcd}	20.40±1.19^{bcd}	26.25±1.19^{bc}	25.03±2.70^{bcd}	38.85±4.37^{a}
5	13.63±0.60^{d}	29.85±4.04b	17.00±2.45^{cd}	23.35±2.8^{bcd}	21.38±4.33^{bcd}	26.43±2.72^{bc}	26.37±0.69^{bc}	40.40±4.08^{a}
6	11.83±0.60^{cd}	27.00±5.58ab	14.17±3.44^{bcd}	19.08±2.35^{abc}	10.33±1.45^{c}	18.90±2.81^{abc}	22.00±4.00^{abc}	29.25±5.91^{a}
7	16.67±3.33^{bc}	27.75±6.33ab	11.67±3.18^{bc}	21.25±2.17^{ab}	12.40±0.00^{b}	20.35±3.61^{ab}	24.07±2.97^{ab}	36.25±7.33^{a}
Total	16.96±1.29^{d}	30.42±1.89a	19.46±1.80^{cd}	26.70±1.50^{ab}	21.98±1.88^{cde}	24.30±1.34^{cd}	24.46±1.52^{bc}	31.94±2.24^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d.

Mean separation done with Duncan Multiple Range test

4.3.2.3 **Haemoglobin Concentration**

Table 4.5 shows haemoglobin concentration in control and infected goats. Haemoglobin levels remained near pre infection levels in control animals but decreased among infected groups through infection period especially in Red Sokoto goats infected with *T. brucei* which had significantly lowest levels. Among Control WAD and RSG, fluctuation during experimental period recorded mostly increases above initial value. Conversely, fluctuations recorded mostly decreases over initial value in infected WAD and RSG except WAD with mixed infection.

Table 4.5 Levels of Haemoglobin (g/L) in West African dwarf and Red Sokoto goats infected with *T.brucei* and *T.congolense*

	Treatments (Mean±SE)							
Week	RSG _*T. congolense*	WAD_*T. congolense*	RSG _*T. brucei*	WAD_*T. brucei*	RSG _mixed infection	WAD_Mixed infection	RSG control	WAD_control
Pre-infection	27.50±5.78^{a}	32.25±6.32^{a}	20.25±4.07^{a}	37.00±4.49^{a}	31.00±7.52^{a}	24.00±5.08^{a}	23.25±3.15^{a}	25.75±7.03^{a}
1	19.50±1.55^{cd}	39.50±7.50^{a}	17.75±3.20^{d}	29.75±1.31^{abc}	21.50±4.44^{bcd}	31.67±2.19^{ab}	16.00±1.78^{d}	24.00±5.12^{bcd}
2	22.75±8.44^{a}	26.75±3.42^{a}	23.00±4.60^{a}	29.75±4.64^{a}	30.5±3.30^{a}	18.75±4.97^{a}	29.50±7.40^{a}	17.25±2.46^{a}
3	21.87±5.23^{bc}	34.25±8.10^{ab}	12.95±2.65^{c}	29.33±5.33^{abc}	20.25±3.49^{bc}	29.36±2.56^{abc}	29.97±4.60^{abc}	43.75±3.08^{a}
4	17.43±2.83^{cd}	30.58±3.85^{ab}	14.87±3.25^{d}	24.08±4.20^{bcd}	20.40±1.19^{bcd}	26.25±1.19^{bc}	25.03±2.70^{bcd}	38.85±4.37^{a}
5	17.00±2.45^{cd}	29.85±4.04^{b}	13.63±0.60^{d}	23.35±2.8^{bcd}	21.38±4.33^{bcd}	26.43±2.72^{bc}	26.37±0.69^{bc}	40.40±4.08^{a}
6	14.17±3.44^{bc}	27.00±5.58^{ab}	11.83±0.60^{c}	19.08±2.35^{abc}	10.33±1.45^{c}	18.90±2.81^{abc}	22.00±4.00^{abc}	29.25±5.91^{a}
7	11.67±3.18^{b}	27.75±6.33^{ab}	16.67±3.33^{b}	21.25±2.17^{ab}	12.40±0.00^{b}	20.35±3.61^{ab}	24.07±2.97^{ab}	36.25±7.33^{a}
Total	19.46±1.80^{de}	30.42±1.89^{ab}	15.82±0.03^{e}	26.70±1.50^{bc}	21.98±1.88^{cde}	24.30±1.34^{cd}	24.46±1.52^{cd}	31.94±2.24^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.2.4 Mean Corpuscular Volume

Table 4.6 shows mean corpuscular volume in all groups remained close to pre infection levels except in week 3 post infection when there was a general rise among infected animals with significantly highest level in RSG having mixed infection. The exception was WAD with T. brucei infection that had significantly lowest level in this week which was below pre-infection value (table 4.6).

Table 4.6 Mean Corpuscular Volume (FL) of West African dwarf and Red Sokoto goats infected with *T.brucei* and *T.congolense*

	Treatments (Mean±SE)							
week	RSG _*T. congolense*	WAD_*T. congolense*	RSG _*T. brucei*	WAD_*T. brucei*	RSG _mixed infection	WAD_Mixed infection	RSG _control	WAD_control
Pre-infection	15.48±0.63^{a}	16.35±0.43^{a}	15.85±0.39^{a}	16.68±0.25^{a}	16.10±0.61^{a}	15.70±0.30^{a}	16.68±0.33^{a}	16.93±0.42^{a}
1	15.13±0.33^{c}	16.93±0.32^{a}	15.13±0.38^{c}	16.45±0.17^{ab}	15.65±.0.31^{bc}	16.40±0.25^{ab}	16.90±0.30^{c}	16.63±0.41^{bc}
2	16.03±0.52^{a}	16.08±0.26^{a}	16.03±0.43^{a}	16.15±0.32^{a}	16.60±0.48^{a}	16.98±0.38^{a}	16.78±0.44^{a}	16.28±0.27^{a}
3	19.00±2.23^{ab}	17.00±0.59^{ab}	19.00±1.67^{ab}	15.35±0.90^{b}	28.93±18.99^{a}	19.03±2.58^{b}	16.83±0.20^{b}	17.18±15.51^{ab}
4	16.14±4.19^{a}	16.29±2.53^{a}	16.14±2.60^{a}	16.64±0.85^{a}	16.56±1.83^{a}	16.97±1.20^{a}	16.85±0.84^{a}	17.14±1.49^{a}
5	19.54±2.22^{a}	17.97±1.49^{a}	16.54±3.57^{a}	18.53±2.67^{a}	19.99±0.79^{a}	17.20±2.71^{a}	17.19±1.79^{a}	17.07±1.98^{a}
6	19.40±2.95^{b}	20.08±0.45^{b}	19.40±11.95^{ab}	19.85±17.35^{a}	18.03±2.03^{b}	17.98±0.40^{b}	17.65±0.30^{b}	17.33±0.30^{b}
7	16.97±0.98^{a}	17.13±0.49^{a}	17.97±0.30^{a}	16.68±0.28^{a}	17.20±0.00^{a}	16.63±0.43^{a}	17.87±0.38^{a}	17.90±0.27^{a}
Total	17.21±0.67^{ab}	17.22±0.37^{ab}	16.96±1.50^{ab}	17.04±2.60^{ab}	18.63±3.13^{a}	17.11±0.48^{ab}	17.09±0.25^{b}	17.05±1.98^{ab}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.2.5 Mean Corpuscular Haemoglobin

Table 4.7 shows mean cell haemoglobin of infected and control goats. It was generally higher in RSG than WAD. An exception was in mixed infected groups when moderately lower values were found in RSG compared to WAD in weeks 2 and 3 Pi, taking a plunge to very low level in week 5 Pi. Among the different groups of RSG, MCH mean values of RSGtc were higher than those of control groups and lowest in RSG with mixed infection. On the other hand, MHC mean was higher in control than infected WAD groups except mixed infected WAD.

Table 4.7 Mean Corpuscular Haemoglobin (Pg) of West African dwarf and Red Sokoto goats infected with *T.brucei and T.congolense*

	Treatments (Mean±SE)							
week	RSG_T. congolense	WAD_T. congolense	RSG _T. brucel	WAD_T. brucel	RSG _mix infection	WAD_Mix infection	RSG _control	WAD_control
Pre-infection	43.88±12.79^{a}	29.85±5.19^{a}	41.44±14.34^{a}	24.91±1.64^{a}	31.56±5.41^{a}	36.03±5.16^{a}	37.37±6.45^{a}	33.58±4.69^{a}
1	45.79±7.56^{ab}	22.42±2.04^{c}	41.65±6.13^{abc}	26.79±1.33^{bc}	37.44±4.27^{abc}	26.07±1.70^{bc}	57.93±11.48^{a}	39.71±7.57^{abc}
2	44.03±14.45^{a}	32.40±3.66^{a}	37.59±7.09^{a}	29.19±3.12^{a}	27.16±2.37^{a}	39.83±5.54^{a}	37.39±7.54^{a}	47.65±7.17^{a}
3	46.41±7.07^{a}	26.43±4.49^{ab}	38.95±3.93^{ab}	29.50±3.74^{ab}	24.53±11.59^{b}	28.98±1.35^{ab}	41.61±6.19^{ab}	22.31±6.12^{b}
4	40.23±2.74^{a}	27.28±3.88^{b}	37.77±2.29^{ab}	32.05±2.35^{ab}	34.27±7.19^{ab}	30.60±1.96^{ab}	38.55±1.86^{ab}	27.58±1.98^{b}
5	38.47±3.38^{a}	25.80±2.90^{b}	37.13±0.53^{a}	31.40±2.47^{ab}	34.15±5.50^{ab}	29.88±2.03^{ab}	35.63±1.37^{ab}	29.30±2.82^{ab}
6	44.87±7.77^{a}	25.54±4.30^{ab}	26.56±8.83^{ab}	20.28±7.04^{b}	33.97±9.11^{ab}	33.85±5.76^{ab}	37.70±2.38^{ab}	31.13±4.42^{ab}
7	38.03±8.17^{a}	20.18±1.50^{bc}	27.25±2.54^{ab}	23.01±3.88^{abc}	10.60±0.00^{c}	28.63±2.55^{ab}	37.97±9.27^{a}	22.50±3.17^{abc}
Total	42.92±3.06^{a}	26.36±1.35^{d}	36.51±2.10^{bc}	27.14±1.32^{d}	30.32±2.54^{cd}	31.91±1.40^{cd}	40.96±2.66^{ab}	31.72±2.15^{cd}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.2.6 Mean Corpuscular Haemoglobin Concentration

Figure 4.3 shows MCHC of infected and control animals. There were fluctuations in values of MCHC across the weeks. Control animals of both breeds had a sharp rise along with infected WAD groups in week 2 Pi. This was less marked in WAD_{tc}. Among infected RSG, RSG_{tc} had values above control (RSG_c).

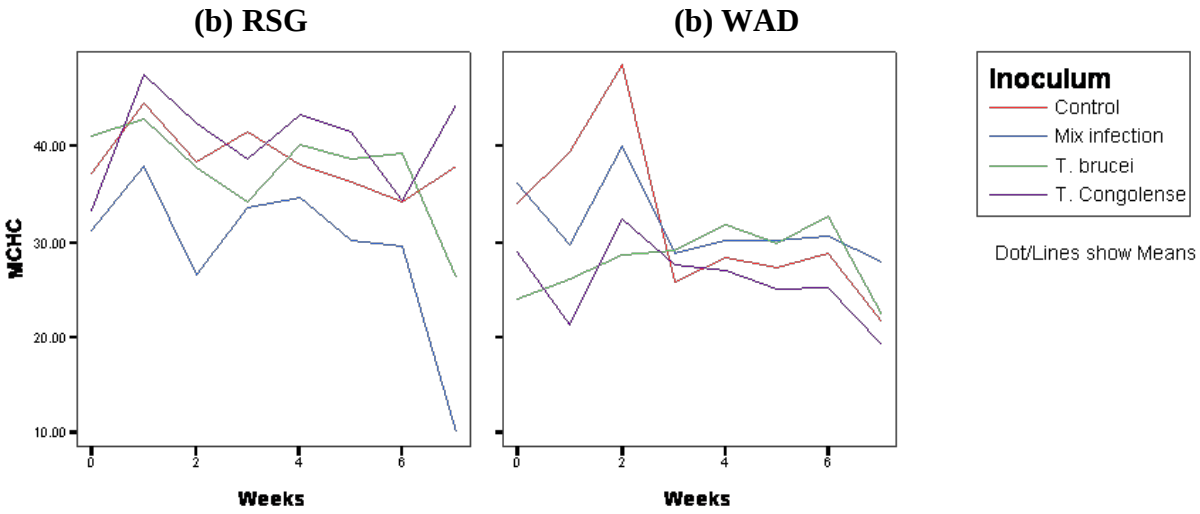


Fig 4.3 Mean Corpuscular Haemoglobin concentration (g/dL) of (b) West African dwarf and (a) Red Sokoto goats infected with *T.brucei* and *T.congolense*

4.3.2.7 Total White Blood Cell Count

Table 4.8 shows Total white blood cell count of control and infected goats. Inconsistent changes characterised values of total WBC. Overall mean drop relative to control was found in infected WAD goats (Leucopaenia) while mean increase occurred in RSG goats (Leucocytosis), except in *T.brucei* infected RSG. Through the infection period, however, values during pre infection had fluctuated to an appreciable rise in week 7 in RSG with *T. brucei,* RSG with mixed infection, WAD with *T. brucei* and WAD with mixed infection but marginal rise in RSG with *T. congolense*. On the contrary, decline was found in WAD with *T. congolense* in week 7.

Despite the general rise in RSG and general drop in WAD values of leucocyte count were still comparatively higher in control and infected WAD goats than RSG on equivalent inoculums, except in *T. congolense* infection.

Table 4.8 Total White Blood Cell Count (WBC)(x 10^3/µl) of West African dwarf and Red Sokoto goats infected with *T.brucei and T.congolense*

WBC

	Treatments (Mean±SE)							
Week	**RSG_*T. congolense***	**WAD_*T. congolense***	**RSG_*T. brucei***	**WAD_*T. brucei***	**RSG _mix infection**	**WAD_Mix infection**	**RSG _Control**	**WAD_ Control**
Pre-infection	24.57±2.64^{a}	39.78±18.09^{a}	20.38±4.10^{a}	26.33±6.50^{a}	14.53±3.31^{a}	27.48±9.09^{a}	16.55±1.21^{a}	37.28±14.48^{a}
1	29.90±8.55^{b}	23.65±2.31^{ab}	21.93±6.40^{ab}	23.43±2.44^{ab}	15.60±1.31^{ab}	28.53±7.04^{ab}	21.65±5.99^{ab}	34.97±4.67^{a}
2	13.93±3.85^{b}	23.20±5.68^{ab}	29.30±3.29^{ab}	33.32±7.19^{a}	19.92±2.41^{ab}	16.93±3.57^{ab}	19.20±4.17^{ab}	28.23±10.34^{ab}
3	35.87±7.75^{a}	23.45±2.15^{abc}	15.20±1.35^{bc}	20.98±2.42^{abc}	25.15±3.41^{abc}	23.70±2.60^{abc}	10.90±5.14^{c}	30.32±8.13^{ab}
4	20.07±3.39^{a}	17.48±3.08^{a}	12.57±1.24^{a}	13.60±3.45^{a}	16.85±9.41^{a}	24.10±7.15^{a}	15.88±3.70^{a}	17.65±3.00^{a}
5	29.73±10.72^{a}	16.05±1.52^{a}	15.77±1.74^{a}	16.37±5.43^{a}	29.90±8.79^{a}	13.23±2.73^{a}	21.10±7.70^{a}	33.15±13.84^{a}
6	22.77±6.16^{a}	17.88±1.19^{a}	16.20±3.26^{a}	17.02±4.58^{a}	20.68±6.73^{a}	17.08±3.34^{a}	20.43±7.40^{a}	14.75±2.91^{a}
7	24.60±3.61^{bc}	29.63±2.83^{abc}	27.67±1.49^{abc}	33.08±4.39^{abc}	23.70±0.00^{bc}	43.28±8.46^{a}	22.43±1.74^{c}	39.20±4.25^{ab}
Total	**25.37±2.38^{ab}**	**23.89±2.54^{ab}**	**20.32±1.60^{b}**	**23.23±1.96^{ab}**	**20.49±2.12^{b}**	**24.29±2.47^{ab}**	**18.39±1.59^{b}**	**29.44±3.14^{a}**

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3 Serum Biochemical Parameters of infected and Non-infected goats

4.3.3.1 Creatinine

Table 4.9 shows creatinine levels in control and infected goats. During pre infection period, creatinine levels were similar across the treatment groups. It remained so in weeks 1and 2 following infection. Significantly, low levels were found in the mixed infected goats of both breeds in week 3, (RSGtbc WADtbc) the significantly lowest level being in the mixed infected Red sokoto goats. The control animals of both West African dwarf and Red Sokoto breed had a fairly constant range of creatinine values through out the duration of experiment. The West African dwarf goat infected with *T.congolense* were also able to maintain fairly constant levels of creatinine while it fluctuated among other infected groups.

Table 4.9 Creatinine (µmol/L) Levels in WAD and RSG infected with *T.brucei* and *T.congolense*

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mix infection	WAD_Mix infection	RSG_control	WAD_control
Pre-infection	105.31±14.28^{a}	119.99±14.52^{a}	111.10±14.19^{a}	114.11.06^{a}	108.25±8.02^{a}	128.99±6.40^{a}	105.08±12.26^{a}	91.83±5.65^{a}
1	120.65±6.46^{a}	108.76±11.41^{a}	104.56±12.44^{a}	125.25±15.03^{a}	116.35±7.66^{a}	120.62±7.45^{a}	98.44±11.52^{a}	112.53±10.08^{a}
2	112.18±6.22^{a}	105.18±13.58^{a}	107.35±16.15^{a}	116.26±6.4^{a}	110.08±5.79^{a}	108.16±5.77^{a}	135.98±10.51^{a}	132.79±2.07^{a}
3	95.71±23.92^{ab}	106.44±30.61^{a}	104.10±20.11^{a}	61.77±1.41^{abc}	40.59±3.83^{c}	54.02±16.67^{bc}	67.55±2.07^{abc}	107.96±15.31^{a}
5	81.99±11.09^{ab}	101.12±15.08^{ab}	79.65±9.88^{ab}	98.19±21.22^{ab}	60.74±25.61^{b}	68.94±21.22^{b}	135.38±12.56^{a}	97.24±12.56^{ab}
6	65.83±6.71^{c}	87.71±8.04^{abc}	66.50±0.40^{c}	96.38±7.96^{a}	72.33±6.10^{bc}	90.32±3.21^{ab}	104.15±15.23^{a}	97.02±2.42^{a}
7	83.38±13.16^{bc}	106.79±0.45^{ab}	73.11±11.47^{c}	112.71±5.88^{ab}	74.29±6.97^{c}	107.83±7.54^{ab}	117.47±16.89^{a}	112.51±4.29^{ab}
Total	97.29±5.87^{ab}	105.18±5.21^{a}	95.29±5.81^{ab}	102.72±5.20^{a}	84.80±6.54^{b}	98.54±6.54^{ab}	107.97±5.98^{a}	106.47±3.83^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.2 Calcium

Table 4.10 shows mean calcium levels in control and infected goats. Mean calcium level was only significantly different between the groups in week 4 Pi, being lowest in mixed infected RSG (RSGtbc). After the transient drop, calcium level in this group picked up to remain similar with other infected groups and controls.

Table 4.10 Calcium (mg/100 ml serum) levels in *T. brucei* and *T. congolense* infected West African Dwarf and Red Sokoto goats

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mixed infection	WAD_Mixed infection	RSG_control	WAD_control
Pre-infection	2.69±0.26^{a}	2.55±0.19^{a}	2.62±0.36^{a}	2.89±0.10^{a}	2.66±0.29^{a}	2.85±0.18^{a}	2.51±0.35^{a}	2.85±0.01^{a}
1	11.16±0.32^{a}	10.63±0.25^{a}	10.41±0.57^{a}	11.19±0.10^{a}	11.34±0.55^{a}	10.32±0.29^{a}	10.65±0.44^{a}	10.33±0.46^{a}
2	5.69±1.55^{a}	7.04±1.66^{a}	6.27±1.57^{a}	7.42±1.26^{a}	5.69±0.55^{a}	6.31±0.74^{a}	6.28±1.20^{a}	5.46±0.32^{a}
3	4.72±0.90^{ab}	4.63±0.61^{ab}	4.44±1.29^{ab}	5.36±0.86^{ab}	4.12±0.82^{b}	4.50±0.42^{ab}	4.56±0.40^{ab}	7.48±2.05^{a}
4	1.25±0.13^{b}	1.17±0.06^{b}	1.41±0.35^{b}	1.44±0.24^{b}	0.95±0.15^{b}	1.19±0.09^{b}	1.17±0.17^{b}	5.42±5.17^{a}
5	13.79±0.47^{a}	14.43±0.35^{a}	14.96±1.09^{a}	14.76±0.29^{a}	13.71±0.59^{a}	14.28±0.17^{a}	14.91±0.80^{a}	14.14±0.26^{a}
6	13.57±0.01^{a}	13.80±0.43^{a}	13.64±0.45^{a}	14.69±0.85^{a}	13.79±0.27^{a}	14.01±0.22^{a}	13.74±0.29^{a}	13.87±0.19^{a}
7	14.29±0.18^{a}	14.32±0.14^{a}	14.76±0.53^{a}	14.96±0.31^{a}	14.49±0.38^{a}	14.51±0.24^{a}	14.73±0.51^{a}	14.55±0.53^{a}
Total	8.03±1.10^{a}	8.56±0.10^{a}	8.22±1.09^{a}	9.33±0.95^{a}	7.59±1.02^{a}	8.38±0.96^{a}	8.20±0.10^{a}	9.52±0.90^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.3 Glucose

Table 4.11 shows Glucose levels in control and infected goats. Significant variation in glucose profiles only occurred in week 4 pi. Control animals of both breeds had profiles in fluctuating manner, maintaining a narrow range. But these and pre-infection levels became significantly lower than profiles of infected animals within this week 3pi.

The significantly lowest level among infected animals in the week 4 was associated with Red Sokoto goats with mixed infection.

Table 4.11 Glucose (Mg/dL) levels in *T. brucei* and *T. congolense* infected West African Dwarf and Red Sokoto goats

	Treatments (Mean±SE)							
Week	**RSG_*T. congolense***	**WAD_*T. congolense***	**RSG_*T. brucei***	**WAD_*T. brucei***	**RSG_mixed infection**	**WAD_Mixed infection**	**RSG_control**	**WAD_control**
Pre-infection	0.13±0.16^{a}	0.13±0.13^{a}	0.18±0.08^{a}	0.43±0.19^{a}	0.23±0.10^{a}	0.45±0.21^{a}	0.10±0.04^{a}	0.05±0.09^{a}
1	1.08±0.35^{a}	1.50±0.04^{a}	1.03±0.45^{a}	1.25±0.16^{a}	1.10±0.33^{a}	1.05±0.35^{a}	0.95±0.34^{a}	1.60±0.11^{a}
2	1.83±0.62^{ab}	1.68±0.30^{ab}	1.10±0.52^{b}	1.80±0.53^{ab}	2.73±0.68^{a}	1.35±0.55^{ab}	1.38±0.20^{ab}	1.57±0.24^{ab}
3	0.80±0.15^{a}	0.40±0.17^{a}	0.40±0.21^{a}	0.55±0.18^{a}	0.48±0.40^{a}	0.50±0.17^{a}	0.23±0.18^{a}	0.40±0.23^{a}
4	2.28±0.05^{ab}	2.33±0.21^{ab}	2.63±0.35^{a}	2.43±0.43^{ab}	1.55±0.64^{ab}	2.38±0.14^{ab}	1.30±0.61^{b}	1.70±0.16^{ab}
5	0.30±0.10^{a}	0.40±0.11^{a}	0.57±0.18^{a}	0.70±0.30^{a}	0.75±0.25^{a}	0.73±0.88^{a}	0.65±0.05^{a}	0.43±0.10^{a}
6	2.60±-0.01^{a}	2.90±0.36^{a}	2.43±0.22^{a}	2.93±0.25^{a}	2.15±0.36^{a}	2.40±0.04^{a}	2.65±0.15^{a}	2.85±0.12^{a}
7	2.50±0.31^{a}	2.38±0.70^{a}	1.53±0.33^{a}	1.40±0.04^{a}	2.00±0.10^{a}	1.70±0.35^{a}	1.93±0.32^{a}	5.57±4.22^{a}
Total	1.29±0.20^{a}	1.45±0.20^{a}	1.18±0.20^{a}	1.43±0.20^{a}	1.37±0.20^{a}	1.34±0.16^{a}	1.07±0.18^{a}	1.65±0.46^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.4 Triglycerides

The table 4.12 shows the level of triglycerides in control and infected goats. Pre infection values of triglycerides ranged from 34 – 53.5 while values in week 7 pi ranged from 0.33 to 22.67. There was depletion of serum triglycerides across the weeks in all infected groups of both breeds. The lowest value was associated with *T. congolense* infected Red Sokoto goat in week 7, when the non- significantly highest level was in *T. congolense* infected West Africn dwarf goats. Triglyceride levels also fluctuated in control animals, being higher in WAD than RSG. Every infected innoculum reflected this higher WAD over RSG values except in mixed infection.

Table 4.12 Triglycerides (Iu/L) levels in *T. brucei* and *T. congolense* infected West African Dwarf and Red Sokoto goats

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mixed infection	WAD_Mixed infection	RSG_control	WAD_control
Pre-infection	50±6.03^{a}	37.50±8.15^{a}	38.25±4.73^{a}	43.50±7.22^{a}	44.25±8.38^{a}	34.75±2.39^{a}	53.50±14.27^{a}	47.50±9.13^{a}
1	38.00±7.45^{ab}	27.00±2.74^{b}	27.50±5.17^{b}	48.25±3.59^{a}	41.25±3.84^{ab}	32.00±7.82^{ab}	30.25±4.48^{b}	32.50±4.87^{ab}
2	8.50±4.50^{b}	17.33±3.53^{ab}	2.25±9.32^{ab}	6.00±2.04^{b}	27.25±2.66^{a}	10.67±13.04^{ab}	7.25±11.74^{ab}	16.33±2.03^{ab}
3	19.33±9.74^{a}	15.50±1.50^{a}	3.25±7.27^{a}	7.50±5.14^{a}	13.50±4.73^{a}	9.50±3.95^{a}	4.75±5.92^{a}	17.50±3.84^{a}
4	10.33±10.41^{ab}	10.25±7.26^{ab}	2.75±4.87^{b}	47.33±26.46^{a}	6.00±6.44^{b}	11.50±7.73^{ab}	8.75±10.50^{b}	27.67±16.34^{ab}
5	4.33±2.03^{a}	4.75±2.39^{a}	4.33±1.20^{a}	3.00±5.05^{a}	1.00±6.00^{a}	0.67±4.63^{a}	3.67±1.86^{a}	1.00±2.74^{a}
6	6.00±-0.01^{b}	26.25±4.71^{ab}	10.00±6.56^{ab}	32.50±12.70^{a}	22.25±7.76^{ab}	17.00±4.14^{ab}	18.00±7.94^{ab}	28.00±3.70^{ab}
7	0.33±3.84^{a}	22.67±11.62^{a}	6.67±5.46^{a}	14.00±6.07^{a}	14.50±8.50^{a}	5.67±7.17^{a}	2.00±10.00^{a}	1.67±5.04^{a}
Total	16.43±4.51^{a}	19.14±3.09^{a}	11.48±3.23^{a}	23.00±4.71^{a}	23.18±3.38^{a}	16.21±2.97^{a}	16.61±4.56^{a}	21.83±3.58^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.5 Alkaline phosphatase

Table 4.13 shows the level of Alkaline phosphatase in control and infected goats. Alkaline phosphatase levels fluctuated in the Red Sokoto goat of control group, pre infection level was lowest in West African dwarf with mixed infection, but the level in this group shot up by several orders of magnitude as from week 1pi. Both the West African dwarf goats of control and infected groups had high levels of alkaline phosphatiase during the course of the infection. Conversely, all the infected Red Sokoto goats had diminishing levels of alkaline phosphatise. In all three infected groups of the Red Sokoto breed, the levels of this enzyme crashed by the second week pi and took a diminishing trend through the weeks up to week 7 pi. Significantly, lowest levels occurred in the Red Sokoto goats infected with *T. congolense* in weeks 5 and 7 those infected with T. brucei had the lowest levels in weeks 2, 3, 4, and 6.

Table 4.13 Alkaline Phosphatase (Iu/L) levels in *T. brucei* and *T. congolense* infected West African Dwarf and Red Sokoto goats

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mixed infection	WAD_Mixed infection	RSG_control	WAD_control
Pre-infection	562.75±285.16^{a}	595.25±487.97^{a}	454.00±199.20^{a}	718.33±637.95^{a}	173.00±83.67^{a}	79.67±23.13^{a}	121.50±53.72^{a}	199.33±80.34^{a}
1	966.25±509.03^{a}	423.25±302.68^{a}	76.75±20.92^{a}	101.67±38.13^{a}	387.50±316.85^{a}	842.67±557.68^{a}	127.00±65.60^{a}	198.50±98.08^{a}
2	72.50±19.50^{a}	286.00±165.56^{a}	49.75±15.13^{a}	307.50±168.95^{a}	57.00±22.00^{a}	510.00±121.25^{a}	120.75±19.27^{a}	332.33±246.69^{a}
3	51.33±12.98^{a}	203.00±133.20^{a}	37.00±13.32^{a}	353.178.70^{a}	62.25±10.26^{a}	749.25±418.23^{a}	95.67±6.74^{a}	741.33±349.82^{a}
4	41.33±11.02^{a}	235.50±177.23^{a}	34.00±11.02^{a}	251.00±107.97^{a}	42.5±3.66^{a}	564.75±448.74^{a}	64.33±15.90^{a}	399.33±335.11^{a}
5	32.33±7.80^{a}	400.75±194.78^{a}	49.00±21.20^{a}	108.50±62.47^{a}	60.00±24.11^{a}	266.33±66.46^{a}	86.67±16.59^{a}	445.25±217.88^{a}
6	35.00±7.55^{b}	276.75±171.43^{b}	34.67±9.60^{b}	316.50±168.78^{b}	52.00±4.10^{b}	1164.75±395.47^{a}	71.67±15.88^{a}	545.00±299.10^{ab}
7	36.33±8.41^{a}	232.00±143.46^{a}	61.33±25.83^{a}	332.25±184.11^{a}	51.00±5.00^{a}	900.00±549.81^{a}	116.00±19.35^{a}	2072.50±1439.41^{a}
Total	274.00±109.91^{b}	331.56±79.82^{b}	110.00±38.75^{b}	306.41±77.32^{b}	120.93±48.87^{b}	664.140.69^{a}	102.96±12.62^{b}	645.07±226.19^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.6 Alanine Aminotransferase (ALT)

Table 4.14 shows the levels of Alanine Aminotransferase in control and infected goats. Pre infection levels defered non significantly among the groups. The RSG_{tbc} had significantly highest level while the lowest level was in RSG_c within week 1. After week 7pi, mean ALT level deferred non significantly between the groups, being highest in $RSG_{tbc.}$

Table 4.14 Alanine Aminotransferase (ALT) (U/L) levels in *T. brucei* and *T. congolense* infected West African Dwarf and Red Sokoto goats

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T Congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mixed infection	WAD_Mixed infection	RSG_control	WAD_control
Pre-infection	20.20±1.89^{a}	22.65±3.39^{a}	26.63±1.26^{a}	24.20±0.25^{a}	21.45±3.46^{a}	23.30±1.55^{a}	20.00±3.08^{a}	25.70±1.65^{a}
1	26.58±2.55^{ab}	29.63±1.90^{ab}	32.10±3.08^{ab}	29.85±0.78^{ab}	39.85±7.72^{a}	26.35±0.78^{ab}	24.18±4.84^{b}	27.18±2.87^{ab}
2	19.55±2.85^{a}	27.60±6.25^{a}	17.80±1.02^{a}	21.73±1.57^{a}	28.33±4.19^{a}	25.93±1.92^{a}	21.20±1.30^{a}	23.23±2.38^{a}
3	19.87±2.20^{a}	23.30±5.36^{a}	15.03±0.77^{a}	19.25±4.48^{a}	15.55±1.99^{a}	15.63±1.14^{a}	18.55±2.07^{a}	17.28±1.83^{a}
4	18.33±1.83^{ab}	25.03±4.08^{a}	12.60±1.76^{b}	19.50±2.00^{ab}	15.70±4.90^{ab}	19.27±4.05^{ab}	19.83±1.45^{ab}	22.45±3.05^{ab}
5	14.37±0.55^{a}	16.53±2.19^{a}	18.37±2.85^{a}	21.33±1.94^{a}	15.70±4.90^{a}	19.27±4.05^{a}	19.83±2.51^{a}	22.45±3.05^{a}
6	13.10±1.10^{b}	17.43±2.42^{ab}	11.80±0.86^{b}	18.78±0.91^{ab}	20.45±3.31^{a}	16.25±1.04^{ab}	18.90±1.62^{ab}	20.50±2.39^{a}
7	15.10±1.50^{b}	17.00±1.41^{ab}	16.20±2.62^{ab}	18.83±1.60^{ab}	16.35±2.75^{ab}	17.20±1.42^{ab}	22.55±1.55^{a}	18.63±2.16^{ab}
Total	19.14±1.09^{a}	22.36±1.42^{a}	19.16±1.41^{a}	21.33±0.10^{a}	22.79±2.25^{a}	20.18±1.00^{a}	20.61±0.93^{a}	22.14±0.96^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.7 Aspartate aminotransferase

Table 4.15 shows the levels of *Aspartate aminotransferase* in control and infected goats. Pre infection levels of *Aspartate aminotransferase* were similar among the different groups of both breeds and remained so up to week 4pi. The levels varied significantly in week 5, becoming lower in all groups constituted by goats of West African dwarf breed, with the exception of controls. The significantly lowest levels in this week were associated with West African Dwarf goats infected with *T.brucei*. The levels increase dramatically in first week of infection, the increase being most in Red Sokoto goats having mixed infection. By the second week of infection, the levels started decreasing in all infected animals. By the fourth week pi the Ast in Red Sokoto goats with mixed infection had more than doubled its pre infection level. Conversely, in this fourth week pi, the West African Dwarf goats carrying mixed infection had AST reduced to half its pre infection level.Conversely, the control animals of both breeds have fluctuating rises and falls in AST levels through all the weeks.

Table 4.15 Aspartate Aminotransferase (AST.) (U/L) levels in *T. brucei* and *T. congolense* infected West African Dwarf and Red Sokoto goats

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mixed infection	WAD_Mixed infection	RSG_control	WAD_control
Pre-infection	117.33±17.33[a]	113.40±9.05[a]	143.15±4.60[a]	142.65±8.06[a]	111.35±8.77[a]	174.95±43.96[a]	123.70±21.39[a]	130.03±15.33[a]
1	155.23±16.03[a]	187.15±38.27[a]	175.60±7.93[a]	167.95±10.51[a]	169.48±15.86[a]	187.55±20.24[a]	151.85±18.76[a]	170.85±29.39[a]
2	118.05±20.95[a]	88.88±4.13[a]	110.53±13.14[a]	88.37±5.64[a]	129.20±31.40[a]	103.68±7.47[a]	104.38±5.96[a]	98.67±18.91[a]
3	94.33±20.52[a]	75.27±1.90[a]	75.70±7.38[a]	72.47±1.47[a]	270.30±162.21[a]	90.78±12.52[a]	105.33±8.77[a]	91.45±75.95[a]
4	104.07±13.34[a]	115.78±21.57[a]	92.63±8.05[a]	93.27±4.81[a]	224.08±101.91[a]	86.85±6.70[a]	103.08±1.33[a]	122.10±7.11[a]
5	87.98±11.46[a]	71.90±7.89[ab]	96.80±2.55[a]	40.83±18.28[b]	98.30±16.50[a]	75.40±2.62[ab]	101.77±10.44[a]	90.93±10.09[a]
6	76.77±8.08[a]	78.88±9.58[a]	87.60±4.31[a]	73.08±10.09[a]	169.90±74.68[a]	73.80±5.38[a]	146.18±54.41[a]	77.70±8.34[a]
7	77.08±9.84[a]	88.30±33.37[a]	86.37±3.13[a]	65.18±8.97[a]	90.70±9.83[a]	69.85±6.04[a]	88.37±11.27[a]	81.53±7.90[a]
total	104.15±6.79[a]	103.82±9.11[a]	112.46±7.08[a]	93.82±8.44[a]	165.60±28.58[b]	108.90±9.94[a]	117.35±8.67[a]	108.62±8.56[a]

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.8 Total Protein

Table 4.16 shows the levels of total protein in control and infected goats.

Whereas between controls, mean total protein was higher in RSG (RSGc) than WAD (WADc), a converse situation occurred among infected goats. In each inoculum, mean total protein of WAD was higher than RSG (WADtc higher than RSGtc), (WADtb higher than RSGtb) and (WADtbc higher than RSGtbc).

Table 4.16 Total Protein (g/L) levels in *T. brucei* and *T. congolense* infected West African Dwarf and Red Sokoto goats

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mixed infection	WAD_Mixed infection	RSG_control	WAD_control
Pre-infection	71.99±4.85^{a}	81.32±3.24^{a}	76.31±4.31^{a}	76.02±2.21^{a}	73.57±3.48^{a}	71.24±1.1.97^{a}	78.45±5.75^{a}	74.25±4.34^{a}
1	74.44±1.90^{a}	70.89±5.28^{a}	64.87±5.58^{a}	72.21±3.62^{a}	70.65±4.06^{a}	66.34±7.86^{a}	70.18±3.92^{a}	65.15±4.43^{a}
2	89.95±7.08^{abc}	104.81±2.87^{a}	88.08±7.25^{abc}	93.74±4.65^{ab}	92.88±6.51^{ab}	101.93±6.02^{a}	76.16±1.46^{c}	79.45±3.52^{bc}
3	88.27±0.18^{abc}	99.21±3.59^{a}	81.91±5.65^{bcd}	89.68±2.98^{abc}	79.84±4.54^{bcd}	93.58±6.45^{ab}	70.70±1.78^{d}	75.94±6.28^{cb}
4	83.97±1.06^{b}	101.85±3.70^{a}	95.87±6.98^{ab}	89.77±3.98^{ab}	82.20±4.03^{b}	94.40±4.53^{ab}	64.23±5.22^{c}	69.52±2.95^{c}
5	89.21±4.54^{ab}	101.96±6.88^{a}	100.44±3.70^{a}	97.65±7.26^{a}	86.88±0.02^{ab}	92.50±2.96^{a}	73.71±1.17^{bc}	68.44±2.34^{c}
6	65.40±0.01^{d}	99±4.76^{a}	90.25±3.60^{ab}	88.74±4.46^{abc}	79.11±3.99^{bcd}	85.46±5.50^{abc}	70.62±2.58^{cd}	77.65±9.03^{bcd}
7	90.03±2.79a	100.24±6.70^{a}	94.69±4.11^{a}	92.57±6.64^{a}	84.80±1.66^{a}	91.25±8.97^{a}	89.34±12.65^{a}	78.86±4.40^{a}
Total	81.97±2.04^{bc}	95.76±2.45^{a}	85.20±2.74^{bc}	87.55±2.09^{b}	80.13±1.85^{cd}	86.91±2.84^{bc}	74.13±2.01^{d}	73.52±1.85^{d}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.9 Albumin

Table 4.17 shows the levels of albumin in control and infected goats. Infected animals generally had progressive fall in albumin reaching lowest levels in all West African dwarfs in week 3 except those infected with *T. brucei* where it occurred later in week 4. The progressive fall in albumin reached lowest level in week 6 in all infected Red Sokoto goats except those with mixed infection where it occurred earlier in week 5. Thus, in each breed, controls had higher albumin levels than infected animals. Between the two breeds, infected WAD had higher mean levels than infected RSG of equivalent inoculums, converse to the situation between control WAD and RSG.

Table 4.17 Albumin (g/L) levels in *T. brucei* and *T. congolense* infected West African Dwarf and Red Sokoto goats

	Treatments (Mean±SE)							
Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG_mixed infection	WAD_Mixed infection	RSG_control	WAD_control
Pre-infection	26.78±3.07^{b}	32.63±2.21^{ab}	32.10±2.59^{ab}	34.48±0.80^{a}	33.98±2.29^{a}	33.13±2.06^{ab}	27.60±1.63^{ab}	31.48±1.32^{ab}
1	27.53±1.10^{a}	31.95±1.16^{a}	26.65±4.48^{a}	30.40±1.31^{a}	30.23±0.99^{a}	27.95±2.26^{a}	25.40±3.18^{a}	28.28±4.39^{a}
2	26.45±0.95^{ab}	27.38±3.57^{ab}	21.97±1.95^{b}	30.88±2.09^{ab}	26.33±3.97^{ab}	26.55±1.74^{ab}	33.53±1.53^{a}	28.50±1.40^{ab}
3	27.07±1.12^{ab}	27.70±3.53^{ab}	22.67±2.37^{b}	28.50±2.04^{ab}	25.65±3.92^{ab}	25.85±0.91^{ab}	31.58±1.37^{a}	29.95±2.05^{ab}
4	26.23±1.97^{a}	25.50±1.90^{a}	27.45±2.05^{a}	30.95±4.48^{a}	23.88±3.74^{a}	28.32±1.68^{a}	30.63±5.10^{a}	31.03±3.82^{a}
5	31.73±8.75^{a}	28.33±3.02^{a}	25.20±1.97^{a}	31.00±2.47^{a}	21.25±5.15^{a}	35.38±5.15^{a}	35.97±2.82^{a}	30.98±1.07^{a}
6	24.07±3.72^{b}	33.70±8.46^{ab}	19.70±1.47^{b}	35.75±15.63^{ab}	23.13±3.17^{b}	26.90±1.26^{ab}	44.93±6.54^{a}	37.63±6.65^{ab}
7	25.23±3.73^{bc}	31.95±0.75^{abc}	26.87±1.77^{bc}	31.53±2.10^{abc}	23.35±6.65^{c}	30.60±1.17^{abc}	37.35±2.95^{a}	33.43±1.71^{ab}
Total	26.92±1.22^{bc}	29.82±1.38^{ab}	25.56±1.15^{c}	31.68±1.18^{a}	26.50±1.32^{bc}	29.29±1.11^{abc}	32.66±1.48^{a}	31.52±1.19^{a}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.10 Globulin

Table 4.18 shows the levels of globulin in control and infected goat. Globulin values were lower in controls than infected animals. Significantly different values ooccured in weeks 2, 3, 4 and 6 Pi when the significantly lowest values were in controls (WAD and RSG). Higher mean value of globulin was found in WAD compared to RSG in each given inoculum.

Table 4.18 Globulin (g/L) values of control and infected goats.

Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG _*T. brucei*	WAD_*T. brucei*	RSG _mix infection	WAD_Mix infection	RSG _control	WAD_control
Pre-infection	45.21±4.14^{a}	43.39±3.65^{a}	44.21±2.65^{a}	46.84±3.82^{a}	39.60±5.46^{a}	38.12±3.25^{a}	50.85±5.23^{a}	42.78±3.24^{a}
1	46.91±2.40^{a}	40.26±2.80^{a}	38.22±2.52^{a}	41.46±4.17^{a}	40.43±4.98^{a}	38.39±5.83^{a}	44.78±4.08^{a}	36.88±3.13^{a}
2	57.37±0.00^{bcd}	66.36±4.78^{abc}	66.11±5.80^{abc}	73.93±3.13^{ab}	66.38±7.98^{abc}	75.38±5.87^{a}	42.64±2.78^{d}	51.41±4.87^{cd}
3	61.20±1.14^{abc}	60.53±3.42^{abc}	56.95±6.36^{abc}	70.71±4.11^{a}	54.19±6.86^{bcd}	67.73±5.76^{ab}	39.13±0.90^{d}	45.99±5.10^{cd}
4	57.73±0.92^{b}	64.27±4.71^{ab}	73.14±10.96^{a}	70.90±3.78^{ab}	58.33±3.42^{b}	66.08±3.30^{ab}	33.60±2.71^{c}	38.06±6.04^{c}
5	57.48±5.81^{a}	69.32±5.22^{a}	75.24±2.84^{a}	70.96±9.08^{a}	65.63±5.13^{a}	64.23±2.59^{a}	37.74±2.30^{b}	37.46±1.93^{b}
6	41.80±6.40^{bc}	55.04±11.24^{ab}	70.55±2.95^{a}	63.87±11.60^{ab}	55.98±5.91^{ab}	58.56±6.22^{ab}	25.68±7.41^{c}	40.03±2.71^{bc}
7	64.79±5.13^{a}	66.68±1.00^{a}	67.82±4.73^{a}	68.72±8.59^{a}	61.45±5.00^{a}	55.74±10.42^{a}	54.82±18.41^{a}	45.43±5.50^{a}
Total	53.61±2.05^{b}	57.57±2.71^{ab}	59.44±3.07^{ab}	64.13±2.94^{a}	53.61±2.60^{b}	57.90±2.98^{ab}	41.12±2.22^{c}	42.09±1.53^{c}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.3.11 Albumin – Globulin Ratio

Table 4.19 shows the levels of albumin – globulin ratio in control and infected goats. Pre-infection albumin-globulin ratio of all groups ranged from 0.56 ± 0.01 in RSGc to 0.93 ± 0.17 in RSGtbc. By week 7 Pi, the ratio ranged from 0.39 ± 0.14 in RSGtbc to 0.77 ± 0.10 in WADc. When mean ratio were higher in control than infected animals. Among infected animals of various inoculums, mean ratios were lower in RSG on single infections than WAD but conversely, mixed infected RSG had higher ratio than mixed infected WAD.

Table 4.19 Albumin: Globulin Ratio of *T. brucei* and *T. congolence*

Week	RSG_*T. congolense*	WAD_*T. congolense*	RSG_*T. brucei*	WAD_*T. brucei*	RSG _mix infection	WAD_Mix infection	RSG _control	WAD_control
Pre-infection	0.61±0.10^{ab}	0.78±0.11^{ab}	0.73±0.06^{ab}	0.75±0.07^{ab}	0.93±0.17^{a}	0.90±0.13^{a}	0.56±0.07^{b}	0.74±0.04^{ab}
1	0.59±0.05^{a}	0.80±0.05^{a}	0.70±0.13^{a}	0.72±0.05^{a}	0.79±0.13^{a}	0.75±0.06^{a}	0.59±0.20^{a}	0.79±0.15^{a}
2	0.45±0.00^{b}	0.42±0.08^{b}	0.33±0.03^{b}	0.42±0.04^{b}	0.43±0.14^{b}	0.36±0.04^{b}	0.80±0.09^{a}	0.57±0.07^{b}
3	0.44±0.03^{bc}	0.46±0.07^{bc}	0.40±0.05^{c}	0.71±0.05^{c}	0.53±0.15^{bc}	0.39±0.02^{c}	0.81±0.04^{a}	0.67±0.07^{b}
4	0.46±0.04^{b}	0.41±0.05^{b}	0.38±0.08^{b}	0.45±0.08^{b}	0.42±0.07^{b}	0.43±0.02^{b}	0.93±0.20^{a}	0.89±0.24^{a}
5	0.59±0.20^{bc}	0.41±0.04^{c}	0.34±0.03^{c}	0.47±0.09^{c}	0.33±0.10^{c}	0.44±0.01^{c}	0.97±0.14^{a}	0.83±0.05^{ab}
6	0.60±0.25^{b}	0.94±0.55^{b}	0.28±0.01^{b}	0.74±0.34^{b}	0.45±0.11^{b}	0.48±0.08^{b}	2.47±1.28^{a}	0.93±0.11^{b}
7	0.40±0.09^{a}	0.48±0.02^{a}	0.40±0.05^{a}	0.49±0.09^{a}	0.39±0.14^{a}	0.60±0.13^{a}	0.75±0.20^{a}	0.77±0.10^{a}
Total	0.53±0.04^{c}	0.60±0.08^{bc}	0.47±0.04^{c}	0.55±0.05^{bc}	0.56±0.06^{bc}	0.55±0.04^{bc}	0.95±0.16^{a}	0.77±0.04^{ab}

Note: Mean±SE across a row with different superscripts are significantly different with a>b>c>d. Mean separation done with Duncan Multiple Range test

4.3.4 Pathological Findings

4.3.4.1 Mortality

Mortalities occurred among RSG to a similar level of 25% in single *T. brucei* and *T. congolense* infection. However, higher level of mortality of 50% was found in mixed infected RSG. The mortalities stretched from weeks 4 Pi to week 7 Pi.

Table 4.20 Post Infection Case Fatality Rate

		Time (Weeks)									
Breed	**Inoculum**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total Mortality**		**Percentage (%)**
WAD	***T. congolense***	-	-	-	-	-	-	-	**0**	**0**	**0**
	T. brucei	-	-	-	-	-	-	-	**0**	**0**	
	Mixed Infection	-	-	-	-	-	-	-	**0**	**0**	
RSG	***T. congolense***	-	-	-	**1**	-	-	-	**1**	**25**	**33.3**
	T. brucei	-	-	-	-	-	-	**1**	**1**	**25**	
	Mixed Infection	-	-	-	-	-	**2**	-	**2**	**50**	

4.3.4.2 Post mortem Findings

Post mortem observation on dead animals revealed some findings.

Ante mortem – observation of infected animals showed starring hair coat, evident weaknesses with some animals on sternal recumbency, lacrinal discharges and sunken eyes.

Post mortem - External examination of dead carcasses showed gross emanciation, dehydration, pallor of eyes and enlargement of pre-scapular lymph node.

Internal examination revealed adherence of skin to sternal angle, emanciation of muscles with cooked appearance of gluteal muscles and prominent ribs.

In the thoracic cavity there was hydrothorax with amber fluid. The heart had blood clot in its chambers. Lungs had white patches with areas of congestion.

In abdominal cavity, voluminous peritoneal fluid appearing dull amber in colour was found. Spleen had blunt edges. The stomach had gelatinous atrophy of fat.

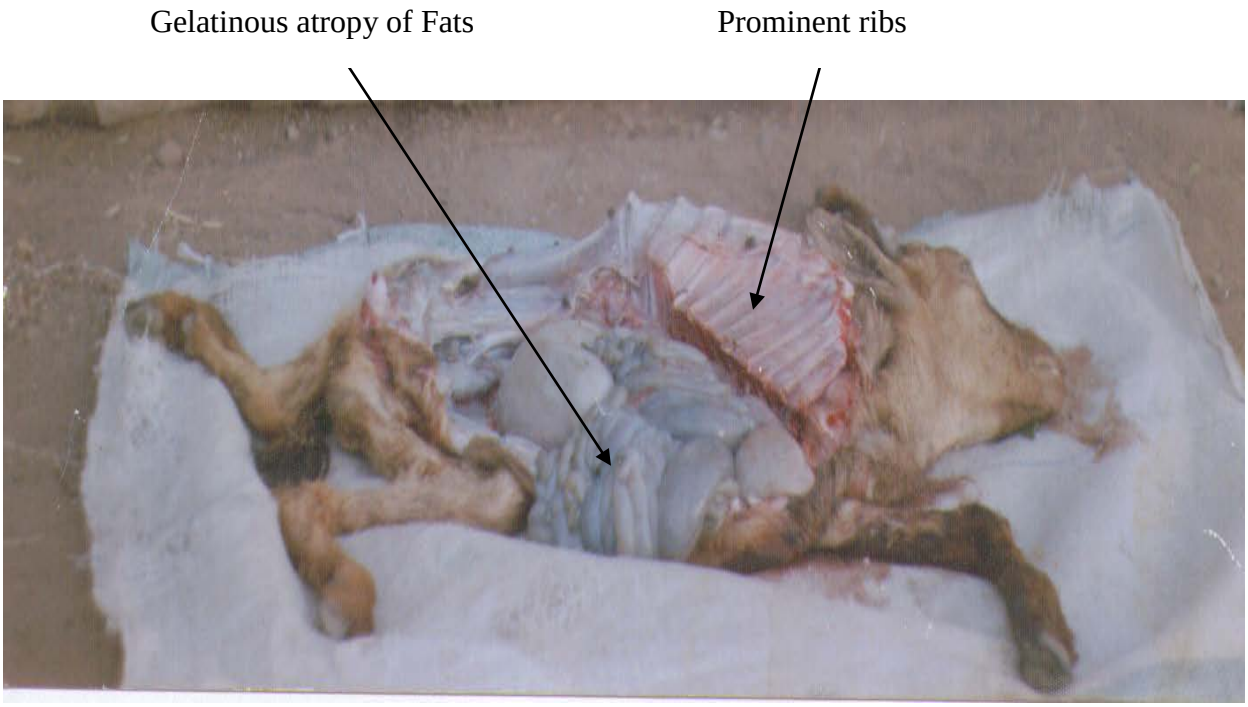


Plate 4.3: Mortality Case – Mixed infected RSG (*T. congolense and T. brucei*)

4.3.4.3 Histopathology

Table 4.21 – 4.24 and Plates 4.4 – 4.12 shows histopathology of infected and control goat. The score of severity based on histopathology reveal equal degree of pathology induced by *T.congolense* and Mix infection in the two breeds.

However, greater severity was induced by *T.brucei* in the Red Sokoto goats. Similar organ pathology appeared to be induced by the infections although periportal lymphocytic infiltration was only seen in *T. brucei* and mixed infected RSG liver. Also centrilobular necrosis was seen in mixed infected liver of WAD and RSG.

Table 4.21 Histopathology in *T. Congolense* infected WAD and RSG goats

	Observed Histopathology						
Breed Sampled	**Liver**	**Spleen**	**Lung**	**Kidney**	**Heart**	**Severity Score**	**Mean Score/breed**
RSG	Sinusoidal dilatation Kupffer cell hyperplasia RSGIC1 ++	Marked lymphoid depletion and Macrophage hyperplasia with haemosiderinRSGIC1 +++	Normal RSGIC2	Normal RSGIC2		+++++	+++++
RSG	Kupffer cell hyperplasia with haemosiderosis RSGIDI ++	Lymphoid depletion RSGID RSGID3 +	RSGID Hyperplasia bronchial associated lymphoid tissue ++	Normal RSGID2	Normal RSGID1 RSGID2	+++++	

Table 4.21 Contd.

	Observed Histopathology						
Breed Sampled	**Liver**	**Spleen**	**Lung**	**Kidney**	**Heart**	**Severity Score**	**Mean Score/breed**
WAD	Sinusoidal dilatation Kupffer cell hyperplasia and shrinkage of hepatic cords DWIB +++	Lymphoid depletion WADIB +	Pulmonary congestion with Macrophage laden with haemosiderin WADIB ++	Normal WADIB	Normal WADIB	++++++	+++++
WAD	Dilated Sinusoids with Kupffer cell hyperplasia and haemosiderosisWADIC3 Inappropriate WADIC1 +++	Marked lymphoid depletion WADIC2 +	Normal WADIC2	Normal WADIC	Normal WADIC3 WADIC	++++	
WAD	Sinusoidal dilatation Kupffer cell hyperplasia and haemosiderosisWADID1 +++	Marked depletion Macrophage hyperplasia with haemosiderin ++	Normal WADID1	-	Normal WADID3	+++++	

Table 4.22 Histopathology in *T.brucei* infected WAD and RSG goats

	Observed Histopathology						
Breed Sampled	Liver	Spleen	Lung	Kidney	Heart	Severity Score	Mean score/breed
	Dilated Sinusoids with macrophage hyperplasia and Haemosiderosis RSG2A2 +++	macrophage hyperplasia and Haemosiderosis marked depletion RSG2A2 +++	--	Normal RSG2A1	Normal RSG2A1	++++++	++++++
RSG	-	Macrophage hyperplasia and Haemosiderosis RSG2B2 ++	Congestion RSG2B2 +	Normal RSG2B1	Normal RSG2B1	+++	
RSG	Kupffer cell hyperplasia with haemosiderosis and periportal lymphocytic infiltration RSG2D2 ++++	Macrophage hyperplasia and haemosiderosis RSG2D3, RSG2D lymphoid depletion RS2D1 +++	Congestion RSG2D3 +		Normal RSG2D1	++++++++	

Table 4.22 Contd.

Breed Sampled	Liver	Spleen	Lung	Kidney	Heart	Severity Score	Mean score/breed
WAD	-	Normal WAD2B2 spleen with macrophage hyperplasia Haemosiderosis and lymphoid depletion. WAD2B3 +++	Normal WAD2B2	Normal WAD2B2	Normal WAD2B3	+++	++++
WAD	Dilated Sinusoids with Kupffer cell hyperplasia and haemosiderosis +++	Normal WAD2C4	Normal WAD2C2 WAD2C3	Proteinaceous cast on Bowman's capsule WAD 2C2 +		++++	
	Dilated Sinusoids with macrophage hyperplasia and haemosiderosis WAD2D1 +++	Normal WAD2D1	Congestion with some foci of oedema WAD 2D2 +			++++	

Table 4.23 Histopathology in mixed *T.brucei* and *T. Congolense* infected WAD and RSG goats

	Observed Histopathology		**Mixed Infection Group**				
Breed Sampled	Liver	Spleen	Lung	Kidney	Heart	Severity Score	Mean score/breed
WAD	Dilated Sinusoids with Kupffer cell hyperplasia and haemosiderosis. WAD3A, WAD3A2 +++	Macrophage hyperplasia with plasmacytosis and haemosiderosis WAD3A +++	-	Normal WAD3A	-	++++++	++++++
WAD	Kupffer cell hyperplasia with haemosiderosis WAD3B1 ++	lymphoid depletion and Macrophage hyperplasia WAD3B1 ++	Normal WAD3B	Normal WAD3B	-	++++	
WAD	Sinusoidal dilatation Kupffer cell hyperplasia with haemosiderosis with some Centrilobular necrosis. WAD3C2 ++++	Macrophage hyperplasia with plasmacytosis and haemosiderosis WAD3C3 +++	-	Normal WAD3C3	-	+++++++	

Table 4.23 Contd.

Breed Sampled	Liver	Spleen	Lung	Kidney	Heart	Severity Score	Mean score/breed
WAD	Sinusoidal dilation with Macrophage hyperplasia and haemosiderosis. WAD3D1 +++	Marked depletion with Macrophage hyperplasia WAD3D3 ++	-	Normal WAD3D2	Normal WAD3D3	+++++	
RSG	Fatty degeneration that is centrilobular in pattern RSG3A3 ++	lymphoid depletion few macrophage with haemosiderin RSG 3A3 ++	Foci of pulmonary oedema,extensive pulmonary congestion RSG3A +++	-	Normal RSG3A2	+++++++	++++++
RSG	Dilation of Sinusoids, Kupffer cell hyperplasia periportal lymphocytic infiltration RSG3B3 ++++		Normal RSG3B3	-	-	++++	
RSG	Sinusoidal dilatation, Kupffer cell hyperplasia and haemosiderosis with Centrilobular necrosis and anaemia. RS3D2 +++++	Depletion of Lymphoid spleen. + RSG3D2	Normal RSG 3D	Normal RSG3D3	Normal RSG3D	++++++	

Table 4.24 Histopathology in control WAD and RSG

	Observed Histopathology					
Breed Sampled	Liver	Spleen	Lung	Kidney	Heart	Severity Score
RSG	Fatty degeneration anaemia RSG 4A2	Macrophage RSG 4A3	-	Normal RSG 4A	Normal	
RSG	Normal RSG 4B2	Normal RSG 4B	Normal RSG 4B	Normal RSG 4B2	Normal RSG 4B3	
WAD	-	-	Pulmonary congestion and macrophages WAD4A1	Normal WAD4A2	Normal WAD4A2	

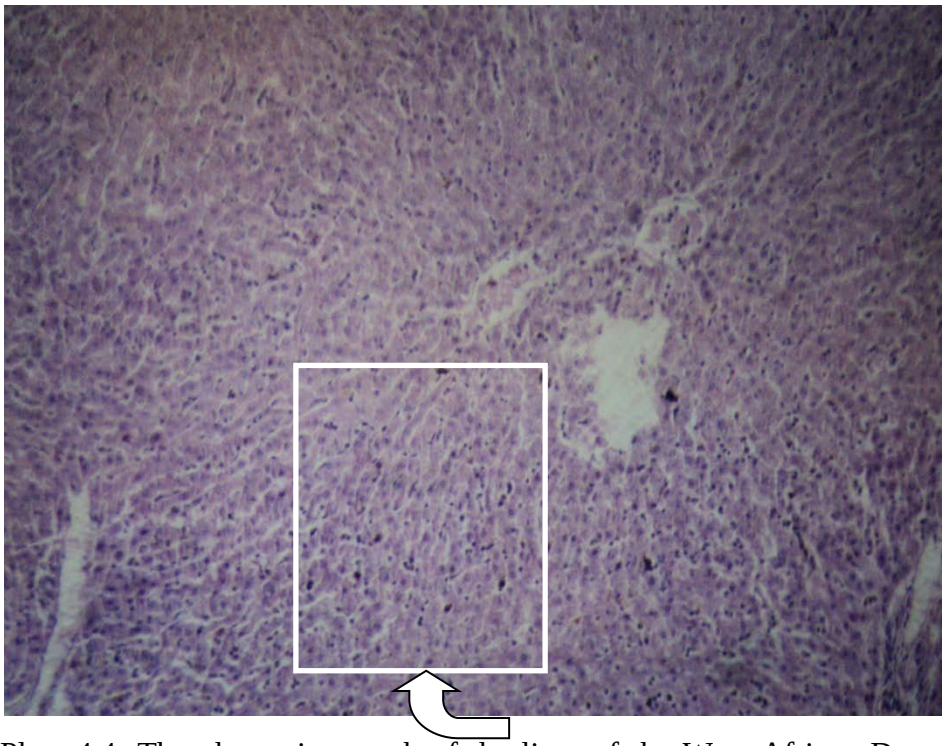


Plate 4.4: The photomicrograph of the liver of the West African Dwarf goat infected with *Trypanosoma brucei* showing kupffer cell hyperplasia and hemosiderosis

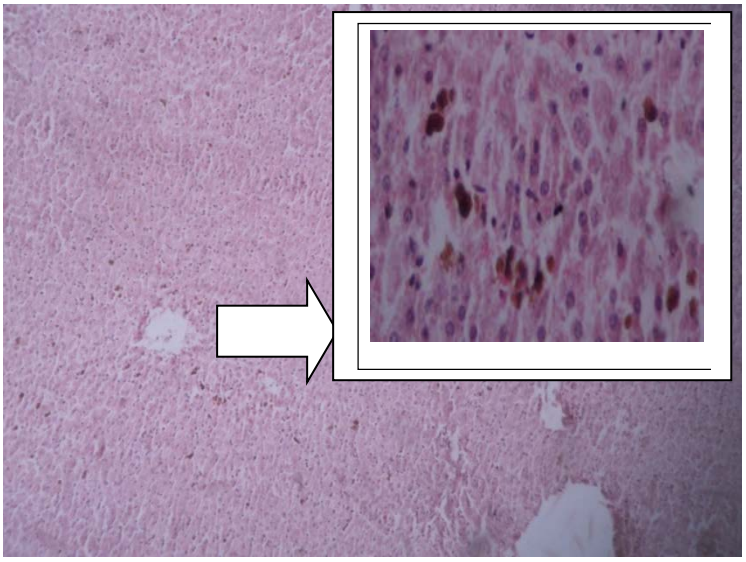


Plate 4.5: The photomicrograph of the liver of the Red Sokoto goat infected with *Trypanosoma brucei* showing kupffer cell hyperplasia and hemosiderosis (arrow)

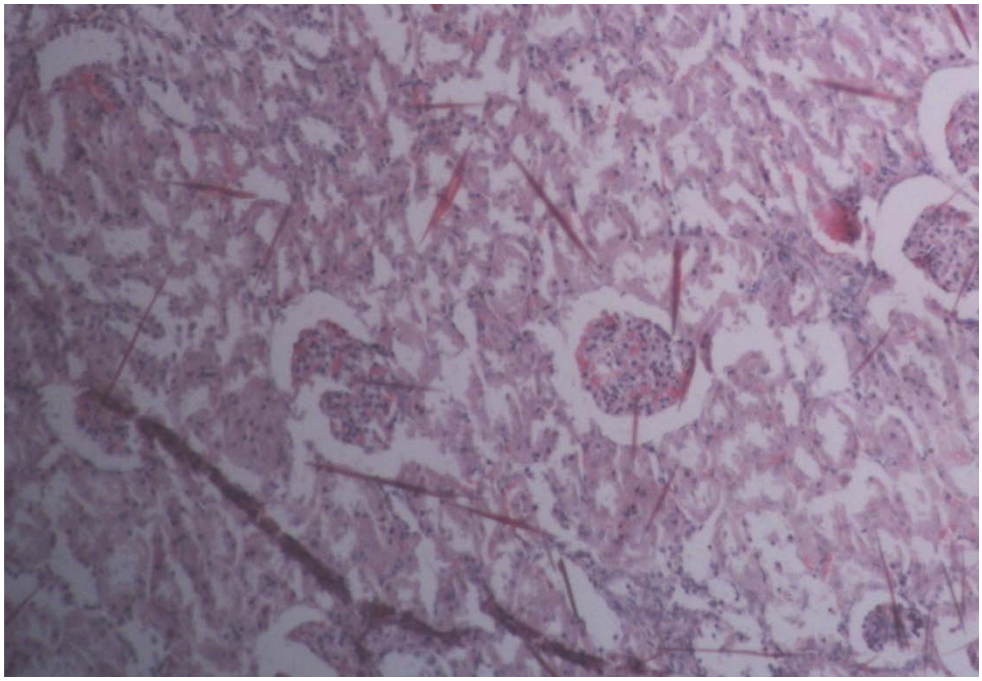


Plate 4.6: The photomicrograph of the kidney of the Red Sokoto goat infected with *Trypanosoma brucei and Trypanosoma congolense* showing no visible lesion

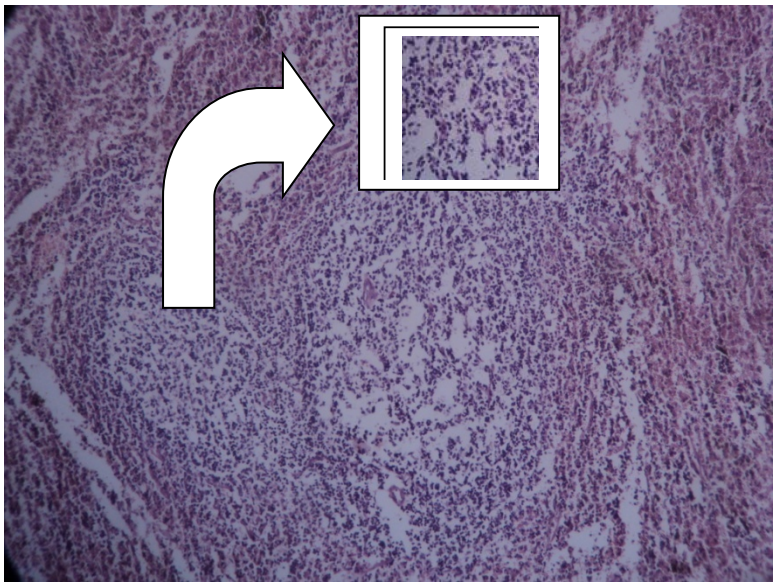


Plate 4.7: The photomicrograph of the spleen of the West African Dwarf goat infected with *Trypanosoma congolense* showing macrophages laddened with hemosiderosis and plasma cell hyperplasia (Mag X 100 400 insert)

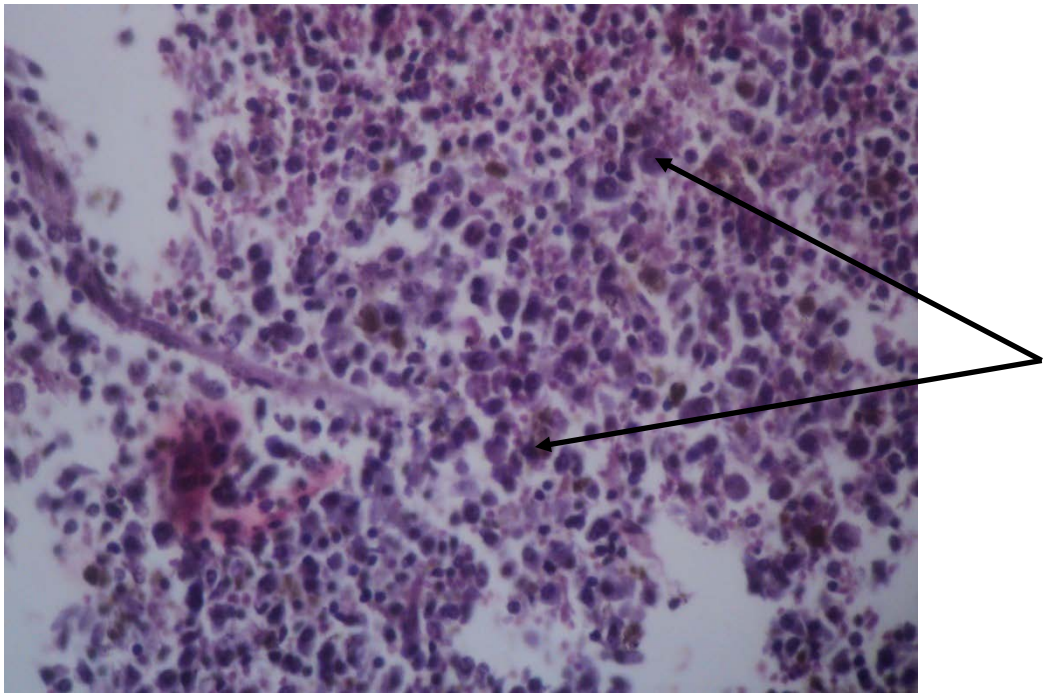


Plate 4.8: The photomicrograph of the spleen of the West African Dwarf goat infected with *Trypanosoma brucei* showing macrophages hyperplasia, some laddened with hemosiderosis Mag X 400)

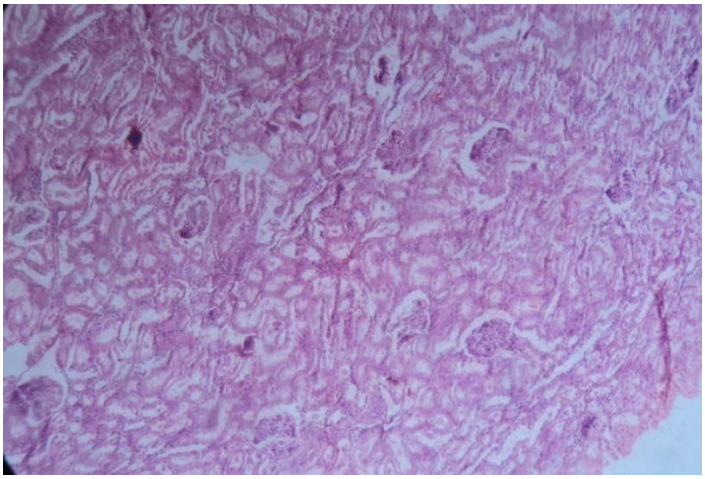


Plate 4.9: The photomicrograph of the kidney of the Red Sokoto goat infected with *Trypanosoma brucei* showing no visible lesion (mag X 400)

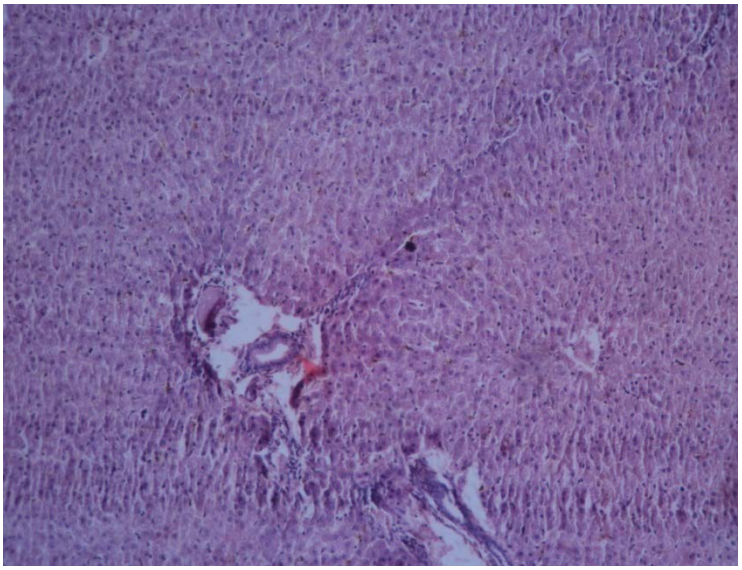


Plate 4.10: The photomicrograph of the liver of the control West African Dwarf goat showing no visible lesion (mag X 100)

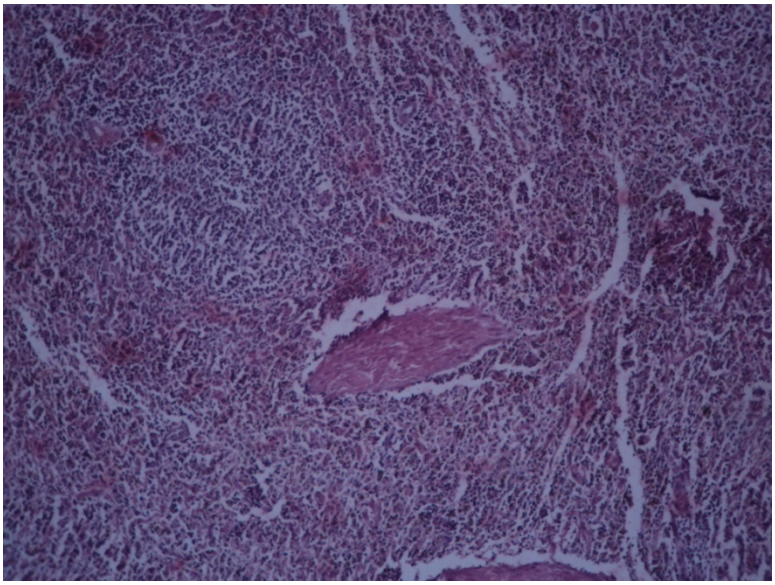


Plate 4.11: The photomicrograph of the spleen of the control West African Dwarf goat showing no visible lesion (mag X 100)

4.4.0 Discussion

Temperature seen in infected goats in this work was significantly ($P < 0.05$) higher than controls and was similar to that reported by Adeiza *et.al.,* (2008) from trypanosome infected goats. Among infected animals, while weight was in *T.brucei* infected Red Sokoto goats. Weight change seen in infected animals in this experiment differed non-significantly ($P > 0.05$) between the groups and was not striking enough to be used as an index of susceptibility or resistance. This differed from Adah *et. al.,* (1993) who found significant difference in weights of *T. congolense.*infected WAD and RSG goats. The result is similar to Faye et. al. 2002 who found no significant difference between the weight of WAD and their crosses with Sahelian breeds under experiemental infection with *T. congolense*. The presence or absence of significant differences in changes of weight under trypanosome infection may relate to differences in tumor necrosis factor which influence weight gain and loss precipitating either obesity or chachexia (Argiles *et. al.* 1997). Blocking the weights of experimental goats under narrow range such as was done in this work where goats were 15kg ± 2 kg may also produce result that differ from experiments where weights were not blocked.

The widest range of fluctuating parasitaemia were associated with the Red Sokoto goat (RSGtbc-RSGtc). This suggests that while both breeds attempted to control parasitaemia as evidenced by its fluctuations, greater fluctuation occurred in the Red Sokoto breed which could signal less control capability in this breed compared to West African dwarf breed. This finding is similar to that of Faye *et. al.,* 2002 who reported higher parasitaemia in crosses with Sahelidin breeds than pure WAD infected with T. congolense.

Anaemia and serum biochemical changes are common features of African trypanosomosis (Ekanem and Yusuf, 2009). In this investigation, *T. congolense* precipitated the lowest PCV value in RSG (RSGtc). This finding was similar to report on East African goats experimentally infected with *T.congolense* and *T.brucei* which showed increased erythrogenesis in response to increased destruction of erythrolytes and this was more in *T.congolense* than *T.brucei* showing its greater effect on this haematological parameter. (Biryomumaisho and Katunguka- Rwakinshaya, 2007). PCV which fluctuated in the infected groups in a decreasing manner as infection progressed and haemoglobin which remained at pre infection levels in control groups but decreased in the infected groups were parameters that mirror anaemia where the infected and non infected animals differed. These could be used to assess susceptibility of the goats to trypanosomosis, and their behavior in this study was in line with earlier reports (Yanan et.al. 2003; Ibrahim *et.al.,* 2005).The values of PCV, MCV, MCHC and MCH show that there was anaemia which was more severe in RSG than in

WAD. These indices show that anaemia found in this work could be characterized as Normocytic normochronic (WADtbc), Normocytic hypochromic (WADtc, WADtb), Normocytic hyperchromic (RSGtc), Macrocytic hypochromic (RSGtbc). Incides in anaemia of RSGtb appeared to assume borderline characteristics between normocytic normochromic and microcytic hypochromic changes. Apart from haemolysis that is induced by trypanosome infection and its depletion of tissue antioxidants (Ibrahim *et. al.*, 2005; Eze and Ochike, 2007), this difference might be due to more blood cells apoptosis in RSG than in WAD as reported by Happi *et. al.*, (2010).

The Findings of Leucocytosis in RSG and Leucopenia in WAD was similar to Adah et. al. 1993. Acting under influence of IL – 1 and TNF, leucocytosis is either induced initially by accelerated release of immature WBC from bone marrow (shift to the left) or later proliferation of precursors in bone marrow caused by increased production of colony stimulating factors (Vegad, 1995).

Lower albumin levels in infected animals compared to control, lower albumin-globulin ratio in infected animals than controls, significantly higher total proteins in infected animals, and relatively lower total proteins in *T. congolense* than in *T. brucei* where similar to report of Biryomumaisho *et al*. (2003) in small East African goats. Trypanosomiasis induces changes in levels of total proteins, globulin and albumin, with altered albumin:globulin ratios (Anosa and Isoun, 1976, Taiwo *et. al.* 2003)

Following infection alanine aminotransferase (Alt) levels fluctuated in a decreasing manner in all infected groups of both breeds. However, an initial sharp rise among infected RSG in first week post infection which was non significantly ($P > 0.05$) highest in mixed infected RSG was seen. Such a rise had been reported by Adah et. al. 1993 in Red Sokoto goats infected with *T. congolense*.

Creatinine was significantly lower in the mix infected goats of both breeds so that using this index, damage of kidney appeared to be mellowed down with mixed infection. Similar interference in mixed infection was reported in goats infected simultaneously with two Serodemes of *T. congolense* which were immune to homologous challenge, had reduced or absent skin reactions compared to single infected groups (Dwinger et. al., 1986). Effect of one parasite on another was observed by Fagbemi (2006) who concluded that multiple or dual parasitism was not a matter of $1 + 1 = 2$.

AST level was significantly high in Red sokoto goats with mixed infection where levels more than doubled pre infection value while in the West African dwarf goat on equivalent inoculum the level had become half of pre infection value. As an index of tissue

damage the significantly higher level of AST in the Red Sokoto goats pointed to greater tissue damage in this breed on mixed infection.

A corollary to this was the response to infection expressed through alkaline phosphate levels by the two breeds. The West African dwarf goats belonging to infected groups maintained high levels of alkaline phosphatase during course of infection. Conversely, all groups of infected Red sokoto goats had diminishing levels of alkaline phosphatase, the levels having crashed in the second week post infection. This hydrolase enzyme is associated with dephosphorylation of many types of molecules including nucleotides, proteins and alkaloids and its levels are usually raised rather than lowered in those disease situations where hepatobiliary damage occurs (Wikipedia, 2011; Davidsohn and Henry 1974). To differentiate whether such damage is from hepatocellular origin or from obstructive jaundice, alkaline phosphatase, levels are lower and aspartate aminotransferase levels are usually higher in hepatocellular disease than in obstructive jaundice (Cheesbrough, 1998). This tally with the situation observed in mixed infected Red Sokoto breed.

Dramatic changes in levels of triglycerides marked by depletion across the weeks in all infected groups of both breeds points to abnormalities of metabolism especially affecting lipids which has been reported in laboratory and domestic animals infected with various species of trypanosomes (Biryomumaisho *et.al.*, 2003). Lipoproteins and triglycerides are secreted from the liver so depletion of triglycerides will among other consequences affect lipoprotein formation and cellular integrity (Vegad, 1995).

Equally low or lower albumin: globulin ratio was found in trypanosome infected Red Sokoto goats compared to WAD goats. This shows that antibodies were produced in the infection of goats of both breeds. However, mortalities occurred particularly in the mixed infected Red Sokoto goats. Antigens from different trypanosome species show considerable cross-reactivity (Seifert, 1992). In mixed infections the impact of such cross-reactivity is expected to show. However in certain highly susceptible strains of mice, mixed infection with trypanosome species lead to high unrelenting parasitaemia with little or no lost antibody response (ILARD, 1985). Taylor *et.al.* (1996) observed that trypanososusceptible animals also produced antibodies but were mostly of immunoglobulin M type that was not as protective as the immunoglobulin G type found in breed of animal that withstood infection of trypanosomosis. The difference therefore lies not in antibody quantity but isotype produced. This is consequent from failure of isotype (class) switch which is the shift of a B cell or its progeny from secretion of antibody of one isotype or class having same V regions but

different heavy chain constant region and therefore different biological activities (Otokunefor and Otokunefor, 2009).

CHAPTER FIVE

5.0 APPLICATION OF POLYMERASE CHAIN REACTION (PCR) IN MOLECULAR IDENTIFICATION OF TRYPANOSOMES ENCOUNTERED IN EXPERIMENTATAL AND SURVEY STUDY

5.1 Introduction

Criteria on which detection of trypanosomosis is commonly based are clinical signs and symptoms of the disease, detection of parasites in body fluids and detection of parasite products in body fluids (Luckins, 2007).

De Almeida *et. al*. (1998) and Ndao et. al. (1998) did works on trypanosome infection of goats comparing parasitological methods such as haematocrit buffy coat examination, versions of wet blood film and polymerase chain reaction (PCR) with the finding that PCR yielded twice as many positive results as did parasitological techniques on the infected animals.

The microscopy techniques which work best for detecting parasites in small numbers are not as well suited for distinguishing trypanosome species (ILRAD, 1988). Genetic analysis of trypanosome species depend on the detection of variation between strains using primers such as those based on sequences of ECORI and Bg/11 (Agbo *et. al.* 2002). Within trypanosome species, variation in pathogenicity depends on strains, so determination of positive samples through PCR could also enable evaluation for presence of specific genetic segments like serum resistance associated (SRA) gene which differentiates human infective *T.brucei rhodesiense* from *T. brucei* as both could be found in sheep, goat and pigs (Ng'ayo *et. al.* 2005). The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions. At the core of the method is use of suitable DNA polymerase able to withstand high temperatures of up to 90^{o}C required to separate the two DNA strands in the DNA double helix after each replication cycle (Mesotheliomalawyers, 2011). Thermus aquaticus which grows best at 70^{o}C has polymerase (Taq polymerase) that is stable at the high temperature and survives PCR process (Maizails *et. al.* 1991)

5.2.0 PCR Method

The step down PCR method was used .

5.2.1 Touch Down PCR (Step-down PCR)

In this variant of PCR annealing temperature in initial cycles is set 3-5^0 c above melting temperature (TM) of primers used while at later cycles it is a few degrees (3-5^0 c) below primer TM. This aims at reducing non-specific background since higher temperatures give greater specificity for primer binding and lower temperatures permit efficient amplification from the specific products formed during initial cycles (Mesotheliona lawyers, 2011).

5.2.2 Preparation of Buffy Coat for DNA

Blood was collected through jugular venipuncture into vacutainers precoated with anti-coagulant. 5ml of whole blood was taken in a test tube and centrifuged at 2,000xg for 5 minutes in labofuge 400R (Heraeus). The serum as supernatant was pipetted while agitating in order to include buffy coat and little R.B.C., the content put into another tube and centrifuged at 5,000xg for 10 minutes. The supernatant was decanted, leaving the pellet which formed as deposit. This was suspended with 400 µl of phosphate buffered Saline (PBS) and stored at –20^{0}c.

5.2.3 Protocol for Extraction of DNA

Extraction of genomic DNA was done using ZR kit according to manufacturer's instructions. 100ml of buffered sample was taken in microcentrifuge tube under thermocool VWR (clear bench) brand, to which was added 95 ml of digestion buffer and 5ml of Proteinase K. The mixture was vortexed on thermolyne mixer (Maxi Mill), incubated at 55^{0}c for 20 minutes in Block heater (Stuart scientific). 700µl of genomic lysis buffer was added, thoroughly mixed by vortexing and transferred to zymo- spin ^{TM} 11c column (having silica membrane at bottom for DNA attachment) placed in a collection tube, centrifuged at 10,000 x g for one minute. 200µl of DNA pre-wash buffer was introduced into the spin column in a new collection tube and centrifuged at 10,000 x g for one minute in eppendorf centrifuge 5417R. Subsequently, 400µl of g-DNA wash buffer added to the spin column was also followed by centrifugation at 10,000 x for 1 minute.

The spin column was transferred to a new microcentrifuge tube and 50µl of Elution buffer or water was added to the spin column incubated at room temperature for 2-5 minutes before centrifuging at top speed (14,000 x g) for 30 seconds to elute the DNA which was then stored at - 20^{o}c for subsequent use.

5.2.4 Preparation of Master Mix

Reagents were kept deep frozen at - 20^{o}c in Haier Thermocool HTF – 319 H1 brand. All volumes were calculated before proceeding to perform master mix under working cabinet (class 11 model Bio- 11-A from Terrassa, spain). Other materials are 0.2ml PCR tubes, 1.5ml tubes, marker, minicentrifuge, ice bucket and VMR galaxy mini mixer for the master mixing.

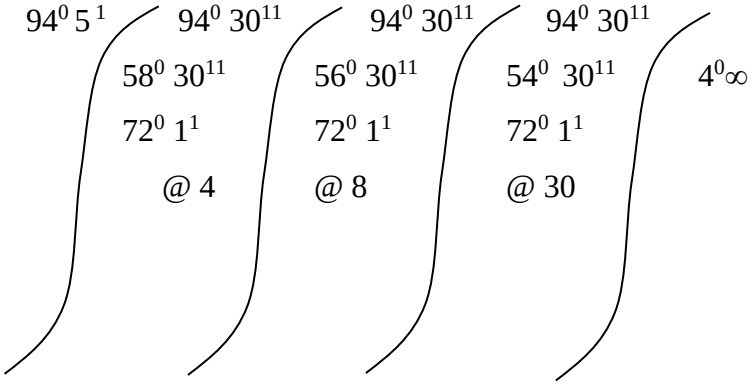
Master Mix	**composition**
	1 X
H20	14.2µl
10x Buffer	2.5
d,NTPs	0.5
MgCl2	1.5
Kin 1	0.5
Kin 2	0.5
Taq Polymerase	0.3
	20.0
DNA	5.0
	25.0

Negative control had nuclease free water while positive controls had *T.brucei* and *T.congolense* respectively.

5.2.5 Amplification

Amplification was done in 25µl reaction mixture containing 10 x reaction buffer. It was programmed based on:

Exp 000 tryps 16 10/29/10



10µl mineral oil overlay was added to each sample before start of amplification if in the Techne Cyclogene Thermacycler:

5.2.6 Resolution of PCR products in Agarose gel Electrophoresis

Materials:

1 x TBE working solution in Schott Duran 1000ml capped bottle.

Agarose (Sigma Chemical Company)

10 x TBE Tris/Boric Acid /EDTA buffer stock (Bio Rad)

Procedure

To make 1.5% Agarose gel, 1.5g agarose powder was weighed on precision scale (ohaus) and 100ml of 1 x TBE /TAE buffer was added to it, mixed, microwaved in oven (National NN 5527 WF brand) for 3 minutes. It was then turned intermittently while waiting to cool and 7µl ethedium bromide was added under PCR work station (Big neat, HP) flitted with chem. Cap filter. Slightly cooled gel was powered in gel casting tray with attached combs and placed at room temperature until full polymerization occurred in about 15 minutes. Combs were retracted and gel casting tray carefully placed in electrophoresis tank (wide mini sub®, Biorad) with the gel totally submerged in TBE buffer solution. Them 10µl of post amplified samples were sequentially mixed with 2µl of loading dye placed on Laboratory film paper (parafilm M brand, American National com.) and loaded into wells. Negative and positive terminals were connected to power source (Power PAC 300 from

Biorad) and gel was run at 120 volts for 45 minutes. Then, gel was transferred to computer system fitted with software for processing, visualization and printing of gel image captured on it (Gene Snap software, Genetreat, syngene / IAEA).

5.3.0 Result

Plate 5.9 and 5.10 show result of PCR used on blood samples from goats. The PCR resolved a 750bp product on agarose gel from samples both from experimental and survey animals. It banded at same molecular mark with *T. congolense* positive control specimen. A 540 bp product was also resolved on the agar rose gel from lanes loaded with some experimental samples. It banded at same molecular range with *T. brucei* positive control sample. None of the survey samples subjected to PCR yielded this product.

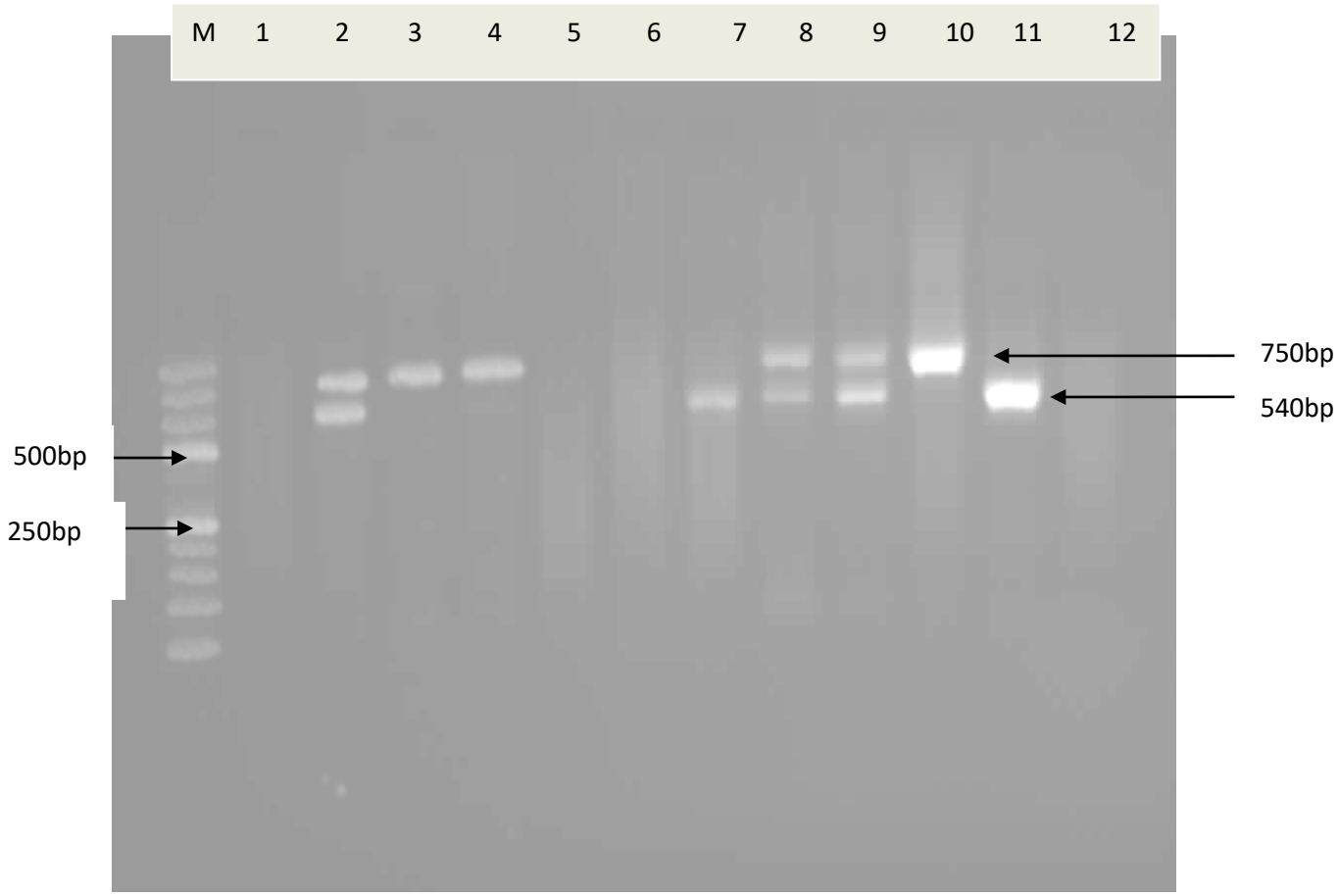


Plate 5.1: Experimental Samples (5,7,21,13,22,30,2, 15,19)

Lane M – 50bp DNA Marker

Lanes 1 – 9 are the experimental samples

Lane 10 – Positive control(T.congo)

Lane 11 - Positive control (T. brucei)

Lane 12 - Negative control

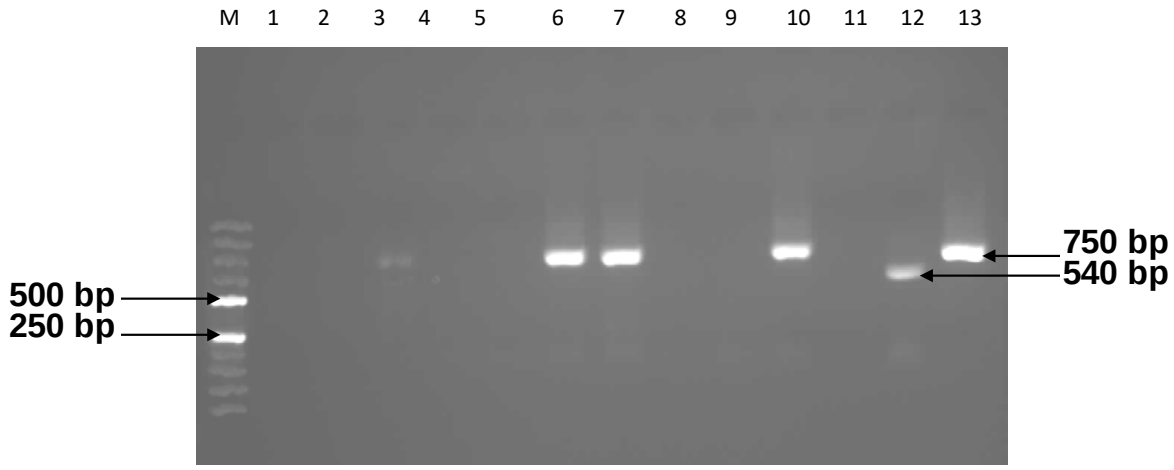


Plate 5.2 Determination of genotype

Lane M – 50 bp DNA Marker

Lanes 1 – 11 samples

Lane 12 – Positive control (*T. brucei*)

Lane 13 - Positive control (*T. congolense*)

5.4.0 Discussion

Genotyping of isolates used in experimental infection revealed 540 bp and 750 bp bands corresponding respectively to *T.brucei* and *T. congolense* positive samples. Both single and multiple bands of the two species were shown by different samples from experimentally infected goats. It gave evidence of the goats being infected by the two trypanosome species in single or mixed inoculums. The finding of these PCR products with kin 1 and kin 2 primers which amplified *T.brucei* and *T.congolense* from the infected goats was similar to that of Enwezor et. al, (2008) who used the same primers to amplified these trypanosome species recovered from ruminants in Kachia grazing reserve.

The nuclear ribosomal internal transcribed spacer 1 i.e. ITS 1 (Dyer *et.al.* 2011) gene is situated between genes in 18S and 5.8S having variable length among trypanosome species but with constant length in each species (Enwezor et. al., 2008). So the ITS 1 sequence of ribosomal DNA provides basis for an efficient and sensitive assay for detection of trypanosomes (Desquesnes *et. al*. 2002)

The prevalence comprised 0% *T. brucei*, 1.3% *T.congolense* was confirmed by PCR. Genotyping of isolates used in experimental infection showed PCR products identical with *T. congolense* and *T. brucei* positive control DNA implying isolates used to infect goats were of these two species.

CHAPTER SIX

SUMMARY AND CONCLUSION

6.1 Summary

This work probed into prevailing status of *Trypanosoma congolense* and *Trypanosoma brucei* in goats around the experimental site, going on to compare susceptibility of the West African Dwarf and Red Sokoto goats to experimental infection with these trypanosome species in Makurdi . The site is in area of overlap in the distribution of populations of WAD and RSG within the Guinea Savannah Vegetation belt.

In the first study, a survey for *Trypanosoma brucei* and *Trypanosoma congolense* from retrospective treatment records of cases in four veterinary clinics in Makurdi was done from April 2005 to March 2009 as well as blood sampling of goats to examine for occurrence of these trypanosome species.

A general trypanosome prevalence of 1.7% from retrospective cases and 1.3% from blood samplings comprising only *T. congolense* were found.

In the second study, experimental infection of WAD and RSG goats obtained from farms and markets around Makurdi in Benue State was done using *Trypanosoma brucei* (Federe Strain) and *Trypanosoma congolense* (Karu Strain). Both trypanosome strains were obtained from cattle within the same agroecological zone. The infection was done to compare susceptibility of the WAD and RSG goats of this zone to the trypanosome strains used. Thirty two goats comprising sixteen of each breed were divided into Four groups of (4) goats which were infected with either *Trypanosoma congolense, Trypanosoma brucei,* Mixed *T. congolense* and *T. brucei* or left as uninfected controls. The goats were blocked against sex, being all males and weight at 1.5kg± 2kg.

It was found that no significant difference ($P > 0.05$) occurred in the weights of infected and control. The infected animals had significantly ($P < 0.05$) higher temperatures and decreased scrotal circumference than the controls. The infected animals developed parasitaemias where higher log parasitaemia and wider range was found in RSG breed.

There was also significant ($P < 0.05$) decreases in PCV, haemoglobin concentration and RBC counts in infected goats with WAD breed having higher values than RSG on equivalent inoculums indicating more severe anaemia in RSG breed. Mixed infection in RSG had highest Mean Cell Volume indicating a macrocytic anaemia unlike the normocytic anaemia that was found in both breeds on other inoculums.

All infected RSG had higher Leucocyte counts than controls indicating Leucocytosis while conversely all infected WAD goats had lower Leucocyte counts than controls indicating Leucopenia.

Only the mixed infected RSG (RSGtbc) had elevated levels of the two enzymes aspartate aminotransferase and alanine aminotransferase which were significant and non-significant respectively.

Creatinine levels of infected animals were not higher than controls, being least in mixed infected RSG. Calcium and glucose did not differ significantly between infected and control goats.

Triglycerides did not vary significantly between infected animals and control but were non significantly depleted in RSG infected with *T. brucei* (RSGtb) and elevated in mixed infected RSG (RSGtbc).

Total protein and glubolin were significantly higher in all infected WAD and RSG than controls which conversely had significantly higher albumin levels than the infected animals. Albumin:globulin ratio was also significantly higher in controls than infected groups with the significantly ($P < 0.05$) least ratio found in *T. brucei* infected RSG(RSGtb)
Infected Red Sokoto goats had mortalities which reached 25% in single T. congolense and T. brucei infection (RSGtc, RSGtb) and 50% in mixed infected RSG (RSGtbc).

Histopathology shown in organs of infected goats was marked by haemosiderosis of spleen and liver lymphocytic infiltration in *T. brucei* infected RSG while mixed infected RSG also had centrilobular necrosis.

In the third study, genotype of trypanosomes found in blood sampling survey and isolates used to infect experimental goats was investigated through polymerase chain reaction. Trypanosomes recovered for blood sampling survey gave 750 bp PCR products corresponding to *T. congolense* positive control. Isolates used to infect experimental goats gave either or both the 750 bp product corresponding to *T. congolense* and 540 bp PCR product corresponding to *T. brucei* positive control, thus confirming species of isolates used in the experimental study.

6.2 **Conclusion**

Natural trypanosomosis of goats occur around the study site in Makurdi area where *T. congolense* was found in survey samples and confirmed by PCR. The PCR technique equally confirmed Trypanosome isolates used to experimentally infect WAD and RSG as *T. congolense* and *T. brucei*.

Experimental infection of WAD and RSG with *Trypanosoma congolense* (Karu Strain) and *Trypanosoma brucei* (Federe Strain) induced metabolic derangement and pathology in both breeds of goats with anaemia occurring in all infected animals. The RSG was more susceptible, showing mortalities to the single and mixed infection of *T. brucei and T. congolense.*

Susceptibility of RSG was more pronounced in the mixed infection (where more mortalities occurred and greater tissue injury was evidenced through elevated mean ALT and AST levels).

Since trypanosomosis in field situation often involves mixed infections, its impact on RSG in Makurdi area could be more devastating or critical to impede production.

6.3 **Suggestion and Recommendation**

In Makurdi area that is at interphase of the WAD and RSG, breed susceptibility to trypanosomosis should be considered when establishing new stock or injecting animals into existing flock following the usual high off-take from flocks during festival seasons.

The findings in this work suggest that WAD could have more difficulties than RSG in adjusting to intensive management in new setting as shown by comparative weight gain of control animals but the WAD survives more in the face of prevailing trypanosomosis and would better improve livestock production in the area.

Following similar experimental infection, further work should be done that includes study of antigen-antibody interaction through method like enzyme linked immunosorbent assay (ELISA), extension in investigation of the parameters examined in this acute infection through a chronic phase, purification of PCR product, sequencing and phylogenetic analysis.

REFERENCES

Abebe G. 1991. Trypanosome Infection Causes dysfunction of the pituitary gland.Ph.D Abstract, Brunel University of London.In *ILRAD Reports* Vol. 9 No. 2 of 1991. p6.

Abenga J.N.,Sanda S.N Ezebuiro O.G.C. 2005. Effect of Trypanosoma congolense and Trypanosoma brucei mixed infection on the pattern of Haematological changes in Murine Trypanosomosis. *African Journal of chemical and Experimental Microbiolog*, Vol 6 No. 2; 193 – 197

Abubakar, A., Onyekwelu N. A., Igweh, A. C., Bot, D. Y. and Ojiegbu, F. N. 1999. Some serum biochemical and Haemotological changes in experimental *Trypanosoma brucei* infection of rabbits. *Proc. Biotec. Soc.* (1999) 86-88.

Adah, I.M., Otesile, E.B. and Joshua, R.A. 1993. Susceptibility of Nigerian West African Dwarf and Red Sokoto Goats to a strain of *Trypoanosoma congolense*. *Veterinary Parasitology*, 47:177 – 188. Elsevier publication B.V. Amsterdam.

Adeiza A.A, Maikai, V.A. and Lawal A.I. 2008. Comparative haematolological changes in experimentally infected Savannah brown goats with Trypanosoma brucei and Trypanosoma vivax. *African Journal of Biotechnology* Vol. 7 (13) pp.2295 – 2298 Issn 1684 – 5315. Http://www.academicjournals.org//Jb.

Agbo, E.C. Majiwa P.A.O., Classes M. and Te Pas M.F.W. 2002. Molecular variation of trypanosoma brucei sub species as revealed by AFLP finger printing.

Agyemang K. 2005. Trypanotolerant Livestock in the Contex of trypanosomiasias intervention strategies.*PAAT Technical and Scientific Series 7*. Food and Agricultural Organisation of the United Nations, Rome.

Ahmed, A.B., Omoogun, G.A. and Shaida, S.S. 2000. Trypanosome infections in *Glossina* species at the Kainji Lake National wild life Park, Nigeria. *ESN Occasional publication 32* (2000) 57-62.

Akinboade, O.A. 2001. Parasites: “The bottom line”. An Inaugural lecture delivered at the University of Ibadan on Thursday 25, June, 1992. First Published 2001 by University of Ibadan, Nigeria.P.4.

Akinyemi, M.O and Salako A.E. 2012. Genetic relationship among Nigerian indigenous sheep populations using blood protein polymorphism. *Agricultural Science and technology*, vol. 4 No. 2 pp. 107-112, 2012. An international journal published by faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

Allsopp.R and Hursey, B.H. 2004. Insecticidal Control of Tse Tse. In: Maudlin, I, Holmes, P.H., and Miles, M. (Eds.) the Trypanosomosis CAB International, P. 491

Ameen S.A., Joshua R.A., Adedeji O.S., Raheem, A.K. Akinbade, A.A., and Leigh, O.O. 2008. Preliminary studies on prevalence of ruminant Trypanosomosis in Ogbomosho area of Oyo state, Nigeria. Middle East Journal of scientific Research, 3(4) 214 –218.

Anderson, I. And Mckay, I.A. 1991. Diagnosis of animal diseases using nuclear and Related techniques; developments and Trends. In: *proceedings of IAEA and FAO symposium on isotope and related techniques in animal production and health* 15 – 19 April, Vienna 1991. PP 491 – 499.

Anosa V.O., Isoun T.T. 1976. Serum proteins, blood and plasma volumes in experimental T. vivax infection of sheep and goats. *Trop. Anim. Health Prod.* 1976 Feb. 8(1) 14 – 19.

Anosa, V.O. 1991. Prevalence, Pathogenesis and Pathology of African trypanosomiasis. In: Ikede, B.O. (Ed.) Control of Animal Trypanosomiasis in Nigeria as a Strategy for increased Livestock Production. *Proceedings of a Preparatory Workshop in Vom.* 5 – 9. June 1989 under EEC Lome III project on the control of animal trypanosomiasis in Nigeria.

Aradaib, E.I. and Majid, A.A. 2006. A simple and rapid method for detection of Trypanosoma evansi in the dromedary camel using a nested polymerase chain reaction. *Kinetoplastid Biology and Disease (2006)* vol. 5:2 published Biomed Central Doi : 10. 1186/1475 – 9292 – 5-2 pubmed ID: 16712737. Retrieved from http://www.Kinetoplastids.com/content 5/1/2

Argiles, J.M., Lopez-Soriano, J., Busquets, S. and Lopez-Soriano, F.J. 1997. Jaourney from Cacheria to obesity by TNF. *FASEB J.* 1997 11/10 (743 – 751).

Audu P.A. and Abifarin O.M. 2001. A survey of blood parasites of some domestic animals at sango Abattoir Tudan-wada, Kaduna. *Sokoto Journal of Veterinary sciences 3* (1) 28 – 33

Audu P.A. and Pila E.A. 2008. The reservoir status of Rabbits for the Maintenance of Ehrlichia ruminantum. Nigerian Journal of Parasitology vol. 29 (2), Sept. 2008 pp. 67-71. ISSN 11174145.

Babu, Y. H. (2003). Serological Reactions. Veterinary Immunology: Basic Concepts and applications. Jay Pee Brothers Medical Publishers, New Delhi. ISBN 81-8061-164-7 P.135

Baker F.J., Silverton R.E. and Pallister C.J. 2001. Baker and Silverton's introduction to Medical Laboratory technology. Seventh Edition. Edward Arnold Publishers, London. Nigerian Edition. ISBN 978 – 2965 – 28 – 6. PP. 337 – 392.

Barret J. 1997. Control strategies of African Trypanosomiasis: Their sustainiability and Effectiveness. In: Hide, G. Mottran J. C., Coombs G. H. and Holmes P. H. (Eds.) Trypanosomiasis and Leishmaniasis, Biology and control. CAB International U. K. pp. 347-359.

Barry J. D., 1997. Biology of Antigenic Variation in African Trypanosomes. In: Hide, G., Mottram, J. C., Coombs, G. H. and Holmes, P. H. (Eds.) Trypanosomiasis and Leishmaniasis, Biology and control. CAB International U. K. pp. 89-107

Barry, D. and Carrington, M. 2004. Antigenic Variation. In: Maudlin, I., Holmes, P. and Miles, A.M. (eds.) CAB International pp. 25 – 37.

Barta O. 1984, Laboratory techniques of Veterinary Clinical Immunology. Charles Thomas Publishers, Illinios. P. 35.

Bastin, P., Matthews, J.R. and Gull, K. 1996. The Paraflagellar rod of Kinetoplastida: Solved and Unsolved questions. *Parasitology Today Vol.* 12 No. 8, 1996, pp. 302 – 307. Elsevier Science Ltd.

Bengaly, Z. Sidibe, I. Boly, H. Sawadogo, L. and Desqueisnes, M. 2002. Pathogenicity of three genetically distinct *T. congolense* type in inbreed Balb/c mice *Vet. Parasitol* 105, 111-118.

Berridge M. 1985 Molecular basis of communication between cells. *Scientific American* vol. 252 No. 4 PP142 – 152.

Biryomumaisho, S. Katunguka – Rwakishaya E. 2007. The Pathogenesis of anaemia in goats experimentally infected with *Trypanosoma congolense* or *Trypanosoma brucei*: use of the myeloid: erythroid ratio. *Vet. Parasital.,* 2007 Feb. 28, 143 (3-4) 354-7. Epub 2006 Sept. 18.

Biryomumaisho, S. Katunguka – Rwakishaya E., Rubaire – Akiiki, C.M. 2003. Serum biochemical Changes in experimental *Trypanosoma Congolense* and *Trypanosoma brucei* infection in small East African goats. *Veterinarski Arhiv* 73 (3) 167 – 180.

Black, S. J., Wang, Q., hamitton, E., Wang J. Van Prag, A and Muranjan M. 1997. Plasma Purines and *Trypanosoma brucei*. *Proceedings of first internet conference on Salivarian Trypanosomes (FAO animal production and health paper* 136).FAO Rome.

Blench, R. 1999. Traditional Livestock Breeds: Geographical Distribution and Dynamics in Relation to the Ecology of West Africa. Working Paper 122. Overseas Development Institute, Portland House, Stad Place London. SWIE 5DP pp. 22, 30-38.

Blood D. C and studdert V. P. 1999. Saunders comprehensive Veterinary Dictionary. Second editrion. W. B. saunders. Printed in great Britain. 1380 pp.

Borst, P. (2002). Antigenic Variation and allelic exclusion. Cell 109, 5 – 8. Cited by Barry, D.and Carrington, M. 2004. Antigenic Variation. In: The Trypanosomiases. Maudlin, I., Homes, P. and Miles, A.M. (eds.) CAB International pp. 25 – 37.

Boulanger, N., Brun, R., Ehret-Sabatier, L., Kunz, C. and Bulet, P. (2002). P. 24

Brem, G. Brenig, B. Muller M. and Springmann K. 1989. Ex Situ cryoconservation of Genomes and genes of endangered cattle breeds by means of modern biotechnological methods. FAO Animal production and Health paper 76. FAO Un. Rome.

Brem, G. and Muller, M. 1991. Introducing Disease Resistant Genes inti the Animal Genome. Proceedings of International Symposium on Nucluear and Related Techniques in Animal Production and Health Organised by IAEA and FAO in Vienna 15-19 Apriel 1991. Pp. 549-554.

Brestcher, P. 1985. Molecules of the cell membrame. Scientific American. Vol. 252 No 4. P 87.

Bungener, W. and Mehhitz, D. 1976. Tropenmed parasitol, 27:405 – 410.

Burleigh, B. 1993. Identification of novel invariant antigens of trypanosomes. Summary of Ph.D thesis reported in ILRAD reports vol.11 No. 3 of 1993. P.6

Buscher, P., Ngoyi, D.M., Kabore, J., Lejor, V., Robays J., Jamonneau, V., Bebronne, N. Veken, W.V. Bieler, S. 2009. Improved models of Mini Anion Exchange centrifugation Technique (mAECT) and modified single centrifugation (MSC) for sleeping sickness Diagnosis and staging. PLOS Negl Trop Dis 3(11) e441.Published November 24, 2009. http://www.finddiagnostics.org/export/sites/default/programs/hat/docs/buscher_improved_models_windows internet E…

CFSPH (Centre for Food Security and Public Health) 2003.Trypanosomiasis. CFSPH, College of Veterinary Medicine Iowa State University Ames. http://www.

CFSPH 2005. Trypanosomiasis; Nagana tse tse fly disease, Trypanosomiasis last updated Aug. 3, 2005. Retrieved from http://64.233.183. 104/search?q=cache:adbLAG118 doJ:www.cfsph.iastate.edu/factsheets/…

Chauhan R.S. and Agarwal D.K. 2006. Text book of veterinary clinical and Laboratory Diagnosis. Second Edition, Jaypee Brothers Medical Publishers, New Delhi. ISBN 81-8061-572-3. Pp. 136-151, 193-206. 246-284.

Cheesbrough, M. 1998. Parasitological tests. In: District Laboratory practice in Tropical Countries. Cambridge low price Editions. P. 266, 310 - 412.

Chiejina S.N., Musongong G.A., Fake, B.B., Behnke, J.M. Ngongeh, L.A. and Wakelin D. (2005) The modulatory influence of *Trypansoma brucei* on challenge infection with Haemonchus contorting in Nigerian West African Dwarf goats segregated into weak and strong responders to the nematode.

Coombs J. 1992. A dictionary of Biotechnology, second edition.The Macmillian Press Ltd.ISBN 0- 333-57822-8.Printed in Great Britain. PP. 254-275

Craompton J.M., Stowell, S. Karaas, M and Sinden, R.E. 1998.Vaccine delivery in the 21st century. In: Towards Livestock disease Diagnosis and control in the 21st century. *Proceedings of an International Symposium on Diagnosis and control of livestock Diseases using nuclear and related techniques* jointly organized by the International Atomic Energy Agency and the Food and Agricultural Organisation of the United Nations, held in Vienna 7-11 April, 1997. Published by International Atomic Energy Agency Vienna, 1998.ISBN 92-0-102498-3.pp.231- 243.

Cross, G.M. 1996. Antigen variation in trypanosomes: Secrets Surface Slowly. *Bioassays* Vol. 8 No. 4, 283 – 291.

d'Ieteren G.D.M., Authie, E., Wissocq, N. and Murray M. 1998.Trypanotolerance, an option for sustainable Livestock production in areas at risk from Trypanosomosis, *Rev. Sci. Tech. off. Int. Epiz.*, 17(1) 154 – 175. Accessed on line Web site: http://www.fao.org/PAAT/paperoie.doc

Daniel, A. D. Joshua, R.A., Kalajaiye, J.O and Dada, A.J. 1994. Prevalence of Trypanosomosis in Sheep and Goats in a region of Northern Nigeria. *Rev. Elev. Med. Vet pays Trop.* 1994, 47(3)295-7.

Davidsohn, I. And Henry, B.J. (Eds.) 1974. Todd-Samford Clinical Diagnosis by Laboratory Methods. WB Saunders Company Philadelphia. 15th Edition ISBN 0-7216-2922-9. Pp559, 580.

De Almeida, P.J.L.P, Ndao, M., Van Meirvenne, N., geerts, S. 1998. Diagnostic evaluation of PCR in goats infected with *Trypanosoma brucei*. Poster presentations. IAEA-SM-348/16P. *Proceedings of Symposium Vienna*,7-11 April, 1997 jointly organised by

IAEA and FAO on Towards Livestock Disease Diagnosis and Control in the 21st Century.

Desquesnes, M., Ravel, S. And Cuny, G. 2002 PCR identification of *Trypanosoma* lewisi a common parasite of laboratory rats. *Kinetoplastid Biology* and Disease 2002, 1:2. http://www.kinetoplastid.com/content/1/1/2.

Dhanotiya R.S. 2004. Textbook of Veterinary biochemistry. Second Edition. Jaypee Brothers Medical Publishers, New Delhi. ISBN 81 – 8081 – 218 – X. PP. 316 – 394.

Dhollander, S., Bos, J. Kora, S. Sanneh, M. Gaye, M. Leak, S. Berkvens, D. and Geerts S. 2005. Susceptibility of West African Dwarf goats and WADA Saanen Crosses to experimental infection with *Trypanosoma congolense*. *Vet. Parasitol.* 130 (1-2): 1-8.

Diallo A. 1991. Use of DNA Probes in Animal Disease Diagnosis *proceedings of International Symposium on Nuclear and Related Techniques in Animal Production and Health* Jointly Organised by the International Atomic Energy Agency and the Food Aid Agricultural Organisation of the United Nationals. Held in Vienna 15-19 April 1991. pp. 521-534.

Diallo, A. and Radwaska M. 2010. Report of the first Research Co-ordination Meeting (RCM) of the Co-ordinated Research Programme (CRP) on "Developing, validating and standardising methodologies for the use of PCR and PCR-ELISA in the diagnosis and monitoring of control and eradication programmes for trypanosomosis". http//www.naweb.iaea.org/nafa /aph/crp/d3202

Drinkwater R.D and Hetzel D.J.S. 1991. Application for Molecular Biology to Understanding Genotype-environment Interactions in Livestock Production. *Proceedings of International Symposium on Nuclear and related Techniques in Animal Prodiction and Health*, FAO and IAEA Vienna, 15-19 April, 1991. Published by IAEA Vienna, Pp. 437-452.

Donelson J.E. 1988. Unsolved mysteries of trypanosome antigenic variation. The Biology of Parasitism. Alan R. Liss, Inc. Pp. 371-400.

Dyer, N.A., Ravel, S. Kwang-Shik C., Darby, A.C., Causse, S., Kapitano, B., Hall, M.J.R., Steen, K., Lutumba, P., Madinga, J., Torr, S.J., Okedi, L.M., Lehane, M.J., Donnelly, M.J. 2011. Cryptic Diversity within the major Trypanosomiasis vector *Glossina Fuscipes* Revealed by molecular markers. *PLoS Neglected tropical diseases* 5(8): e 1266. Doi:10.1371/journal.pntd.0001266. http:/www.Plosntds.org/article/ info%3adoi %2f10.1371%2fjournal.pntd.0001266

Egan A. R. 1976. metabolism of Nitrogen compounds. In: Philips J. W. (Ed.) Veterinary Physiology. Wright- scientechnica Publishers. Bristol. P. 612.

Egbe – Nwiyi, T.N., Igbokwe, I.O. and Onyeyili, P.A. 2005. Pathogenicity of *Trpanosoma congolense* infection following oral calcium chloride Administration in Rats. *African Journal of Biomedical Research* Vol. 8 No. 3, 197 – 201.

Eisler, M.C., Dwinger, R.H., Majiwa, P.A.O. and Picozzi, K. 2004. Diagnosis and Epidemiology of African Animal Trypanosomiasis. In: The Trypanosomiasis. (eds. I. Maudlin, P.H. Holmes and M.A. Miles). CAB International. Pp. 253 – 267.

Ekanem, J.T. and Yusuf, O.K. 2007. Some liver function indices and blood parameters in *T.brucei* infected rats treated with honey.

El-Sayed, N.M.A. and Donelson, J.E. 1997. sequencing and Mapping the African Trypanosome Genome. In: Hide. G. Mottram, J.C. coombs, G.H. and Holmes, P.H. (Eds.) Trypanosomiasia nd Leishmaniasis Biology and Control. CAB International Oxon, UK. ISBN O 85199 139 4 pp. 51 – 63.

ENCYCLO 2011. Nagana Online encycopaedia http://www.encyclo.co.uk/define/nagama Assessed 19 Feb. 2011.

Encyclopedic References of Parasitology (1997) Trypanocidal drugs, man. *First Internet conference on Salivarian trypanosomes.*

Enwezor, F.N.C., authie, E., Bossard, G., Esievo, K.A.N. and Umoh, J.U. (2008). Molecular characterization of bovine trypanosomes from the kachia Grazing Reserve, North-west Nigeria. *Nigerian Journal of Parasitology.* Pp. 98-102

Everything 2006. Anaemia.Online.Anaemia @ Everything 2.comhttp:// www.e verything 2.com/index. P/?node-id=184277. Accessed 11/1/2006.

Eze J.I. and Ochike B.O. 2007. Effect of Dietary Vitamin E supplementation on Trypanosoma brucei infected mice *Trop.Vet.* Vol. 25(1) 18-25 (2007)

FAO 2005.Trypanosomiasis. FAO document repository (on line). Access June 15th 2005

Faye, D., Osaer, S., Goossens, B. Van Winghem J., Dorny, P., Lejon, V., Losson, B. and Geerts, S. 2002. Susceptibility of trypanotolerant West African Dwarf goats and F1 Crosses with the susceptible sahalian breed to experimental trypanosoma Congolense infection and interactions with helminth infections and different levels of diet. *Vet. Parasitol.* 108(2):117-36.

Fagbemi B.O 2006. Tapestry of parasitism. An inaugural lecture delivered at the University of Ibadan on Thursday 2nd November, 2006. University of Ibadan Press. Pp.70

Fayemi, O. 2006, Association of Trypasome infection with sperm antibodies production in Red Sokoto (Maradi) goats. *Pakistan Vet. J.*, 2006, 26(1): 30 32

Frandson R.D., Wilke, W.L. and Fails A.D. 2003. Anatomy and physiology of Farm Animals. Sixth Edition. Pp. 233 – 288. lippincott, Williams & Wilkins, Baltimore, USA.

French M.H. 1970. observation on the goat FAO Agricultural series No. 80.FAO Rome 204 pp.

Garcia, A., Jamonneau, V., Magnus, E., Laveisiere, C., Legion, V., N'guessan, P., N'dri, L., Meirvenne, N.V., and Buscher, P. 2000. Follow-up of Card Agglutination Trypanosomosis Test (CATT) Positive but Apparently Aparasitaemic Individuals in Cote d'Ivoire: evidence for a complex and heterogenous Population. *Tropical Medicine and International Health* Volume 5 P. 786. November, 2000. doi.10.1046/ j.1365-3156.2000.00623.x

Geerts, S., Kageruka, P., DeDeken, R., Brandt, J.R.A., Kazadi, J.M., Diarra, B., Eisler, M.C., Lemmouchi, Y., Schacht, E. and Holmes, P.H. 1997. Extension of the prophylactic effects of isometamidium and Ethidium Using sustained release devices. *Proceedings of the First Internet Conference on Salivarian Trypanosomes* (FAO Animal Production and Health Paper 136) 1997.

Geerts, S., Osaer, S., Goossens, B. And Faye, D. 2009. Trypanotolerance in small ruminants of sub-saharan Africa. *Trends parasitol.* 2009 mar 25(3)132-8. E.pub.feb.4,2009.

Georgi J. R and Georgi m. E. 1990. Protozoa.Parasitology for Veterinarians W. B. Saunders & Co., Philadephia, PA.

Gethring W.J. 1985. The Molecular basis of development. *Scientific American.* Vol. 253 No. 4 PP 153 – 160.

Gibson, W. 2001. Sex and evolution in Trypanosomes (T. brucei). International Journal for parasitology, 31 (5-6): 643-647. *In Tse tse and trypanosomias information Quarterly (TTIQ)* 34 (3) No. 11989.P. 110.FAO Rome.

Goodhead, I., Archibald, A., Amwayi, P. Brass, A., Gibson, J., Hall, N., Hughes, M.A., Limo, M., Iraqi., F., Kemp, J. S. and Noyes H.A. 2010. A comprehensive genetic analysis of candidate genes regulating response to trypanosome congolense infection in Mice *Plos Neglected Tropical Diseases*. November 2010. /volume 4/issue 11/e880 retrieved on 15/7/2011 from www.Plosntds.org.

Goossens, B., Osaer, S. Kora, S. and Ndo, M. 1998. Haematological changes and antibody response in trypanotoloerant sheep and goats following experimental *Trypanosoma congolense* infection. *Vet. Parasitol.* 79(4): 283 – 97.

Goossens, B., Osaer, S., Van Winghem, J., Faye, D., Dnollander, S. and Geerts, S. 2001. Sustainability of Small Ruminant Production in the Gambia with special reference to improved hustandry of trypanotolerant sheep (on line).

Gould A.R., MacColl, K.A., Bashiruddin, J.B and Taylor, T.K 1991 Applifying Genes using the polymerase chain reaction: A promission diagnostic tool. *Proc. of international symposium on nucluear and related techniques in animal production and health* on Isotope and related techniques in animal production and health organised by Internation Atomic Energy agency and the Food and Agricultural Organisation of UNO held in Vienna, 15-19 Apriel, 1991.

Griffin, K. 2011. Nested RT-PCR Available on line, last modified on 27th April 2011. http://openwetware.org/wiki/Griffin:Nested_RT-PCR

Happi, A.N., Miller, D.A., Antia R.E. (2010) Trypanosoma brucei induced leucocyte apoptosis and trypanosusceptibility in animals. OASIS – The online Abstract submission and invitation system. Coe-Truman Technologies, Inc CTT. *American Society of Tropical Medicine and Hygiene,* 59, Nov. 3 -7, 2010. Atlanta Georgia. http://www.abstractsonline.com/plan/view/abstract.aspx? m/D=26 25/s key=5e0da339-7c754b05-98ba-e029c44df5f28/c key=8752e631-aac9-4ba7-ab21-7fee51ac56!

Harper, H. A. 1975. The blood, Lymph and cerebrospinal fluid. Review of Physiological Chemistry.15th Edition.Lange medical Publications. Pp. 194-219.

Harris, T.H., Mansfield, J.M. and Paulrock, D. M., 2007. CPG Oligodeoxynucleotide treatment enhances innate resistance and acquired immunity to African trypanosomes. *Infection and immunity,* May 2007. Vol. 75, No. 5, pp. 2366 – 2373. Doi 10.1128/IAI.01649-06. Accessed online on 5/7/2011. http:www.springerlink. com/content/72o27314n613443

Herbert, W. J. and Lumsden, w. H. R. 1976. *Tryponosoma brucei* and rapid " matching" method for estimating the Host's parasitaemia. *Experimental Parasitology*. 40: 427-431.

Hide, G., Mottram, J.C., Holmes, P.H. and Coombs G.H. 1997. Preface: Current Research into the Biology and Control of Trypanosomiasis and Leishmaniasis In: Hide, G.,

Mottram, J.C., Holmes, P.H. and Coombs G.H. (Eds.) Trypanosomiasis and Leishmaniasis Biology and Control. CAB International Oxdon. UK. P.xvii.

Hide, G. and Trait, A. 2004. Genetic and Molecular Epidemiology of Trypanosomes. In: Maudlin, I., Holmes, P.H. and Miles, M. (Eds). The Trypanosomiases. CAB International. Pp. 77-93

Hoffman, J. A., Dafatos, F. C., Faneway Jr. C. A. Ezekowitz R. A. B. 1999. Phylogenetic perspectives in innate immunity. *Science Vol* 284 21 May 1999. Pp. 1313 – 1318 www.sciencemag.org

Holmes, P.H., Eisler, M.C. and Geerts, S. 2004. Current Chemotherapy of Animal Trypanosomiasis. In: Maudlin, I., Holmes, P.H. and Miles, M. (Eds). The trypanosomiases. CAB International P. 431.

Hussain, K., Brodie, B., Ott, R.S., Montealegre, F. 1985. Prevalence of Trypanozoma Theileri in Cows and Fetuses at Slaughter. *Am. J. Vet. Res.* Vol 46. No 6, June 1985. Pp. 1256-1258.

Hutchinson, O.C., Fevre, E.M. Carrington, M. and Welburn, S.C. 2003. Lessons learned from the emergence of a new *Trypanosoma brucei rhodesiense* sleeping sickness focus in Uganda. (Editorial comment) *Lancet Infectious Diseases, 3* (1): 42-45. In: Tsetse and Trypanosomiasis Information Quarterly Vol. 26(1) 2003 S/No. 12496 page 12.

Hutchinson, R, 1999. Horseflies. Online version, last updated: 08/18/1999 23:19:02

Ibrahim, N. D. G, Esievo, K. A. N. and Umar I. A. 2005. The effects of Lactose Infusion on the Pathological Manifestations of *Trypanosoma vivax Nig. Vet. Journal* Vol. 26 (1), 2005. Pp. 25-33.

Igbokwe, I.O., Esievo, K.A.N., Saror, D.I. and Obagaiye, O.K. 1994. Increased susceptibility of erythrocytes to in vitro peroxidation in acute *Trypanosoma brucei* infection of Mice. *Vet. Parasitology* 55: 279 – 286.

Ignacimuthu, J. S. 1995. Basic Biotechnology. Tata McGraw Hill. ISBN 0-07462162-9. P. 76.

Ikede, B.O. 1991. EEC trypanosomiasis project – scope, objectives and responsibilities. In: Ikede, B. O. (Ed.) control of animal trypanosomiasis as a strategy for increased livestock production. Published 1991, based on *proceedings of a preparatory workshop held in Vom* 5 – 9 June, 1989 under the Auspicies of the European Economic Communidty Lome 111 project on the control of animal trypanosomiasis in Nigeria, P. 10

ILARD 1985. Mechanism of host resistance to trypanosomiasis. *ILRAD reports*. Vol. 3 No. 4 Oct. 1985. PP. 1 – 6.

ILCA 1979. Trypanotolerant Limestock in West and Central Africa, *Monograph 2*. Addis Ababa, Ethiopia: International Livestock Centre for Africa, 1979.

ILCA 2005. Trypanotolerant Livestock in West and Central Africa Volume 2.Country Studies.Chapter 13, Nigeria. (online).

Ilemobade, A. 2002. Report on further PAAT – PAATEC harmonization Tsetse and *Trypanosomiasis Information Quarterly* 25(3) Pp. 72 – 73.

Ilemobade, A.A. 2001. Continental Appraisal of tsetse and trypanosomiasis problem. Paper presented at a *workshop on "strategic planning of area – wide tsetse and trypanosomiasis control in West Africa*", Ouagadougou, 21 – 24 May 2001. Vienna, International Atomic Energy Agency.

ILRAD 1985. Mechanism of Host resistance to trypanosomiasis. *ILRAD Reports* vol. 3 No. 4, 6 pp.

ILRAD 1984. Anaemia in Trypanosomiasis; Haemorrhagic *T. vivax* May provide a Clue. *ILRAD Reports* Vol. 2 No. 4, pp. 1 – 6.

ILRAD 1987. Highlights. *ILRAD Reports* ISBN 92 – 9055 – 288 – 3 p. 3

ILRAD 1987. Towards improved disease resistance; the bovine major hiscompatibility complex. *ILRAD Reports* Vol. 5(3) 1 – 8 ISSN 0255 – 4585

ILRAD 1988. Changes in respiration during the trypanosome life cycle. *ILRAD Reports* April 1988 vol. 6 No. 2 p, 4 ISBN 0255 – 4585

ILRAD 1989. N'dama cattle: Managing Africa's Genetic Resources *ILRAD Reports* Vol. 7 No. 4 P.1

ILRAD 1991. Recombinant DNA Technology in the Diagnosis of African Livestock Disease. *ILRAD Report* Vol. 9 No. 1 Pp. 1 – 8. ISSN 0255 – 4585

ILRAD 1991. The mechanism by which cattle acquire immunity to trypanosomiasis. *ILRAD Reports* (1991) vol. 9 No. 2 pp. 1-6.

ILRAD 1992. Transfecting genes. *ILRAD reports* vol. 10 No. 1 Jan. 1992. PP. 1 – 6.

ILRAD 1993a. Estimating the cost of Animal Trypanosomiasis in Africa. *ILRAD Reports*, April 1993. Vol. 11 No 2 P. I – 6

ILRAD 1993b. Decoding the language of cellular interactions. *ILRAD Reports* July 1993 vol. 11 No. 3. P. 5

ILRI 2009. Trypanosomiasis. http://www.ilri.org/infoserv/webpub/Fulldocs/ilri88/trypanosomiasis.htm Accessed 2/19/2009., 26 pp.

Immunopeptides in the defence reactions of *Glossina morsitans* to bacterial and *Trypanosoma brucei brucei* infections. Insect Biochemistry and Molecular Biology, 32(4): 369 – 375. In: *Tse Tse and Trypanosomiasis Information quarterly* Vol. 25(2) p. 41 FAO Rome.

Infonet (2011) African Animal Trypanosomiasis/Nagana. Assessed on www.infonet biovision .org/default/ct/167/ *animal diseases* on 19 feb. 2011.

Itard, J. (1989) Epizootiology of the African Trypanosomes. In: Shah – Fischer, M. and say, R.R. (Eds.) *Manual of Tropical veterinary parasitology.* CAB International, Oxon Pp. 179 – 293.

Jacobson, R.H. and Crowther, J. R. 1998.Elisa and the joint FAo/JAE division, Accomplishments and opportunities. In:Towards livestock disease diagnosis ant control in the 21st centur. *Proceedings of an international symposiym on diagnosis and control of livestock disease using nuclear and related techniques* jointly organized by the international atomic energy Agency and the food and Agriculture organization of the United Nations, held in Vienna, 7 – 11 April, 1997. Published by IAEA in Austria, August 1998. (ISSN) 0074 – 1884). Sti/pub/1023. Pp 15 – 26

Jeffcoate, I.A. and Holmes, P.H. 1997. Effects of Trypanosomiasis on Reproduction in Domestic Ruminants. In: Hide, G. Motttram, J.C., Coombs, G.H. and Holmes, P.H. (Eds.) Trypanosomiasis and Leishmaniasis Biology and control, C.A.B. International, Oxon, UK. Pp. 335 – 346.

Jones, T.W. 1997. Serodeme Analysis – Past, Present has it any future? *Proceedings of first internet conference on salivarian trypanosomes*, 1996 (online) published by FAO Rome, 1997.

Jordan A.M. 1986. Trypanosomosis control and African rural development. Longman group limited. ISBN: 0-582-46356-4. P.38

Kahn and Scott L. 2010.Collection and submission of Laboratory samples. The Merck Veterinary Manual, tenth Edith Merck & Co. Inc. ISBN 0 – 911910 – 93 – x

Kalu A.U., Edeghere H.U. and Lawani F.A. 1985.The Role of Parasitological diagnostic techniques in the Epizootiology of trypanasomiasis in small Ruminants. In: Adu F, Taiwo, B.A. Osinowo G.A. and Alhassan WS (Eds.) Small Ruminant of *National Conference on small ruminant potential Zaria*, NAPRI Publications p. 269.

Kalu, A. U., Edeghere, H. U. and Lawani F. A. 2002. Comparison of diagnostic techniques during subclinical single infections of trypanosomiasis in goats.Accepted 28 October

1985.Available online 12 November 2002.http://www.sciencedirect.com/ science/ article/pii/0304401786900051

Kalu, A.U. and Lawani, I.A. 1996. Observations on the Epidemiology of Ruminant trypanosomosis in Kano State, Nigeria. *Revue Elev. Med. Vet. Pays trop.*, 49(3): 213 – 217.

Kalu A.U., Uzoukwu, M., Ikeme, M.M. and Magaji, Y. (1991). Trypanosomosis in Nigeria, High Prevalence among Ruminants in Gboko Local Government Area. *Bull. Anim. Hlth. Prod.* Afr. 39:3-8, 1991.

Koslowsky, D. J. 2004. A historical perspective on RNA editing; how the peculiar and bizarre became mainstream, *methods mol. Biol*, 2004, 265: 161 – 97. PubMed. Retrieved on 15/7/2011 from http://www.ncbi./hi.gov/pubmed/15103074

Kumar, S., Chaudhury, K., Sen, P. and Guha, S. K. 2005. Atomic force microscopy; a powerful tool for high – resolution imaging of spermatozoa. *Journal of Nano biotechnology*. 10.1186/1477.3155-3-9 Accessed on 23/6/2011 http.//www. springerlink.com/content/g466n120x4417483/full text.html

Kumshe, A.H., Otesile, E.B., Sonibare O.A., Mbaya, A.W., Oladosu, L.A. 2010. Some indices of trypanotolerance in three breeds of Nigerian goats that differ in susceptibility to trypanosomosis. *The internet journal of Veterinary Medicine* 2010 volume 8 No 2. ISSN 1937-8165.

Ladd Prosser C. (Ed.) 1991. Environmental and Metabolic Animal Physiology, 4th edition. Wiley-liss: New York. P.111 In: Body Temperature and the AAT/H: Does the human condition indicate an aquatic past? http://www.aquatic ape.or/bodytemp.html

Lale N.E.S. 2006. Dictionay of Entomology and Acarology, second Edition ISBN 978 – 32369 – 2-x. Mole publications (Nig.) Ltd. PP. 249 and 279.

Lamorde, G.A 1991. Welcome Address. In: Ikede B.O. (Ed.) Control of Animal Trypanosomiasis in Nigeria as a Strategy for increased Livestock Production. Published in 1991 based on *proceedings of a preparatory Workshop held in Vom* 6 - 9 June, 1989 under the Auspices of the European Community Lome 111 project on the control of Animal Trypanosomiasis in Nigeria. P 1

Lawal A. I., Ameya F. Ajanusi, O.J., Jatau I. D. and Bala, M. 2007. Distribution and morphological forms of Trypanosomea brucei in Tissues/organs of experimentally infected Albino rats. *Proceedings of 44th Annual congress of the Nigerian Veterinary Medical Association* 22nd – 25th October 2007. Effurum – Delta state, Nigeria. ISSN 0331 – 3026, PP. 257 – 260.

Leak, S., Faye, D. and Geerts, S. 2002. Genetic resistance to vectors and vector – borne diseases in indigenous West African Livestock. *Newsletter on Integrated control of pathogenic Trypanosomes and their vectors (ICPTV)* No. 6 September, 2002.

Leigh, O.O. and Fayemi, O.E. 2011. The prevalence of trypanosomosis in female WAD Goats in Three Local Government Area of Ibadan, Nigeria. *Global Veterinaria* 6(1):11-15, 2011. ISSN 1992-6197. IDOSI Publications, 2011. http://www.idosi.org/gv/ gv6(1)11/26.pdf

Lodish, H., Berk, A., Matsudaira, P., Kaiser, A.C., Krieger, M., Scott, P.M., Zipursky S.L and Darnell J. 2004. Molecular cell biology. Fifth Edition, W.H. Freeman and Co. New York. ISBN 0-7167-4366-3 Pp. 533-570.

Lonsdale-Eccles, J.D. and Grab D.J 2002 Trypanosome Hydrolases and the blood-brain-barrier. Trends in Parasitology 18(1) 17-19.

Losos. G. J. 1986 Trypanosomiasis. Infectious Tropical Diseases of Domestic Animals.Longman England.

Luck S. (Ed,) 1998. Philip's Sccience and Technology Encyclopaedia. First Edition, George Philip Limited, London. ISBN 1-85613-486-5. P. 119

Luckins A.G. 2007 New Diagnosis for the detection of animal Trypanosomoses-A short review. In: "Developing Methodologies for the use of Palymerase Chain Reaction in the Diagnosis and Monitoring of Trypanosomosis" *IAEA-TEC DOC*-1559. Pp.11-13.

Luckuis A.G. 1998. Application of Enzayme Linked Immunosorbent Asseys to the Diagnosis of the Pathogence Animal Trypanomes. In: Towards Livestock Disease Diagnosis and control in the 21st Century: *Proceedings of an International Symposium on Diagnosis and control of Livestock Diseases using Nuclear and Related Techniques* jointly organised by the VInternational Atomic Energy Agency and the Food and Agricultural Organisation of the United Nations and held in Vienna, 7-11 April 1997. Published by International Atomic Energy Agency Vienna, 1998. ISSN 0074-1884, ISBN 92-01-102498-3. Pp 47-64.

Lumsden, W. H. R., Herbert, W. J.M., M'Neillage G. J. C 1973. Technique with Trypanosomes.Churchill Livingstone, Edinburg and London.

Magez, S. Radwanska, M., Drennan, M., Fick, L., Baral, T.N. Brombacher, F. and DeBaetselier P., 2006. Interferon – T and Nitric Oxide in combination with Antibodies Are key Protective Host immune factors during Trypanosoma Congolense Te 13 Infections. *The Journal of Infections Diseases*, Volume 193 (2006) Pp. 1575 – 1583.

Magez, S. Stijlemans B., Caljon G., Engster, H.P. and De Baekselier, P. 2002. Control of experimental *Trypanosoma brucei* infections occurs independently of Lymphotoxin – and induction. *Infection and immunity*, 70 (3): 1342 – 1351. Tsetse and Trypanosomiasis Information Quarterly 25(1) 2002. No Page FAO, Rome.

Mahmoud, M.M and Elmalik, K. H. 1977. Trypanosomiasis: Goats as a possible reservoir of *Trypanosoma congolense* in the Republic of the Sudan. Trop. Anim. Hlth Prod. (1977) 9, 167-170

Maichomo, M.W., Olubai, W., Mwendia, C.M.T. and Mapenay, I.M. 2001. Farmer responses on acceptability of the Orma Boran, a trypanotolerant breed of cattle introduced into Nguruman, Kajiado district in Kenya. In: OAU/STRC, 2001. Published in *Tsetse and Trypanosomiasis Information Quarterly* (TTIQ) 25(3)12351. FAO Rome.

Maizels, R.M., Blaxter, M.L., Robertson, B.D. and Selkirk, M.E. 1991 *Parasite Antigens, Parasite Genes. A Laboratory Manual for Molecula parasitology*. Cambridge University Press New York. ISBN 0-521-419271 pp. 141

Majiwa, P.A.O., Maina, M., Waitumbi, J.N., Mihor, S. and Zweygarth, E. 1993. *Trypanosoma (Nannomonas) congolense*: Molecular Characterisation of a new genotype from Tsavo, Kenya. *Parasitology* 1151 – 162..Cambridge University Press.

Majiwa, P.A.O., 1998. Application of the Polymerase chain Reaction for detection of Trypanosome. Pp. 177-183

Mare, C.J. 1998. African Animal Trypanosomiasis. In: Part IV Foreign Animal Disease. United States Animal Health Association (USAHA) Richmond, USA pp. 29 – 40.

Masake R.A. and Nantulya V.M. 1991. Sensitivity of an Antigen Detection Enzyme Immuno assay for Diagnosis of *Trypanosoma Congolense* infections in Goats and Cattle. *The Journal of Parasitology*, vol. 77, No. 2 (April 1991) pp. 231 – 236.

Masake R.A., Majiwa, P.A.O., Moloo, S.K., Makau, J.M., Njuguna, J.T., Maina, M., Kabata, J., Olemoi Yoi, O.K. and Nantulya V.M. 1997. Sensitive and Specific detection of *Trypanosoma vivax* using the polymerase chain reaction. *Experimental Parasitology* 85, 193 – 203. Cited by Eisler, C.M., Dwinger, R.H., Majiwa, P.O.A. and Picozzi, K. 2004. Diagnosis and Epidemilogy of African animal Trypanosomiasis. In: Maudlin, I., Holmes, P.H. and Miles, M.A. (eds.) (2004). The Trypanosomiases CAB International pp. 253 – 267.

Masiga, D.K., McNamara, J.j. Laveissiere, C. Truc, P. and Gibson, W.C. 1996. A high prevalence of mixed trypanosome infections in tsetse flies in sinfra, cote d' ivoire, detected by DNA amplification. *Parasitology* 112:75-80.

Masiga, D.K., Okech, G., Irungu, P., Ouma, J., Wekesa, S., Ouma, B., Growth and Mortality in sheep and goats under high Tsetse challenge in Kenya. *Tropical Animal health and production.* Vol. 34 No. 6 Pp. 489 – 501. springer Netherlands. ISSN 1573 – 7438 (online) online date Tuesday Novemebr 02, 2004.

Masocha, W., Rothenberg, M.E. and Kristensson K. 2006. Minocycline Impedes African Trypanosome Invasion of the brain in a Murine Model. J. American Soc. For microbiology vol. 50 No 5 Pp. 1798 – 1804.

Masocha, W., Rottenberg, M.E. and Kristensson, K. 2006. Minocyline impedes African Trypanosome Invasion of the brain in a murine model. *Journal of American Soci.For microbial*. Vol. 50 No. 5, 1798 – 1804.

Mathews J. 1999. Diseases of the goat.Second Edition. Pp. 266 – 349. Blackwell Science Ltd. Oxford.

Maudlin, I., Holmes, P.H. and Miles, M.A. 2004.The Trypanosomiases CAB International Oxfordshire.

McDermott, J., Woitag, T., Sidibe, I., Bauer, B., Diarra, B., Quedraogo, D., Kamuanga, M., Peregrine, A. Eisler, M., Zessin, K.H., Mehlitz, D. and Clausen, P.H. 2003. Field Studies of drug resistant Cattle trypanosomes in Kenedougou Province, Burkina Faso. *Acta Trop*. 86: 93 – 103.

Mehlitz, D. 1982. Typanosomes in African Wild mammals. Baker J.R. (Ed.) Research Studies Press, Herts, England Pp. 25 – 35.

Mekasha, Y., Tegegne, A. And Rodriguez-Martinez, H. 2007. Sperm Morphological attributes in indigenous male goats raised under extensive husbandry in Ethiopia. Anim. Reprod. V.4, n. 1/2., p.15-22 Jan/June 2007.

Merriam-webster 2011. An encyclopaedia Britannica Company. Accessed on 8/7/2011 http://www.merriam-webster/dictionary/enzyme.linked+immunosorbent+assey

Mesotheliomalawyers, 2011. Polymerase Chain Reaction. Accessed on 7/15/2011 http://mesotheliomalawyersusa.net/polymerase-chain-reaction.

Milon, G., (1994). Drugs or Vaccines? *Parasitology Today*, vol. 10, no. 10, 1994. Pp. 402-404

Minter, D.M. 1989. Geographical distribution of anthropod-borne diseases and their principal Vectors.Unpublished document.WHO/VBC/89.967 Geneva.World health organization. Reproduced on line with permission from WHO. http://www.ciesin.org/docs/001-613/001-613.html accessed 13/10/2005.

Mowlem, A. 1992. Goat Farming. Second Edition. Butler & Tarner Ltd., London. ISBN 0-85236-235-8. Pp. 1, 8, 34.

Muller, N., Imboden, M., Detmer, E., Masfield, J.M. and Seebeck, T. 1993. Cytosketeton associated Antigens fron African Trypanosomes are recognised by self-reactive antibodies of unifected Mice *Parasitol.* Pp. 107,411-417

Murphy, N.B. and Pelle R. 1997. Differential gene expression during the life cycle of Trypanosomes. *Proceedings of the first internet conference on salivarian trypanosomes* (FAO animal health and production paper 136) FAO Rome.Online 1997.

Murray, *et al*.(2005) Trypanosomiasis, Parasitology, chapter two Pp. 632 – 645. In: Abdul Ghaffar (Ed.) *Microbiology and Immunology online.* http://pathmicro.med.sc.edu/parasitology/blood-proto.htm. Accessed on 13/10/2005.

Murray, M. Murray, P.K. McInteyre, W.I.M. 1977. An improved parasitological technique for the diagnosis of African Trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* 71,325 – 326.

Murray, M., d'Ieteren, G.D.M. and Teale, A.J. (2004). Trypanotolerance. In: Maudlin, I., Holmes, P.H. and Miles, M.A. (Eds.). The Trypanosomiases.CAB International Oxfordshire pp. 461 – 477.

Murray, M., Trail, J.C.M., Davis, C.E. and Black, S.J. 1984. Genetic resistance to African Trypanosomiasis. *The Journal of Infect. Diseases* Vol. 149 No 3 Pp. 311 – 319.

Mutayoba, B. M., Gombe, S. Waindi, E. N. and Kaaya, G. P. 1989. comparative Trypanotolerance of the small East African breed of goats from different localities to *Trypanosoma congolense* Infection. *Vet. Parasitol.* 31 (2) 95-105.

Mutugi, M.W., Ries,R., Mudenge, D., and Van den Bossche, P. 2006. The use of enzyme-linked immunosorbent asseys (ELIAS) for Trypanosomosis surveys and surveillance. Retrieved on 10/13/2006 from http://www.ilri.org/ Infoserv/Webpub/ Fulldocs/Ag_ Elisa/Mutugi.htm

Ndao, M., De Almeida, P.J.L.P., Van Meirvenne, N., Geerts, S. 1998. Diagnostic evaluation of PCR in goats experimentally infected with *Trypanosoma vivax.* Poster presentations IAEA-SM-348/17P *Proceedings of Symposium in Vienna.* 7-11 April, 1997, Jointly Organised by IAEA and FAO on Towards Livestock Disease diagnosis in the 21st Century, Published by IAEA Vienna 1998. Pp. 541-542

N'Doutamia, G., Mbakasse, R.N., Brahim, A., Khadidja, A. 2002. Influence de la trypanosomose a T. Congolense sur les parameters haematologiques, mineraux et

proteo – energetique, chez les chevres saheliennes du ichad. *Revue med. Vet.* 153,395 – 400 In:

Naessens J. Teale, A. J. and Sileghem M. 2002. identification of mechanisms of natural resistance to African Trypanosomiasis in Cattle. Veterinary Immunology and immunopathology, 87(3-4): 187-194. In: Tse- Tse and trypanosomiasis Information Quarterly (TTIQ), 25 (2) 2002 p. 53.

Naessens J., Leak, S. G. A., Kennedy, D. J. Kemp S. J., Teale A. J. 2003. Response of bovine Chimeras combining Trynosomosis resistant and susceptible genotypes to Experimental infection with *Trypanosoma congolense*. *Veterinary parasitology* 111: 125-142.

Ng'ayo, M.O., Njiru, K.Z., Kenya, E.U., Muluvi G.M. Osir, O.E. and Masiga, K.D. 2005. Detection of trypanosomes in small ruminants and pigs in Western Kenya. Important reservoirs in the epidemiology of sleeping sickness? *Kinetoplastid Biology and Disease* 2005 4:5 vol; 10.1186/1475 – 9292-4-5.

Njiru, Z.K., Kinyua, J.K, Consstantaine, C.C., Guya, S., Crowther, J.R., Kiragu J.M., Thompson C.A. Davila, A.M.R, (2007). The use of ITS 1 RDNA PCR in detecting Pathogenic African Trypanosomes. IN: IAEA 2007. Development megthodologies for the use of polymerase chain Reaction in the diagnosiss and monitoring of trypanosomosis. IAEA, Vienna, 2007. *IAEA-TECDOC-1559*. ISBN:978-92-0-105507-1. ISSN: 1011-4289. Pp. 76-79

NOK, A.J. and Balogun E.O. 2003. A bloodstream *Trypanosoma congolense* Sialidase could be involved in anaemia during experimental trypanosomiasis. *Journal of Biochemistry*. 133 (6) 725 – 730.

Nuru S. 1985. Trends in small ruminant production in Nigeria. In: Small Ruminant Production in Nigeria: *Proceedings of National conference on small ruminant production, Zaria, Nigeria*, October 6th – 10th, 1985, p. 42.

Nwosu, C.O., Ogunrimade, A.F., Fagbemi B.O. 2006. Effect of Concurrent *T. brucei* infection on haemonchosis in Red Sokoto (Maradi) goats. *Sahel J. Vet. Sci*. 5, 45-54

Odoya, E.M. Omotainse, S.O. and Thompson, G.A. 2003. Prevalence of Trypanosomosis in Goats in Abraka, Delta State, Nigeria. *Nig. Vet. Journal 24* (3): 42 – 43.

Offor, I.I. 2000. "The role of veterinary entomology in Nation Building: The proceedings of ESN 30th Annual Conference held 4th – 7th October, 1999 at Kano. *ESN Occasional Publication* 32 (2000): 23 – 29.

Ogunsanmi O.A. and Taiwo V.O. 2004. Comparative Studies on erythrocyte calcium, potassium, haemoglobin concentration, osmotii resistance and sedimentation rates in grey duiker (*Sylvicapra grimmia*), sheep and goats experimentally infected with *Trypanosoma congolense*. *Veterinarski Arhiv* 74 (3), 201 – 216, 2004.

Ohaeri, C.C. 2010. Prevalence of Trypanosomosis in Ruminants in Parts of Abia State, *Nigeria Journal of Animal and Veterinary Advances* 9 (18) 2422-2426. Medwell Journals ISSN 1680-5593

Okonkwo J.C., Okonkwo I.F., Ebuh, G.U. 2011. Effect of breed sex and source within breed on haematological parameters of Nigerian goats. *On line journal of animal and feed Research.* Vol. 1 issue 1 : 8 -13. ISBN 2228 – 7701.

Olatoye, I.O. and Ogundipe, G.A.T. 2003. Comparative study of two diagnostic techniques and status of sheep trypanosomosis in south west Nigeria. *Nig. Vet. Journ.* Vol. 24(3): 44 – 48.

Omoogun, G.A. 1991. The tsetse fly: Biology, Ecology, Trapping and Dissection. In: Ikede, B.O. (ed.) Control of Animal Trypanosomiasis in Nigeria as a Strategy for increased Livestock production. Based on *Proceedings of a Preparatory Workshop held in Vom, Plateau State Nigeria,* 5 – 9 June 1989 under EEC Lome III Project on the Control of animal trypanosomiasis in Nigeria. Pp. 90 – 102.

Omotainse, S.O., Edeghere, H., Omoogun, G.A., Alhassan, E.O., Thompson, G., Igweh, C.A., Ukah, J.A.C., Ikenga, M.A. and Halid, I. 2000. The Prevalence of Animal Trypanosomosis in Konshisha Local Government Area of Benue State, Nigeria. *Israel J. of Vet.Med.* 55:142 – 144.

Omotainse, S.O., Kalejaiye, J.O., Dede, P. and Dadah, A.J. 2004. the current status of Tsetse and Animal Trypanosomosis in Nigeria. *Vom Journal of Veterinary Science* vol. 1 No. 1. Pp. 1-7.

Ortega, C., I. De Blas, K. Frankena, J. Noordhuizen (1996): Win Episcope 1.0: Su aplicacion de tipo cuantitativo. In Reunion Cientifica de la Sociedad Espanola de Epidemiologia. Zaragoza.

Osir, E.O., Abakar, M. and Abubakar L. 2001. The role of trypanolysin in the development of trypansomes in tsetse. In: OAU/STRC, 2001.

Otesile, E.B., Fagbemi, B.O., Adeyemo, O. 1991. The effect of *Trypanosoma brucei* infection on serman biochemical parameters of boars on different planes of dietary energy. *Veterinary Parasitology*, 40:207-216.

Otokurefor, T and Otokurefor, K. 2009. Antigen and Antibody. Malthouse Press Ltd. Lagos. ISBN 978 978 023 270 2 PP. 151

Overath, P., Haag J., Lischke, A. and O'h uigin, C. 2001. The Surface Structure of Trypanosomes in relation to their Molecular Phylogeny (Incl. *T. brucei*) *Internation Journal for Parasitology*, 31 (5-6): 468-471. In TTIQ vol. 24 Part 3, No. 11991. Pp 111. FAO Rome.

Paris, J., Murray, M. and Mcodimba, F. 1982. An evaluation of the sensitivity of current parasitological techniques for the diagnosis of bovine African trypanosomiasis. *Acta Tropica* 39,307-316.

Pays, E., Rolin, S. And Megez., S. 1997. Cell Signalling in trypanosomatids. In: Hide.

Pollock J.N. 2000. Training manual for tse tse control personnel Vol. 1.M/P5178/ E/1.82 /1 /2000 FAO, Rome

Rabo, J.S. and Oyejide, A. 1996. Humoral immune response of West African Dwarf sheep to infection with T*rypanosoma congolense* (Gboko Strain) *journal of small ruminant research*, Elsevier publishers.

Radostits, O. M. Gray, C. C., Blood, D. C. and Hinchcliff K. W. 2000. Disease caused by Trypanosomes. A textbook of the Diseases of cattle, sheep, pigs, Goats and Horses.Ninth Edition. W. B Saunders Company Ltd. Printed in China pp. 1329-1337.

Rahman, A.H.A. 2002. Report on Tsetse and Trypanosomiasis activities in sudan FAO TCP/SUD/0069) In *Tsetse and Trypanosomiasis information quarterly* (TT1Q) 25 (3) P. 77 FAO Rome.

Reagan, W.J., Sanders, T.G. and DeNicola D.B. 1998. Veterinary Haematology Atlas of Common Domestic Species.Manson Publishing/The Veterinary Press. First Edition, Pp. 3 – 69.

Reifenberg, J.M., Solano, P., Cuissance, D., Duvallet, G., 1997. Contribution of the PCR (Polymerase Chain Reaction). Technique for a better understanding of the Epidemiology of animal trypanosomosis in West Africa. *Proc. 1st Internet Conference on Salivarian Trypanosomes. Tryplink-L Discussion list* 9-14 December, 1996. Published by FAO Animal Production and Health Paper 136, 1997. http//www4.fao.org/cgi-bin.

Robinson, N.P., Burman, N., Melville, S.E. and Barry, D.J. 1999. Predominance of Duplicative VSG gene Conversion in Antigenic Variation in African Trypanosomes. *Molecular and Cellular Biology* Vol. 19 No. 9: 5839 – 5846.

Rudenko, G., Cross, M. and Borst, P. 1998. Changing the end: antigenic Variation Orchestrated at the Telomeres of African Trypanosomes.Trends Microbiol. 6: 113 – 117 [Medline]. Cited by Robinson, N.P., Burman, N., Melville, S.E. and Barry, D.J. 1999. Predominance of Duplicative VSG gene Conversion in antigenic Variation in African Trypanosomes. *Molecular and Cellular Biology* Vol. 19, No. 9, 5839 – 5846.

Russo, D.C.W. 1992. Clearance of antibody – VSG complexes by trypanosomes. *ILRAD Reports*, vol. 10 (1): 6.

Saror D.I. 1980 Observation on the course and pathology of *T. vivax* in Red Sokoto goats. *Res. Vet. Sci.* 28:36-38.

Schalm, O.W. 1971. Veterinary Haematology Second Edition 664 pp lea and Febiger USA.

Seifert S.H. 1992. Tropical Animal Health Kluwer Academic Publishers Group, The Netherlands.

Shaw A.P.M. 2004, Economics of African trypanosomosis in: Maudlin I. Holmes, P. And Miles M. (eds) The trypanosomiases CAB International Pp. 345

Smadi, S. M., Fajinmi, A.O., Kalejaye J.O., Wayo, B., Haruna, M.K., Yarnapr J.E., Usman, O.A., Hamra, S.M., Jijitar, A., Ogunwale, R., Bizi., R., Ovbagbedia, R.P. and Abenga, J.N. 2010. Period Variation in Trypanosoma Infection Rates in Trade Small Ruminants at Slaughter in Kaduna Central Abattoir. *Research Journal of Veterinary Science*, 3(3) 139-193, 210 ISSN 1819-1908, Academic-Journalns Inc.

Smyth J.D. 1996. Animal Parasitology.Cambridge Low Price Edition, Cambridge University Press pp. 57 – 89.

Snyder S.H. 1985. The Molecular basis of communication between cells. *Scientific American.* Vol. 253 No 4. PP 152.

Steel M., 1996. Goats. The Tropical Agriculturist Series. CTA/Macmillan Pubishers limited, Oxford. www.Macmillan-africa.com. ISBN 978-0-333-523094 p.2, 9, 68.

Stevens, J.R. and Brisse, S. 2004. Systematics of trypanosomes of Medical and Veterinary importance. In: Maudlin I., Holmes P. and Miles, M. (eds) The Trypanosomosis : PP. 1 – 24

Taiwo, V.O., Olaniyi, M.O. and Ogunsanmi, O.A., 2003. Comparative plasma biochemical changes and susceptibility of erythrocytes to invitro peroxidation during experimental Trypanosoma congolense and T. brucei infections in sheep. Israel Journal of Veterinary Medicine Vol. 584

Takeda, K., Tsuneyasu, K. and Shizua, A. 2003. Toll-like receptors. *Annu. Rev. Immunol.* 2003, 21:335-76. Doi:10.1146/annurev.immunol.21.120601.141126. Accessed online on July 5th,2011

Targan S.R and Shanahan F. 1990. Immunology and Immunopathology of the liver and gastrointestinal track. Igaku - Shion Medical Publishers., Inc. New York. ISBN 0 – 89640-168-5. PP 5 – 10.

Taylor K.A. 1998. Immune responses of cattle to African trypanosomes protective or pathogenic? *Int. J. Parasitol.,* 28,219-240.

Taylor K.A., Lutje, V. Kennedy, D. Authie, E. Boulange, A. Logan – Henfrey L., Gichuki, B. and Gettinby, G. 1996. *Trypanosoma congolense*: B-Lymphocyte Responses Differ between trypanotolerant and trypanosusceptible cattle. *Experimental Parasitology* 83,106-116.

Taylor, K. and Authie, E. M. L. 2004. Pathogenesis of African Trypanosomiasis. In: Mandlin, I., Holmes, P.H., and Miles M.A. (Eds) The Trypanosomiases. CABI Publishing. ISBN: 0-85199-475X pp. 331-353.

Thrusfield, M.V., 1991. Epidemiology – Data Collection. In; Ikede, B.O. (Ed). Control of Animal trypanosomiasis in Nigeria as a strategy for increased Livestock Production. *Proceedings of a preparatory workshop held in VOM, Nigeria.* 5-9 June, 1989 under EEC lome III Project on the control of Animal Trypanosomiasis in Nigeria. Published, 1991, Pp. 19-37.

Tibayrenc, M. 1995. Population genetics of parastic protozoa and other microorganisms. *Advances in parasitology* 36, 47 – 115.

Tibayrenc, M. 1997B. molecular epidemiology of Salivarian trypanosomes. In: Roberto Aguilar M.S. Silva Alberts m.R. Davila (Eds.) *proceedings of the first salivarian trypanosomes. Tryplink – L discussion list* 9-14 December 1996. published by FAO Rome, 1997.

Tibayrence, M. 1997A. Evolutionary Genetics of Trypanosoma, Leishmania and other microorganisms: Epidemiological, Taxonomical and Medical implications. In: Hide, G. Mottran J.C., Coombs G.H. and holmes, P.H. (Eds.) Trpanosomiasis and Leishmaniasis Biology and Control CAB International. Pp. 305 – 314.

Tizard, I., 2004. Veterinary immology, an introduction. Seventh Edition. San 0-7216-0136-7. Pp. 95, 120.

TTIQ (Tsetse and Trypanosomiasis Information Quarterly) News vol. 24 part 3 p. 80, 2001.

Turner, C. M. R., Aslam, N. and Dye C 1995. Replication, Differentiation, growth and the virulence of *Trypanosoma brucei* infections. *Parasitology* 111: 289-300.

Tyler, K.M., Higgs P.G., Mathews R.K. and Gull K. 2001. Limitation of *Trypanosoma brucei* parasitaemia results from density dependent parasite differentiation and parasite killing by the host immune responses. *Proceedings of the royal society of London*, series B. Biological Sciences 268 (1482): 2235 – In TTIQ vol. 24 part 4 No. 12047 – 12133 p.

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. and Jennings F. W. 1998. Veterinary protozoology. Veterinary Parasitology.Second Edition, Blackwell Science Ltd. Oxford. Pp. 212-218.

Uza, D.V., Barde, R.E. and Ayoade, J.A., 2005. The Effect of Urea treated cassava peels as supplement to West African Dwarf (WAD) goats grazing natural pasture. *Nig. Vet. Journal*, Vol. 26(1) 2005 pp. 1-9.

Uzcanga, G., Mendoza, M., Aso, P.M. and Bubis, J. 2002. Purification of 64 Kda antigen from *Trypanosoma evansi* that exhibits cross – reactivity with *Trypanosoma vivax*. *Parasitology* 2002., 124: 287 – 299. Cambridge University Press.

Vale G.A. and Torr. S.J. 2004. Development of Bait Technology to Control tse tse. In: Maudlin, I., Holmes P.H., and Miles M. 2004. P.513.

Vegad J. L. 1995. complement system. In: A textbook of veterinary General pathology. Vikas publishing House Pvt Ltd Delhi pp 94-96.

Ventura, R.M., Takeda, G.F. and Teixeira, M.G. 1997. Molecular markers for characterization and identification of Trypanosoma evansi: In: Roberto Aguilar M.S. Silva Alberto M.R. Davila (Eds.) *Proceedings of the First internet Conference on Salivarian Trypanosomes Typlink – L discussion list* 9-14 Deecmber 1996. published by FAO, 1997.

Verhulst, A. and Pandey V. S 1998. Technical problems in the estimation of Trypanotolerance Within Cattle and Small Ruminant Breeds. *Proc. of the FAO expert consultation on The genetic aspects of Trypanotolerance.* FAO Animal Production and Health (Rome) paper 94, 20 – 43.

Vickerman K. 1997. Landmarks in Trypanosome research in: Hide, G., Mottram, J.C., Coombs, G.H. and Holmes P.H. (Eds.) Trypanosomiasis and Leishmanias Biology and Control, C.A.B. International Oxford, UK. Pp. 1-37.

Viswambharan, H, Seebeck, T. and yang, Z.H. 2003. Enhanced endothelial nitric oxide synthase activity in mice infected with *Trypanosoma brucei*. *International Journal for parasitology*, 33 (10): 1099 – 1104.

Walker, J.M. and Rapley, R. 2005. Medical Biomethods. Humana Press, New Jersey. ISBN 1 – 58829 – 334 – 3. PP. 4 – 12.

Walker P.J. 1969. Standardisation of routine observations of blood trypanosomes. In: *Annual Report of the East African Trypanosomiasis Research Organisation* 1968 pp. 15 – 16.

Whitelaw, D.D., Kaaya, G.P., Moutlon, S.K. and Max Murray 2006. Susceptibility of different breeds of goats in Kenya to Experimental infection with *Trypanosoma congolense*.*Tropical Animal Health and Production* vol. 17 (3) 155 – 165 Springer Netherlands, 1985.online. Online Date Tuesday, April 11, 2006.

Wikipaedia, 2012. *Trypanosome brucei*, wikipaedia, the free encyclopaedia. On line modified on 29/12/2012. http://webcache.goggleusercontent.com/search.

Williamson D.J.L., Taylor K.A., Newson, J., Gichuki, B. and Naessens, J. (1996).The role of anti-variable surface glycoprotein antibody responses in bovine trypanotolerance.

Parasite immunology, in press. In: Taylor K.A., Lutje, V. Kennedy, D., Authie E. Boulange, A., Logan – Henfrey L., Gichuki B. and Gettinby, G. 1996. *Trypanosoma congolense*: B – Lymphocyte Responses Differ between trypanotolerant and Trypanosusceptible cattle. *Experimental Parasitology*, 93,106-116.

Wilkinson, J.F., 1975. Introduction to Microbiology. Blackwell Scientific Publications, Oxford. Second edition. P. 99.

Wilson, T.R. 1991. FAO Animal Health and Production Paper no. 88. p. 231.

Wilson, A.C. 1985. The Molecular basis of evolution. Scientific American. Vol 253 No 4. PP 164 - 173.

Witola, W.H. and Lovelace C.E. 2001. Demonstration of erythrophagocytosn in *Trypanosoma Congolense* infected goats. *Vet. Parasitol*.

Woo P.K.T. The haematocrit Centrifuge technique for the diagnosis of African trypanosomosis. *Acta tropica* 27, 384 – 386.

Yanan, G.E., Dede, P.M. and Uzoigwe N.R. 2003. A Survey of animal trypanosomiasis in selected muturu herds on the Jos plateau, Plateau State, Nigerian, *Nig. Vet. Journal* 24 (3):39 – 41.

Zahang, G.W. Song, H.D. and Cheen Z. 2004. Molecular Mechanism of mRNA alternative splicing. Yi Chuan Xue Bao 2004, Jan. 31(1): 102-7. Retrieved on 15/7/2011 from http://www.ncbi.nih.gov/pubmed/15468927.

Zakpa, G., d'Ieteren, G., Diedhou, M., Leak, S.G.A. and Coulibaly, L. (2001) Breed Choice and tryupanosomosis risk. In: OAU/STRC, 2001 (See 25: no 1229). Pp. 299-300. In: Tsetse and Trypanosomiasis information quartly vol. 25 (3) No 12348. P. 124

ZVan Kampen R.K. 1998. immpordulator in: Aiello E.S. (Ed.) The Merck Veterinary Mamal. Eight Edition, Merck and Co. Inc.

Appendix I

Assembly of Improvised Scale

Appendix II

Tse-Tse Fly Biconical Trap Mounted at Zoological Garden, Fiidi Council Ward

Appendix III

Appendix III shows biconical trap catches

Block	Glossina sp	Hippobosca sp.	Tabanids	Stomoxys sp.
Modern market ward	0	0	350	11
Agan ward	3	0	150	4
Mbalagh ward	2	0	217	0
Fiidi ward	2	0	31	0
North Bank ward 1	0	0	20	23
Total	7	0	768	38

Appendix IV

SEQUENCE OF PRIMER

KIN1 = 5' GCGTTCAAAGATTGGGCAAAT 3'

KIN2 = 5'CGCCCGAAAGTTCCCAACC 3'

Source: Njiru et. al.,2007.

Appendix V

In DNA Extraction LAB. DNA Extraction in Progress. The Researcher (Dr. Nongo N.N) with Dr. Pam, a staff of NVRI VOM

Appendix VI

ZR Genomic DNA Extraction Kit

Appendix VII

PCR Master Mix Room

Appendix VIII

PCR Amplification Laboratory

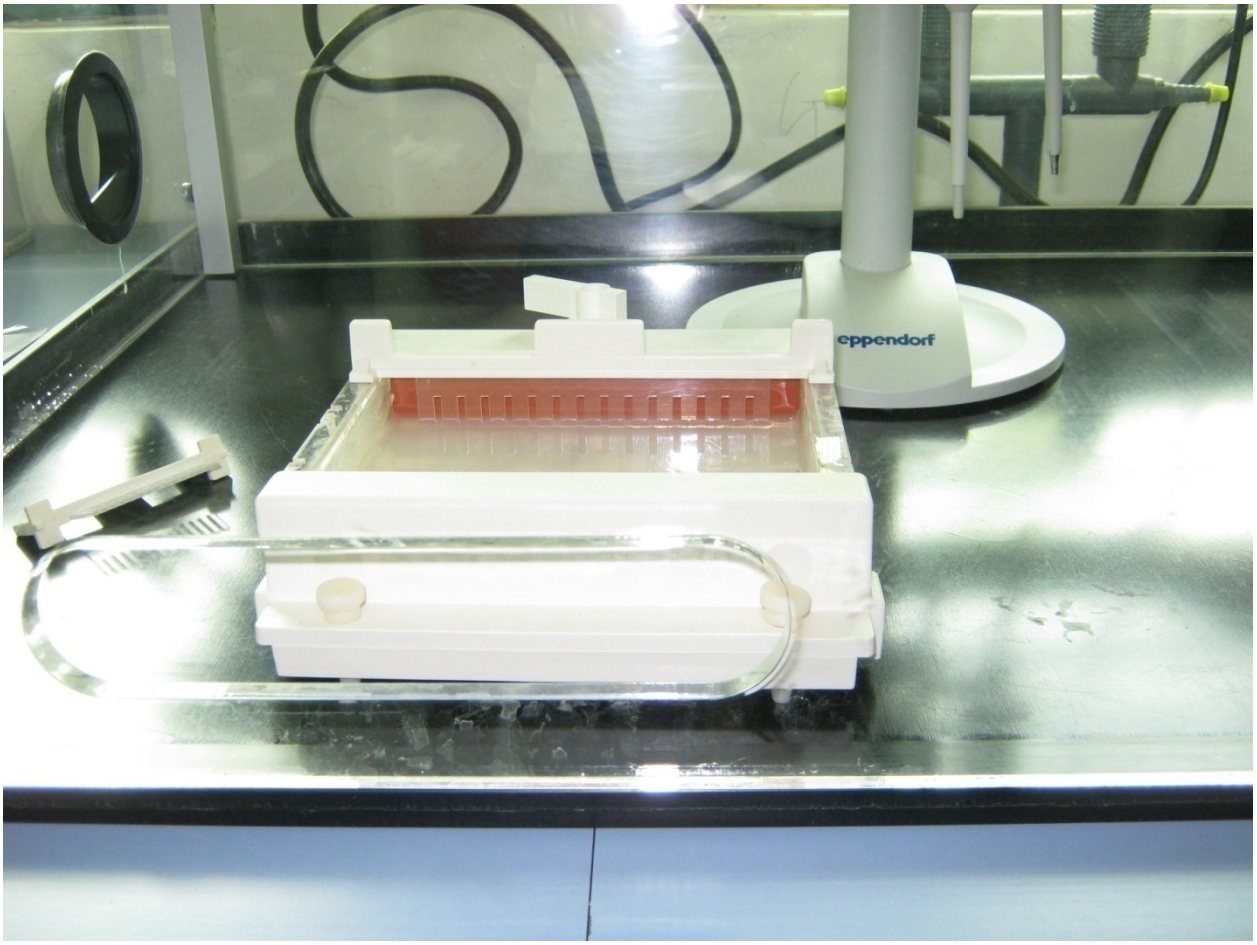
Appendix IX

GeneAmp PCR Thermal Cycler. In the PCR Amplification Laboratory

Appendix X

Electrophoresis Laboratory

Appendix XI



Gel Tank

Appendix XII

Syngene Gentreat Gel Imaging System