EFFECT OF EXPERIMENTAL *TRYPANOSOMA EVANSII* INFECTION ON HAEMATOLOGICAL PARAMETERS AND SEMEN CHARACTERISTICS OF YANKASA RAMS

By

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ABSTRACT

This study was designed to evaluate the effect of experimental *Trypanosoma evansi* infection on haematological and reproductive parameters of Yankasa rams. Twelve rams allotted to two groups were used; Group A (Infected, n=7) and group B (Control, n=5). Each animal in the infected group was inoculated via the jugular vein with 1mL of blood from the donor rats containg 1 X 10^6 trypanosomes. The infected rams were allowed to run the course of the disease for fourteen weeks. Experimental rams were monitored for clinical manifestations of the disease. Temperature and body weight changes were observed and recorded daily and weekly respectively. Scrotal circumferences were also monitored. Haematological parameters (PCV, RBC, WBC and differential leucocyte counts) and total protein were determined weekly. Similarly, semen volume, gross motility, live - dead sperm ratio, sperm morphological abnormalities and concentration as well as reaction time of infected and control rams were evaluated on a weekly basis. The testes and epididymides in each group were harvested for gross and histological examination. The data obtained were analysed using Student's t-test on Graph Pad Prism version 5.0., P □ 0.05 was considered significant. Infected rams had a pre-patent period of 5.3±0.29 days and the parasitaemia was intermittent. Pale mucous membrane, rough hair coat, loss of weight, dullness, edema of the face and lower jaw, corneal opacity, lacrimation were the clinical signs observed. There was progressive decrease in mean PCV and RBC count from first week post infection (28.9 ± 0.51%)(9.05 ± 0.26 x10^6 cells/mm^3)and culminating in anaemia in infected animals (21.2 ± 1.99%) and (5.42 ± 0.49 x10^6 cells/mm^3). There was a transient decrease in total plasma protein reaching its least mean value of 5.1± 0.20 g/dl at 8 weeks post infection. There wassignificant (p□ 0.05)increases in leucocyte countin the infected post infection as compared to the control rams.There were no significant (P□ 0.05) differences in the differential leucocyte counts in the infected group compared to the control. The results showed non significant

(p □ 0.05) decreases in semen volume (pre-infection (0.47 ± 0.05 to 0.36 ± 0.09mL at 13 weeks post infection)and sperm concentration was significantly lowerfrom (2.87 ± 0.29 x10^6 cells/mm^3) at pre-infection to (1.01 ± 0.17 x10^6 cells/mm^3) at 14 weeks post infection. Reaction time showed significant (p□ 0.05) increase from pre infection value(26.7 ± 4.54 to 94.7 ± 7.54 secs) 13 weeks post infection. Furthermore, the gross motility for infected rams was significantly (p□ 0.05)lower. There was a drastic surge in sperm morphological abnormalities from pre-infection (20.86 ± 0.52%) to (90.75 ± 2.73%) at 13 weeks post infection. Following post mortem examination no pathologies were observed grossly on the testes and epididymides but histologically, the testes showed extensive fibrosis and thickening of the seminiferous tubules with necrotized spermatogonic cells. Seminiferous tubules were devoid of spermatids. The epididymis revealed extensive fibroplasia and necrosis of epididymal cells with depleted epididymal sperm reserves. Conclusively,*Trypanosoma evansi* infection produced deleteriouseffects onhaematological values and testicular functions thus affecting reproductive performance of Yankasa rams.

CHAPTER ONE

INTRODUCTION

1.1 Background

Trypanosomosis is a widespread and economically important disease of human and animals caused by species of flagellate protozoa belonging to the genus *Trypanosoma*, family *Trypanosomatidae* that multiplies in and inhabit the blood stream, lymphatic vessels, and tissues including the cardiac muscle and central nervous system (Gebreyohans and Gangwar, 2010). These trypanosomes survive in the blood stream of the host by complex evasion mechanisms, antigenic variation of the variant surface glycoprotein (Cross, 1990) and immunosuppression (Taylor, 1998). The condition is a serious, often fatal disease of mainly domestic animals and humans, and a major constraint to livestock productivity and agricultural development in areas of Africa, Latin America, the Middle East and Asia (Mihret and Mano, 2007; El-Metanewey *et al*., 2009). Trypanosomosis is transmitted mainly by Tsetse flies cyclically and biting flies mechanically (Molalegne *et al*., 2011). *T. equiperdum* the causative agent of dourine in horses is transmitted by coitus (Wanga and Munga, 2011). Transplacental transmissions have also been reported in both natural and experimental infections in cattle and sheep (Ogwu *et al*., 1986).

Trypanosoma evansi is undoubtedly one of the most widespread pathogenic trypanosomes in the world (Njiru *et al.*, 2010), infecting a variety of both wild and domestic animals including camel, equines, buffalo, cattle, deer, dogs, sheep and goats (Luckins, 1988; Sekoni, 1994). It success is attributed to it transmissibility by biting flies (Njiru *et al.*, 2010).

T. evansi causes an epidemic of a disease called “Surra”, which is of great economic importance in Africa (Luckins, 1988; Gebreyohans and Gangwar 2010). It is primarily

transmitted mechanically by flies like *Tabanus, Stomoxys, Haematopota*, *Lyperosia* and *Chrysops* (Luckins, 1999) and vampire bats (*Desmodus rotundus*) (Hoare, 1972). Transmission is enhanced when animals congregate (at water hole or river areas) or are closely herded, and when they have high number of parasites in their blood. It is very difficult to control this insect population, because they exist in very large number (Gebreyohans and Gangwar, 2010). Therefore control measures are aimed at the host and they include detection and treatment of infected animals, prophylactic treatment of susceptible animals and their protection from biting flies (Radostits *et al.*, 1994).

In Africa, camel is the most important host of *T. evansi* (Dia *et al*., 1997), but cattle has also been reported as the next most highly susceptible animal (Mahmoud and Gray, 1980). Due to various reasons, small ruminants do not show overt clinical signs of the disease under field condition as cattle (Gebreyohans and Gangwar, 2010). However, infection in small ruminants has been reported from field surveys and the economic impact has been substantial (Luckins, 1996).

The clinical signs of surra are non-specific and may likely be confused with other diseases. The clinical signs seem to vary amongst hosts depending on the virulence of the isolate, the susceptibility of the hosts and the presence of concurrent infections, stress, malnutrition or adverse climatic conditions (Hoare, 1972; Losos, 1980; Luckins, 1998a; Reid, 2002). The most common signs amongst infected animals include progressive anaemia, intermittent fever, poor body condition, weakness, anorexia, oedema, lymphadenopathy, nervous signs, low reproduction and death (Losos, 1980; Brun *et al.*, 1998). In horses and camels, *T. evansi* infection is usually fatal, (Enwezor and Sackey, 2005). In small ruminants, infection is usually chronic but acute cases may also occur with high mortality. In addition, abortion,

infertility, testicular enlargement, diarrhoea, coughing and ocular lesions have been reported in goats (Jacquiet *et al.*, 1993; Dargantes *et al.*, 2005b; Morales *et al.*, 2006).

Diagnosis of *T. evansi* is based on demonstration of trypanosomes in tissues or blood of hosts by light microscopy. This is done using direct microscopic examination (DME) of fresh or Giemsa-stained thick or thin blood smears, micro-haematocrit centrifugation technique (MHCT), the miniature anion-exchange centrifugation technique (MAECT) and the rodent or mouse inoculation test (MIT) (OIE 2012). Other methods include serology and polymerase chain reaction (PCR) (OIE 2012).

Treatment involves the use homidium bromide (Novidium®), Suramin, although its production for animal use is limited because of its narrow margin of safety and potential problems with drug-resistance (El Rayah *et al.*, 1999). Diminazene aceturate is another drug which is very safe and effective (Kashiwazaki *et al.*, 1998). Quinapyramine (Luckins 2000), melarsomine hydrochloride originally produced to treat surra in camels, was later found to be highly effective and safe for the treatment of surra in other domestic animals. Isometamidium Chloride is also being used (Youssif *et al.*, 2008).

1.2 Statement of Research Problem

The physiology of animals is altered when they are infected with trypanosomes (Biryomumaisho *et al.*, 2003). This is usually due to the wide range of biochemical changes that take place in the infected animals (Katunguka-Rwakishaya, 1996). Haematological aberrations do occur in animals infected with trypanosomes (Anosa and Isoun, 1980a; Singla and Juyal, 2000). The severity of the haematological and biochemical changes that take

place in infected animals is determined by the strain of the infecting trypanosome and the host (Anosa, 1983).

Evaluation of blood indices and parameters is required to enable the health status of animals to be determined (Coles, 1986). This gives an indication of the degree of degenerative changes that have occurred to host tissues as well as the severity of the infection (Otesile *et al*., 1991).

Sheep like other domestic livestock can be infected with several species of trypanosomes. The trypanosomes that cause tyrpanosomosis in sheep are *Trypanosoma vivax, T. brucei, T. congolense and T. evansi*. The disease has been shown to result in a wide range of reproductive disorders in animals due to the induced pathological effects on endocrine glands and gonads, which disrupts the secretion and plasma concentration of hormones necessary for normal reproductive process in both sexes (Sekoni, 1994).

In male animals, delayed puberty, loss of libido, severe degenerative changes of the genitalia manifested by production of very poor quality semen or even cessation of semen production are some of the reproductive disorders resulting from Trypanosomosis (Sekoni *et al.*, 1990b, Sekoni 1994, Okubanjo *et al.*, 2014a, 2014b). Increased scrotal diameters, scrotal inflammation, testicular degeneration, peri-orchitis, epididymitis, abnormal spermatogenesis/aspermatogenesis and preputial haemorrhages have all been reported in rabbits infected with *T. brucei* (Ikede and Akpavie, 1982). Massive scrotal enlargement, severe testicular and epididymal degeneration and deteriorated semen characteristics have been reported in bucks and rams infected with *T. vivax* (Agu *et al.*, 1986; Isoun and Anosa, 1974; Anosa and Isoun, 1980b; Sekoni, 1992). Similarly *T. vivax* infection was reported to have caused testicular and epididymal degeneration and poor semen quality in the bovine

(Isoun *et al.,* 1975). Deteriorated semen characteristics were reported in West African short horn bulls resulting from *T. congolense* infection (Grundler and Djabakou, 1985; Grundler *et al.,* 1988). In Zebu bulls, infection with *T. vivax and T. congolense*, was observed to have caused progressive enlongation of ejaculation time and poor semen quality such as lack or decreased semen volume, oligospermia, aspermia, poor sperm motility, high rates of spermatozoa death and sperm morphological abnormalities (Sekoni *et al.*, 1988; Sekoni *et al.,* 1990b).

Some of the effects of trypanosomosis on the male gonads are through the effects of pyrexia, accompanying waves of increasing parasitaemia during infection (Mutayoba *et al.,* 1995, Okubanjo *et al* ., 2014b). Jeffcoate and Holmes (1997) reported that circulatory disturbances in the scrotum and testes resulting from elevated body temperature and the reduction in the temperature gradient between the testis and body may be responsible for altered testosterone synthesis and secretion.

Unlike most pathogenic trypanosomes that have consistently maintained their endemic foci and hosts, *T. evansi* has been emerging in non-endemic areas and infecting new hosts (Njiru *et al*., 2010). It is therefore pertinent to study the pathogenic effects of this organism on haematology and some reproductive aspect of animals

1.3 Justification of the Study

Trypanosomosis figures among the first three priority veterinary diseases (FAO,1992) and has been ranked the fourth most important disease of cattle in Nigeria after rinderpest, contagious bovine pleuropneumonia (CBPP) and dermatophilosis (streptothricosis) (Ademosun, 1973).

For the years, there has been an increasing interest shown in this infection because it militates against the development of livestock potential of Africa (Moluneux and Ashford, 1983). Trypanosomosis has direct impact on livestock productivity, reducing meat and milk take by 20%, calving rate by 20%, decreases both lambing and kidding rates in sheep and goat livestock management especially the number of livestock kept by farmers, the breed and species composition of the livestock herd, the way the livestock are grazed, cost of trypanocidal drugs and cost of insecticides (Swallow, 2000).

Trypanosomes cause a wide range of reproductive disorders in animals. In the male animal, the reproductive disorders include delayed puberty, loss of libido, severe degenerative changes of the genitalia manifested by the production of very poor quality semen or cessation of semen production (Sekoni, 1994, Okubanjo *et al.,*2014a, Okubanjo *et al*., 2014b). Trypanosomoses cause pathological effects on the endocrine glands thus interfering with the endocrine control of reproduction and reproductive processes in both sexes. (Ogwu *et al.*, 1992; Sekoni, 1994).

The role of trypanosomosis in limiting livestock production and its economic impact on the livestock industry in Nigeria is widely recognised. Despite the widespread distribution of the disease, and several researches on *T. evansi* in small ruminants in Nigeria (Audu *et al*., 1999; Shehu *et al*., 2006; Ogbaje *et al*., 2010), little has been done on the effects of *T. evansi* on reproduction.

Several reports from experimental studies have shown that infection with *T. vivax, T. congolense* and *T. brucei* cause testicular damage (Sekoni *et al.*, 1990b, Adamu *et al*., 2007, Okubanjo *et al.*, 2014b) resulting in reduction in the number and motility of live spermatozoa in ejaculates from bovine (Anosa and Isoun, 1980a; Sekoni *et al*., 1988) and

rams (Agu *et al*., 1986; Akpavie *et al*., 1986, Okubanjo *et al.,* 2014a). However, there is paucity of information on the effect of *T. evansi* infection on reproduction in indigenous Yankasa rams. It is therefore desirable to investigate the effect of experimental *T. evansi* infection on reproduction in Yankasa rams.

1.4 Aim of the Study

This study is aimed at determining the effects of experimental *T. evansi* infection on haematological parameters and semen characteristics in Yankasa rams.

1.5 Objectives of the Study

I. To evaluate the effect of *T. evansi* infection on haematological parameters of Yankasa rams.

II. To evaluate the effect of *T. evansi* infection on semen characteristics of Yankasa rams.

1.6 Research Question

Does experimental *Trypanosoma evansi* infection affect reproduction in Yankasa rams ?

CHAPTER TWO

GENERAL LITERATURE REVIEW

2.1 Background

Haemoparasitic diseases, like Trypanosomosis, have adverse impact on the health, productivity and working capacity of animals in large areas of Africa. *Trypanosoma evansi* the causative agent of Surra constitutes one of the major veterinary problems worldwide. The disease causes significant morbidity and mortality in many farm animals. Trypanosomosis occurs in sub acute, acute and chronic forms. The sub acute and acute forms are usually fatal, whereas the chronic form is more common and associated with secondary infection.

According to Sekoni (1994), Trypanosomosis is among the important diseases which cause various reproductive disorders in both male and female animals. Although, the protozoan parasites localize in internal organs of the infected host, the gonads are apparently their preferred site (Omeke and Igboeli., 2000)

Trypanosome infections are known to cause immune suppression responsible for the host's inability to eliminate the trypanosomes even after administration of trypanocidal drugs (Radiostis, *et al.,* 1994). Fluctuations in levels of parasitaemia could be attributed to variant surface glycoproteins (VSG) coats of the parasites which make them elusive to host immune system and chemotherapy (Donelson *et al*., 1998).

Trypanosomes are obligate endoparasites infecting most vertebrate animal groups. The importance of trypanosomosis in medicine, veterinary medicine and livestock production lies in the enormous economic losses they cause.

Trypanosoma evansi was first reported as the causative agent of mammalian Trypanosomosis when Griffith Evans in 1880 discovered this parasite in the blood of camels and equines (Mahmoud and Gray, 1980). The parasite has since been known to infect other mammalian hosts. Both natural and experimental infection with this parasite has been described in sheep, dogs, goats, cattle, rabbits, mice and rats.

Recently *T evansi* was reported to infect humans in India (Joshi *et al*., 2005). It has the broadest geographical and host range among the pathogenic mammalian trypanosomes (Sallau *et al*., 2008). This may be explained by its transmission by biting flies mechanically as opposed to the dependence by other trypanosomes on cyclical transmission by tsetse flies as vectors which limits them to sub-Saharan Africa, with the exception of T. *equiperdum* which is transmitted by sexual contact (Brun *et al*., 1998) and T. *cruzi* the agent of Chagas disease, which is transmitted by triatome bugs restricted to the Americas.

Sheep constitute a major source of protein in Nigeria. However, in spite of the large population of these domestic species, animal protein consumption is far below the national requirements. This shortfall in animal protein availability has been linked to low productivity of Nigeria domestic livestock. (Akerejola *et al.*, 1979).

According to the Federal Department of Livestock (FDL) 2010 report, sheep population in Nigeria was 34.69 million in 2009, and the value was only a slight increase from the 2004 figure despite the increasing population and intensifying demand for animal protein. This rather slow growth is attributable to limited technological inputs, weak animal health services, poor access to market and credit, and production losses from endemic diseases. Sheep is an important livestock species in the socio-economic lives of people around the world including Nigerians (Agaviezor *et al*. 2012)

There are generally four breeds or races of sheep considered to be native to Nigeria, the Balami, Uda, Yankasa and West African Dwarf (WAD) (Adu and Ngere, 1979).

The livestock industry is the principal source of animal proteins (meat, milk and eggs) which are vital for body growth and maintenance. According to the Food and Agriculture Organization of the United Nations (FAO, 1996), 35g of animal protein per person per day is considered a basic requirement which can only be sourced from livestock (FAO, 1996). The difference in animal protein availability and consumption had become a big differentiating factor between developed and developing countries, thus while an average Nigerian gets about 4.5 g of animal protein per person per day, the animal protein consumption per person per day in the developed countries of Europe is about 52 g (Atinmo and Akinyele 1983; ILRI, 2013). The livestock industry also provides employment, source of income, draught power, fuel, fertilizer and hides and skin for the large majority of human population, and in most low-income settings ownership of livestock is a repository of saved money that can be drawn on by sale of the livestock when the need arises (FAO 1996, ILRI, 2013). This aspect of the role of livestock in the socio-economy of low-income people cannot be ignored if the first of the Millennium Development Goals (eradication of extreme poverty and hunger) is to be attained.

The occurrence and endemicity of certain animal diseases, followed by poor nutrition, stand above all other factors in their contribution towards poor productivity and output from the livestock sector (ILRI 2013).

2.2 Origin and Classification of *Trypanosoma evansi*

Trypanosoma evansi is a salivarian trypanosome, a protozoan belonging to the family Trypanomastidae, order Kinetoplastida, genus Trypanosoma and subgenus Trypanozoon (Wei *et al*., 2011). It is believed to have arisen and closely related morphologically and

genetically to T. *brucei*. (Brun *et al*., 1998, Wei *et al*., 2011). *T evansi* became adapted to mechanical transmission by insect vectors among mammalian hosts resulting in it being devoid of the capacity to develop within its vectors (Hoare 1956, 1957). Hoare (1940) had earlier postulated that *T. evansi* had once been *T. brucei* carried outside the tsetse zone by camels. Recent evidence gives credence to the close genetic relationship between *T. brucei* and *T. evansi* (Jensen *et al*., 2008; Lai *et al*., 2008).

2.3 Morphology of *T. evansi*

T. *evansi* is generally monomorphic, occurring in a long slender form probably due to lack of cyclical transmission within insects (Wei *et al*., 2011). Although polymorphic forms may occur sporadically in some isolates especially during serial passage in experimental animals, the slender form is the most consistent in most isolates (Losos 1980, Wei *et al*., 2011). *Trypanosoma evansi* is an enlongated cell with a pointed posterior end, a sub-terminal or marginal kinetoplast, centrally positioned single nucleus, undulating membrane and like T. brucei a free flagellum is present. Measuring between 14-33µm in length and 1.5-2.2µm in width (Brun *et al*., 1998, Wei *et al*., 2011). Loss of kinetoplast known as akinetoplastidy is a feature that has been noticed in some wild strains of T. *evansi* (Wei *et al*., 2011), in brazil, some isolates from livestock were identified without kinetoplast (Ventura *et al*., 2000). Mutation, chemotherapy, exposure to certain dyes and in vitro cultivation are some factors responsible for this loss of kinetoplast (Schanufer *et al*., 2002)

2.4 Host Range

T. evansi is the most widely spread pathogenic trypanosomes in the world, where infection leads to a wasting disease called Surra in a wide range of mammalian hosts, causing great economic losses in areas of Africa, Asia and South America (Giardina *et al.,* 2003, Gillingwater *et al*., 2007). *T. evansi* is pathogenic in most domestic animals and some wild

animals (Lun *et al*., 2004). Infection with this parasite is usually fatal in camels, horses and dogs. Other animals susceptible to both natural and experimental *T. evansi* include cattle (Tuntasuvan *et al., 1997)*), sheep (Audu *et al*., 1999), goat (Ngeranwa *et al*., 1993), pigs (Allam *et al*., 2011) and cats. It has also been shown to infect some wild animals such as elephants, deer and capybaras (Herrera *et al*., 2004).Camels are the most affected in the middle east and Africa (Dia *et al*., 1997). Thus, the assertions that movement of infected African camels led to the spread of the disease to middle east and Asia. It is believed that the importation of animal from West Africa in the 16th century introduced *T. evansi* to Latin America (Wells, 1984). Similarly, occurrences of *T. evansi* infection in Europe such as the incursion of Surra in the canary islands of Spain was due to the importation of infected camels from Africa (Gutierrez *et al.*, 2004). Nevertheless, the OIE (2012) puts the endemic geographic distribution of this disease to range from China, the Philippines and parts of Indonesia across southeast Asia and parts of the former USSR, into India, the middle east and Africa until it reaches central and South America.

2.5 Life Cycle of *T. evansi*

The parasite multiplies by longitudinal binary fission only in the blood and tissue fluids of animal host (Brun *et al*., 1998), no intermediate hosts are present and there is no insect developmental cycle available (Foil 1989, Gillingwater *et al*., 2007).

Transmission of T. *evansi* is by mechanical transfer of trypanosomes by hematophagous flies from an infected to a susceptible host. Biting flies of the genera *Tabanus, Stomoxys, Haematopota, Lyperosia* and *Chrysops* have been implicated in the transmission of *T. evansi* (Luckins 1999). Successful transmission depends on time taken for a contaminated vector to find a new host, as the viable trypanosomes remain within the mouth parts of the flies only

and are therefore susceptible to rapid desiccation (Gillingwater *et al* 2007). Trypanosomes do not survive for more than 10-15 minutes in the proboscis of a fly (Soulsby, 1983).

Tabanids have been identified as the most effective transmitter of *T. evansi,* because their aggressive feeding behavior cause them to elicit significant avoidance behavior in hosts which leads to half fed flies moving to other hosts (Luckins, 1992). The Tabanids are widely distributed all over the world. Not more than 15 minutes of feeding interval between animals was observed for successful transmission of *T. evansi* by *Tabanus striatus* (Mitzmain, 1914). Sumba *et al.* (1998) observed that *T. evansi* would not survive for more than seven minutes in the mouth part of *Stomoxys niger niger.* Other studies in Philippines and India have shown *Stomoxys calcitrans* as unimportant in the transmission of *T. evansi* (Choudhuri *et al.,* 1966). Other biting insects are considered not very significant in the transmission because they tend to complete their blood meal from a single animal (Luckins 1998b). In Africa, tsetse fly like other blood sucking flies can act as mechanical vector (Brun *et al*., 1998). In south and central America Vampire bats (*Desmodes rotundus*) can serve as both vectors and reservoir host. Besides mechanical transmission by biting insects and vampire bats, *T. evansi* can be transmitted through milk or during coitus (Wang, 1988). Also Canids are likely to be infected by eating infected animals (Brun *et al*., 1998).

Transmission is greatly enhanced when animals congregate or become closely herded, and when they have high number of parasites in their blood (Gebreyohans and Gangwar, 2010).

2.6 Antigenic Variation

Trypanosomes survive in the blood stream of the host by a complex evasion mechanism (Cross 1990). Antigenic variation of the variant surface glycoproteins (VSG) is the ability of trypanosomes to evade the host's immune system (Baron 1996, Donelson *et al*., 1998) by producing generations of trypanosomes in the course of infection that each expresses a

different variable surface glycoproteins (VSG). VSG is the external monolayer that covers the entire trypanosomes as a protective shield against host antibodies. (Pays *et al.*, 2001, Machado *et al*., 2006). It is highly immunogenic, once a host is infected with a trypanosome manifesting a specific antigen known as variable antigen type (VAT), the body responds by producing specific antibodies to eliminate the proliferating trypanosomes. Subsequently, waves of parasitaemia occurs and a new VAT becomes predominant. There are over 1000 genes encoding different VSGs but only one gene coding for a particular VSG is expressed at any point in time (Baron 1996).

There has been some extensive work on trypanosome antigenic variation but much of the work centres on *T. brucei* (Boid *et al*., 1996).

2.7 PATHOGENESIS OF SURRA

Luckins *et al* (1992) described the pathogenesis of the disease caused by *T. evansi* to involve an initial replication phase at the site of inoculation triggering the development of a necrotic oedematous cutenous swelling and sore called "chancre". The trypanosomes then spread to the lymph nodes, blood circulation and other body tissues eg (muscles, liver, heart, brain, kidney, testes) and produce inflammatory lesions subsequently. Immunological response in the host may mediate cellular damage, production of immune complexes (Al-Qarawi *et al* 2004, Saleh *et al*., 2009). A significant and complicating factor in the pathogenesis is the profound suppression of both humoral and cellular immunity of host thus lowering the host resistance, which might lead to complication with other infections (Radostits *et al.*, 1994, Ngeranwa *et al*., 1993, Dargantes *et al*., 2005a,). Anemia, an ever present feature of trypanosomosis may further aggravate organ failure and final death of host (Audu *et al.*, 1999; Dargantes *et al.*, 2005a).

2.8 Prepatent Period

Pre-patent period is the length of time taken for trypanosomes to appear in peripheral blood following inoculation. The pre-patent period following infection with *T. evansi* varies from species to species and this depends on the susceptibility of the host, pathogenecity of the isolate, route and dose of infection and the test used for detection of the parasite (Ilemobade, 1971, Jones *et al*., 1997). Pre-patent period in Yankasa rams infected with $2x10^6$ *Trypanosoma evansi* intravenously was 3 days using wet mount and buffy coat (Audu *et al*., 1999). Similarly, studies by Ogbaje *et al*., (2009) revealed 3 days prepatent period in intravenously infected West African dwarf goats. The pre-patent period of as early as 24 hours have been reported in horses (Wernery *et al.*, 2001), 5 days in rats (Al-Mohammed, 2006) and 9 days in dogs (Aquino *et al.*, 1999) after intravenous infection with *T. evansi*. Also, following oral challenge of dogs a pre-patent period of between 8 and 10 days was observed (Raina *et al*., 1985).

Although, various factors affects the establishment of infection in various host. It is generally accepted that infection via intravenous route is more rapid when compared to the subcutaneous route or by bite by infected vectors. (Maxie *et al.*, 1979; Saror 1979)

2.9 Origin and Classification of Sheep

Sheep (*Ovis aries*) are quadruped ruminant mammals typically kept as livestock. Like all ruminants, sheep are members of the even toed ungulates. It is one of the earliest animals to be domesticated for agricultural purposes, and is raised for fleece, meat (lamb, hogget or mutton) and milk.

Domestic sheep are thought to descend mainly from two wild stocks: the Moufflons (*Ovis musimon* and *Ovis orientalis*), and the Asiatic urial (*Ovis vignei*) (Khan *et al*.,2003)

There are two wild stocks of the Moufflon, the Asiatic Moufflon (*O. orientalis*), a wild sheep still found in Asia Minor and the Caucasus, and the European Moufflon (*O. musimon*), which is native to Europe and still found in certain parts of Europe (Khan e*t al*., 2003). Both of the Moufflon stocks are considered as ancestors of domestic sheep. The Asiatic urial (*O. vignei*), which is a smaller race of sheep than the Moufflon, is native to the grassy open plains of central Asia. It lives in large flocks and is much less a mountain animal than the Moufflon. Most of our familiar breeds of sheep are thought to be descendents of this wild stock (Khan *et al*., 2003).

2.10 Clinical Signs

The clinical signs of *T. evansi* infection (Surra) are generally not regarded as being specific and may be confused with other disease syndromes, since interaction of several factors are involved in the production of clinical symptoms (Maikaje, 1985). The clinical signs seem to vary amongst hosts depending on factors such as virulence of the isolate, the susceptibility of the hosts and presence of concurrent infections, stress, malnutrition or adverse climatic conditions (Luckins 1998; Reid 2002). Certain genetic factors such as species and breeds may influence the observable presentation of the disease

The disease is most severe in horses, camels and dogs (Enwezor and Sackey, 2005). Progressive anaemia, intermittent fever, poor body condition, weakness, anorexia, oedema, lymphadenopathy, nervous signs, low reproduction and death are the most common signs amongst infected animals. (Losos, 1980, Brun *et al*., 1998). Infection due to *T. evansi* may be hyper acute, acute or chronic. Chronic cases of the infection may persist for months (OIE, 2008) and if left untreated may lead to death (Eisler *et al.*, 2004).

In horses and camels, the disease is usually fatal (Ewenzor and Sackey, 2005) and characterized by fever, severe anaemia, conjunctivitis, edema of the legs and lower parts of

the body, progressive weakness, paresis of hindquarters, staggering gait, loss of condition (Silva *et al*., 1995, Wernery, 2001, Ewenzor and Sackey 2005). Rodrigues *et al*., (2009) reported certain neurologic signs in natural *T. evansi* infection in horses. They include ataxia, head tilting and circling, hyper excitability, head pressing and paddling movements.

2.11 Anaemia

Anaemia is a major component of the pathology of *Trypanosoma evansi* infection and has also been identified as one of the principal features and major cause of death of infected animals (Logan-Henfrey *et al*., 1992, Faye *et al*., 2005, Adamu *et al*., 2008). It is also highly linked to overall losses in production and reproduction (D'leteren *et al*., 1998., Murray *et al*., 1991).

The breakdown of erythrocytes (RBC) begins at infection and progresses over the following weeks. Reports have indicated that the onset of anaemia is related to the appearance of trypanosomes in the periphery blood (Goosens *et al*., 1998). Within a week of infection, there is usually a pronounced decrease in packed cell volume, haemoglobin concentration and red blood cell counts.

The anaemia has been described as normocytic normochromic in goats, donkeys, horses and buffaloes (Damayanti *et al*., 1994, Dargantes *et al*., 2005a, Rodrigues *et al*., 2009). Sharma *et al*. (2000) reported a macrocytic anaemia with reticulocytosis in experimentally infected barbari goats. In camels the anemia is reportedly macrocytic and hypochromic (Enwenor and Sackey, 2005). Silva *et al*.(1995) in their studies with *T. evansi* reported microcytic normochromic and microcytic hypochromic anaemia in horses and dogs respectively. Likewise, leucocytic changes were characterized by lymphocytosis and monocytosis amongst infected animals (Damayanti *et al*., 1994, Onah *et al*., 1996, Wernery *et al*., 2001, Dargantes *et al*., 2005b). Leucopaenia may occur in prolonged infection (Holland *et al*.,

2003) as noted in goats (Dargantes *et al*., 2005a) Dogs and horses (Silva *et al*., 1995, Marques *et al.,* 2000) which may suggest immunosupression.

Reviewing effect of *T. evansi* infection on other haematological parameters revealed most studies demonstrate normal total serum protein concentration even though increased value have been reported by some investigators. Marques *et al.* (2000) noted no significant changes in total serum protein of horses experimentally infected with *T. evansi* .

2.12 Pathology of *Trypanosoma evansi* Infection

There are numerous studies that thoroughly describe the pathology of *T. evansi* infection in goats (Dargantes *et al*., 2005a), sheep (Audu *et al.*, 1999), camels (Sackey and Enwezor, 2005) and donkeys (Cardioli *et al.*, 2006). However, these pathologies are not pathognomonic.

In experimentally infected bufalloes the major pathological changes were emaciation, serous atrophy of fat, hydropericardium, petechial to larger haemorrhages in the pericardium, pneumonia, congested liver and spleen. Enlarged and edematous lymph-nodes and bone marrow hyperplasia (Damayanti *et al*., 1994). Histologically, the most consistent lesions were interstitial pneumonia, interstitial myocarditis, splenic multifocal necrosis, interstitial myositis and hyperplastic bone marrow (Damayanti *et al*., 1994).

Tuntasuvan *et al.*,(2000) reported congestion and petechial haemorrhages in the brain of deer naturally infected with *T. evansi*. In addition, severe to moderate necrotizing meningo-encephalitis with edema, haemorrhage and perivascular cuffing was reported in horses showing nervous signs (Rodrigues *et al*., 2009).

Dargantes *et al* (2005a) and Shehu *et al.*, (2006) observed similar gross pathological changes in goats infected with *T. evansi*, including pale carcass with hydroperitoneum, generalized atrophy of body fat, catarrhal enteritis, hepatomegally, congested kidney and lungs.

Inaddition, testicular enlargement, hyperplasia of the spleen, haemosiderosis of spleen, liver, kidney and bone marrow as well as depopulation of cells of bone marrow and lymphnodes (Dargantes *et al*.,2005a). Degenerated seminiferous tubules and degenerated spermatids in duct of epididymis (Shehu *et al* 2006) have been observed histologically. Similarly, lesions have been reported in donkeys (Sooden *et al*., 1996 and Cardioli *et al* 2006)

2.13 Diagnosis

A wide range of clinical, parasitological and serological techniques are presently employed for the diagnosis of trypanosomosis. The non specific nature and the absence of pathognomonic signs and the tendency of a sub-clinical course of the disease are major hurdles in clinical diagnosis of trypanosomosis in livestock (Ravindran *et al*., 2008, OIE 2012).

Parasitological diagnosis is based on demonstration of trypanosomes in tissues or blood of host by microscopy. This is considered the most definitive means of diagnosis for trypanosomosis (Tran *et al*., 2009). Parasitological diagnosis involve direct microscoipic examination of fresh or Geimsa-stained thick or thin blood smears (OIE, 2012), although these methods have inherent limitations owing to the flunctuating nature of parasitaemia. However, other parasitological techniques such as Micro haematocrit centrifugation technique (MCHT) and mouse or rodent inoculation test (MIT) (OIE, 2012) can be used to overcome this limitations in parasitological diagnosis.

Unfortunately, parasite detection methods cannot always detect infections, particularly during chronic stages of the disease (Mahmoud and Gray, 1980, Nantulya, 1990). Even though demonstration of *T. evansi* in the blood of animals is a sure method of diagnosis, the sequential destruction of parasites by antibodies and subsequent growth of new parasites

with a new antigenic specificity leading to cyclical fluctuation of parasitaemia especially in chronic infections necessitates the use of highly sensitive and specific diagnostic tests.

Immuno diagnostic techniques based on the detection of circulating antibodies and antigens of *T. evansi* have been developed, because of the difficulties in demonstrating parasites in subclinical infection. (Aslam *et al.,* 2010). ELISA/ *T. evansi* immune trypanolysis (TL) assay, latex agglutination test, indirect fluorescent antibody test, card agglutination test CATT/*T. evansi* (Tran *et al*., 2009) have been used to diagnose *T. evansi* infection. These serological test are important in detecting carrier status of infection which posses great challenge in the epidemiology of the disease. However, these serological test still lack specificity and are unable to differentiate on-going from previous infection (Aslam *et al.,* 2010).

Finally because of the need for a more sensitive test, molecular diagnosis using polymerase chain reaction (PCR) with high sensitivity and specificity, targeting specific fragments of *T. evansi* genomic DNA have been employed for surveying the disease in epidemiological studies (Shahzad *et al*., 2012) and has been found to be a superior test for diagnosis, especially in field conditions (Aslam *et al*., 2010).

2.14 Male Reproductive System

The male reproductive system consists of testicles, which produce sperm and sex hormones, a duct system for sperm transport, accessory sex glands, and the penis, or male organ of copulation, which deposits semen in the female (Hafez., 2000).

2.14.1 Testes: The testes are paired organs which descend from the abdominal cavity during fetal development to lie in the scrotum. They produce the male gametes (spermatozoa) and secrete the male sex hormone, testosterone. Testosterone is essential for the development of male characteristics, maintaining normal sexual behavior and sperm production (Ritar,

1990). The mammalian testis consist of two compartments, the seminiferous tubules and the interstitium. The interstitium is responsible for blood supply, immunological responses and contains the Leydig cells which mediate endocrine signals of the pituitary to the testis and back. The tubules contain androgen-sensitive Sertoli cells and the entire germ line (Hafez, 2000, Wistuba *et al*., 2007).

Peritubular epithelial cells outside the tubule divides the testis into two intratesticular components. These peritubular cells are myoid and drive the peristalsis necessary to move the non motile elongated spermatozoa released from the nourishing sertoli cells in the direction of the efferent ducts from where they are forced into the epididymis where they mature until capable of fertilization (Hafez, 2000, Wistuba *et al*., 2007).

2.14.2 Scrotum: The scrotum is a muscular sac containing the testes. It supports and protects the testes and also plays a major role in temperature regulation. It maintains the temperature, 3 to $5^{O}C$ below body temperature for optimal function (Ritar, 1990, Hafez, 2000).

2.14.3 Epididymis: The epididymis is located in the testes and is a long and convoluted tube in which sperm cells produced by the testicles are stored and mature to a stage capable of fertilization (Ritar, 1990). The epididymis has three recognized anatomic parts: The caput epididymidis (Head) in which a number of efferent ductules join the epididymis. The corpus epididymides (Body) a narrow tube connecting the head and the tail in a terminal expanded Cauda epididymides (Tail). The first two segments are concerned with sperm maturation, whereas the terminal segment is for sperm storage providing factors that enhance fertilizing ability. Transport of sperm through the epididymis takes about 9 to 13 days (Hafez, 2000).

2.14.4 Vas deferens: The vas deferens is the duct that rises from the tail of the epididymis into the abdomen, where it joins the urethra at the neck of the bladder. It is often referred to

as the ‘spermatic cord.’ Removal of a section of the vas deferens in each testis is known as a vasectomy, preventing passage of sperm from the epididymis (Ritar, 1990, Hafez, 2000).

2.14.5 Accessory sex glands: The accessory sex glands include the bulbourethral glands, prostate gland, and seminal vesicles. Accessory glands secrete additional fluids, which when combined with the sperm and other secretions from the epididymis, form the semen. Some of the secretions contain nutrients like fructose and other substances like citric acid, ergothioneine and inositol while others produce alkali secretion to raise the pH of the ejaculate. These secretions are added quickly and forcibly during the mating to propel sperm into the urethra (Ritar, 1990, Hafez, 2000) .

2.14.6 Penis: This is the final part of the male reproductive tract and its function is to deposit semen into the vaginal tract of the female (Ritar, 1990). Three cavernous bodies aggregate around the penile urethra. The corpus spongiosum penis which surrounds the urethra and is covered by bulbospongiosus muscle. The corpus cavernosum covered by the ischiocavernousus muscle. A thick covering (tunica albuginea) encloses the cavernous bodies. (Hafez, 2000).

2.14.7 Prepuce: The preputial sheath protects the penis, except during mating (Ritar, 1990, Hafez, 2000).

2.15. Puberty

Puberty is a gradual process, during which animal reproductive competence is attained with respect to physiology, morphology and behaviour. Generally it is defined as the end point of series of events affecting the development of the hypothalamo-pituitary-gonadal axis leading to reproductive competence (Senger, 2003, Valasi *et al* 2012).

Multiple determinants of pubertal development have been identified, including genetic factors, endogenous signals (metabolic state) and environmental cues, such as photoperiod

and socio-sexual signals (Valasi *et al*., 2012). Onset of puberty in small ruminants differs between sexes, due to early sexual differentiation in the control of steroid feedback systems and, thus, GnRH secretion. A complex interplay of genetic factors as well as endogenous signals, such as energy balance and environment is responsible for the onset of puberty.

Attainment of puberty by ewe-lambs/doe-kids is under the control of internal and external influence and depends mainly on three factors:

(i) Age of the animals (6–12 months depending on breed and month of birth). Spermatogenesis has been found to begin as early as 56 days of age, with spermatozoa present in the epididymis at 112 days of age in suffolk rams (Skinner *et al* 1968).

(ii) Appropriate season of the year (within the reproductive period of each breed). In temperate latitudes, photoperiod is one of the most important factors controlling seasonal reproductive activity and puberty onset (Malpaux *et al*., 1989). Photoperiodic information exerts its effects on the hypothalamo–pituitary axis through the rhythmic, diurnal secretion of melatonin by the pineal gland (Arendt, 1986). An annual rhythm in the pattern of melatonin secretion functions as the neuroendocrine signal that links the reproductive system to the hypothalamo–pituitary axis, modifying its neuroendocrine activity in response to the negative feedback action of oestradiol (Malpaux *et al*., 1989). However, the response is sexually differentiated, because male animals attain puberty at a smaller size and under longer photoperiods compared to females, as a result of the organising action of testicular androgens during foetal development. Thus, males have an inherent ability to begin reproductive activity, regardless of photoperiodicity, provided an appropriate physiologic size has been attained (Foster, 1994, Wood and Foster, 1998).

(iii) Body weight–size and metabolic state (at least 65% of adult weight). Acquisition of a minimum body weight is a determinant for onset of puberty. The onset of puberty is more

closely related to body weight and size than to age (Foster and Nagatani, 1999) especially in the tropics, irrespective of the season of birth, compared with subtropical and temperate latitudes (Mukasa-Mugerwa *et al.*, 1991). Small ruminants reach puberty when their body weight is 60–65% of the adult weight (Bizelis *et. al*., 1990, Santiago-Moreno *et al*., 2000).

2.16 Spermatogenesis

The formation of mature spermatozoa is one of the most essential functions in life. A concerted sequence of events is needed to proliferate, maintain and mature germ cells starting with spermatogonial stem cells and culminating in mature gametes (Wistuba *et al*,. 2007). Spermatogenesis is a complex, cyclic and highly coordinated biological process of cellular transformation in which diploid spermatogonial stem cells differentiate into mature haploid spermatozoa (Neves *et al*., 2002,) within seminiferous tubules in the testis. Spermatogenesis is one of the most productive self-renewing systems in the body, lasting from 30 to 75 days in mammals (Russell *et al.*, 1990). These processes include mitotic multiplication and propagation of the spermatogonial stem cells, meiotic recombination of genetic material and testicular maturation of spermatozoa (Ehmcke *et al*., 2006). Spermatogonia are derived from primordial germ cells (PGCs) which after entering the testis, develop into gonocytes (Wistuba *et al*,. 2007).

2.16.1 Phases of Spermatogenesis

2.16.1.1 Mitosis

In the fully developed mammalian testis, the majority of undifferentiated cells of the germ line are type A spermatogonia. This population of cells also includes spermatogonial stem cells (SSCs). These are the most important cells for spermatogenesis because their task is to provide both self renewal of SSCs and Spermatogonia type B that differentiates and divides

mitotically into cell stages able to enter meiosis. (Wistuba *et al.*, 2007). Spermatogonia are diploid germ cells that divide by mitosis and reside on the basement membrane. There are 3 types of spematogonia. Type A- the undifferentiated spermtogonium, the intermediate spermatogonia and type B- differentiated spermotogonium. (Russell *et al.*, 1990). In fully developed mammalian testis, the majority of undifferentiated cells of the germ line are type A spermatogonia , important in both self renewal of SSCs and spermatogonia type B that differentiates to produce primary spermatocytes which undergoes meiosis, reducing its genomic content in the secondary spermatocytes (Wistuba *et al.*, 2007).

2.16.1.2 Meiosis

Differentiated spermatogonia type B undergoes mitotic division forming two spermatocytes, cells representing the beginning of meiotic prophase. These cells become transit and move through the blood-testis barrier (Sertoli-Sertoli barrier) (Wistuba *et al.*, 2007). This cellular division goes through three categories:

(a) Meiosis 1, the division of the 4n cells

(b) Formation of secondary spermatocytes (2n) which are larger than step1 spermatids but are rarely found as the only spermatocyte in a tubular cross section.

(c) Meiosis 2, the division of 2n secondary spermatocyte to form haploid (1n) round spermatids (Garner and Hafez, 2000,Wistuba *et al* 2007).

2.16.1.3 Spermiogenesis

The secondary spermatocytes differentiates, producing haploid spermatids, which is the beginning of spermiogenesis. During this period the spermatids drastically alters their shape

and content, after further transformantion and elongation of the spermatids, a typical spermatozoa is formed.

The transformation of spherical, haploid spermatids (1n) into elongated, highly condensed and mature spermatozoa with a head bearing the nucleus, acrosome, a midpiece and the flagellum (de Rooij and Grootegoed, 1998, Wistuba *et al*,. 2007) that are released into the seminiferous tubule lumen. The differentiation of spermatids proceeds through 4 steps or phases:

1. Golgi Phase: Involves the development and differentiation of the proacrosomic system of the spermatozoa within the golgi apparatus. Golgi apparatus is very important during early spermiogenesis . The formation of the acrosome is dependent on the organelles' ability to produce vesicles and granules containg the enzymatic components of the acrosomic system that will cover the developing sperm nucleus (Garner and Hafez, 2000).
2. Capping Phase: Involves the touching and spreading of the acrosomic granules to the nuclear envelope, thus causing a flattening of the vesicle into a small cap over the nuclear surface covering approximately two-thirds of the nuclear surface. (Garner and Hafez, 2000).
3. Acrosomal phase: Involves the migration of the acrosomal system over the ventral surface of the enlogating spermatid nucleus with condensation of chromatin into dense granules (Garner and Hafez, 2000) .
4. Maturation: Involves final transformation of elongated spermatids into cells to be released into the lumen of the seminiferous tubule. The reshaping of the nucleus

and acrosome of each spermatid, initiated during the previous phase, produces spermatozoa characteristic for each species (Garner and Hafez, 2000).

2.17 Reproductive Endocrinology

Reproduction function of animals is controlled by a well coordinated and efficient neuro endocrine system (Nalbandor, 1976). Hypothalamus, Pituitary gland and the gonads are three important structures involved in reproductive hormone production (Sekoni 1994).

Testosterone, the male reproductive hormone belongs to a class of steroids called androgens. It is the most important of all the steroids produced. Testosterone is produced mainly by the Leydig cells of the testis with a limited amount by the adrenal cortext (Hafiz 1987). The Leydig cells are the main sites of testosterone synthesis (Hooker 1970, Cook *et al.,* 1972,) and are stimulated by pulse of pituitary gonadotropins, lutenizing hormone (LH) or interstitial cell stimulating hormone (ICSH) to secret this androgens (Verhoeven *et al., 2010*). Released androgens have a negative feedback effect on the hypothalamic pituitary axis in the control of LH and FSH release.

Testosterone is responsible for the masculinization characteristics and development of secondary male sex characteristics. It promotes growth and development of accessory sex organs (prostate, vesicular gland, bulbourethral gland) and external genitalia. Testosterone together with other androgens stimulate the latter stages of spermatogenesis and prolong the life of the epididymal sperm, a critical factor for spermatogenesis (Verhoeven *et al., 2010*).

Sekoni (1994) stated that a disease having pathological effect on the endocrine glands especially anterior pituitary gland and the gonads are most likely to interfere with the endocrine control of reproduction and normal reproductive process. Releasing factors are

produced by the hypothalamus which stimulates the anterior pituitary gland to produce the gonadropins (FSH, LH and ICSH) in male. These gonadotrophins act on the testes where it stimulates spermatogenesis and production of androgen (Testosterone) (Sekoni *et al.*, 1994).

Endocrine dysfunction have been reported in both human and animal trypanosomosis. Adrenal insufficiency, hypopituitarism, hypogonadism and hypothyroidism as well as polyglandular endocrine failure in humans and animals due to local inflammation of the pituitary, thyroid, adrenal and gonadal glands have been reported in *T. brucei* infection (Reincke *et al*., 1998).

There have been reports of low levels of plasma testosterone in goats and laboratory animals infected with *T. congolense* (Wainde *et al*., 1986). Adeyemo *et al.*,(1990) reported a decline in serum testosterone in West African Dwarf rams infected with *T. brucei* and *T. congolense,* while Otesile *et al.,* (1991) reported same in pigs infected with *T. brucei*.

Mutayoba *et al* (1994) reported a decline in plasma LH and testosterone concentration in rams infected with *T. congolense*. This decline is partly due to reduce sensitivity of the Leydig cells to circulating LH and impairment of enzymes involved in leydig cell steroid biosynthesis.

Similarly, some endocrine dysfunction were reported in *T. congolense* infected cattle (Ogwu *et al*., 1992) and goats (Mutayoba *et al.*, 1988). Fatihu *et al.* (2009) reported a significant decline in level of serum thyroxine (T4) in zebu bulls infected with *T. vivax*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Animals

Twelve matured, healthy and sexually intact Yankasa rams of 1 ½ to 2 years of age were bought from livestock markets in Zaria Local Government Area of Kaduna State, Nigeria.

3.2 Housing and Feeding

The experimental animals were kept in clean fly- proof pens provided by the Department of Vet. Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. This was to prevent transmission of *T. evansi* infection by hematophagous flies and spread of infection in the environment. On arrival the rams were screened for haemoparasitic and helmintic infections as well as ectoparasites, after which they were treated appropriately. The rams were fed on hay, maize offal and concentrates, multimineral salt lick and water was provided *ad libitum*.

3.3 *Trypanosoma evansi* Stock

A field strain of *T. evansi* was obtained from the pooled blood of camels slaughtered in kano abattoir in Kano state, Northwestern Nigeria and inoculated into Laboratory rats. Patency was detected in the rats after 31 days and from thence the parasite was maintained in laboratory rats until inoculation into experimental rams.

3.4 Experimental Design (Infection / Post infection Monitoring)

The rams were allowed to acclimatize for a period of two months and were allotted to two groups. A (infected) and B (control), consisting of seven and five rams respectively. The infected group A (n=7) rams were each inoculated with $1x10^6$ *Trypanosoma evansi* trypomastigotes in 1ml of donor blood via the jugular vein on week 6(week 0) of the study.

The infection was allowed to run through its course for fourteen weeks. Group B (n=5) served as control.

All the animals were monitored daily for clinical signs.

3.4.1 Temperature/Weight of animal

Rectal temperature was determined in all the experimental animals daily by 7am with the use of a hand-held digital thermometer inserted into the rectum, it was withdrawn after 3 minutes and read. A weighing (Bathroom) scale was used to determine the weights of the animals.

3.4.2 Scrotal circumference

The scrotal circumference was measured with a flexible tape at the point of greatest diameter on a weekly basis for twenty weeks.

3.4.3 Semen collection and evaluation

Semen was collected weekly, into pre-warmed graduated bottles using an electro-ejaculator (Bailey ®) consisting of a small probe with longitudinal electrodes delivering six volts. The semen volume was measured directly by reading from the graduated test tube used for collection. It was then maintained at 37^{o}C until evaluations were complete. Spermatozoa concentration was determined using the red cell counting chamber of a haemocytometer in a manner similar to that used in red blood cell count. Formol-saline was used as diluent. Live/Dead sperm ratio, was determined by examining thin smears of semen stained with Eosin-Nigrosin. The same stained slides used to determine the live/dead ratio were used to determine the sperm morphological abnormalities and it involved counting at least 400 sperm cells per slide.

3.5 BLOOD COLLECTION

Two milliliters of blood was collected daily from the jugular vein of each of the experimental animals, into sample bottles containing ethylene diamine tetra acetic acid (EDTA) (1mg/ml) and used for haematological and parasitological evaluations.

3.5.1 Parasitaemia Scoring

Parasitaemia was determined using the Microhaematocrit centrifugation technique and scored according to methods described by Dargantes *et al*., (2005a).

3.5.2 Determination of Packed cell volume (P.C.V).

Packed cell volume (P.C.V) in percentage was determined using the haematocrit centrifugation technique (Coles, 1986) and the value read from a micro hematocrit reader. Two readings per sample were taken and the average of both used

3.5.3 Determination of Total plasma protein

Total plasma protein in g/dl was read from a Goldberg hand refractometer (Coles, 1986).

3.5.4 Determination of Blood Cell Counts

White and red blood counts were determined using a Neubauer hemocytometer and the values expressed as 10^9/L and 10^{12}/L of blood respectively.

Differential leucocyte count of Geimsa-stained blood smear was determined.

3.6 Gross and Histopathology

The heart, lungs, kidneys, liver, spleen, testes and epididymis from four of the infected and two of the control rams were harvested after the experimental period (14 weeks post infection) and examined for gross pathological changes. Sections of the caput, corpus and cauda of the testis and the epididymis were prepared and fixed in Bouin's solution while those of the other organs were prepared and fixed in 10% formaline solution. All organ sections were processed by standard histological techniques and then stained with

Haematoxylene and Eosin (H&E) stain. The degrees of testicular degeneration observed were scored according to Sekoni *et al*., (1990b).

3.7 Data Analyses

The data generated from parasitaemia, body weight, temperature, blood and semen evaluations were expressed as mean ± S.E.M and differences in the mean values between infected and control groups were analyzed using the Student's t-test of graph pad prism version 5. Values of $p < 0.05$ were considered to be statistically significant.

CHAPTER FOUR

RESULTS

4.1 Clinical Signs

On the basis of clinical examination, all infected animals showed varying clinical signs of the disease. These signs became obvious at the 10^{th} day post inoculation. Appetite was not affected but rams tagged numbers 4 and 7 that died in the course of the experiment showed inappetence and recumbence shortly before death. Pale mucous membrane, rough hair coat, loss of weight, dullness, edema of the face and lower jaw, corneal opacity, lacrimation were some of the common signs observed in the course of the study. Four of the infected rams had scrotal oedema manifested by enlargement of the scrotum with the disappearance of normal scrotal folds. By the 4^{th} week post-infection, all infected animals showed no interest in sexual activities manifested by absence of Flehmen's activities present at onset of experiment which could still be noticed in the control group. Rams 4 and 7 died on days 74 and 88 respectively post inoculation.

4.2 Obsevation on Rectal Temperature

The mean rectal temperatures of the infected rams started rising from day 4 post infection. Individual animals showed fluctuations in temperature attaining different peaks ranging from 39.9 to $40.6^{O}C$ during the period of infection. The mean temperatures ranged from 37.94 ± 0.09 to 39.36 ± 0.25^{OC} in the infected rams. The peak mean rectal temperature ($39.26 \pm 0.25^{O}C$) was recorded on the 27^{th} day post-infection. Rams 4 and 7 showed hypothermia shortly before they died with their rectal temperatures dropping to $37.4^{o}C$ and 37.5^{oC}, respectively. The mean rectal temperature in the uninfected control was 37.58 ± 0.04 to 38.42 ± 0.05. The results of rectal temperature were as shown in figure 1.

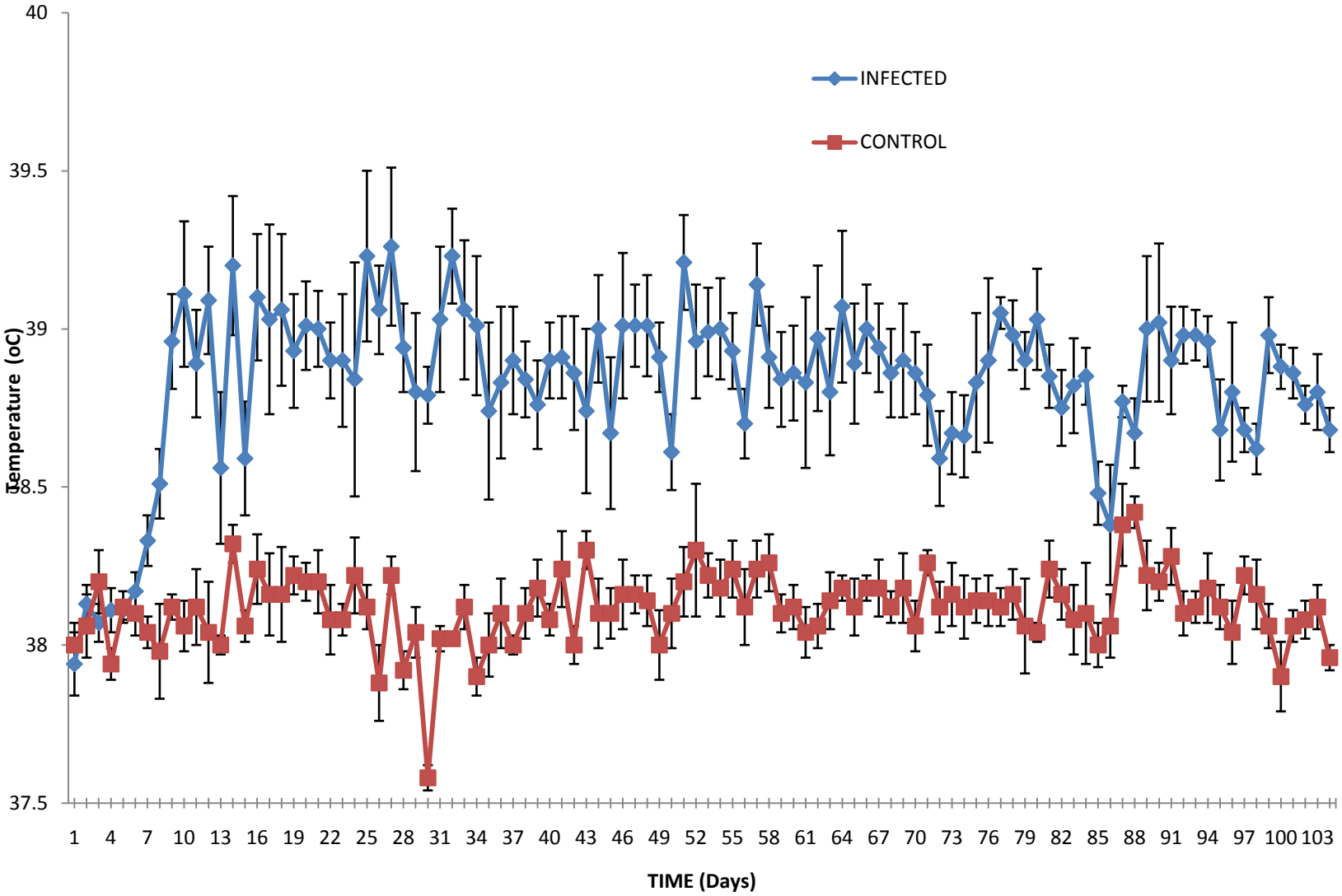

Figure 1: Mean (±SEM) Rectal Temperature of *T. evansi* infected and control Yankasa rams

4.3 Observation on pre-patent period and parasitaemia

Parasitaemia was first noticed in six of the seven infected rams at day 5 post infection and by day 7 post infection, trypanosomes could be detected in wet mount in all infected rams. A low persistent parasitaemia not exceeding 1- 2 trypanomastigote per 20 microscopic fields was noticed in wet mount for all the infected rams. Beginning from Day 91 post infection, the parasite could not be detected from the blood. Figure 2 shows flunctuating parasitaemia.

4.4 Observation of Blood parameters

4.4.1 PCV

The PCV (%) shown in Figure 3. There were significant ($p \square 0.05$) decreases in most blood parameters of infected rams beginning from the first week post- infection and culminating in severe anaemia in infected rams at the termination of the study 14 weeks post infection. Mean PCV of infected rams had dropped below normal range by the 4^{th} week post infection and then stabilized at values below the normal from 5^{th} week to 9^{th} week post-infection. At termination of the study 14 weeks post infection the mean PCV was (21.2 ± 1.99%). The mean PCV of infected rams ranged between 21 ± 1.45 and 29.3 ± 0.61% post infection which was lower than the pre infection level in the infected animal and the uninfected control group at the same period.

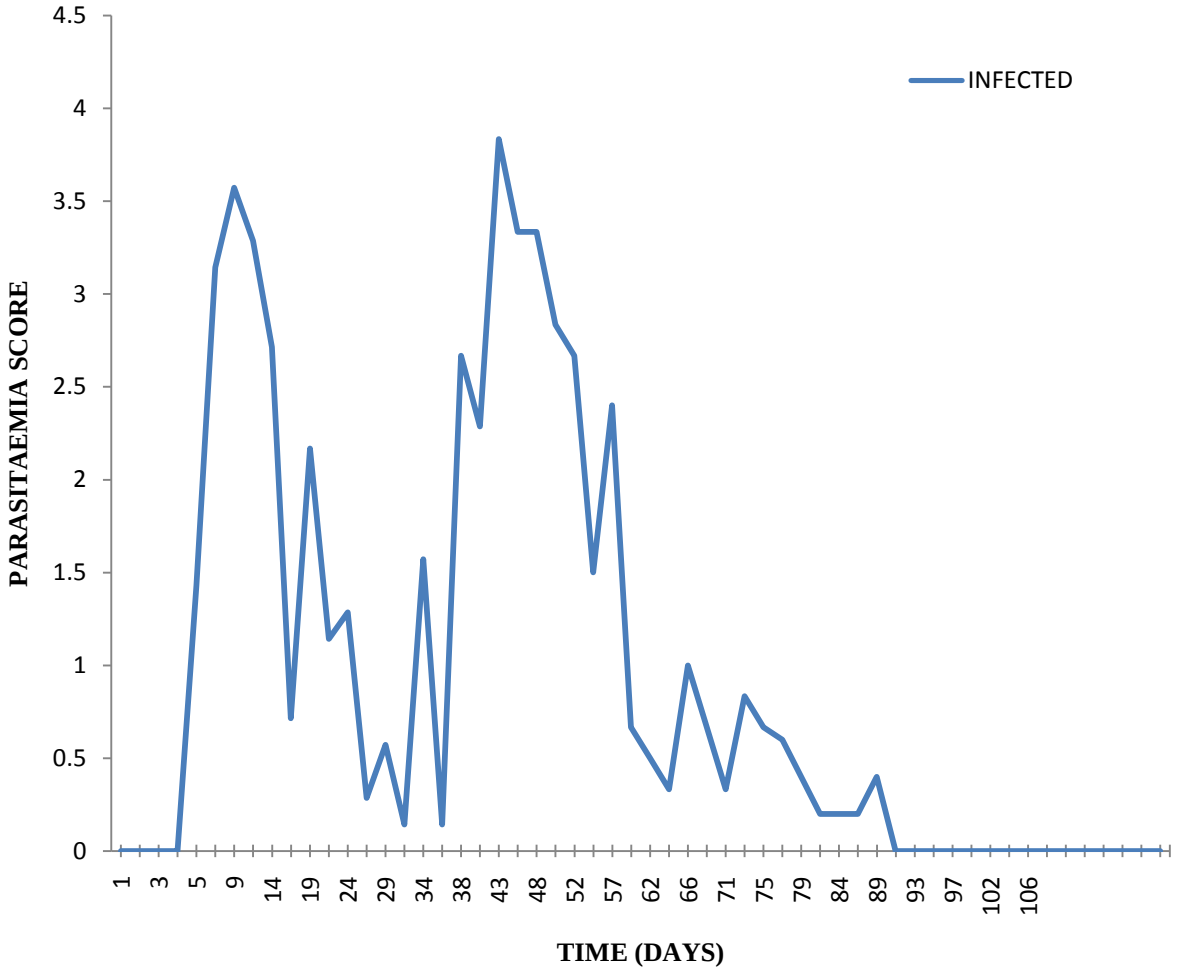

Figure 2: Mean (±SEM) Parasitaemia scores in *T. evansi* infected Yankasa rams

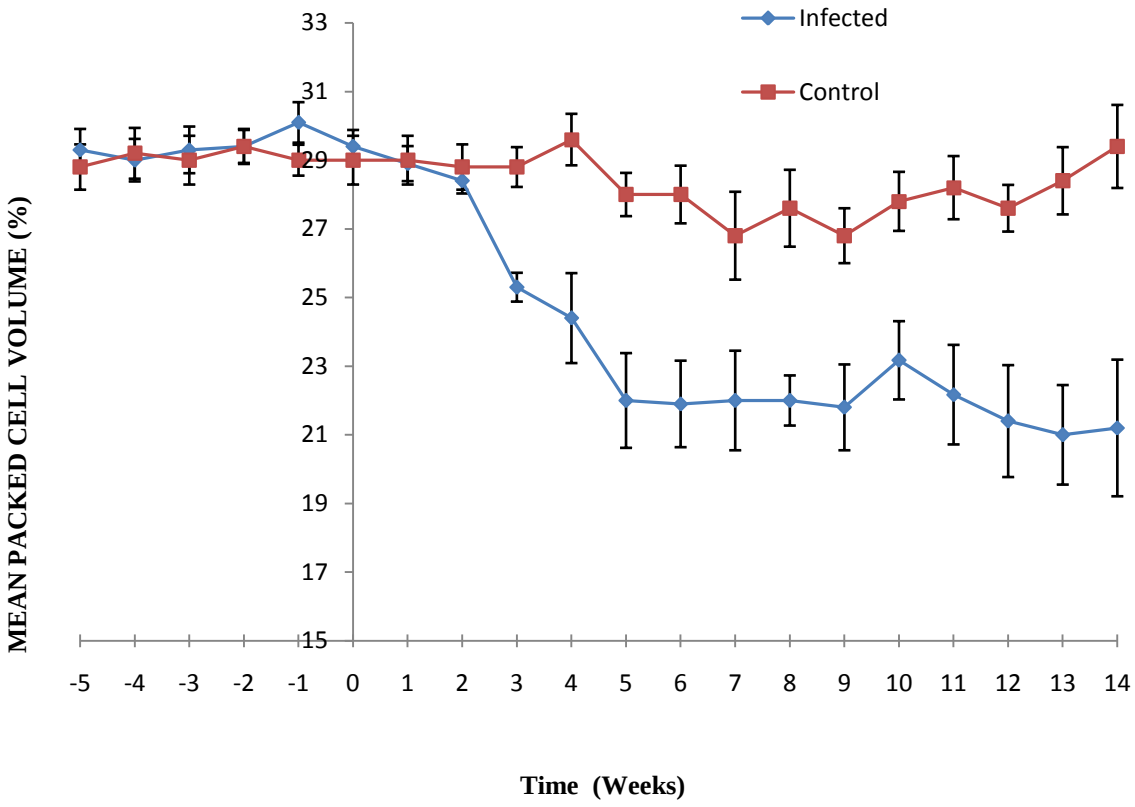

Figure 3: Mean (±SEM) Packed Cell Volume values in *T. evansi* infected and control Yankasa rams.

Key: Arrow (Point of infection)

4.4.2 Erythrocyte count

The mean erythrocyte count decreased in a similar pattern as PCV in infected rams. This decline continued to the end of study as indicated by (5.42 ± 0.50 x 10^6cells/mm^3) at termination from a pre infection value of 9.17 ± 0.23x10^6cells/mm^3. The mean erythrocyte count in infected rams was significantly (p□ 0.5) lower when compared to the uninfected control group which showed no significant change through the entire study. Figure 4 shows mean erythrocyte count for infected and control rams.

4.4.3 Total Plasma Protein

The mean total plasma protein was as shown in Figure 5. There was a gradual non-significant (p □ 0.05) decrease in the mean total plasma protein in infected rams reaching its lowest level by the 8th week post-infection before gradually increasing. The post-infection mean total protein in infected animals ranged between 5.1 ± 0.20 and 6.1 ± 0.22 g/dl compared to the control animals which had values ranging between 5.6 ± 0.10 and 6.2 ± 0.28g/dl.

4.4.4 Red Blood Cell Indices (Mean Corpuscular Volume and Mean Corpuscular Haemoglobin Concentration)

The mean values of MCV (Figure 6) differed significantly (P < 0.05) between the two groups beginning from week 5 post-infection until termination of the experiment at 14 week post infection. In the infected rams, the mean MCV ranged between 31.94 ± 0.6 and 42.07 ± 1.9 fl, remaining within the normal reference range for sheep (23-48 fl). The mean MCHC (Fig. 7) did not differ among groups.

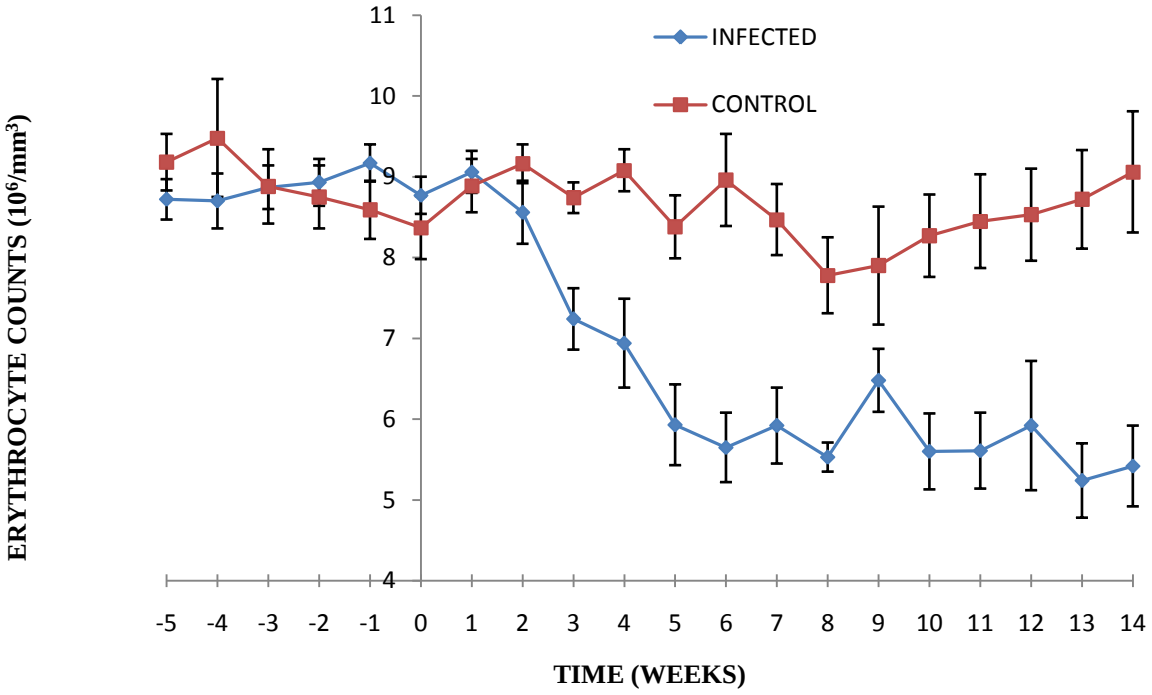

Figure 4: Mean (±SEM) Erythrocyte counts in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

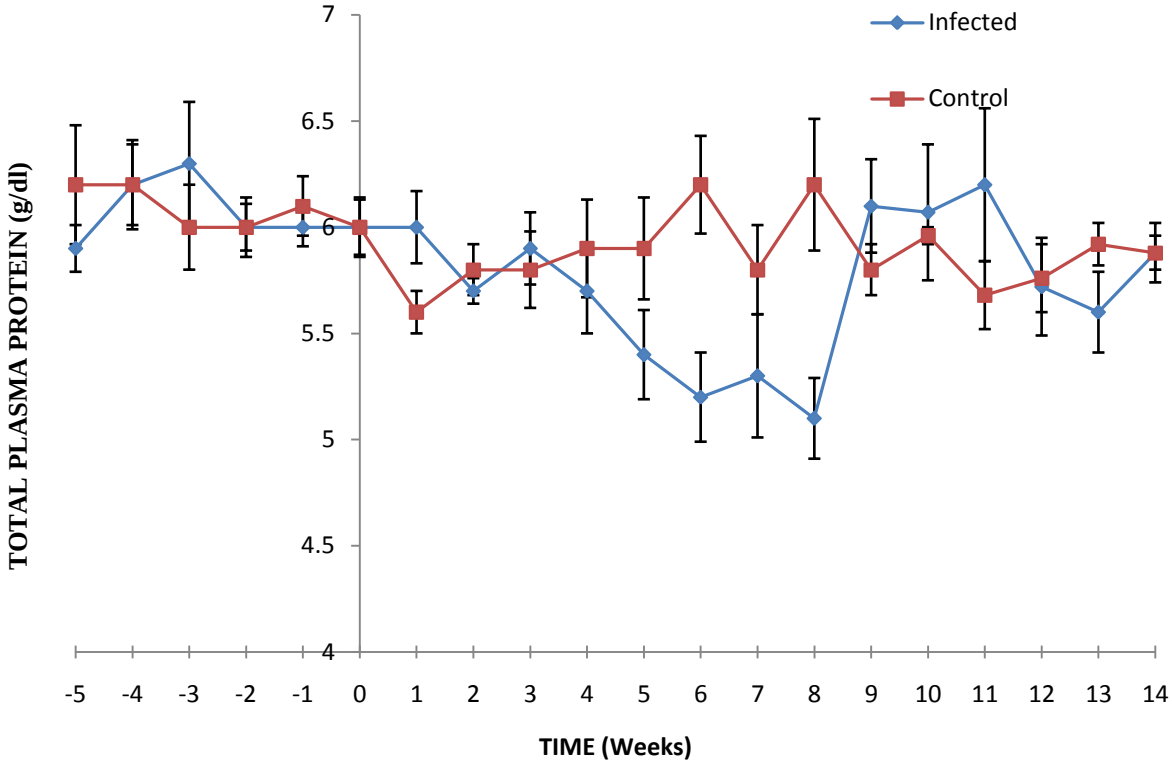

Figure 5: Mean (±SEM) Total Plasma Protein (g/dl) in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

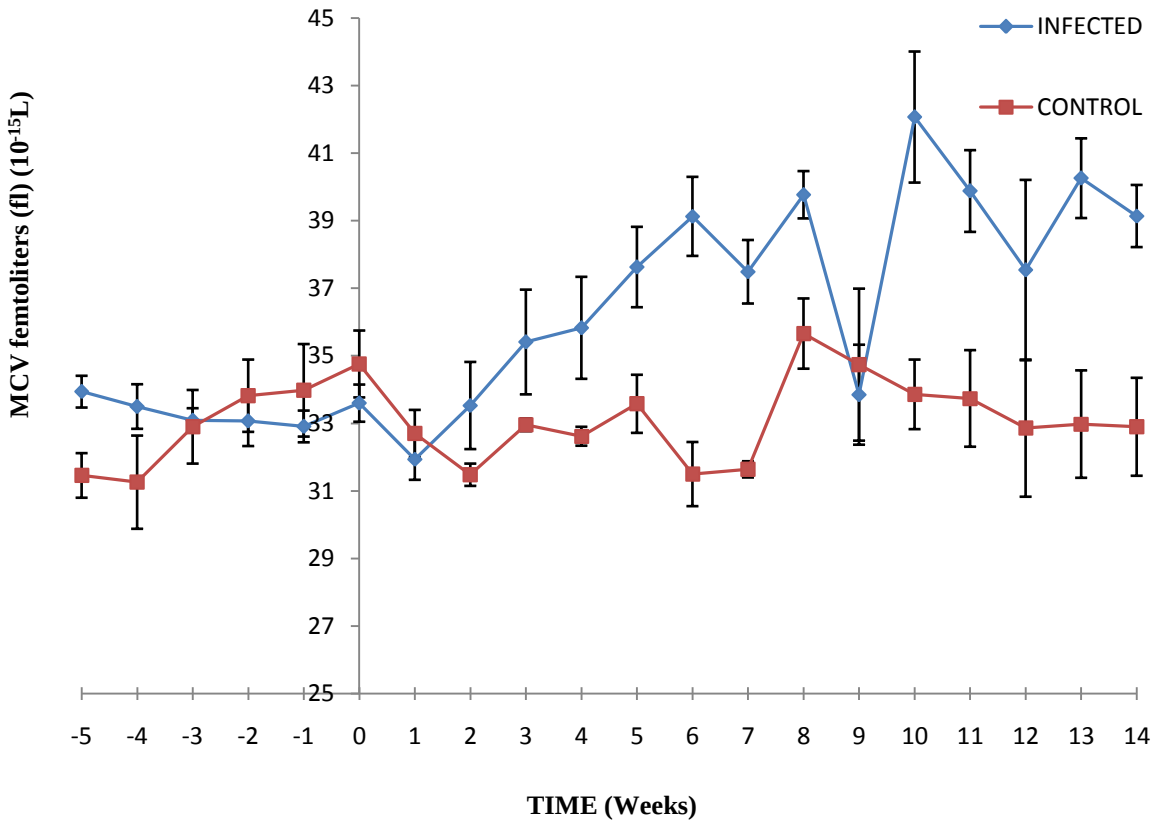

Figure 6: **Mean Corpuscular Volume values in *T. evansi* infected and control Yankasa rams**

Key: Arrow (Point of infection)

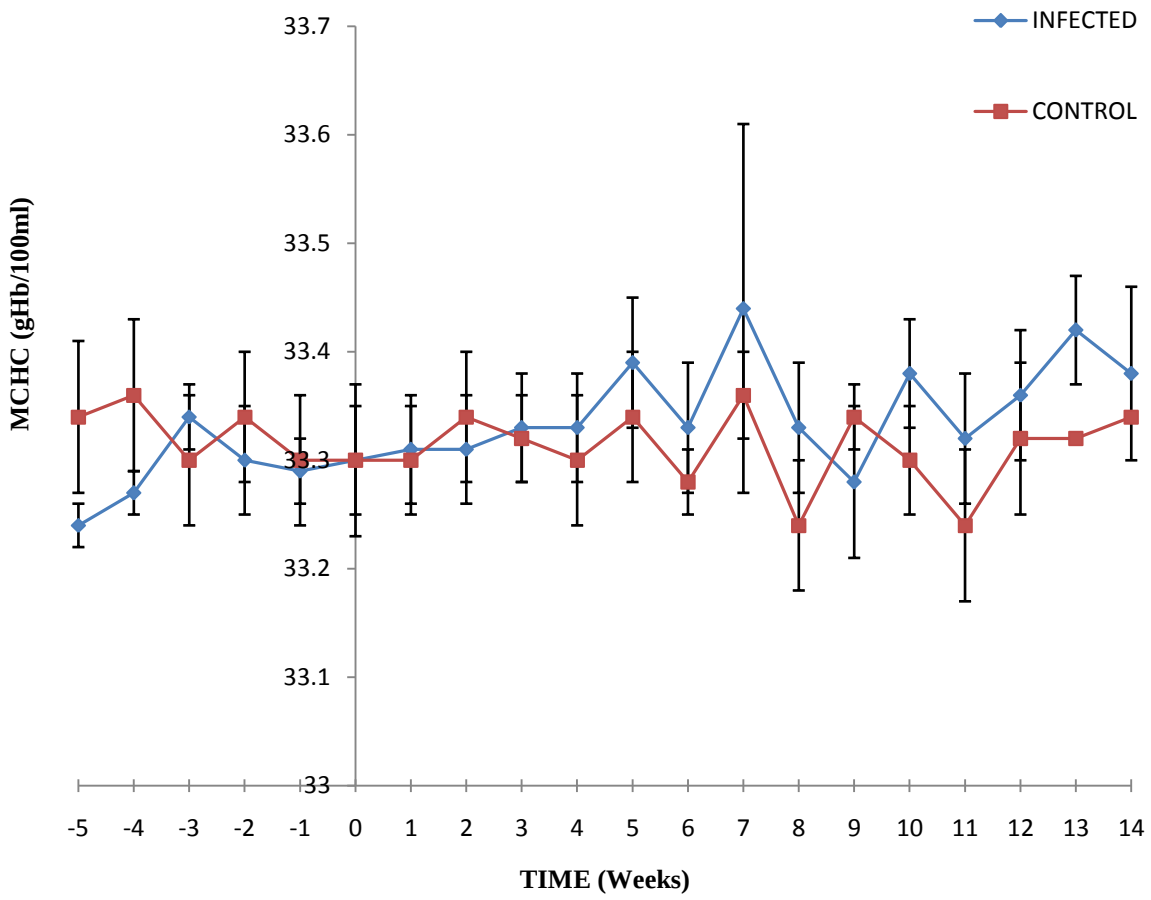

Figure 7: Mean Corpuscular Hemoglobin Concentration (MCHC) values in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

4.4.5 Leucocyte (Total White Blood) count

Figures 8 show mean values for total leucocytes. There were significant differences (p □ 0.05) in leucocyte counts of rams in the infected group on weeks 4, 7, 10, 11, 12 and 14 post infection.

4.4.6 Differential leucocyte

Figure 9 Differential leucocyte count revealed no significant differences in lymphocytes between the groups

Figure 10 Differential leucocyte count revealed no significant differences in monocytes between the groups.

Figure 11 Differential leucocyte count revealed no significant differences in neutrophils between the groups.

Figure 12 Differential leucocyte count revealed no significant differences in eosinophils between the groups.

Figure 13 Differential leucocyte count revealed no significant differences in basophils between the groups.

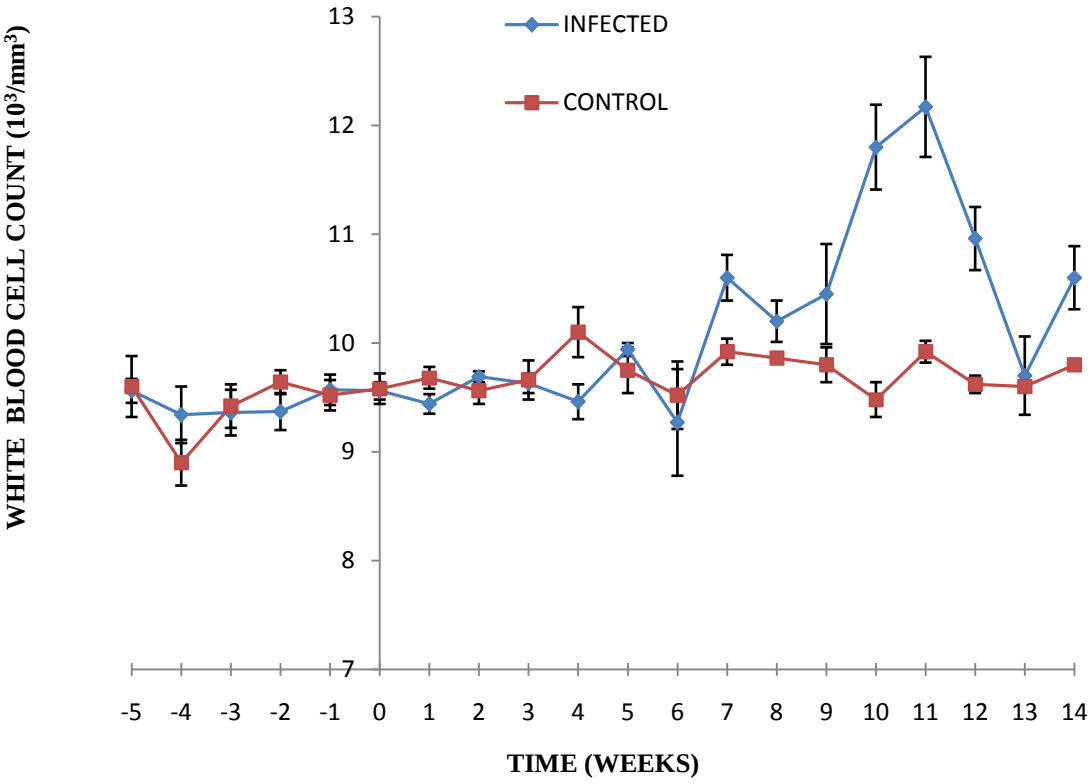

Figure 8: Mean(±SEM) Total White Blood Cell counts (WBC) in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

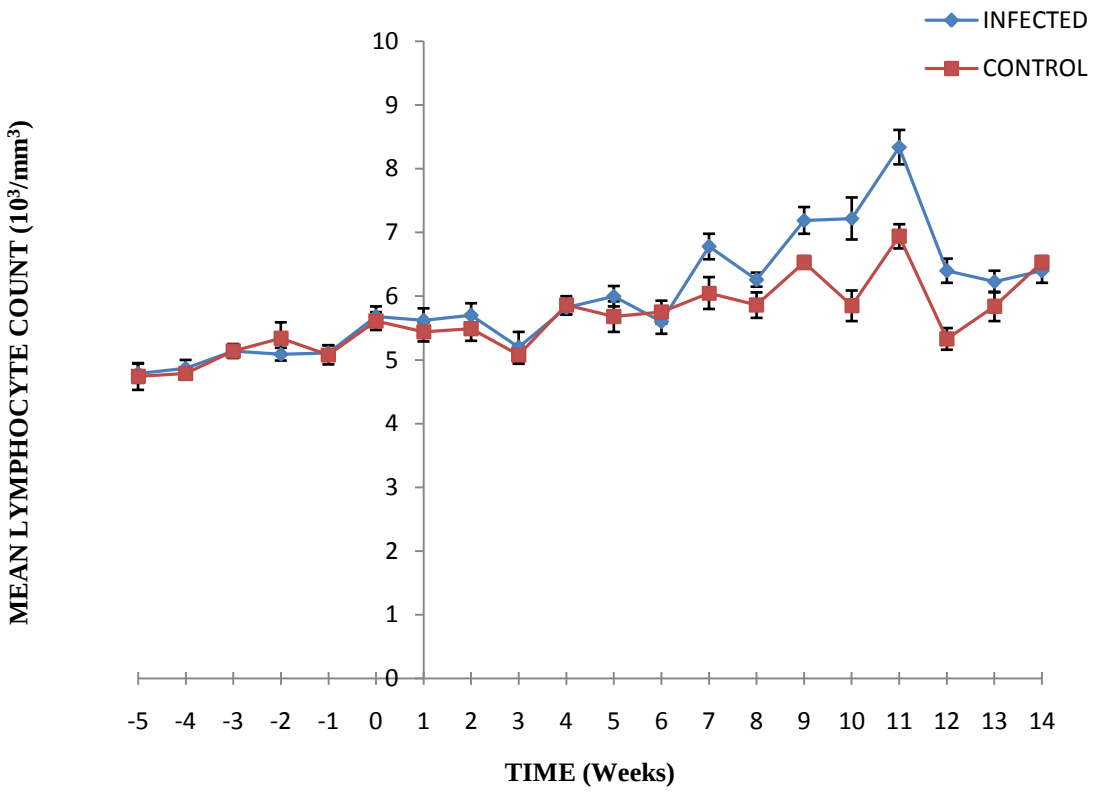

Figure 9: Mean (±SEM) Lymphocyte counts (%) in *T. evansi* infected and control Yankasa rams.

Key: Arrow (Point of infection)

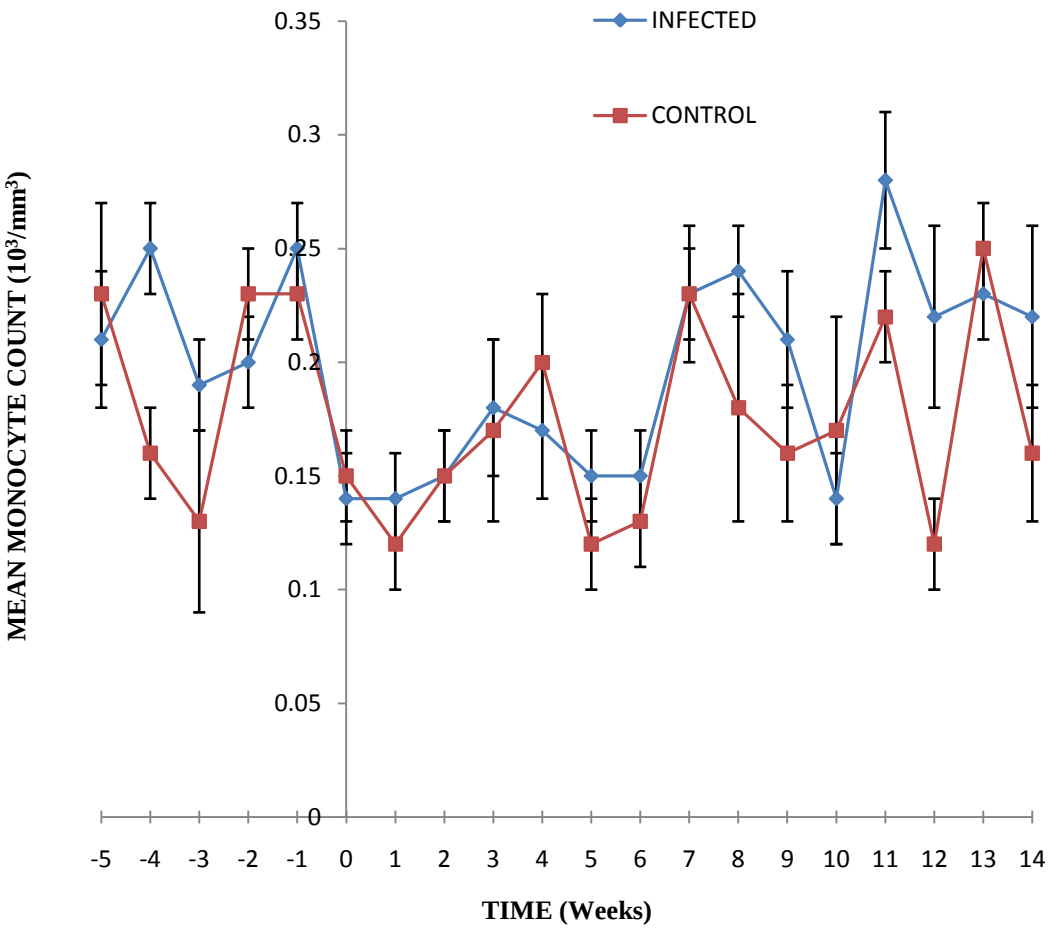

Figure 10: Mean (±SEM) Monocyte counts (%) in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

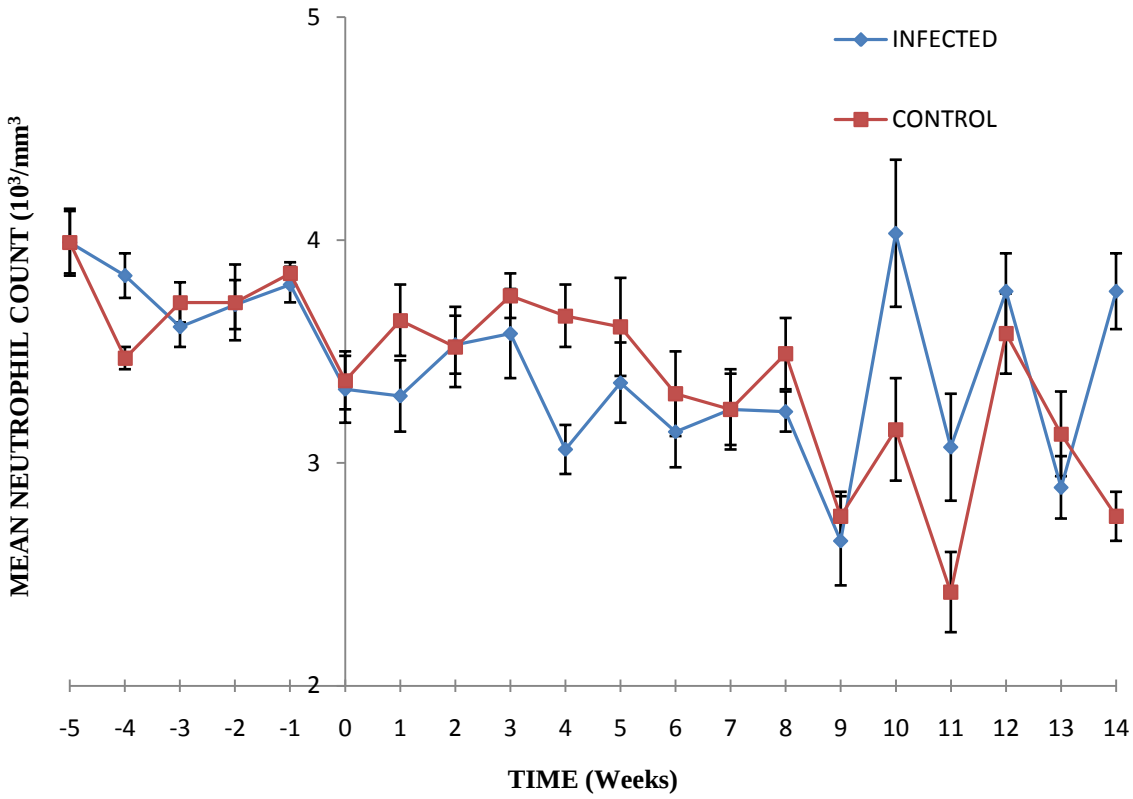

Figure 11: Mean (±SEM) Neutrophil counts (%) in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

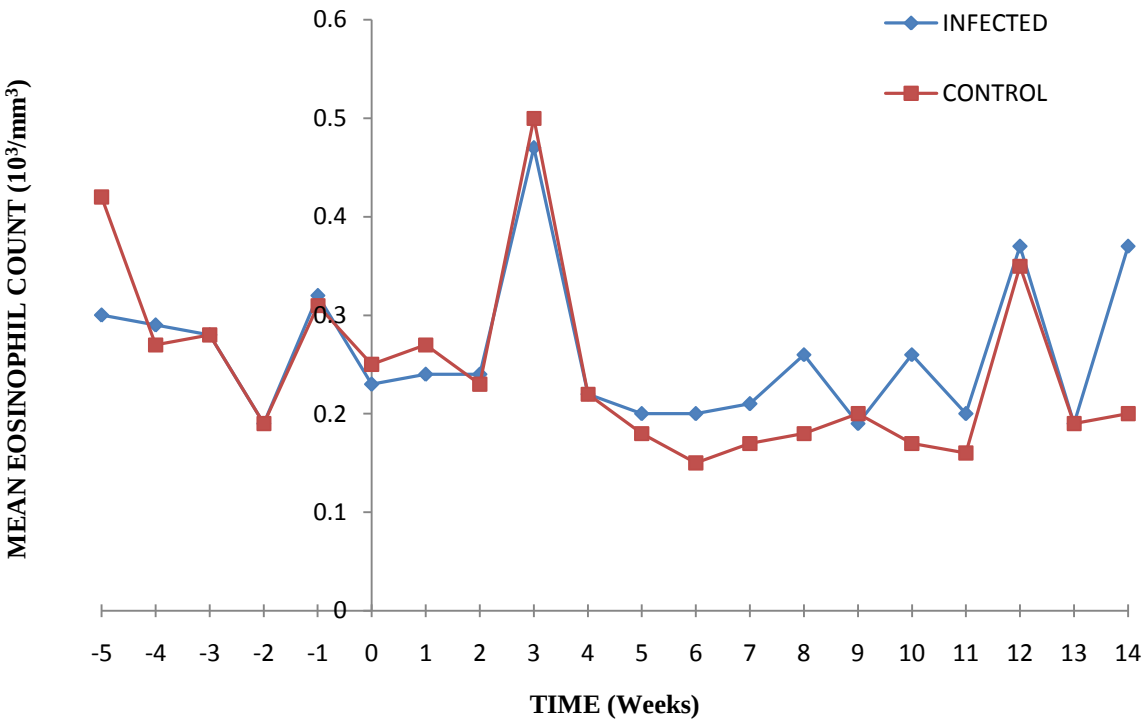

Figure 12: Mean (±SEM) Eosinophil counts (%) in *T. evansi* infected and control Yankasa rams.

Key: Arrow (Point of infection)

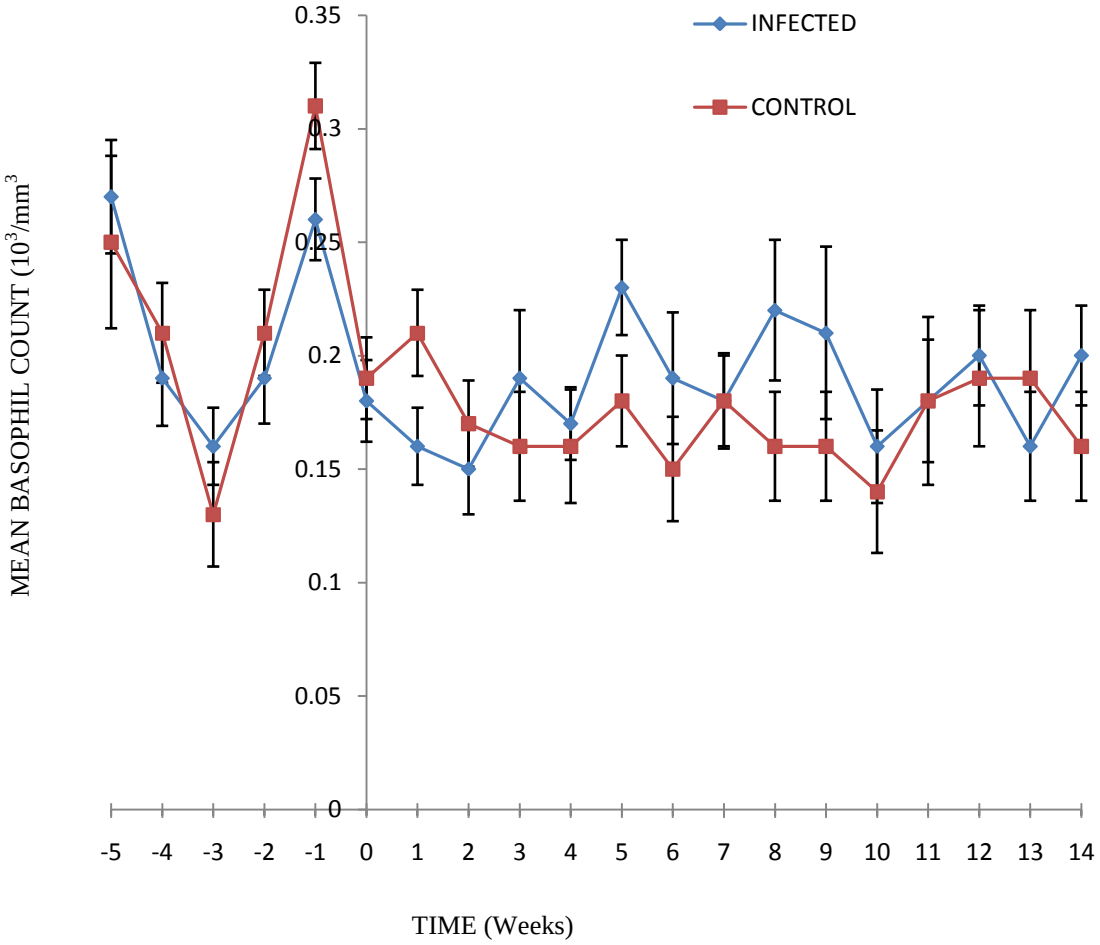

Figure 13: Mean (±SEM) Basophil counts (%) in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

4.5 Observation on Body Weight

Figure 14 shows the mean weight of animals. There was a significant ($p < 0.05$) difference between the mean body weights of the infected and the control. The initial mean body weights prior to infection were 23.3 ± 1.19 Kg for the infected and 21.4 ± 2.44 Kg for the controls and the terminal weights at week 20 (14 weeks post infection) were 17.5 ± 2.36 Kg for the infected and 21.2 ± 2.64 Kg for the control. Rams 4, 7 and 16 became severely emaciated with rams 4 and 7 weighing 13kg just before dying.

4.6 Observation on Scrotal Circumference

The mean scrotal circumference values obtained in the infected group post infection were significantly ($p < 0.05$) lower compared to the pre-infection mean values. Throughout the duration of infection, the mean values in both groups did not differ significantly ($p < 0.05$) despite a decrease in the infected group from pre infection (25.8 ± 0.39 cm) to 21.1 ± 1.28 , compared to the controls 23.3 ± 1.34 to 23.3 ± 1.54. (Figure 15).

4.7 Observation on Reaction time

Mean values and SEM of Reaction time is shown in Figure 16. Infection with *T. evansi* had profound effect on the reaction time in the infected group compared to the control group. The mean reaction time of the infected group was significantly higher ($p < 0.05$) at weeks 9, 11, 18, 19 and 20 compared to the controls with the following corresponding values, (48.9 ± 5.11; 32.2 ± 1.50), (68.3 ± 8.87; 31.2 ± 2.42), (65.6 ± 10.84; 33.4 ± 4.78), (94.7 ± 7.54; 31.2 ± 3.60) and (76.6 ± 17.55; 33.4 ± 4.78) seconds for infected and controls respectively. From week 3 to week 14 post infection, the mean reaction time of infected group were significantly higher ($p < 0.05$) compared to their pre-infection value.

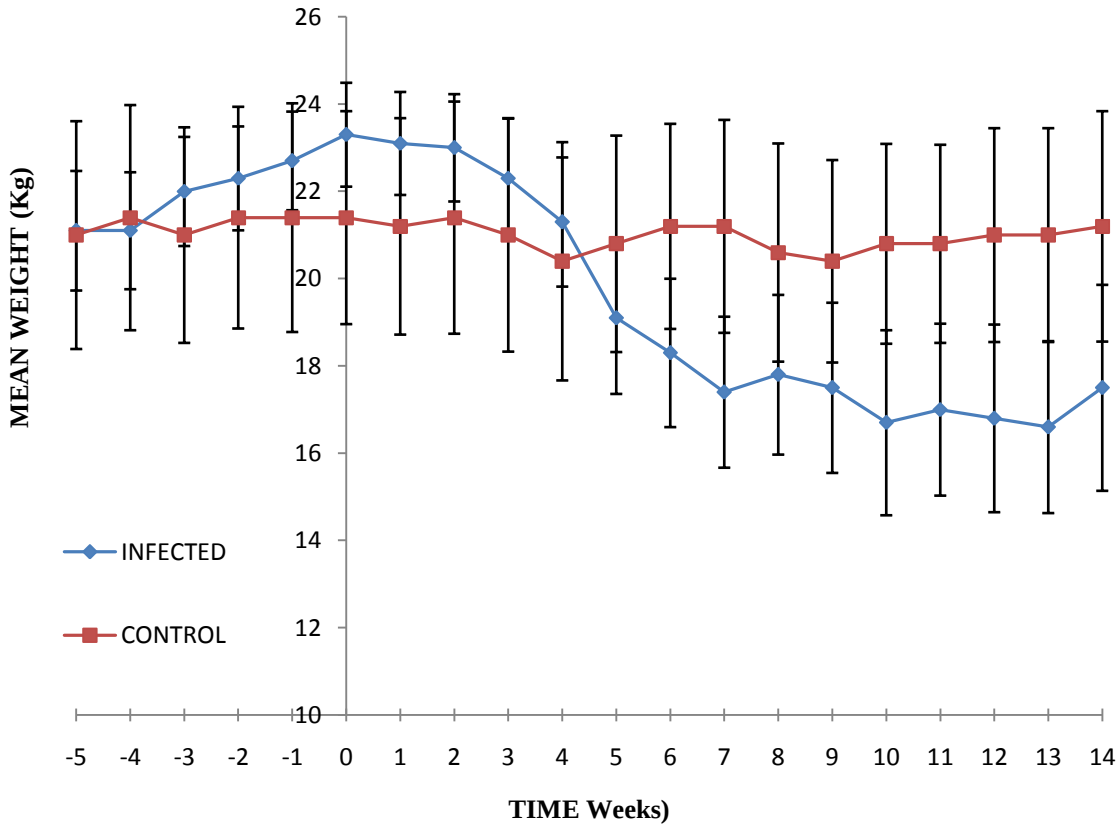

Figure 14: Mean (±SEM) Weight in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

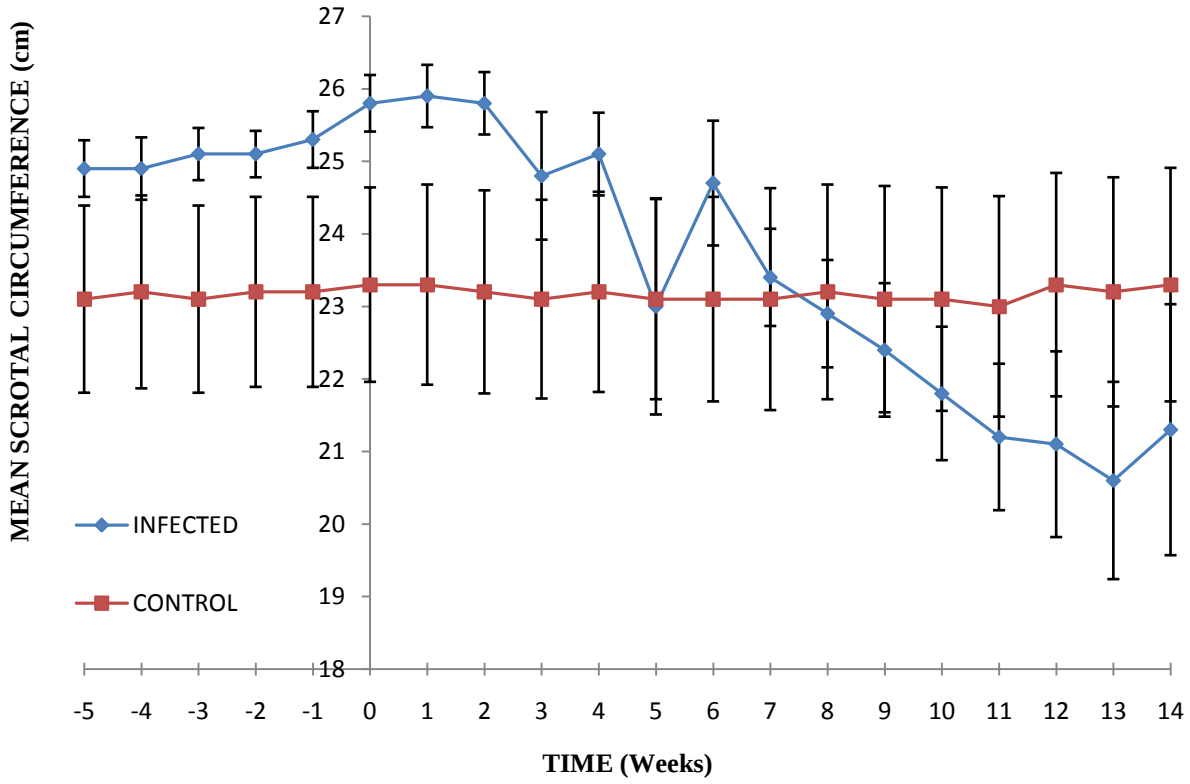

Figure 15: Mean (±SEM) Scrotal Circumference in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

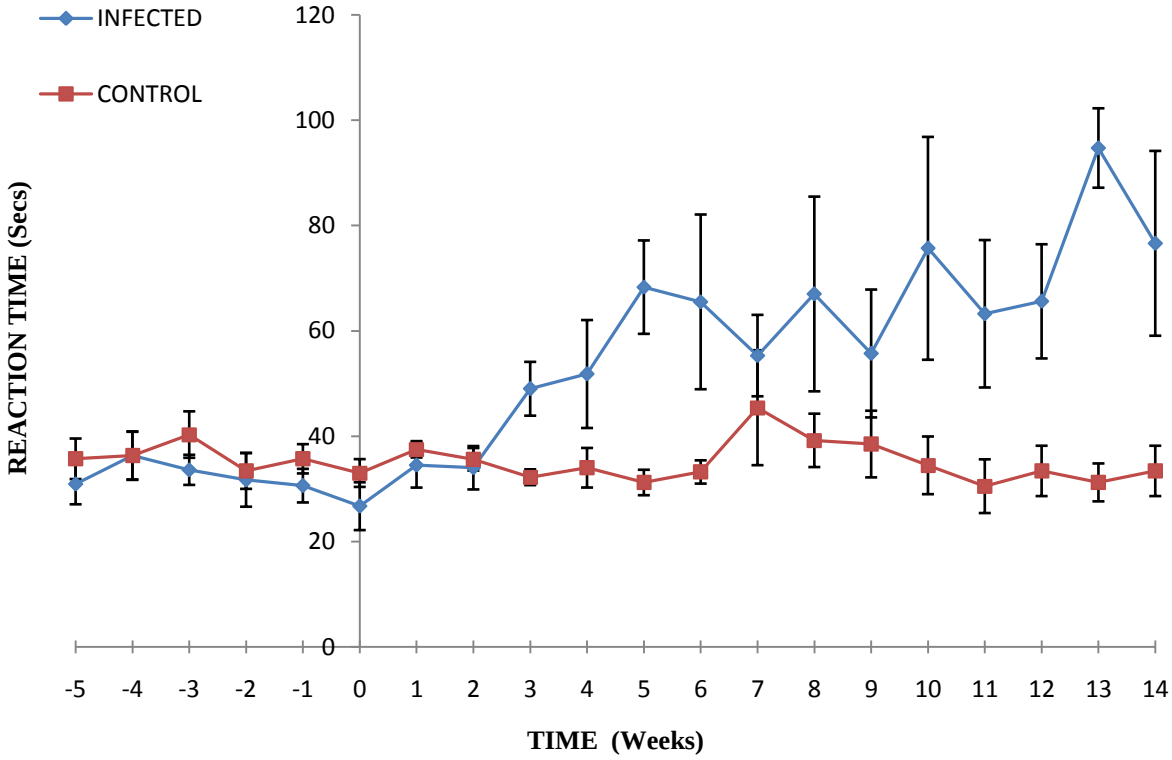

Figure 16: Mean (±SEM) Reaction time (Sec) in *T. evansi* infected and control Yankasa rams

Key: Arrow (Point of infection)

4.8 Semen Characteristics

4.8.1 Semen Volume

Mean and SEM of semen volume are shown in Figure 17.There was no significant (P □ 0.05) decrease in semen volume in the infected group when compared to the control. By the time of termination (14 weeks post infection) of the study, mean semen volume had a value of 0.43 ± 0.09 in the infected group and 0.54 ± 0.05 in the control.

4.8.2 Sperm Concentration

Mean values and SEM of semen concentration is shown in Figure 18. Compared to the uninfected control group, the sperm concentration of the infected group was significantly lower (P □ 0.05) at weeks 17, 18, 19, and 20.The mean values for the control at these periods were 2.25 ± 0.24 x 10^6, 2.41 ± 0.25 x 10^6, 2.28 ± 0.18 x 10^6 and 1.97 ±0.14 x 10^6 compared to 1.08 ±0.30 x 10^6, 0.79 ± 0.18 x 10^6, 0.86 ± 0.18.x 10^6 and 1.01±0.17 x 10^6 for the infected. The mean values ranged between 2.92 ± 0.18 x 10^6 and 0.83 ± 0.15 x 10^6 in the infected, while that in the control rams ranged between 2.72 ± 0.28 and 1.56±0.44.

4.8.3. Gross Sperm Motility

Mean values and S.E.M of semen gross motility is shown in Figure 19. The motility varied at various points with a consistent decrease beginning at the fourth week post infection. This progressive decrease continued to 0 % in some of the infected rams with the mean value dropping to as low as 43 ± 11.14 % by the tenth week post infection which was consistent till termination of the study. The motility in individual animals in the infected group ranged between 75 and 95% in the first ten weeks post infection, 0% and 75% in the final six weeks post infection. Overall, showing a significant decrease ($P < 0.05$) compared to the control group.

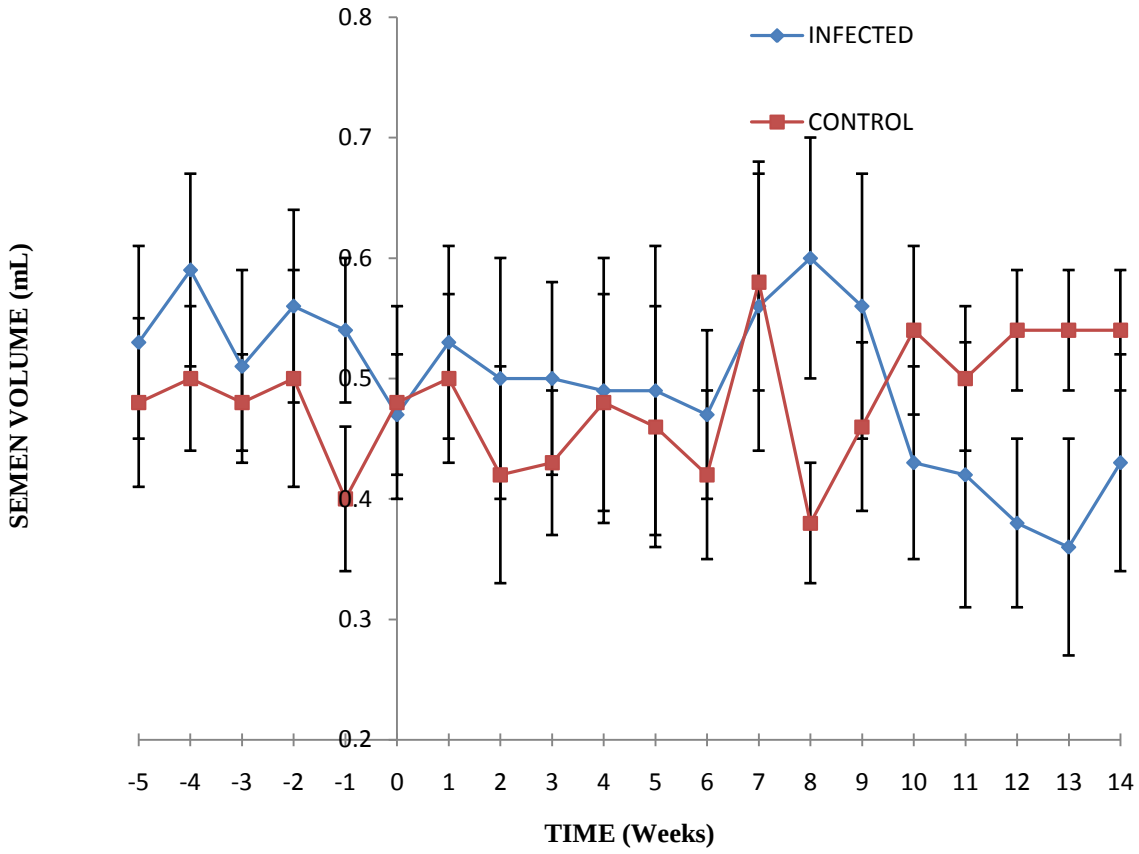

Figure 17: Mean (±SEM) Semen Volume (ml) in *T. evansi* infected and uninfected control Yankasa rams

Key: Arrow (Point of infection)

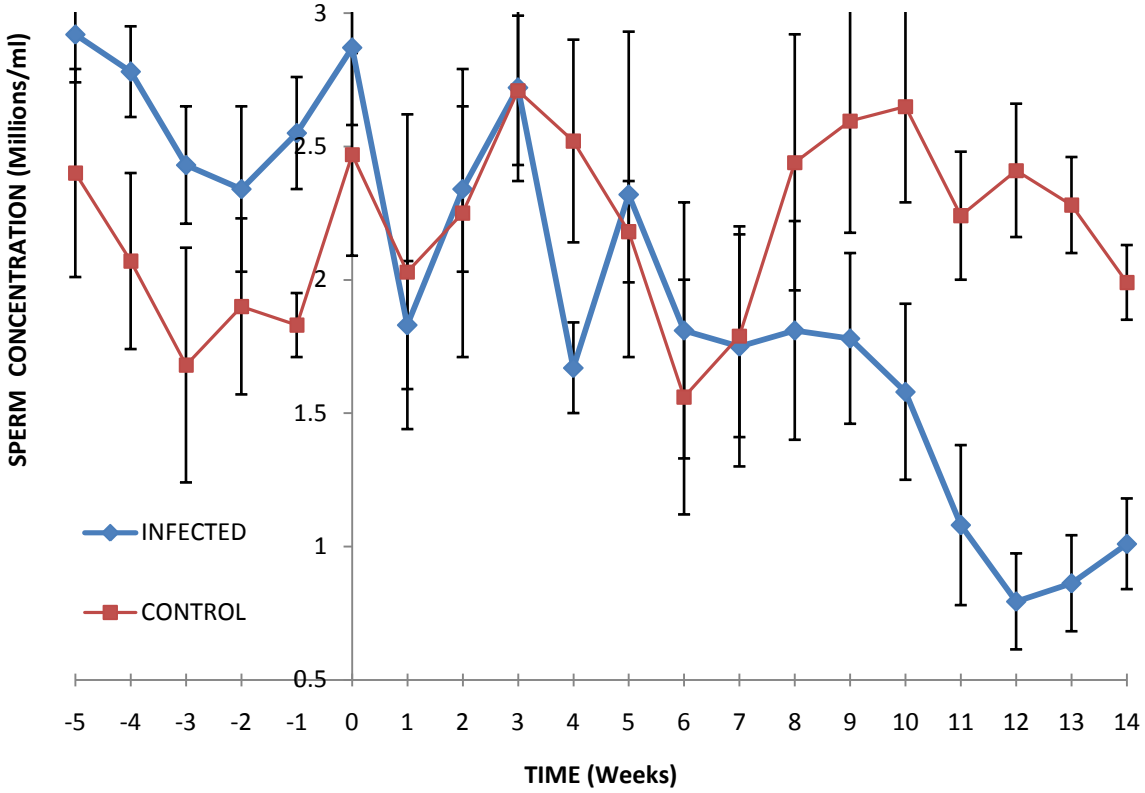

Figure 18: Mean (±SEM) Sperm Concentration (10^6/ml) in *T. evansi* infected and uninfected control Yankasa rams

Key: Arrow (Point of infection)

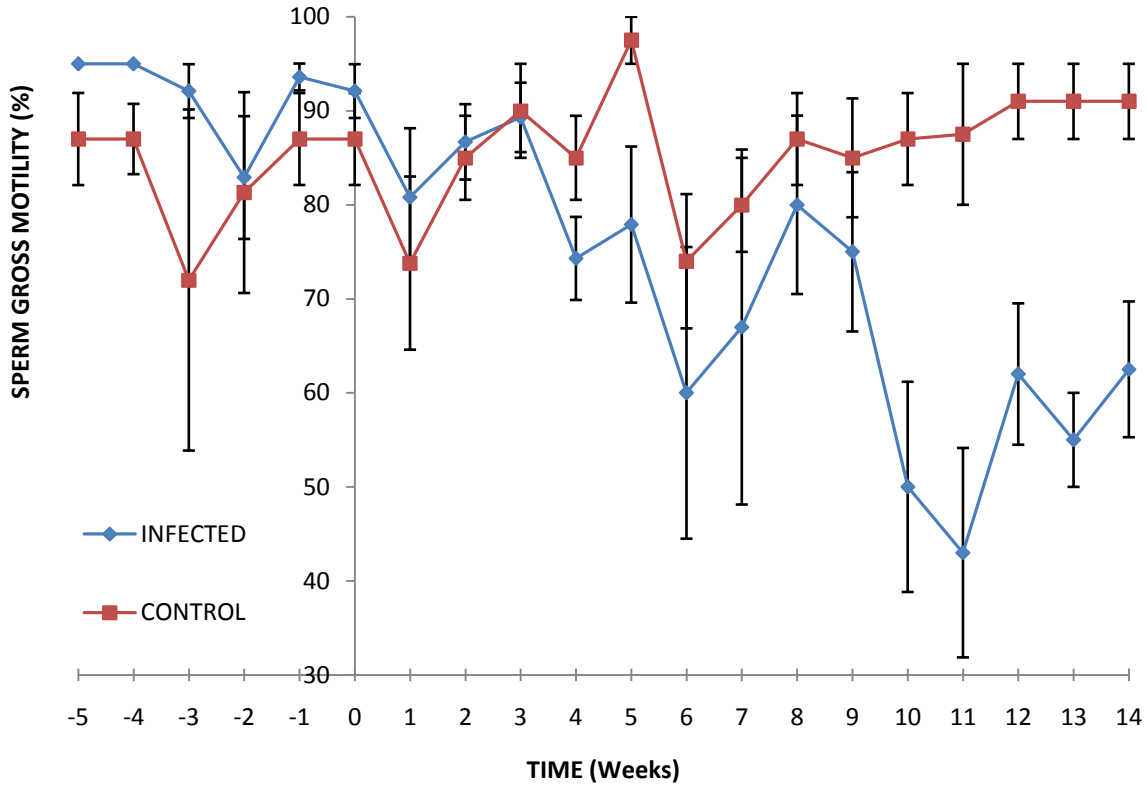

Figure 19: Mean (±SEM) Sperm Gross Motility (%) in *T. evansi* infected and uninfected control Yankasa rams

Key: Arrow (Point of infection)

4.8.4 Total Sperm Morphological Abnormalities

Infection in group A caused a progressive increase in mean total sperm morphological abnormalities. The mean values for the infected group were significantly higher (P □ 0.05) from week 8 to the end of the experiment. The mean values ranged between 28.6 ± 1.07 and 90.8 ± 2.73 % in the infected rams compared to 20.7 ± 1.02 and 26.8 ± 1.71 % in the control which were within the normal range. (Figure 20)

Appendices (XXI and XXII) shows the relationships between all the aforementioned parameters in relation to time in weeks

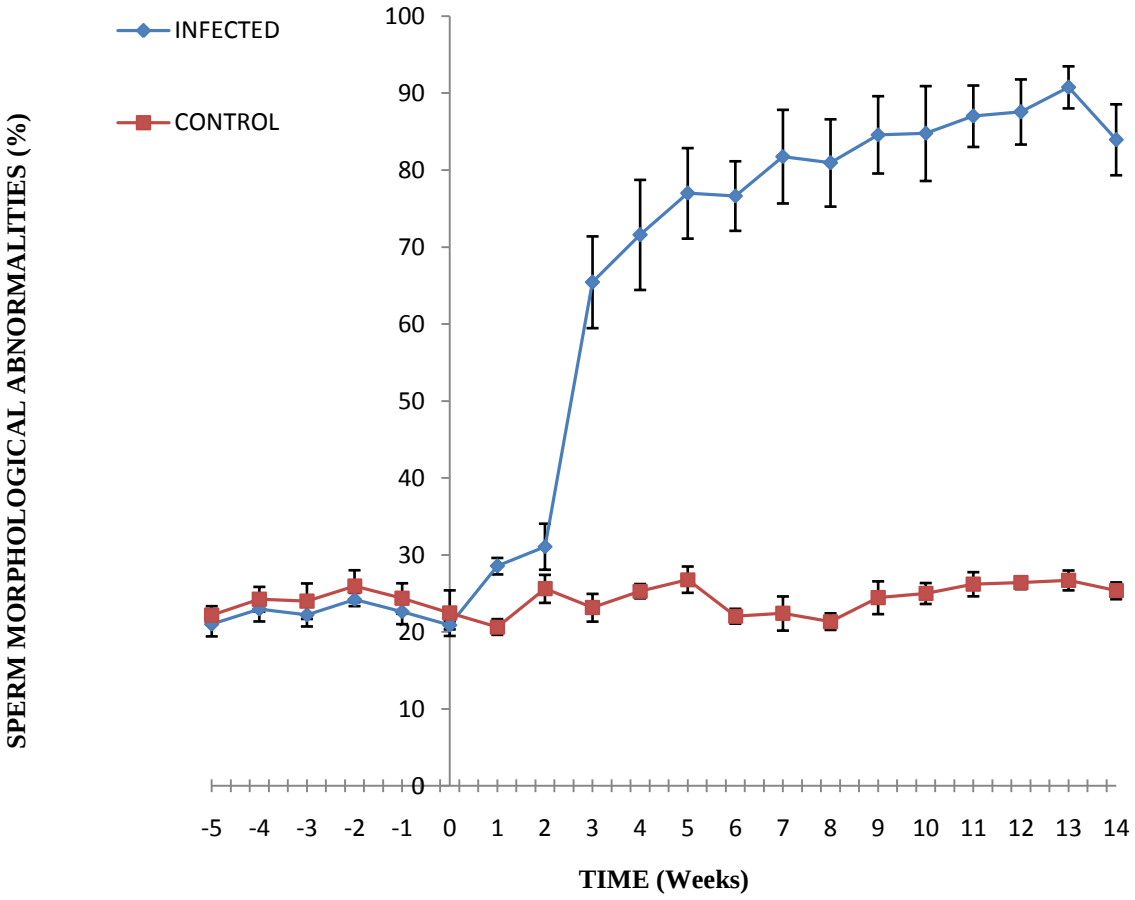

Figure20: Mean (±SEM) Total Sperm Morphological Abnormalities (%) in *T. evansi* infected and uninfected control Yankasa rams

Key: Arrow (Point of infection)

4.8.4.1 Sperm Head Abnormalities

The sperm head abnormalities rose from a mean pre infection value of 0.6 ± 0.13% to 6.2±0.89% by the 13th week post infection, the highest mean value. The control group had a mean value that ranged between 0.05% and 0.7%. The mean values for the infected rams differed significantly from the control ($P < 0.05$). (Table 1).

4.8.4.2 Detached Head

There was an increase in mean values for detached head beginning from the second week post infection. The values ranged between 1.5 ± 0.22% and 17.8 ± 3.55% post infection compared to 0.6 ± 0.17% and 1.5 ± 0.28% range for the control. There was a significant ($p < 0.05$) difference between infected and control. (Table 1) .

4.8.4.3 Acrosome Abnormalities

Acrosome abnormalities increased in the infected group within the period of this study. The values ranged between 1.1 ± 0.10% and 2.5 ± 0.41% pre infection and 2.5 ± 0.36% and 10.5 ± 0.79% post infection. Compared to the control rams whose value ranged between 1.4 ± 0.13% and 3.0 ± 0.22% for the same period. The increase in mean values of acrosomal abnormalities in the infected was significant ($p < 0.05$) compared to the control. (Table 1).

4.8.4.4 Proximal Cytoplasmic Droplets

Proximal cytoplasmic droplets increased from a pre infection mean value of 0.3 ± 0.08% and peaked at 2.6 ± 0.41% on week 9 post infection compared to the control rams with values ranging between 0.0 ± 0.00% and 0.4 ± 0.06% within the same period. The infected rams had statistically higher ($P<0.05$) values than the control. (Table 1).

4.8.4.5 Distal Cytoplasmic Droplets

Following infection there was an insignificant increase in the mean values of distal cytoplasmic droplets ($p > 0.05$) from a pre-infection value of 0.04 ± 0.00% to a post infection value of 0.3 ± 0.16% compared to the control which had its mean value range between 0.0 ± 0.00% and 0.2 ± 0.10% throughout the study period. (Table 1)

4.8.4.6 Middle Piece Abnormalities

There was a drastic increase in mid piece abnormalities from the second week (3.5 ± 1.09%) post infection. Significant ($p \square 0.05$) differences in the mean values occurred on week 10 through to week 20. The mean value peaked on the tenth week (20.0 ± 6.67%) post infection. The mean mid piece abnormalities ranged between 1.8 ± 0.42% and 20.0 ± 6.67% post infection compared to 0.4 ± 0.19% and 1.7 ± 0.38% for the control. There was significant difference ($p \square 0.05$) in the mean values of the infected compared to the control. (Table 1).

4.8.4.7 Tail Abnormalities

There was a statistically significant ($p \square 0.05$) increase post infection. Spermatozoa with bent tails had a range of 17.8 ± 0.90% and 36.4 ± 7.54% post infection compared to the control rams with mean values ranging from 14.5 ± 0.64% to 20.9 ± 0.62% in the same study period. There was a statistically significant ($p \square 0.05$) increase compared to the control rams. (Table 1).

Table 1a: Sperm morphological abnormalities of Yankasa rams pre and post infection with *Trypanosoma evansi*

	Abnormal Heads (%)		Detached Head (%)		Mid Piece Abnormalities (%)		Bent Tail (%)	
WEEKS	GROUP A	B	GROUP A	B	GROUP A	B	GROUP A	B
1	0.4±0.05	0.4±0.13	0.1±0.10	0.6±0.37	0.3±0.13	0.15±0.1	17.4±1.18	18.6±1.21
2	0.3±0.21	0.7±0.19	0.3±0.15	0.8±0.34	0.1±0.14	0.5±0.35	18.5±1.05	19.3±0.76
3	0.6±0.17	0.4±0.22	0.6±0.32	0.9±0.51	0.3±0.18	0.2±0.12	17.8±1.20	19.3±1.30
4	0.6±0.22	0.7±0.12	1.3±0.40	0.4±0.36	0.1±0.11	0.4±0.37	19.0±0.83	21.3±1.82
5	0.5±0.22	0.4±0.15	1.0±0.42	0.8±0.09	0.2±0.10	0.6±0.17	18.9±1.13	19.4±1.61
6	0.6±0.13[a]	0.2±0.10[b]	0.4±0.16	1.1±0.78	0.5±0.23	0.9±0.64	17.3±0.39	18.2±1.07
7	0.6±0.12	0.3±0.33	1.5±0.22	1.4±0.26	1.8±0.42	1.25±0.54	20.3±0.39[a]	14.5±0.64[b]
8	0.6±0.11	0.7±0.15	1.8±0.45	1.5±0.28	3.5±1.09	1.4±0.55	17.8±0.90	19.3±0.98
9	2.2±0.53[a]	0.5±0.50[b]	8.0±2.21[a]	0.6±0.38[b]	11.7±4.28	1.1±0.23	31.0±2.80[a]	19.2±1.11[b]
10	5.1±0.63[a]	0.5±0.50[b]	11.1±2.08[a]	0.6±0.17[b]	14.6±4.84[a]	1.0±0.21[b]	24.5±2.65	20.0±0.55
11	4.3±1.18[a]	0.5±0.14[b]	11.5±2.70[a]	1.1±0.30[b]	19.4±5.16[a]	1.2±0.27[b]	21.5±3.95	20.2±0.62
12	3.9±0.76[a]	0.6±0.06[b]	14.4±3.95[a]	1.3±0.21[b]	8.5±2.35[a]	0.4±0.19[b]	32.3±4.90[a]	17.7±1.03[b]
13	4.6±0.63[a]	0.4±0.15[b]	12.2±4.85	1.2±0.20	14.6±3.21[a]	0.9±0.26[b]	30.5±5.09[a]	16.9±1.21[b]
14	5.2±0.59[a]	0.4±0.17[b]	13.5±2.93[a]	0.5±0.15[b]	15.3±2.60[a]	1.1±0.30[b]	28.4±3.98[a]	16.3±1.08[b]
15	5.4±0.70[a]	0.5±0.08[b]	17.8±3.55[a]	1.1±0.31[b]	17.5±3.04[a]	1.3±0.45[b]	24.5±4.02	18.0±0.97
16	4.7±0.61[a]	0.6±0.09[b]	14.3±3.53[a]	1.4±0.19[b]	20.0±6.67[a]	0.9±0.25[b]	25.5±6.08	18.9±0.84
17	4.5±0.90[a]	0.2±0.15[b]	9.7±1.98[a]	0.9±0.10[b]	18.8±6.34[a]	1.2±0.52[b]	30.6±6.97	20.9±0.62
18	5.0±0.65[a]	0.4±0.17[b]	14.3±1.73[a]	1.2±0.17[b]	15.0±3.15[a]	1.7±0.38[b]	25.7±2.59[a]	19.3±0.62[b]
19	6.2±0.89[a]	0.2±0.15[b]	10.0±2.13[a]	0.9±0.10[b]	13.2±1.76[a]	1.2±0.52[b]	36.4±6.68[a]	20.9±0.62[b]
20	3.4±0.80[a]	0.5±0.11[b]	9.1±2.30[a]	0.8±0.14[b]	12.4±1.89[a]	1.4±0.06[b]	36.4±7.54[a]	19.0±0.49[b]

[a,b]Means in the same columns of each parameter with different superscript alphabets are statistically (p □ 0.05) different.

Table 1b: Sperm morphological abnormalities of Yankasa rams prior to and post infection with *Trypanosoma evansi*

WEEKS	Coiled Tails (%)		Acrosomal abnormalities (%)		Proximal Cytoplasmic Droplets (%)		Distal Cytoplasmic Droplets (%)	
	GROUP A	B	GROUP A	B	GROUP A	B	GROUP A	B
1	0.7 ± 0.21	0.6±0.24	1.7 ± 0.25	1.4 ±0.44	0.36 ± 0.12	0.4 ±0.17	0.1 ±0.05	0.2±0.1
2	0.8±0.29	1.2±0.52	2.5 ± 0.41 [a]	1.3 ± 0.35[b]	0.36±0.07	0.5±0.15	0.0±0.00	0.2 ± 0.1
3	0.2±0.14	1.0±0.65	2.1 ± 0.22	2.1 ± 0.26	0.5±0.16	0.2±0.12	0.1±0.07	0.0±0.00
4	0.6±0.24	1.0±0.50	2.2 ± 0.22	1.6 ± 0.51	0.3±0.12	0.6±0.16	0.1±0.05	0.0±0.00
5	0.8±0.24	0.7±0.35	1.1 ± 0.23 [a]	2.2 ± 0.35[b]	0.11±0.07	0.3±0.07	0.0±0.00	0.0±0.00
6	0.8±0.26	1.1±0.7	1.1 ± 0.10	1.1 ± 0.41	0.3 ±0.08	0.0±0.00	0.04±0.00	0.0±0.00
7	1.2±0.5	0.2±0.17	2.5 ± 0.36 [a]	2.1 ± 0.29	0.3±0.07	0.2±0.07	0.08 ± 0.08	0.1 ± 0.06
8	1.5±0.37	0.5±0.30	2.8 ± 0.45	2.8 ± 0.45	0.4±0.14	0.3±0.14	0.04 ± 0.04	0.05 ± 0.05
9	3.4±0.52 [a]	0.2±0.09 [b]	5.5 ± 0.76	2.0 ± 0.34	1.1±0.24 [a]	0.1±0.06 [b]	0.29±0.07	0.0±0.00
10	5.6±1.19 [a]	0.3±0.25 [b]	7.5 ± 0.58 [a]	3.0 ± 0.22[b]	1.5±0.23 [a]	0.1±0.06 [b]	0.2±0.06	0.0±0.00
11	7.1±1.36 [a]	0.7±0.29 [b]	8.9 ± 1.01 [a]	2.4 ± 0.35[b]	1.6±0.41 [a]	0.2±0.09 [b]	0.2 ± 0.09	0.05 ± 0.05
12	9.5±1.62 [a]	1.3±0.29 [b]	7.6 ± 0.75 [a]	1.4 ± 0.13[b]	2.3±0.29 [a]	0.2±0.09 [b]	0.2 ± 0.04[a]	0.05 ±0.05[b]
13	7.6±1.82 [a]	0.6±0.2 [b]	8.3 ± 0.53 [a]	2.1 ± 0.09[b]	1.9±0.57 [a]	0.3±0.12 [b]	0.2 ± 0.09 [a]	0.05 ± 0.05 [b]
14	9.5±1.85 [a]	0.7±0.30 [b]	8.5 ± 0.83 [a]	1.6 ± 0.17[b]	1.0±0.29	0.2±0.07	0.3 ± 0.10 [a]	0.05 ± 0.05 [b]
15	8.8±1.66 [a]	1.4±0.56 [b]	9.5 ± 1.51 [a]	2.5 ± 0.29[b]	2.6±0.41 [a]	0.3±0.19 [b]	0.3 ± 0.16	0.10 ±0.10
16	7.1±2.02 [a]	0.9±0.25 [b]	9.0 ± 0.80 [a]	2.6 ± 0.22[b]	1.8±0.59 [a]	0.3±0.1 [b] 9	0.3±0.09 [a]	0.0±0.00 [b]
17	9.4±1.85 [a]	0.6±0.20 [b]	10.5 ± 0.79 [a]	2.1 ± 0.20[b]	1.6±0.42 [a]	0.1±0.1 [b]	0.2±0.09 [a]	0.0±0.00 [b]
18	11.3±4.15 [a]	1.0±0.38 [b]	8.7 ± 1.03 [a]	3.0 ± 0.15[b]	2.3±0.52 [a]	0.4±0.06 [b]	0.2±0.06 [a]	0.0±0.00 [b]
19	16.5±1.95 [a]	0.7±0.25 [b]	9.8 ± 0.28 [a]	2.5 ± 0.35[b]	1.6±0.28 [a]	0.1±0.06 [b]	0.2 ± 0.06	0.1 ± 0.06
20	13.4±4.62[a]	1.0±0.38 [b]	7.4 ±1.65 [a]	2.8 ±0.24 [b]	2.3 ±0.46 [a]	0.1 ±0.06 [b]	0.2±0.05 [a]	0.05± [b]

[a,b]Means in the same columns of each parameter with different superscript alphabets are statistically (p □ 0.05) different.

Key: Group A- Infected Group
Group B- Control Group

4.8.4.8 Live / Dead Sperm Ratio.

There was a gradual decrease in the mean values of live sperms from a pre infection value of 96.6 ± 0.61% to 32.3 ± 15.17% post infection in the infected compared to the control with a range between 94.0 ± 0.84% and 99.6 ± 0.25%. The difference was statistically significant (p□0.05). Table 1 shows the trend.

Table 2. Live / Dead sperm ratio of Yankasa rams pre and post experimental infection with ***Trypanosoma evansi***

WEEKS	INFECTED	CONTROL
1	98.0 ± 0.49	98.6 ± 0.87
2	99.3 ± 0.36a	96.8 ± 0.86 b
3	98.0 ± 0.72	96.3 ± 2.46
4	96.9 ± 0.63	98.0 ± 1.08
5	99.6 ± 0.30	99.6 ± 0.25
6	96.6 ± 0.61	96.2 ± 0.74
7	96.2 ± 0.60	97.0 ± 0.45
8	96.4 ± 0.69	96.6 ± 0.98
9	69.3 ± 7.00 a	95.4 ± 0.68 b
10	57.0 ± 10.75 a	96.6 ± 1.17 b
11	51.1 ± 8.76 a	97.4 ± 0.68b
12	48.7 ± 9.70 a	97.4 ± 0.25 b
13	41.8 ± 10.35 a	96.6 ± 0.68 b
14	36.5 ± 10.22 a	97.6 ± 0.25 b
15	40.2 ± 9.81 a	96.0 ± 1.14 b
16	33.8 ± 11.44 a	94.0 ± 0.84b
17	36.4 ± 10.24 a	98.4± 0.75 b
18	33.6 ± 7.95 a	97.4± 0.40 b
19	40.3 ± 7.88 a	98.4± 0.75 b
20	32.3 ±15.17 a	97.0 ±0.45 b

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p □ 0.05) different

4.9 Pathological Observation

4.9.1 Gross pathological observations

At necropsy, all infected rams showed non-specific changes including emaciation, shrunken scrotum, pale mucous membranes, enlarged and edematous lymph nodes, depleted coronary fats. Ram 4 and 16 had areas of necrosis in the abomasum. Plates 17-21 show some of these gross pathological changes.

4.9.2 Histopathological observations

Epididymis: There was extensive fibroplasia and necrosis of epididymal cells. Many segments of the tubules were devoid of sperm reserves. There was also infiltration of inflammatory cells in the interstices. (Plates 23, 25 and 27)

Testes: The testes showed extensive fibrosis and thickening of the seminiferous tubules, with necrotized spermatogonic cells. Apparently normal seminiferous tubules were either devoid of spermatids or had scanty spermatids. In-addition, interstitial oedema and calcification of the semiferous tubules and chronic orchitis were observed. (Plates 22, 24 and 26).

Other organs: The sections of the liver showed diffuse congestion, perivascular infiltration of inflammatory cells and multifocal areas of coagulative necrosis, calcification and slight vacuolation. In the kidneys, the observed histological lesions included, congested blood vessels, diffuse renal tubular necrosis and enlargement of the glomerulus (glomerulonephritis) in most cases obliterating the entire Bowman's capsules. Infiltration of inflammatory cells and congestion was also noticed. The heart showed areas of multifocal necrosis of myocardial fibres and infiltration of inflammatory cells (macrophages) while showing diffuse congestion . There was severe congestion and collapse of the alveoli, infiltration of alveolar

macrophages, edematous alveoli and presence of fluid within the bronchioles of the lungs.

Plate 1: Newly acquired experimental rams in good condition before infection

Plate 2: Rams showing signs of good libido

Plate 3: Infected ram showing Oedema of the face and lower jaw at day 61 post infection

Plate 4: A ram showing Emaciation and Listlessness at day 86 post infection

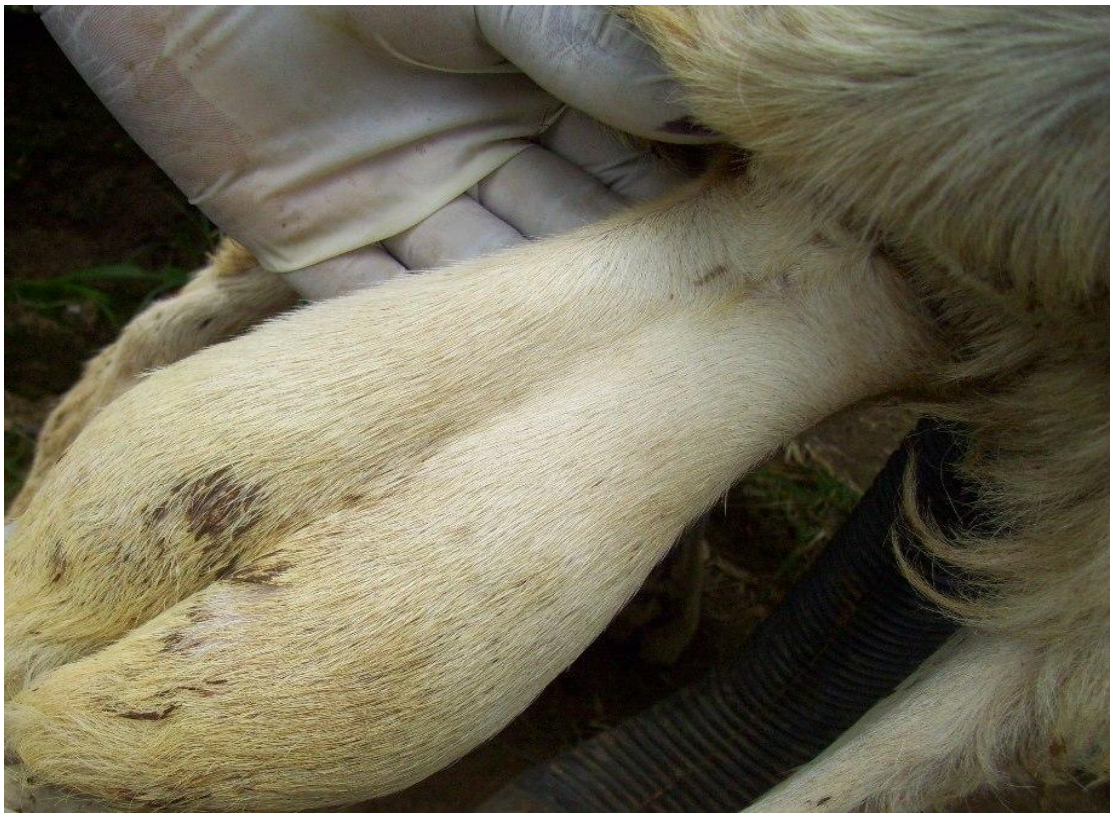

Plate 5: Infected ram showing scrotum lacking scrotal folds at day 44 post infection.

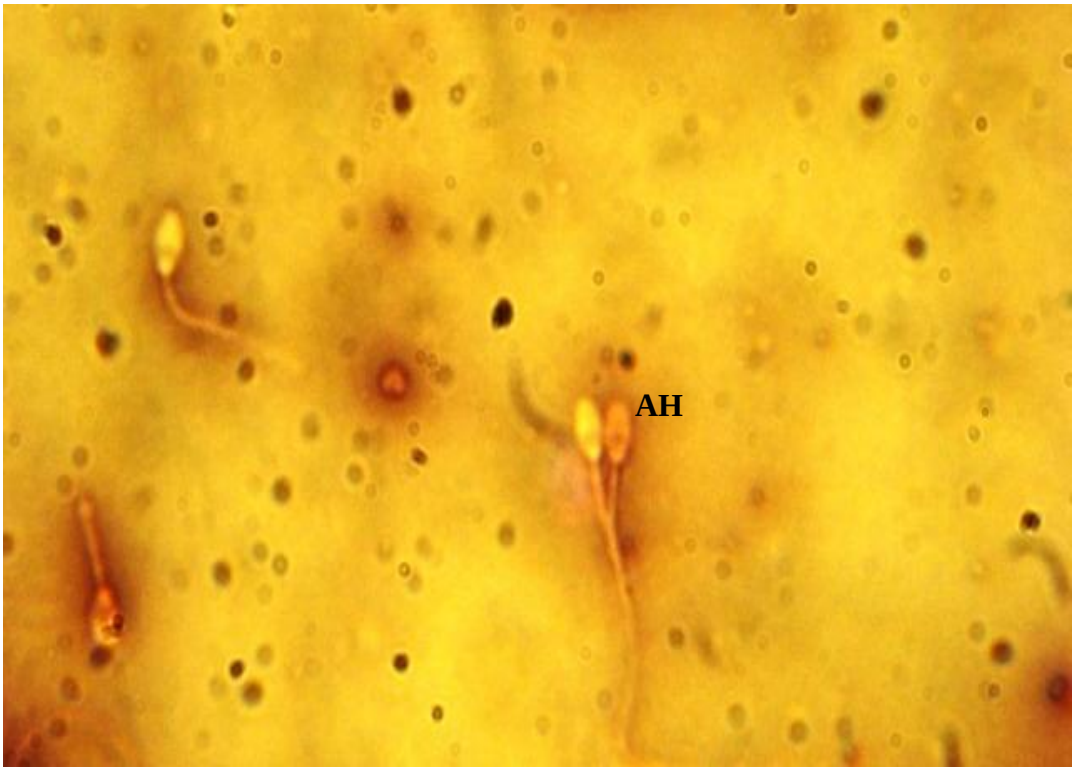

Plate 6: Eosin-Nigrosin stained semen smear from an infected ram showing sperm with Double Head (AH).

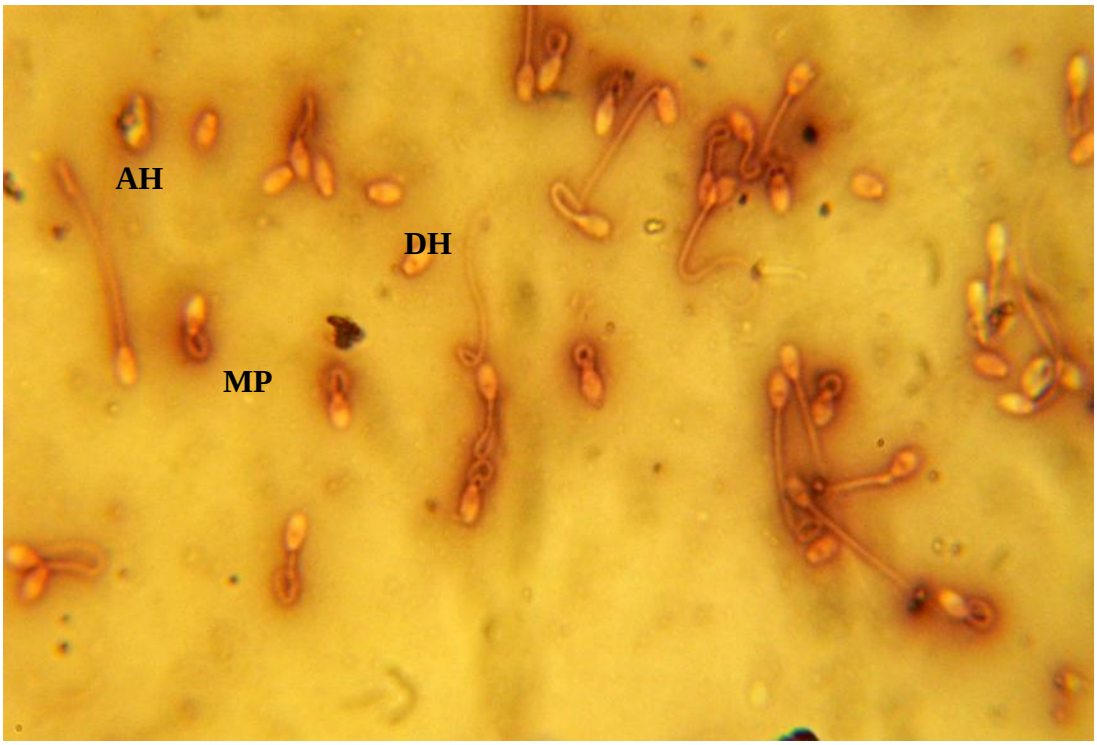

Plate 7: Eosin-Nigrosin stained semen smear from an infected ram showing an abnormally enlarged sperm head(AH); Warped middle Piece (MP) and Detached Head; (DH)

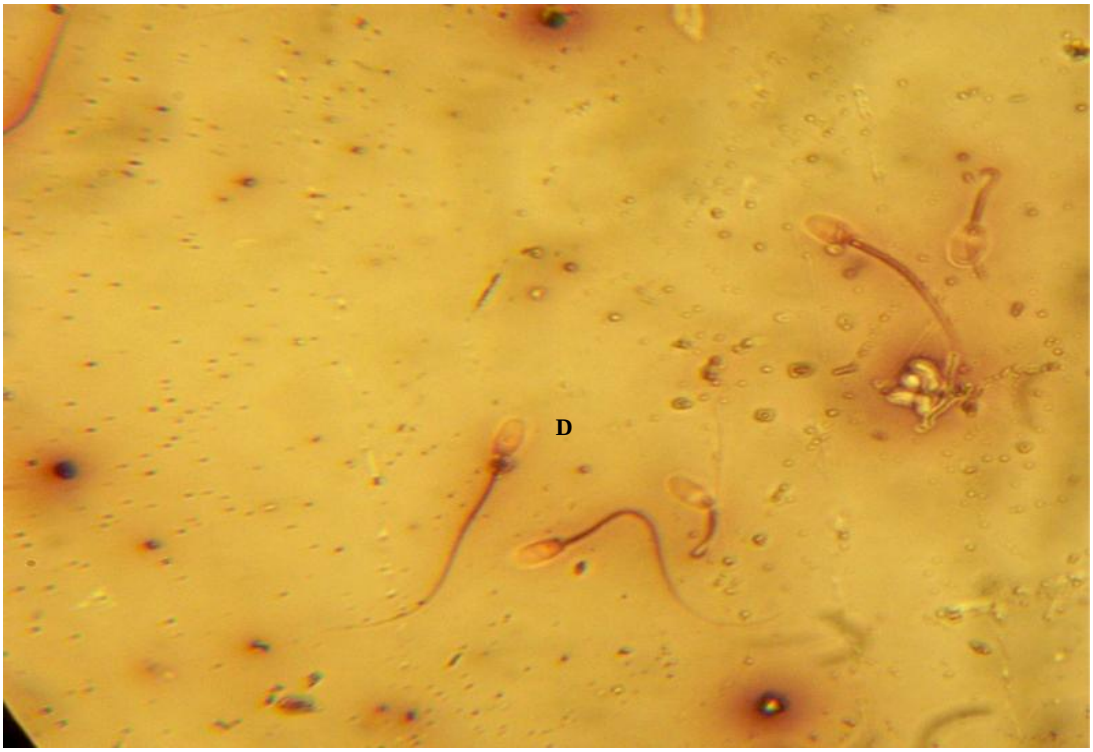

Plate 8: Eosin-Nigrosin stained semen smear from an infected ram showing Sperm with Proximal Cytoplasmic Droplet (D) and Normal Sperm (N)

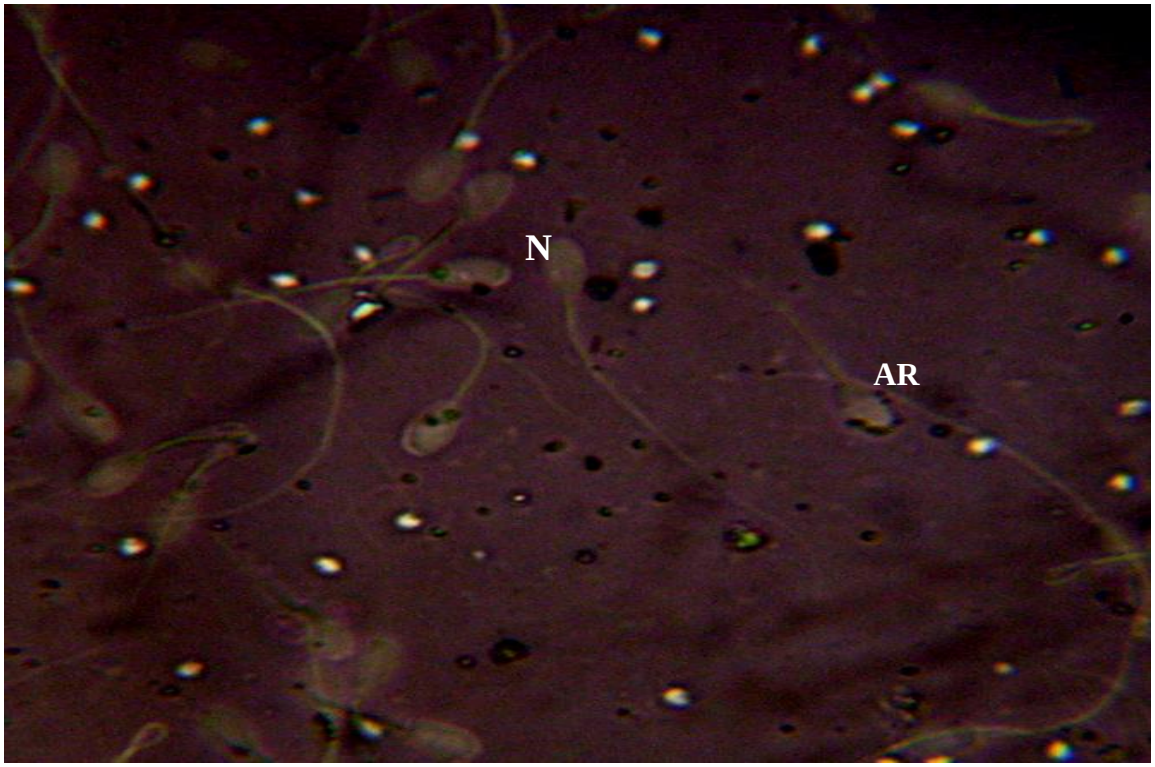

Plate 9: Eosin-Nigrosin stained semen smear from an infected ram showing an abnormal (Serrated) Acrosome (AR) and Normal Sperm (N)

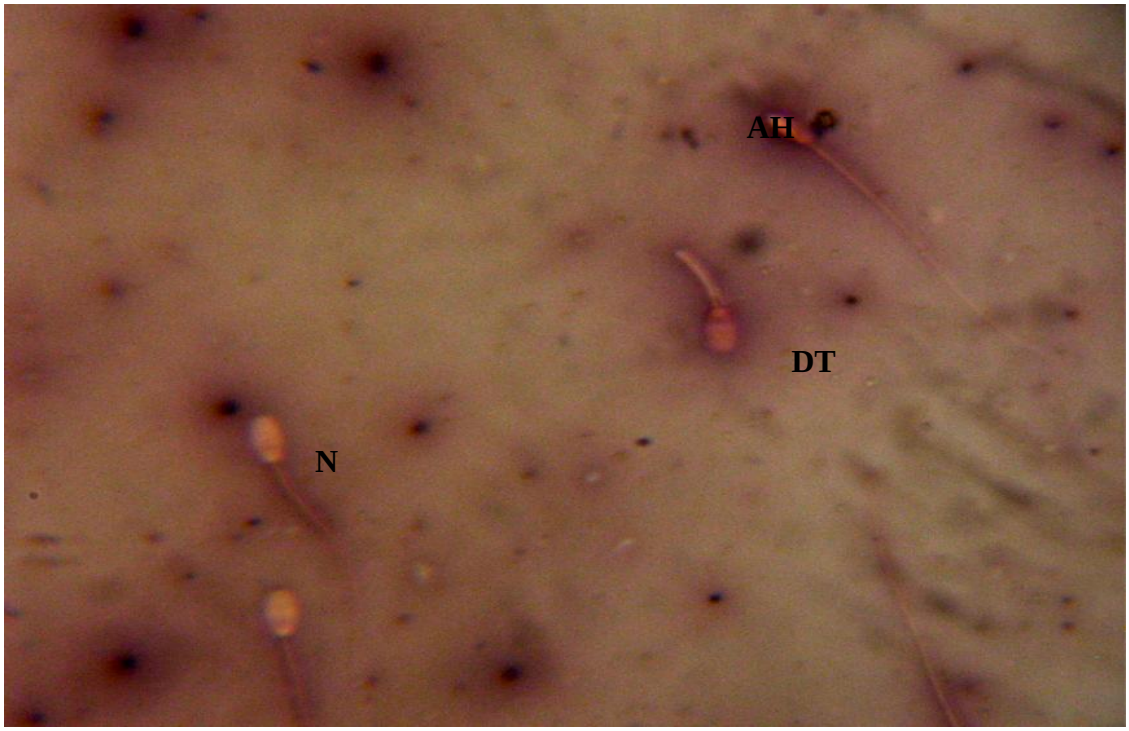

Plate 10: Eosin-Nigrosin stained semen smear from an infected ram showing Abnormal (Narrow Pin) Head (AH); Normal Sperm (N) and Detached Tail (DT)

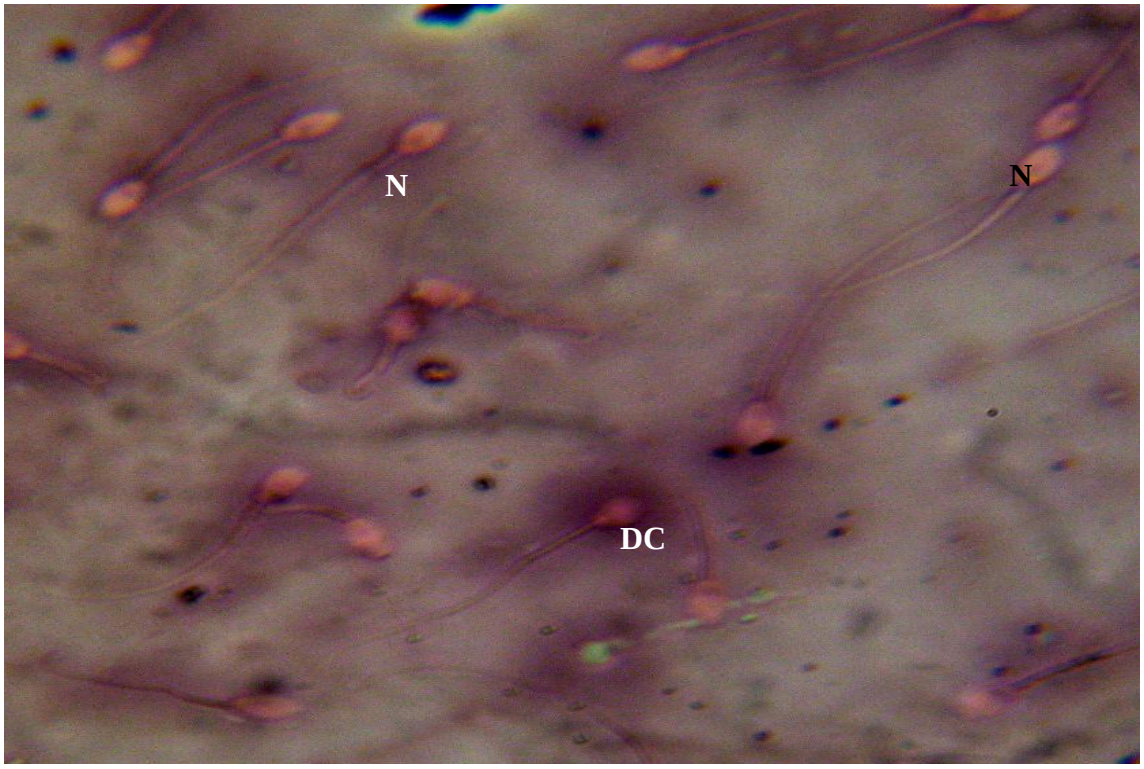

Plate 11: Eosin-Nigrosin stained semen smear from an infected ram showing many Dead Sperm (DC-) and Normal Sperm (N)

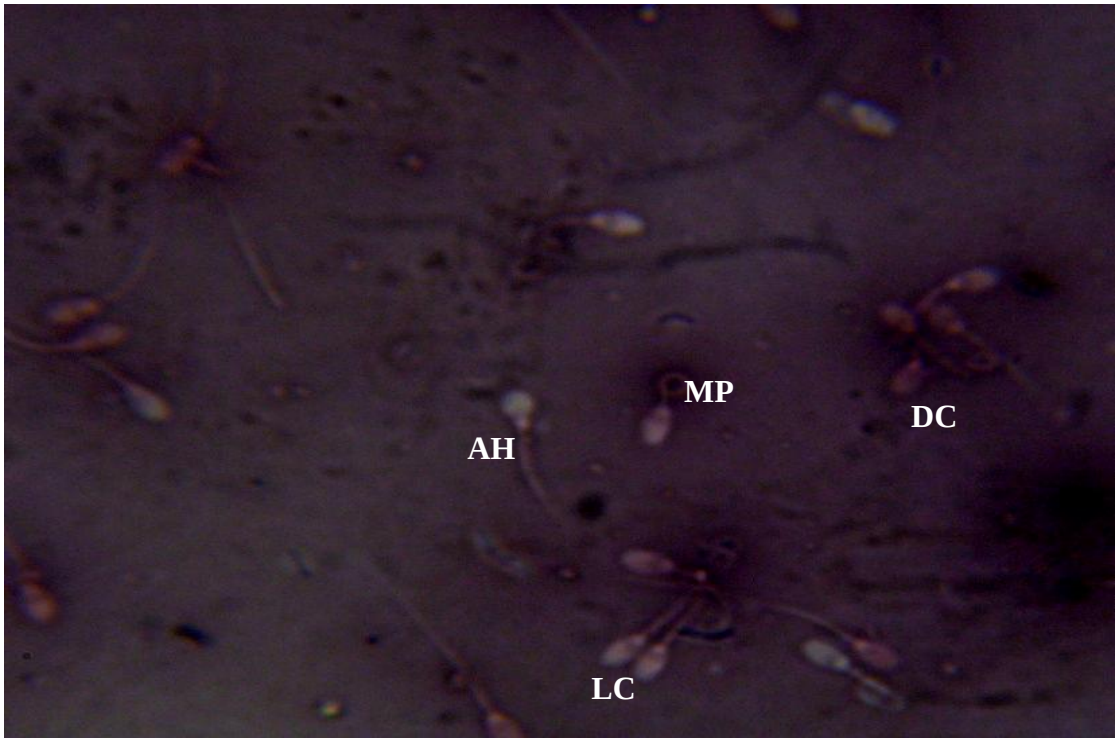

Plate 12: Eosin-Nigrosin stained semen smear from an infected ram showing Abnormal Head (AH); Abnormal (Warped) Middle piece (MP); Dead Sperm (DC) and Live Sperm (LC)

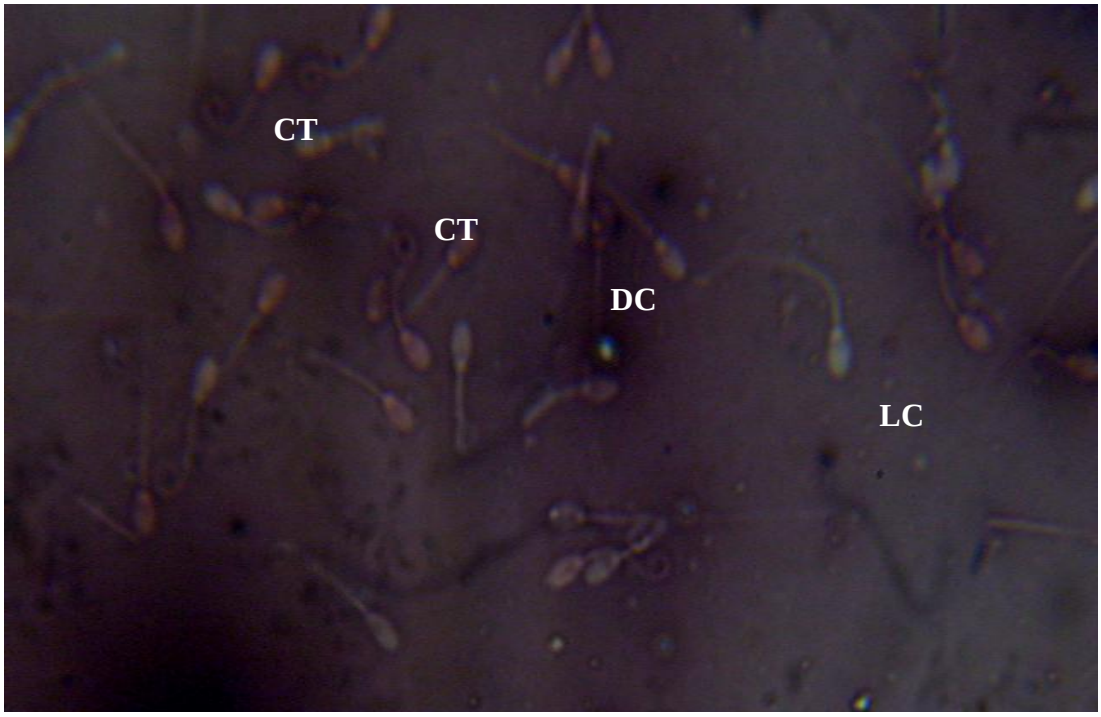

Plate 13: Eosin-Nigrosin stained semen smear from an infected ram showing sperm with Coiled Tail (CT); Dead Sperm (DC) and Live Sperm (LC)

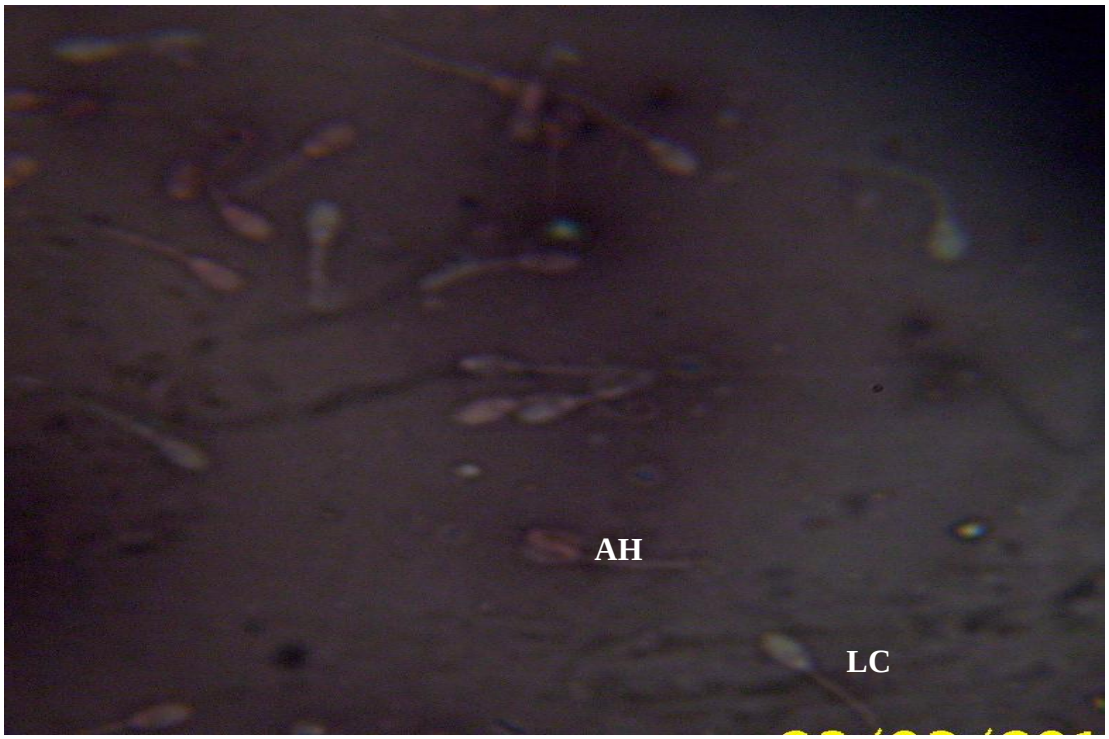

Plate 14: Eosin-Nigrosin stained semen smear from an infected ram showing sperm with Abnormal (Corked) Head (AH); Live Sperm (LC) with Normal Head.

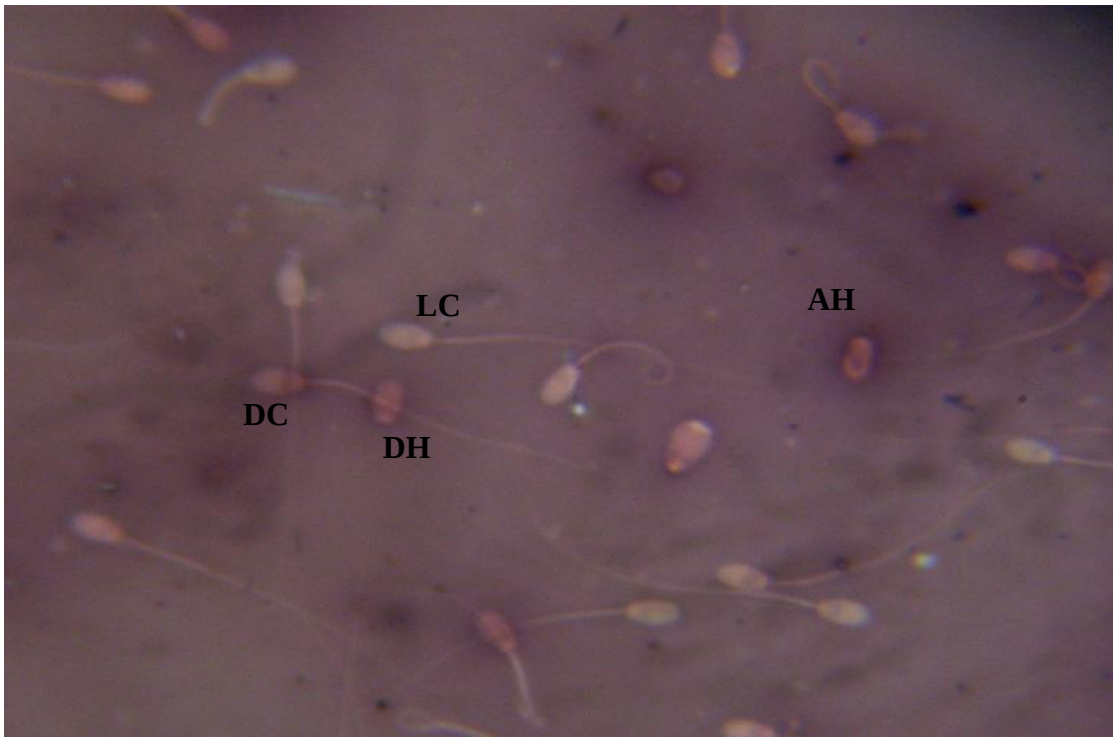

Plate 15: Eosin-Nigrosin stained semen smear from an infected ram showing sperm with Abnormal (Enlarged) Head (AH-); Detached Head (DH-); Live Sperm (LC) and Dead Sperm (DC)

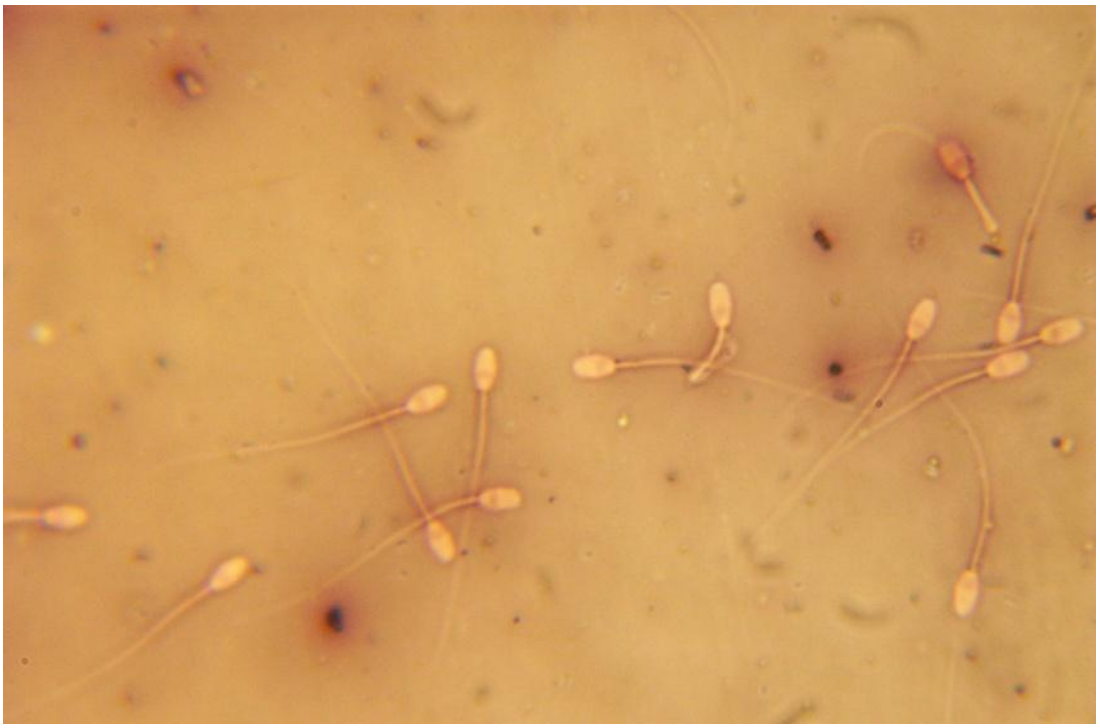

Plate 16: Eosin-Nigrosin stained semen smear from a control ram showing Normal Live Spermatozoa

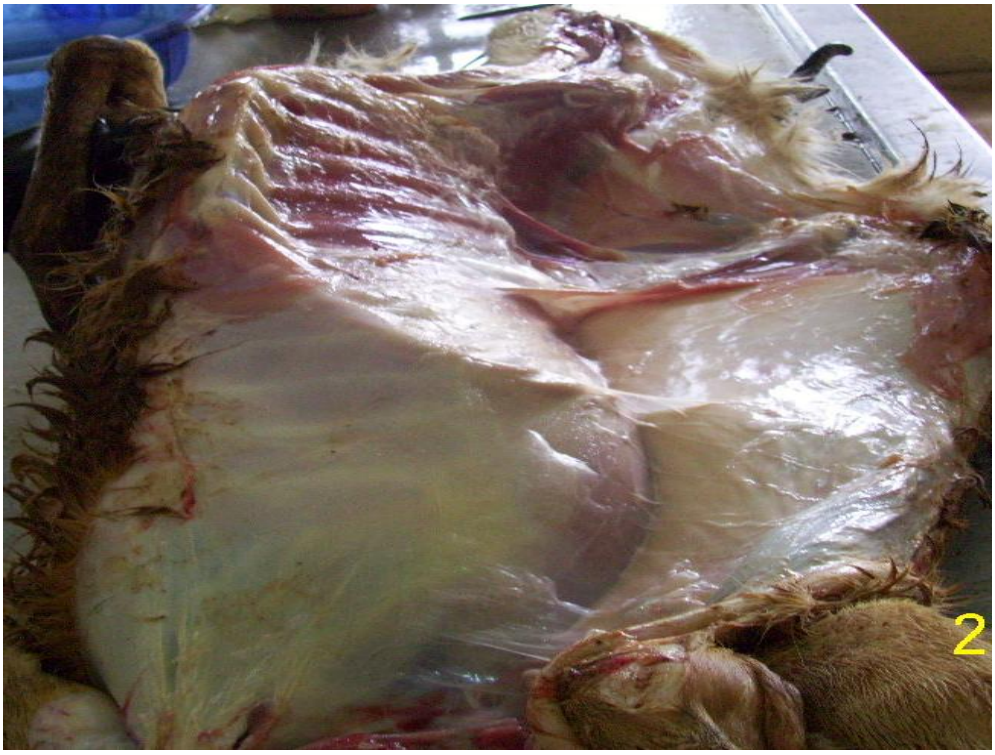

Plate 17: Pale Emaciated Carcass of a *T. evansi*- infected ram

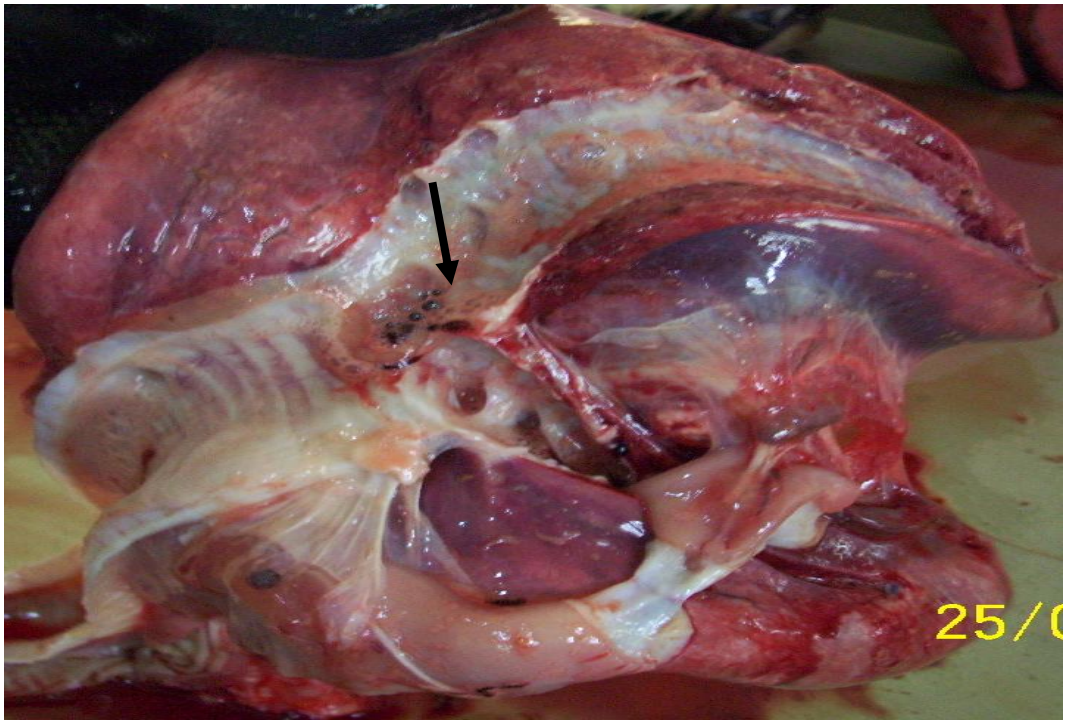

Plate 18: Congested Lungs with Froth in the Trachea and Bronchus from a *T. evansi* infected ram

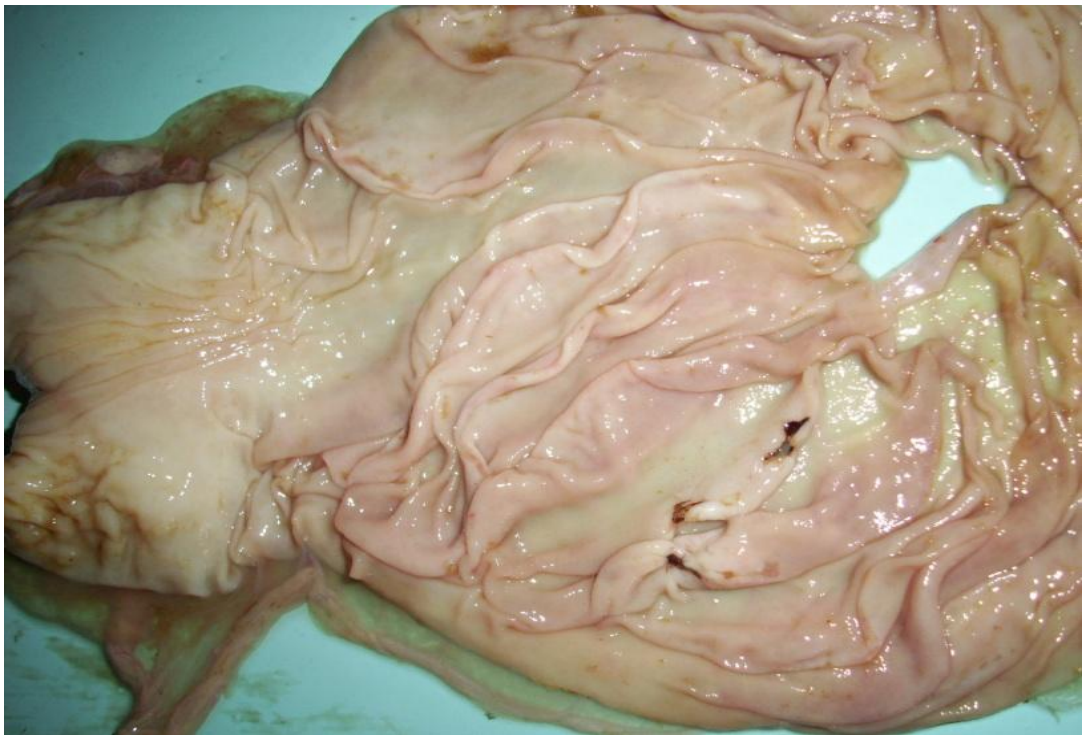

Plate 19: Abomasum with patches of Necrosis from a *T. evansi* infected ram

Plate 20: The Pale Carcass of a *T. evansi* infected Yankasa ram showing enlarged pre-Scapular Lymph Node

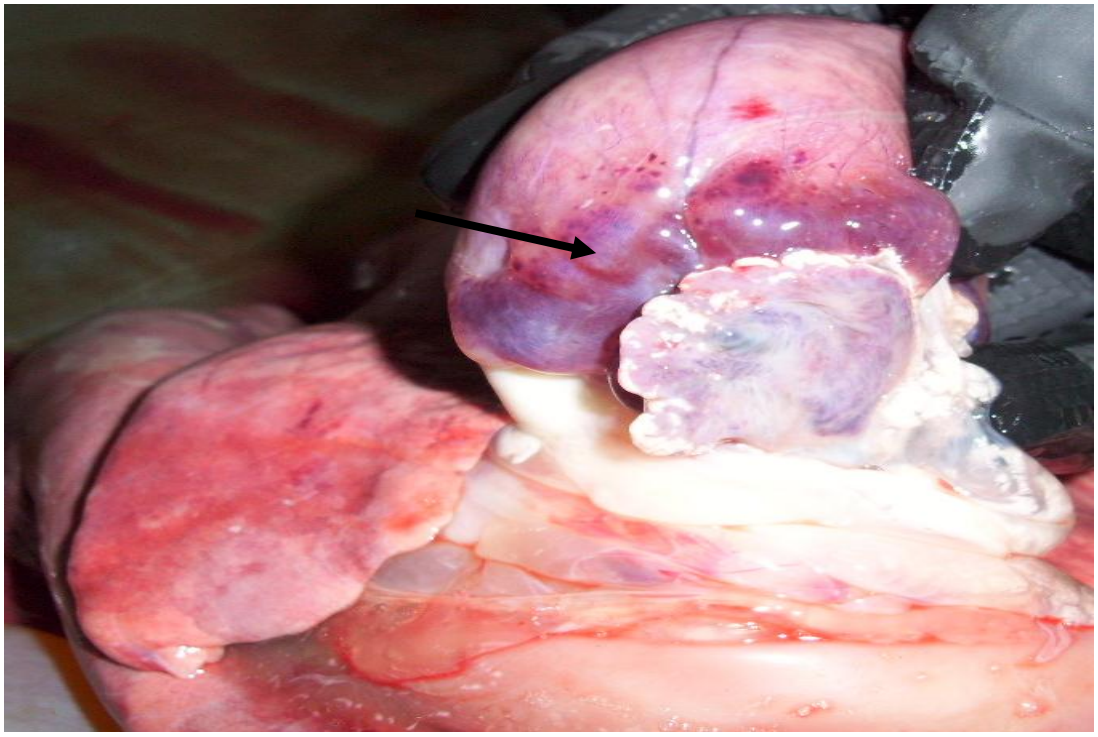

Plate 21: Heart of an infected ram with Depleted Coronary Fats

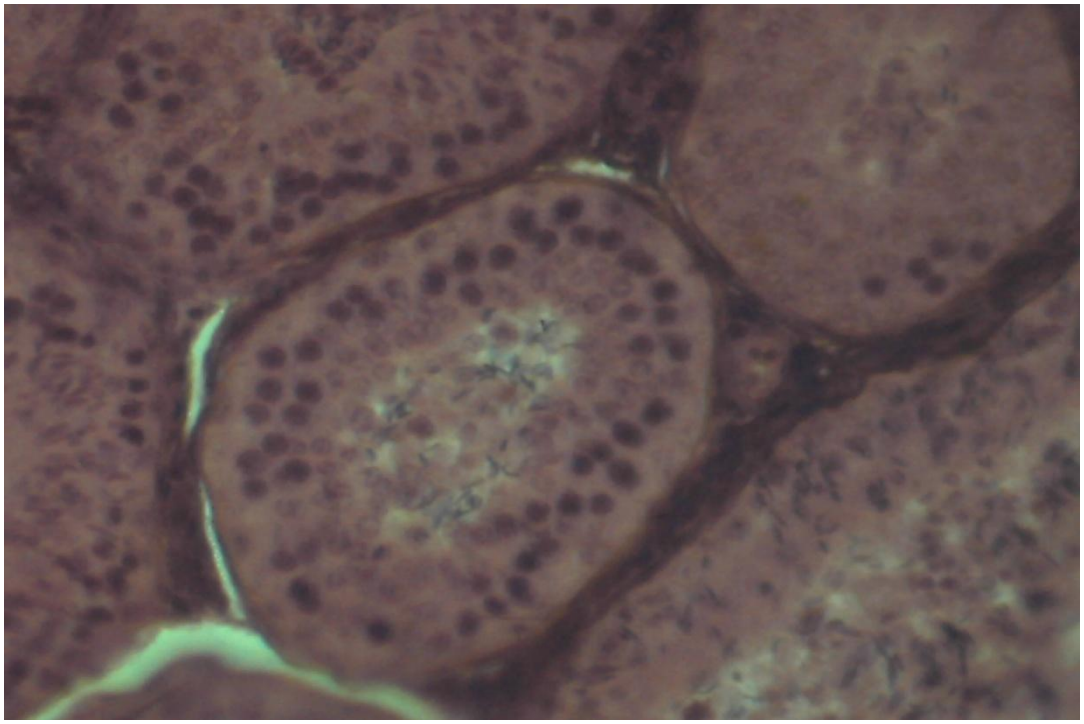

Plate 22: Photomicrograph of the testis of an infected ram 4 that died on day 74 post infection showing moderate necrosis of the spermatogonic cells (H&E stain x 400)

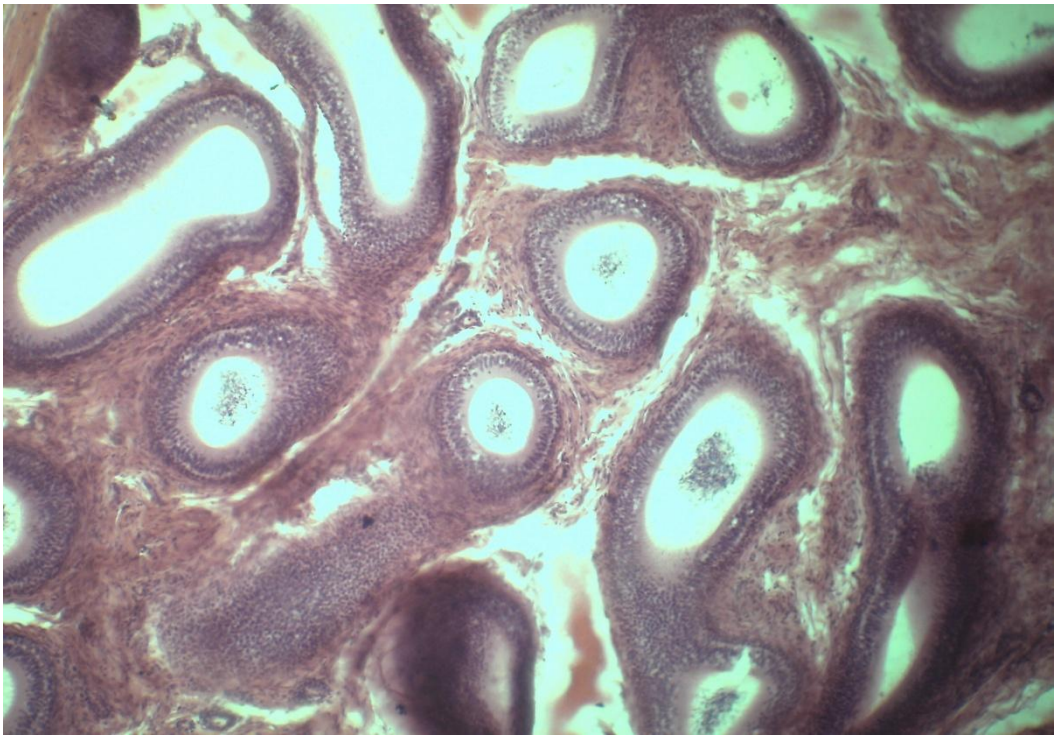

Plate 23: Photomicrograph of the epididymis of ram 4 that died on day 74 post infection showing scanty and depleted epididymal sperm reserves within the tubules (H&E stain x 100)

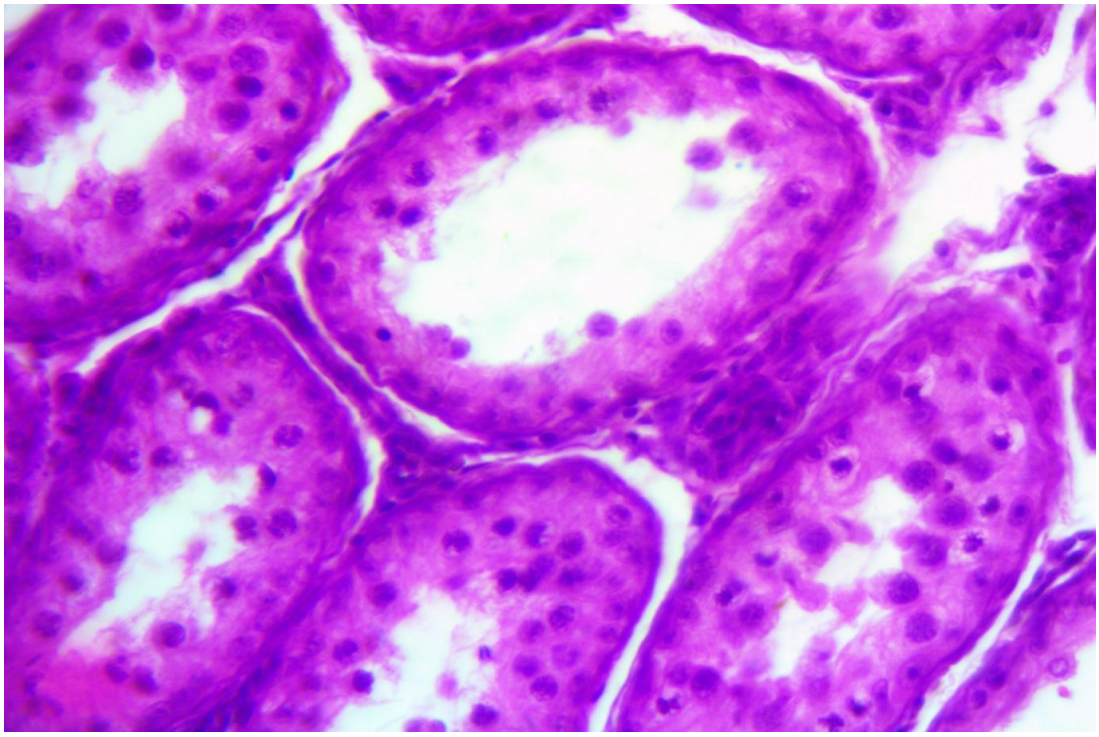

Plate 24: Photomicrograph of the testis of ram 7 that died on day 88 post infection showing extensive fibrosis and thickening of the seminiferous tubules with severe necrosis of the spermatogonic cells (H&E stain x 400)

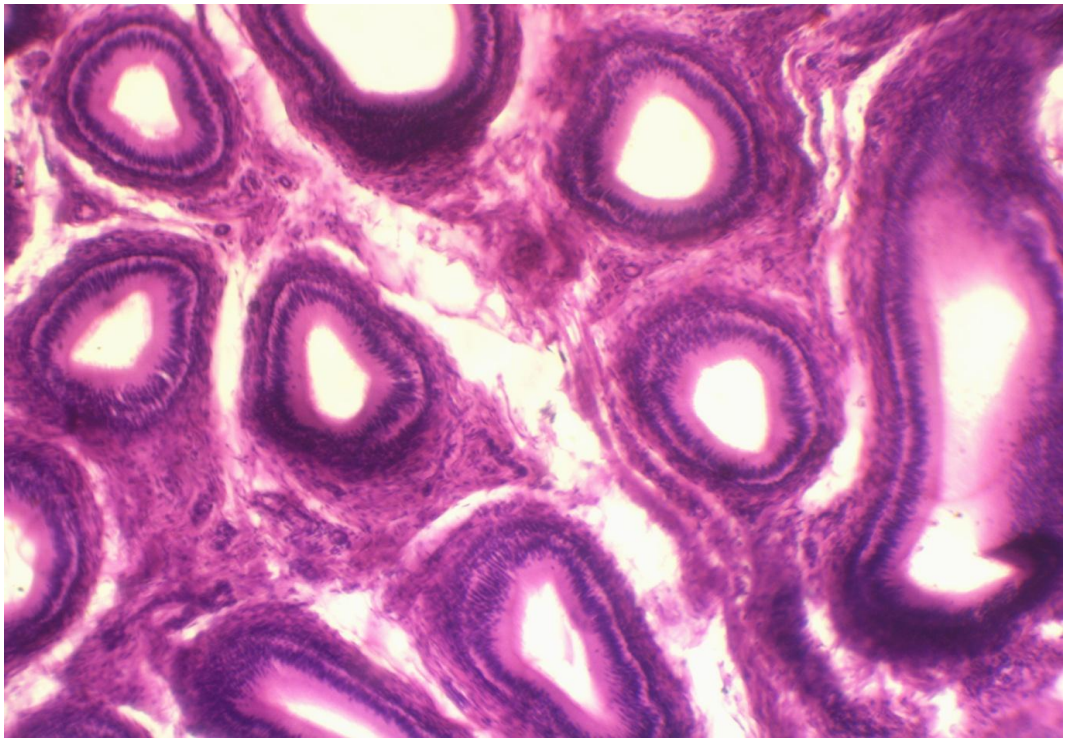

Plate 25: Photomicrograph of the epididymis of ram 7 that died on day 88 post infection showing fibroplasia and necrosis of epididymal epitheliail cells, infiltration of inflammatory cells in the inter spaces with depleted epididymal sperm reserves (H&E stain x)

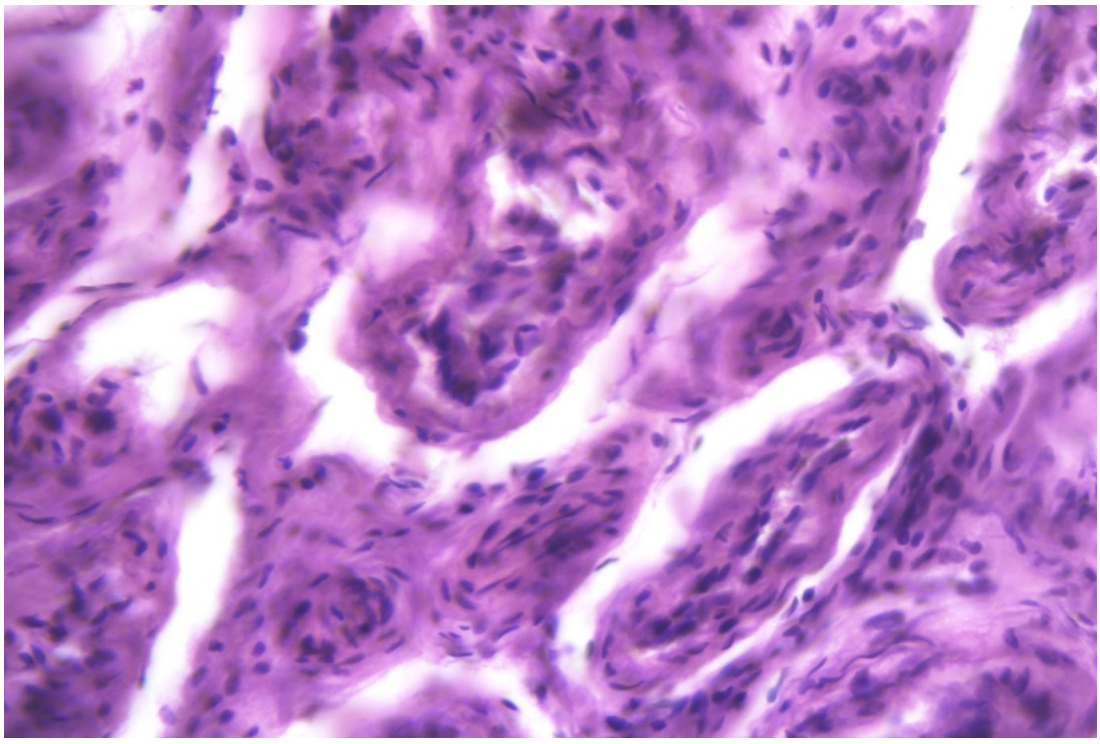

Plate 26: Photomicrograph of the testis of ram 17 that was sacrificed at termination of the study showing extensive fibroplasias and loss of architecture with severe necrosis of the spermatogonic cells (H&E stain x400)

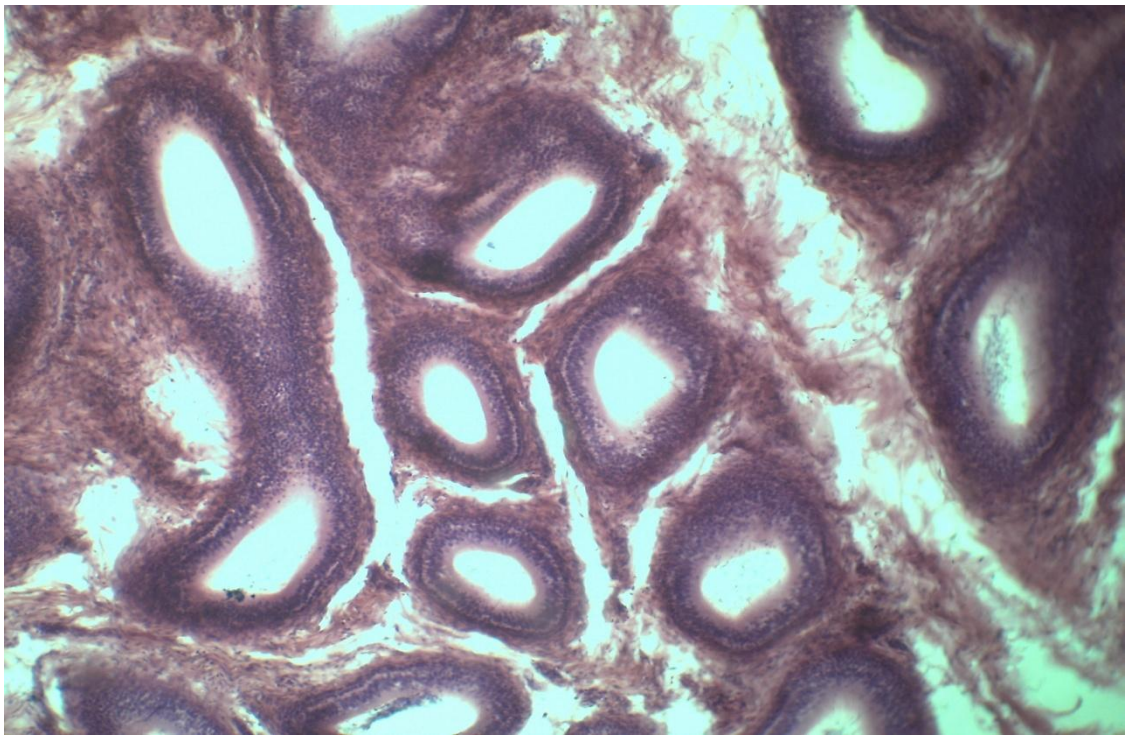

Plate 27: Photomicrograph of the epididymis of ram 17 that was sacrificed at termination of the study showing fibroplasia and necrosis of epididymal cells, infiltration of inflammatory cells in the inter spaces with depleted sperma reserves (H&E stain x400)

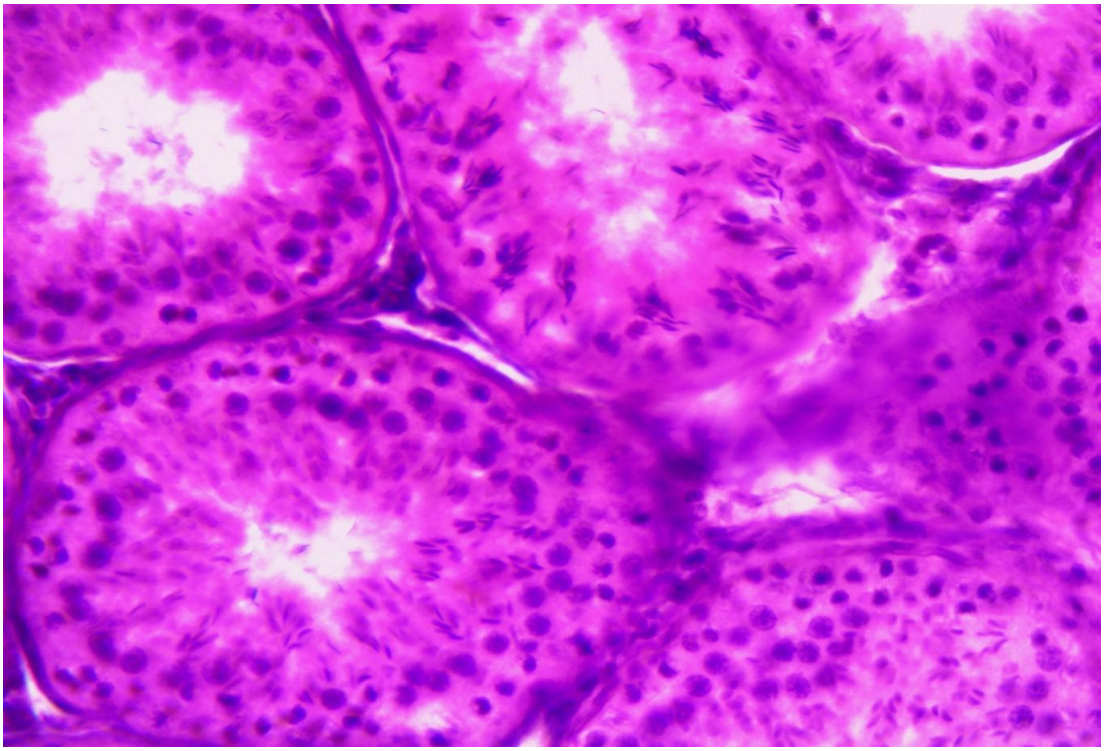

Plate 28: Photomicrograph of the testis of the control animal showing normal architecture (H&E stain x)

CHAPTER FIVE

DISCUSSION

Results of this study showed that *Trypanosoma evansi* infection in Yankasa rams have severe effects on blood parameters and some reproductive parameters.

In the present study, the clinical signs and gross pathological lesions encountered in the infected animals such as straw - coloured fluid in the pericardium and thoracic cavity, diarrhoea, laboured breathing, loss of weight, anaemia, pale mucous membranes, enlarged lymphnode decreased growth rate, unthriftness, corneal opacity, in-coordination, nervous manifestations like convulsion and death in one or more of the infected animal were consistent with the findings of other investigators (Audu *et al.,* 1999.; Marques *et al.,*2000.; Dargantes *et al.,* 2005a, Morales *et al., 2006*).

The rise and fluctuation in temperature of infected rams following inoculation is consistent with *T. evansi* infection in goat (Dargantes *et al.,* 2005a) and may be attributed to the activities of trypanosomes in the circulation and in tissues, triggering the release of pyrogenic cytokines such as tumour necrosis factor α (TNF-α) and interleukins 6 (IL6). The concentration of these cytokines is suggested to be related to the fluctuation in temperature observed (Dargantes *et al.,* 2005a). The undulant fever may as well be a result of toxic metabolites released by dying trypanosomes as suggested by Tizzard *et al*., (1978).

The decrease in the body weight of infected rams while still maintaining normal appetite is an indication of the wasting nature of the disease and agrees with the reports of Audu *et al.*, (1999) and Dargantes *et al.,*(2005a). The decrease in weight may be attributed to increased maintenance requirements due to infection, leading to a decreased nitrogen and energy retention and thus a reduction in weight (Akinbamijo *et al*., 1994, Osaer *et al.,* 1999a). Impaired feed conversion efficiency, increase in metabolizable energy and reduction in the proportion of protein used for growth have been suggested as possible

cause of weight loss (Pathak, 2009, Eghianruwa *et al.,* 2012). Verstegen *et al*., (1991) had reported that fever in trypanosomosis changes energy and nitrogen metabolism.

Anaemia is a consistent feature of trypanosomosis in domestic animals and a common characteristic of the pathogenicity of *Trypanosoma evansi* infection in several animals. The mechanism of anaemia in trypanosomosis is complex and multifactorial in origin (Nassens *et al.,* 2005.; Mbaya *et al.*,2012). Murray and Dexter (1988) reported that the severity and onset of anaemia in trypanosomosis is directly proportional to the appearance of the parasite in peripheral blood and the level of parasitaemia. However, the result of this study disagrees with the assertion. Although, the onset of anaemia in this study coincided with parasite appearance, a persistent low- level parasitaemia characterized this study. The low level parasitaemia in this study have been reported in rabbits, dogs, horses and wild animals infected with *T. evansi* (Dela Rue *et al.,* 2000; Herrera *et al.,* 2004; Colpo *et al.,* 2008). Factors responsible for this low level parasitaemia have not yet been elucidated due to lack of information. However, the strain of the parasite and the method of estimation of parasitaemia might be contributory factors.

The progressive decline in Packed cell volume (PCV) and RBC counts of infected rams in this study is consistent with the findings of several researchers on *T. evansi in* sheep (Onah *et al.,* 1996), goats (Ngeranwa *et al.*, 1993., Sharma *et al*., 2000, Dargantes *et al*., 2005a), donkeys (Cardioli *et al*., 2006), bufalloes (Damayanti *et al*., 1994), dogs (Aquino *et al*., 2002) and cats (Da Silva *et al*., 2009) but contrasts those of Otieno and Gachanya (1976) who found no significant change in any of these blood parameters in goats. This decline in blood parameters of infected rams may be attributed to increase in erythrocyte destruction (haemolysis) from mechanical damage resulting from the movement of trypanosomes within the blood stream and may also result from a massive

erythrophagocytosis from active mononuclear phargocytic system of the hosts (Igbokwe and Nwosu, 1997). Amongst other factors are inadequate erythrocyte productions. Hemolysin and sialidase enzyme produced by *Trypanosoma* species have been fingered in erythrocyte destruction (Fiennes *et al*., 1946, Shehu *et al.*,2006). Nassens *et al*., (2005) described intravascular haemodilution as responsible for anemia in *T.b. rhodensiense* infection in rats. Sharma *et al.,* (2000) suggested that the anemia encountered in Babari goats infected with *T. evansi* could be a result of cumulative effects of haemodilution, extravascular haemolysis and dishaemopoiesis. The mean MCV and MCHC values obtained in the study characterizes a normocytic-normochromic anaemia. This is consistent with *T. evansi* infection as reported in dogs, cats and horses (Franciscato *et al.*, 2007; Da Silva *et al.,* 2009) but contradicts the report of macrocytic normochromic anaemia in experimental *T. evansi* infection in dogs (Aquino *et al*., 2002) and macrocytic hypochromic anaemia in rabbits (Da Silva *et al*.,2008).

The transient increase observed in leucocyte counts in this study contradicts the report of Aquino *et al*. (2002) who had reported leukopaenia with neutropaenia in experimental *T. evansi* infection in dogs but corroborates the report of leucocytosis with lymphocytosis in experimental *T. evansi* infected horses (Monzon *et al*., 1991), and sheep (Onah *et al*., 1996). Chaudhary and Iqbal in experimental *T. evansi* infection in camels and Marques *et al.,*

(2000) in horses both reported a decrease in lymphocyte which concurs with the observation in this study. The eosinophilia noticed in this study agrees with observations in *T. evansi* infected cattle by Sivajothi *et al.,* (2014) which is a constant feature of parasitic disease often associated with immediate hypersensitivity reactions. Researches have shown no particular pattern for leucocyte changes in T*rypanosoma evansi* infection. The eventual rise in mean total leucocyte counts encountered in this study can be attributed to recovery in infected animals (Allam *et al*.2011).

The transient drop in mean total plasma protein in this study agrees with reports of Losos and Ikede (1972) and Audu *et al*., (1999) Taiwo *et al*., (2003) which corroborates a report by Boero, (1974) that a decrease in plasma protein occurs in chronic stage of the disease. This decrease are suggestive of increased protein breakdown, urea loss, haemodilution and serum extravasations which could cause edema in infected sheep (Audu *et al*., 1999). Cardioli *et al*., (2006) reported decline in serum albumin of *T. evansi* infected donkeys which suggested severe degenerative hepatic changes. An increase in total protein due to increase in serum globulin levels have been reported in trypanosomosis and this usually results from immune response of animals to infection (Anosa and Isoun, 1976; Singh and Gaur, 1983, Rajora *et al*., 1986).

The results of this study revealed that reproduction capacity of Yankasa rams were severely affected by experimental *T evansi* infection. Spermatogenesis is a lengthy sequential process occurring in the seminiferous tubules by which stem cell spermatogonia divide by mitosis to cyclically produce primary spermatocytes that undergo meiosis to produce haploid spermatids, which differentiate into spermatozoa. The reproductive capacity through spermatogenesis is assessed according to the volume, colour, concentration and number of abnormal spermatozoa produced in ejaculates of infected rams.

The prolonged reaction time observed in this study agrees with the results of Sekoni *et al*. (2004) in bulls infected with *T. vivax* and *T. congolense* and Okubanjo *et al*., (2014) in Yankasa rams infected with *T. congolense*. This prolongation of reaction time have been attributed to stress of infection and possible damage to the male genitalia (Sekoni *et al.*, 2004). Another probable cause of this prolongation is the loss of libido (ability to detect and service female on estrus). Libido tends to reduce in trypanosomosis infection (Sekoni

et al., 2004) making animals less responsive to teasing or stimulation (Raheem *et al*., 2009).

The findings regarding semen volume of infected rams compared to the control is in agreement with the report of Okubanjo *et al.,* (2014) who, working with *T. congolense* in Yankasa rams in the same geographical region showed no significant difference in semen volume. However, Sekoni *et al.,* (2004) reported a decrease in semen volume of bulls infected with *T. congolense* and *T. vivax,* Ikede *et al.,* (1979) made a similar report in rams infected with *T. brucei*

Semen concentration in infected rams showed a non-significant decrease compared to those of the control and agrees with the report of Al Qawari *et al*., (2004) who noted lower mean sperm concentration in dromedary camels infected with *T. evansi* while also corroborating Sekoni *et al*. (2004) who reported a progressive decline in semen concentration which tended towards cessation in bulls infected with *T. congolense* and *T. vivax*. The finding contrasts those of Osaer *et al.,* (1997) who reported that *T. congolense* infection in Djallonke rams had no effect on sperm output. The decrease in sperm concentration could be attributed to degeneration of seminiferous tubules, spermatic degeneration in the epididymis and reduction in number of spermatogonic cells in tubular lumen (Adamu *et al.*, 2007; Faccio *et al.,* 2014).

Increase in sperm morphological abnormalities such as dead sperm, skewed and narrow pin heads, detached heads, bent and coiled tail, warped middle piece, proximal and distal cytoplasmic droplets and acrosomal abnormalities were highly significant and show obvious deviation from the accepted values. These findings are similar to previous reports in camels (Al Qawari *et al.,* 2004), bulls (Sekoni *et al*., 2004), rams (Okubanjo *et al.,* 2014) and goats (Raheem *et al.,* 2009). Progressive increase in morphological abnormalities in this study may be due to impairment of spermatogenesis resulting from

several factors including fluctuating pyrexia and severe gonadal degeneration resulting from this. The ability of the *Brucei* group of trypanosomes to invade tissues and localize in organs thereby causing severe damage is widely recognized (Raheem *et al.*,2009*)*.

The rams in the infected group showed flabby and shrunken testes with no gross pathological signs. Histologically, moderate to severe testicular degeneration, epididymitis, hyperplasia of the epithelium and wide spread infiltration by mononuclear inflammatory cells were observed. These lesions could be associated with the presence of the parasite in these tissues and anoxic condition created in these tissues as a result of the persistent anaemia. *T. evansi* is a member of the Brucei group of trypanosomes known to have preference for testicular and epididymal tissues in addition to their tissue dwelling affinity (Ashman and Seed, 1974). It is suggested to participate in the pathophysiological mechanism of the reproductive damage. Tissue anoxia is created by the persistent anaemia resulting in a fall in tissue pH and vascular damage (Eyob *et al.,* 2013). Further tissue damage is enhanced by the large quantities of cytoplasmic and mitochondrial enzymes released into the blood stream by dying trypanosomes (Eyob *et al.,* 2013). Another factor implicated in tissue and organ degenerative changes is oxidative stress imposed by trypanosomes and macrophageal activities (Igbokwe *et al.,* 1997; Umar *et al.*,2009)

Most effects of trypanosomosis on male reproduction stems from the circulatory disturbances in the scrotum and testes resulting from pyrexia and reduction in temperature gradient between the testes and body which may also alter testosterone synthesis and secretion (Mutayoba *et. al.,* 1995b, Jeffcoate and Holmes, 1997).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

This study showed that the isolate of *Trypanosoma evansi* used was pathogenic, causing severe effects in infected Yankasa rams. Parasitaemia was low but persistent. Packed cell volume, erythrocyte counts and total protein, were significantly lowered, while, white blood cell counts were altered by the infection. Semen volume, semen concentration,

sperm motility, live and dead % ratio and morphological sperm abnormalities, such as abnormal /detached heads, mid-piece abnormalities, cytoplasmic droplets, coiled and bent tail, were significantly increased as a result of the infection. Testicular circumference and body weight were also significantly reduced during the period of infection. The infection also caused death in some infected rams.

6.2 Conclusion

In conclusion, the study has demonstrated that exposure of Yankasa rams to chronic trypanosomosis caused by *T. evansi* induced a diseased state characterized by a normocytic normochromic anaemia, judged by the severe depression in the haematological parameters monitored and reproductive problems reflected by the surge observed in sperm morphological abnormalities, increase in reaction time and decrease in sperm concentration of semen. The magnitude of these deviations from normal in infected rams is capable of hampering the reproductive performance thus productivity of this breed since reproduction is an integral part of production. Therefore, it is conceivable that *Trypanosoma evansi* infection poses great reproductive risk to Yankasa breed of sheep and other animals that are been herded with these animals as is becoming the practice in areas in and around the Guinea Savannah region of Nigeria.

6.3 Recommendations

It is therefore recommended that a thorough and wholesome screening is carried out when selecting or buying breeding rams from open markets in endemic areas. This work could not assess the effect of treatment on semen characteristics of infected rams, it is therefore suggested that studies to identify these effects be carried out. Farmers should ensure that adequately steps are taken to control vectors involved in spread of this organism so as to minimize spread to other animals, especially when these small

ruminants are herded with other animals. There should be provision of adequate veterinary services in strategic areas where camels, the major host of *T. evansi* are being herded with other small ruminants. Finally, further studies should be carried out on ewes to establish the effect of *Trypanosoma evansi* on reproduction.

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APPENDIX I

DAILY TEMPERATURE OF YANKASA RAMS POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

DAYS	INFECTED	CONTROL
1	37.9 ± 0.10	38.0 ± 0.07
2	38.1 ± 0.06	38.1 ± 0.10
3	38.1 ± 0.06	38.2 ± 0.10
4	38.1 ± 0.07	37.9 ± 0.05
5	38.1 ± 0.03	38.1 ± 0.05
6	38.2 ± 0.06	38.1 ± 0.07
7	38.3 ± 0.08 [a]	38.0 ± 0.05 [b]
8	38.5 ± 0.11 [a]	37.9 ± 0.15 [b]
9	39.0 ± 0.15 [a]	38.1 ± 0.04 [b]
10	39.1 ± 0.23 [a]	38.1 ± 0.08 [b]
11	38.9 ± 0.17 [a]	38.1 ± 0.12 [b]
12	39.1 ± 0.17 [a]	38.0 ± 0.16 [b]
13	38.6 ± 0.24	38.0 ± 0.03
14	39.2 ± 0.22 [a]	38.3 ± 0.06 [b]
15	38.6 ± 0.18 [a]	38.1 ± 0.05 [b]
16	39.1 ± 0.20 [a]	38.2 ± 0.11 [b]
17	39.0 ± 0.30 [a]	38.2 ± 0.13 [b]
18	39.1 ± 0.24 [a]	38.2 ± 0.15 [b]
19	38.9 ± 0.18 [a]	38.2 ± 0.06 [b]
20	39.0 ± 0.14 [a]	38.2 ± 0.06 [b]
21	39.0 ± 0.12 [a]	38.2 ± 0.10 [b]
22	38.9 ± 0.12 [a]	38.1 ± 0.11 [b]
23	38.9 ± 0.21 [a]	38.1 ± 0.05 [b]
24	38.8 ± 0.37	38.2 ± 0.12
25	39.2 ± 0.27 [a]	38.1 ± 0.07
26	39.1 ± 0.14 [a]	37.9 ± 0.12 [b]
27	39.3 ± 0.25 [a]	38.2 ± 0.06 [b]
28	38.9 ± 0.09 [a]	37.9 ± 0.06 [b]
29	38.8 ± 0.23 [a]	38.0 ± 0.08 [b]
30	38.8 ± 0.15 [a]	37.6 ± 0.04 [b]
31	39.0 ± 0.22 [a]	38.0 ± 0.04 [b]
32	39.2 ± 0.22 [a]	38.0 ± 0.02 [b]
33	39.1 ± 0.28 [a]	38.1 ± 0.07 [b]
34	39.0 ± 0.24 [a]	37.9 ± 0.06 [b]
35	38.7 ± 0.17 [a]	38.0 ± 0.10 [b]
DAYS	INFECTED	CONTROL
36	38.8 ± 0.12 [a]	38.1 ± 0.11 [b]
37	38.9 ± 0.14 [a]	38.0 ± 0.03 [b]

38	38.8 ± 0.12 [a]	38.1 ± 0.08 [b]
39	38.8 ± 0.13 [a]	38.2 ± 0.09 [b]
40	38.9 ± 0.18 [a]	38.2 ± 0.05 [b]
41	38.9 ± 0.26	38.2 ± 0.12
42	38.9 ± 0.17 [a]	38.0 ± 0.06 [b]
43	38.7 ± 0.24	38.3 ± 0.06
44	39.0 ± 0.23 [a]	38.1 ± 0.11 [b]
45	38.7 ± 0.13 [a]	38.1 ± 0.08 [b]
46	39.0 ± 0.16 [a]	38.2 ± 0.11 [b]
47	39.0 ± 0.11 [a]	38.2 ± 0.06 [b]
48	39.0 ± 0.12 [a]	38.1 ± 0.08 [b]
49	38.9 ± 0.15 [a]	38.0 ± 0.11 [b]
50	38.6 ± 0.18	38.1 ± 0.11
51	39.2 ± 0.14 [a]	38.2 ± 0.11 [b]
52	39.0 ± 0.16 [a]	38.3 ± 0.21 [b]
53	39.0 ± 0.12 [a]	38.2 ± 0.07 [b]
54	39.0 ± 0.11 [a]	38.2 ± 0.09 [b]
55	38.9 ± 0.13 [a]	38.2 ± 0.09 [b]
56	38.7 ± 0.16 [a]	38.1 ± 0.12 [b]
57	39.1 ± 0.15 [a]	38.2 ± 0.09 [b]
58	38.9 ± 0.15 [a]	38.3 ± 0.09 [b]
59	38.8 ± 0.27 [a]	38.1 ± 0.06 [b]
60	38.9 ± 0.23 [a]	38.1 ± 0.07 [b]
61	38.8 ± 0.20 [a]	38.0 ± 0.08 [b]
62	39.0 ± 0.24 [a]	38.1 ± 0.07 [b]
63	38.8 ± 0.19 [a]	38.1 ± 0.09 [b]
64	39.1 ± 0.14 [a]	38.2 ± 0.04 [b]
65	38.9 ± 0.14 [a]	38.1 ± 0.09 [b]
66	39.0 ± 0.14 [a]	38.2 ± 0.04 [b]
67	38.9 ± 0.18 [a]	38.2 ± 0.09 [b]
68	38.9 ± 0.13 [a]	38.1 ± 0.05 [b]
69	38.9 ± 0.16 [a]	38.2 ± 0.11 [b]
70	38.9 ± 0.15 [a]	38.1 ± 0.08 [b]
71	38.8 ± 0.13 [a]	38.3 ± 0.04 [b]
72	38.6 ± 0.13 [a]	38.1 ± 0.08 [b]
73	38.7 ± 0.22	38.2 ± 0.10
74	38.7 ± 0.26	38.1 ± 0.10
75	38.8 ± 0.05 [a]	38.1 ± 0.07[b]
DAYS	INFECTED	CONTROL
76	38.9 ± 0.11 [a]	38.1 ± 0.08 [b]
77	39.1 ± 0.09 [a]	38.1 ± 0.06 [b]
78	39.0 ± 0.16 [a]	38.2 ± 0.08 [b]
79	38.9 ± 0.10 [a]	38.2 ± 0.15 [b]
80	39.0 ± 0.12 [a]	38.0 ± 0.03 [b]
81	38.9 ± 0.15 [a]	38.2 ± 0.09 [b]

82	38.8 ± 0.09 [a]	38.2 ± 0.08 [b]
83	38.8 ± 0.10 [a]	38.1 ± 0.11 [b]
84	38.9 ± 0.20 [a]	38.1 ± 0.16 [b]
85	38.5 ± 0.05 [a]	38.0 ± 0.07 [b]
86	38.4 ± 0.11	38.1 ± 0.10
87	38.8 ± 0.23	38.4 ± 0.13
88	38.7 ± 0.25	38.4 ± 0.05
89	39.0 ± 0.17 [a]	38.2 ± 0.11 [b]
90	39.0 ± 0.09 [a]	38.2 ± 0.06 [b]
91	38.9 ± 0.08 [a]	38.3 ± 0.09 [b]
92	39.0 ± 0.08 [a]	38.1 ± 0.07 [b]
93	39.0 ± 0.16 [a]	38.1 ± 0.05 [b]
94	39.0 ± 0.22 [a]	38.2 ± 0.11 [b]
95	38.7 ± 0.07 [a]	38.1 ± 0.07 [b]
96	38.8 ± 0.08 [a]	38.0 ± 0.10 [b]
97	38.7 ± 0.12 [a]	38.2 ± 0.06 [b]
98	38.6 ± 0.07 [a]	38.2 ± 0.11[b]
99	39.0 ± 0.08 [a]	38.2 ± 0.07 [b]
100	38.9 ± 0.06 [a]	37.9 ± 0.11 [b]
101	38.9 ± 0.12 [a]	38.2 ± 0.05 [b]
102	38.8 ± 0.07 [a]	38.1 ± 0.06 [b]
103	38.8 ± 0.08 [a]	38.1 ± 0.07 [b]
104	38.7 ± 0.12[a]	37.9 ± 0.04 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX II

PARASITAEMIA SCORE OF YANKASA RAMS POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

DAYS	INFECTED	CONTROL
1	0.00 ± 0.00	0.00 ± 0.00
2	0.00 ± 0.00	0.00 ± 0.00
3	0.00 ± 0.00	0.00 ± 0.00
4	0.00 ± 0.00	0.00 ± 0.00
5	1.43 ± 0.30	0.00 ± 0.00
7	3.14 ± 0.46	0.00 ± 0.00
9	3.57 ± 0.30	0.00 ± 0.00
12	3.29 ± 0.36	0.00 ± 0.00
14	2.71 ± 0.57	0.00 ± 0.00
16	0.71 ± 0.57	0.00 ± 0.00
19	2.17 ± 0.54	0.00 ± 0.00
21	1.14 ± 0.55	0.00 ± 0.00
24	1.29 ± 0.52	0.00 ± 0.00
27	0.29 ± 0.29	0.00 ± 0.00
29	0.57 ± 0.30	0.00 ± 0.00
31	0.14 ± 0.14	0.00 ± 0.00
34	1.57 ± 0.75	0.00 ± 0.00
36	0.14 ± 0.14	0.00 ± 0.00
38	2.67 ± 0.62	0.00 ± 0.00
41	2.29 ± 0.81	0.00 ± 0.00
43	3.83 ± 0.17	0.00 ± 0.00
45	3.33 ± 0.67	0.00 ± 0.00
48	3.33 ± 0.50	0.00 ± 0.00
50	2.83 ± 0.65	0.00 ± 0.00
52	2.67 ± 0.72	0.00 ± 0.00
55	1.50 ± 0.81	0.00 ± 0.00
57	2.40 ± 0.98	0.00 ± 0.00
DAYS	INFECTED	CONTROL
59	0.67 ± 0.67	0.00 ± 0.00
62	0.50 ± 0.22	0.00 ± 0.00

64	0.33 ± 0.21	0.00 ± 0.00
66	1.00 ± 0.45	0.00 ± 0.00
69	0.67 ± 0.33	0.00 ± 0.00
71	0.33 ± 0.21	0.00 ± 0.00
73	0.83 ± 0.54	0.00 ± 0.00
75	0.67 ± 0.33	0.00 ± 0.00
77	0.60 ± 0.40	0.00 ± 0.00
79	0.40 ± 0.25	0.00 ± 0.00
82	0.20 ± 0.20	0.00 ± 0.00
84	0.20 ± 0.20	0.00 ± 0.00
86	0.20 ± 0.20	0.00 ± 0.00
89	0.40 ± 0.25	0.00 ± 0.00
91	0.00 ± 0.00	0.00 ± 0.00
93	0.00 ± 0.00	0.00 ± 0.00
95	0.00 ± 0.00	0.00 ± 0.00
97	0.00 ± 0.00	0.00 ± 0.00
99	0.00 ± 0.00	0.00 ± 0.00
102	0.00 ± 0.00	0.00 ± 0.00
104	0.00 ± 0.00	0.00 ± 0.00
106	0.00 ± 0.00	0.00 ± 0.00
109	0.00 ± 0.00	0.00 ± 0.00

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different.

APPENDIX III

PACKED CELL VOLUME OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	29.3 ± 0.61	28.8 ± 0.66
2	29.0 ± 0.62	29.2 ± 0.74
3	29.3 ± 0.68	29.0 ± 0.71
4	29.4 ± 0.48	29.4 ± 0.51
5	30.1 ± 0.59	29.0 ± 0.45
6	29.4 ± 0.48	29.0 ± 0.71
7	28.9 ± 0.51	29.0 ± 0.71
8	28.4 ± 0.37	28.8 ± 0.66
9	25.3 ± 0.42[a]	28.8 ± 0.58 [b]
10	24.4 ± 1.31 [a]	29.6 ± 0.75 [b]
11	22.0 ± 1.38 [a]	28.0 ± 0.63 [b]
12	21.9 ± 1.26 [a]	28.0 ± 0.84 [b]
13	22.0 ± 1.45 [a]	26.8 ± 1.28 [b]
14	22.0 ± 0.73 [a]	27.6 ± 1.12 [b]
15	21.8 ± 1.25 [a]	26.8 ± 0.80 [b]
16	23.2 ± 1.14 [a]	27.8 ± 0.86 [b]
17	22.2 ± 1.45 [a]	28.2 ± 0.92 [b]
18	21.4 ± 1.63 [a]	27.6 ± 0.68 [b]
19	21.0± 1.45 [a]	28.4 ± 0.98 [b]
20	21.2 ± 1.99[a]	29.4 ± 1.21[b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX IV

ERYTHROCYTE COUNTS OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	871.9 ± 24.75	917.6 ± 35.15
2	869.9 ± 33.83	947.6 ± 73.21
3	886.9 ± 26.96	887.8 ± 45.47
4	893.3 ± 28.76	875.0 ± 39.28
5	917.1 ± 23.13	859.0 ± 36.10
6	877.1 ± 22.55	837.4 ± 38.48
7	905.6 ± 26.04	889.4 ± 32.52
8	856.4 ± 38.77	915.6 ± 23.65
9	723.9 ± 38.11[a]	874.4 ± 18.76 [b]
10	693.7 ± 55.09 [a]	907.8 ± 26.26 [b]
11	593.3 ± 49.68 [a]	837.8 ± 38.83 [b]
12	564.6 ± 42.80 [a]	896.2 ± 57.09 [b]
13	591.6 ± 46.93 [a]	847.2 ± 43.90 [b]
14	553.8 ± 18.34 [a]	778.4 ± 47.05 [b]
15	648.3 ± 39.37	790.0 ± 73.01
16	560.3 ± 47.30 [a]	826.8 ± 50.48 [b]
17	560.8 ± 46.47 [a]	845.0 ± 57.48 [b]
18	592.6 ± 80.10 [a]	853.2 ± 56.55 [b]
19	525.4 ± 45.92 [a]	872.4 ± 60.49 [b]
20	542.2 ± 49.78 [a]	906.4 ± 75.31 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX V

TOTAL PROTEIN OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	5.9 ± 0.11	6.2 ± 0.28
2	6.2 ± 0.19	6.2 ± 0.21
3	6.3 ± 0.29	6.0 ± 0.20
4	6.0 ± 0.14	6.0 ± 0.11
5	6.0 ± 0.09	6.1 ± 0.14
6	6.0 ± 0.13	6.0 ± 0.14
7	6.0 ± 0.17	5.6 ± 0.10
8	5.7 ± 0.06	5.8 ± 0.12
9	5.9 ± 0.17	5.8 ± 0.18
10	5.7 ± 0.20	5.9 ± 0.23
11	5.4 ± 0.21	5.9 ± 0.24
12	5.2 ± 0.21	6.2 ± 0.23
13	5.3 ± 0.29	5.8 ± 0.21
14	5.1 ± 0.19[a]	6.2 ± 0.31 [b]
15	6.1 ± 0.22	5.8 ± 0.12
16	6.1 ± 0.32	5.9 ± 0.04
17	6.2 ± 0.36	5.7 ± 0.16
18	5.7 ± 0.23	5.8 ± 0.16
19	5.6 ± 0.19	5.9 ± 0.10
20	5.9 ± 0.14	5.9 ± 0.08

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX VI

MEAN CORPUSCULAR VOLUME (MCV) OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	33.94 ± 0.47	31.46 ± 0.66
2	33.50 ± 0.66	31.26 ± 1.38
3	33.09 ± 0.36	32.90 ± 1.09
4	33.07 ± 0.74	33.82 ± 1.07
5	32.91 ± 0.48	33.98 ± 1.37
6	33.60 ± 0.55	34.76 ± 0.99
7	31.94 ± 0.61	32.70 ± 0.70
8	33.53 ± 1.30	31.48 ± 0.33
9	35.41 ± 1.55	32.96 ± 0.19
10	35.83 ± 1.51	32.62 ± 0.28
11	37.63 ± 1.19 [a]	33.58 ± 0.86 [b]
12	39.13 ± 1.17 [a]	31.50 ± 0.95 [b]
13	37.49 ± 0.94 [a]	31.64 ± 0.24 [b]
14	39.77 ± 0.70 [a]	35.66 ± 1.04 [b]
15	33.85 ± 1.48	34.74 ± 2.25
16	42.07 ± 1.94 [a]	33.86 ± 1.03 [b]
17	39.88 ± 1.21 [a]	33.74 ± 1.43 [b]
18	37.54 ± 2.67	32.86 ± 2.03
19	40.26 ± 1.18 [a]	32.98 ± 1.59 [b]
20	39.14 ± 0.92 [a]	32.90 ± 1.45 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX VII

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC) OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	33.24 ± 0.02	33.34 ± 0.07
2	33.27 ± 0.02	33.36 ± 0.07
3	33.34 ± 0.03	33.30 ± 0.06
4	33.30 ± 0.05	33.34 ± 0.07
5	33.29 ± 0.03	33.30 ± 0.06
6	33.30 ± 0.05	33.30 ± 0.06
7	33.31 ± 0.05	33.30 ± 0.06
8	33.31 ± 0.05	33.34 ± 0.07
9	33.33 ± 0.05	33.32 ± 0.05
10	33.33 ± 0.05	33.30 ± 0.06
11	33.39 ± 0.06	33.34 ± 0.04
12	33.33 ± 0.06	33.28 ± 0.06
13	33.44 ± 0.17	33.36 ± 0.06
14	33.33 ± 0.06	33.24 ± 0.03
15	33.28 ± 0.07	33.34 ± 0.04
16	33.38 ± 0.05	33.30 ± 0.06
17	33.32 ± 0.06	33.24 ± 0.03
18	33.36 ± 0.06	33.32 ± 0.05
19	33.42 ± 0.05	33.32 ± 0.07
20	33.38 ± 0.08	33.34 ± 0.07

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX VIII

LEUCOCYTE COUNTS OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	9.6 ± 0.11	9.6 ± 0.19
2	9.3 ± 0.26	8.9 ± 0.70
3	9.4 ± 0.21	9.4 ± 0.36
4	9.4 ± 0.17	9.6 ± 0.13
5	9.6 ± 0.14	9.5 ± 0.21
6	9.6 ± 0.08	9.6 ± 0.12
7	9.4 ± 0.09	9.7 ± 0.06
8	9.7 ± 0.05	9.6 ± 0.18
9	9.6 ± 0.09	9.7 ± 0.06
10	9.5 ± 0.16 [a]	10.1 ± 0.18 [b]
11	9.9 ± 0.06	9.8 ± 0.07
12	9.3 ± 0.49	9.5 ± 0.28
13	10.6 ± 0.21 [a]	9.9 ± 0.15 [b]
14	10.2 ± 0.19	9.9 ± 0.08
15	10.5 ± 0.46	9.8 ± 0.17
16	11.8 ± 0.39 [a]	9.5 ± 0.05 [b]
17	12.1 ± 0.46 [a]	9.9 ± 0.08 [b]
18	11.0 ± 0.29 [a]	9.6 ± 0.12 [b]
19	9.7 ± 0.36	9.6 ± 0.09
20	11.0 ± 0.29 [a]	9.8 ± 0.17 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX IX

LYMPHOCYTE COUNTS OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	4.79±0.15	4.74±0.21
2	4.87±0.13	4.79±0.06
3	5.14±0.08	5.14±0.11
4	5.09±0.10	5.34±0.25
5	5.11±0.12	5.08±0.15
6	5.68±0.16	5.61±0.14
7	5.62±019	5.44±0.15
8	5.70±0.19	5.49±0.19
9	5.20±024	5.08±0.14
10	5.82±0.11	5.86±0.14
11	6.00±0.16	5.68±0.24
12	5.60±0.19	5.75±0.18
13	6.78±0.20	6.05±0.25
14	6.26±0.11	5.86±0.20
15	7.19±0.21	6.53±0.10
16	7.22±0.33	5.85±0.24
17	8.34±0.27	6.94±0.19
18	6.40±0.19	5.33±0.17
19	6.23±0.17	5.84±0.23
20	6.40±0.19	6.53±0.10

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX X

MONOCYTE COUNTS OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	0.3±0.014	0.42±0.058
2	0.25±0.02	0.16±0.022
3	0.28±0.029	0.28±0.03
4	0.19±0.035	0.19±0.031
5	0.32±0.018	0.31±0.036
6	0.23±0.019	0.25±0.024
7	024±0.019	0.27±0.019
8	0.24±0.02	0.23±0.024
9	0.47±0.075	0.50±0.048
10	0.22±0.031	0.22±0.020
11	0.20±0.026	0.18±0.02
12	0.2±0.013	0.15±0.024
13	0.21±0.04	0.17±0.025
14	0.26±0.023	0.18±0.037
15	0.19±0.032	0.2±0.031
16	0.26±0.02	0.17±0.024
17	0.20±0.04	0.16±0.024
18	0.37±0.056	0.35±0.058
19	0.19±0.031	0.19±0.030
20	0.37±0.056	0.2±0.031

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XI

NEUTROPHIL COUNTS OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	3.99±0.14	3.99±0.15
2	3.84±0.10	3.47±0.05
3	3.61±0.09	3.72±0.09
4	3.71±0.11	3.72±0.17
5	3.80±0.08	3.85±0.05
6	3.33±0.15	3.37±0.13
7	3.30±0.16	3.64±0.16
8	3.53±0.13	3.52±0.18
9	3.58±0.20	3.75±0.10
10	3.06±0.11	3.66±0.14
11	3.36±0.18	3.61±0.22
12	3.14±0.16	3.31±0.19
13	3.24±0.16	3.24±0.18
14	3.23±0.09	3.49±0.16
15	2.65±0.20	2.76±0.11
16	4.03±0.33	3.15±0.23
17	3.07±0.24	2.42±0.18
18	3.77±0.17	3.58±0.18
19	2.89±0.14	3.13±0.19
20	3.77±0.17	2.76±0.11

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XII

EOSINOPHIL COUNTS OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	3.1 ± 0.14 [a]	4.4 ± 0.60 [b]
2	3.1 ±0.26	3.0 ± 0.00
3	3.0 ± 0.31	3.0 ± 0.32
4	2.0 ± 0.38	2.0 ± 0.32
5	3.3 ± 0.18	3.2 ± 0.37
6	2.4 ± 0.20	2.6 ± 0.25
7	2.6 ± 0.20	2.8 ± 0.20
8	2.4 ± 0.20	2.4 ± 0.25
9	4.9 ± 0.77	5.2 ± 0.49
10	2.3 ± 0.33	2.2 ± 0.20
11	2.0 ± 0.26	1.8 ± 0.20
12	2.1 ± 0.14	1.6 ± 0.25
13	2.0 ± 0.38	1.8 ± 0.25
14	2.5 ± 0.22	1.8 ± 0.37
15	1.8 ± 0.31	2.0 ± 0.32
16	2.2 ± 0.17	1.8 ± 0.25
17	1.7 ± 0.33	1.6 ± 0.25
18	3.4 ± 0.51	3.6 ± 0.60
19	2.0 ± 0.32	2.0 ± 0.32
20	3.4 ± 0.51 [a]	2.0 ± 0.32 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XIII

BASOPHIL COUNTS OF YANKASA RAMS PRIOR AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	0.27±0.025	0.25±0.038
2	0.19±0.021	0.21±0.022
3	0.16±0.017	0.13±0.023
4	0.19±0.0.020	0.21±0.019
5	0.26±0.018	0.31±0.019
6	0.18±0.018	0.19±0.018
7	0.16±0.017	0.21±0.019
8	015±0.02	0.17±0.019
9	0.19±0.03	0.16±0.024
10	0.17±0.016	0.16±0.025
11	0.23±0.021	0.18±0.02
12	0.19±0.029	0.15±0.023
13	0.18±0.028	0.18±0.019
14	0.22±0.031	0.16±0.024
15	0.21±0.038	0.16±0.024
16	0.16±0.025	0.14±0.027
17	0.18±0.027	0.18±0.037
18	0.20±0.022	0.19±0.030
19	0.16±0.024	0.19±0.030
20	0.20±0.022	0.16±0.024

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XIV

LIVE WEIGHT OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	21.1 ± 1.37	21.0 ± 2.61
2	21.1 ± 1.34	21.4 ± 2.58
3	22.0 ± 1.25	21.0 ± 2.47
4	22.3 ± 1.19	21.4 ± 2.54
5	22.7 ± 1.13	21.4 ± 2.62
6	23.3 ± 1.19	21.4 ± 2.44
7	23.1 ± 1.18	21.2 ± 2.48
8	23.0 ± 1.23	21.4 ± 2.66
9	22.3 ± 1.38	21.0 ± 2.67
10	21.3 ± 1.48	20.4 ± 2.73
11	19.1 ± 1.74	20.8 ± 2.48
12	18.3 ± 1.70	21.2 ± 2.35
13	17.4 ± 1.73	21.2 ± 2.44
14	17.8 ± 1.83	20.6 ± 2.50
15	17.5 ± 1.95	20.4 ± 2.32
16	16.7 ± 2.12	20.8 ± 2.29
17	17.0 ± 1.97	20.8 ± 2.27
18	16.8 ± 2.15	21.0 ± 2.45
19	16.6 ± 1.97	21.0 ± 2.45
20	17.5 ± 2.36	21.2 ± 2.64

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XV

SCROTAL CIRCUMFERENCE OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	24.9 ± 0.39	23.1 ± 1.29
2	24.9 ± 0.43	23.2 ± 1.33
3	25.1 ± 0.36	23.1 ± 1.29
4	25.1 ± 0.32	23.2 ± 1.31
5	25.3 ± 0.39	23.2 ± 1.31
6	25.8 ± 0.39	23.3 ± 1.34
7	25.9 ± 0.43	23.3 ± 1.38
8	25.8 ± 0.43	23.2 ± 1.40
9	24.8 ± 0.88	23.1 ± 1.37
10	25.1 ± 0.57	23.2 ± 1.38
11	23.0 ± 1.49	23.1 ± 1.38
12	24.7 ± 0.86	23.1 ± 1.41
13	23.4 ± 0.67	23.1 ± 1.53
14	22.9 ± 0.74	23.2 ± 1.48
15	22.4 ± 0.92	23.1 ± 1.56
16	21.8 ± 0.92	23.1 ± 1.54
17	21.2 ± 1.01	23.0 ± 1.52
18	21.1 ± 1.28	23.3 ± 1.54
19	20.6 ± 1.36	23.2 ± 1.58
20	21.3 ± 1.73	23.3 ± 1.61

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XVI

REACTION TIME OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	30.9 ± 3.84	35.7 ± 3.85
2	36.3 ± 4.53	36.3 ± 4.60
3	33.6 ± 2.86	40.3 ± 4.41
4	31.7 ± 5.10	33.4 ± 3.41
5	30.6 ± 3.19	35.7 ± 2.78
6	26.7 ± 4.54	32.9 ± 2.64
7	34.5 ± 4.27	37.5 ± 1.55
8	33.9 ± 4.12	35.6 ± 2.14
9	48.9 ± 5.11 [b]	32.2 ± 1.50 [b]
10	51.8 ± 10.25	34.0 ± 3.76
11	68.3 ± 8.87 [a]	31.2 ± 2.42 [b]
12	65.5 ± 16.59	33.2 ± 2.21
13	55.3 ± 7.73	45.4 ± 10.91
14	66.9 ± 18.49	39.2 ± 5.07
15	55.7 ± 12.14	38.5 ± 6.33
16	75.7 ± 21.16	34.5 ± 5.48
17	63.2 ± 14.00	30.5 ± 5.10
18	65.6 ± 10.84 [a]	33.4 ± 4.78 [b]
19	94.7 ± 7.54 [a]	31.2 ± 3.60 [b]
20	76.6 ± 17.55 [a]	33.4 ± 4.78 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XVII

SEMEN VOLUME OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	0.53 ± 0.08	0.48 ± 0.07
2	0.59 ± 0.08	0.50 ± 0.06
3	0.51 ± 0.08	0.48 ± 0.04
4	0.56 ± 0.08	0.50 ± 0.09
5	0.54 ± 0.06	0.40 ± 0.06
6	0.47 ± 0.05	0.48 ± 0.08
7	0.53 ± 0.08	0.50 ± 0.07
8	0.50 ± 0.10	0.42 ± 0.09
9	0.50 ± 0.08	0.43 ± 0.06
10	0.49 ± 0.11	0.48 ± 0.09
11	0.49 ± 0.12	0.46 ± 0.10
12	0.47 ± 0.07	0.42 ± 0.07
13	0.56 ± 0.12	0.58 ± 0.09
14	0.60 ± 0.10	0.38 ± 0.05
15	0.56 ± 0.11	0.46 ± 0.07
16	0.43 ± 0.08	0.54 ± 0.07
17	0.42 ± 0.11	0.50 ± 0.06
18	0.38 ± 0.07	0.54 ± 0.05
19	0.36 ± 0.09	0.54 ± 0.05
20	0.43 ± 0.09	0.54 ± 0.05

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XVIII

SEMEN CONCENTRATION OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	292.4 ± 17.87	240.4 ± 39.28
2	278.1 ± 16.53	206.6 ± 32.58
3	243.1 ± 21.61	167.6 ± 43.76
4	233.6 ± 31.00	189.8 ± 32.55
5	255.3 ± 21.36 [a]	183.4 ± 12.05[b]
6	287.1 ± 28.92	247.2 ± 38.34
7	183.3 ± 23.49	203.3 ± 58.93
8	234.2 ± 30.66	225.5 ± 53.73
9	271.6 ± 35.10	271.8 ± 28.25
10	166.5 ± 16.76	252.0 ± 38.38
11	232.7 ± 61.29	218.3 ± 19.00
12	181.4 ± 48.22	156.0 ± 43.93
13	175.2 ± 45.21	179.5 ± 38.33
14	181.4 ± 40.56	244.2 ± 47.97
15	177.8 ± 32.35	259.6 ± 42.41
16	157.8 ± 33.27	265.4 ± 35.58
17	108.2 ± 29.57 [a]	224.5 ± 23.64 [b]
18	79.40 ± 18.08 [a]	241.4 ± 24.87 [b]
19	86.20 ± 17.89 [a]	228.4 ± 18.13 [b]
20	101.0 ± 16.52 [a]	198.6 ± 14.27 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XIX

SEMEN GROSS MOTILITY OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	95 ± 0.00	87 ± 4.90
2	95 ± 0.00	87 ± 3.74
3	92.1 ± 2.86	72.0 ± 18.14
4	82.9 ± 6.53	81.3 ± 10.68
5	93.6 ± 1.43	87.0 ± 4.89
6	92.1 ± 2.86	87.0 ± 4.89
7	80.8 ± 7.35	73.8 ± 9.21
8	86.7 ± 4.01	85.0 ± 4.47
9	89.3 ± 3.69	90.0 ± 5.00
10	74.3 ± 4.42	85.0 ± 4.47
11	77.9 ± 8.30	87.5 ± 2.50
12	60.0 ± 15.51	74.0 ± 7.14
13	67.0 ± 18.88	80.0 ± 5.00
14	80.0 ± 9.49	87.0 ± 4.89
15	75.0 ± 8.47	85.0 ± 6.32
16	50.0 ± 11.18 [a]	87.0 ± 4.89 [b]
17	43.0 ± 11.14 [a]	87.5 ± 7.50 [b]
18	62.0 ± 7.52 [a]	91.0 ± 4.00 [b]
19	55.0 ± 5.00 [a]	91.0 ± 4.00 [b]
20	62.5 ± 7.22 [a]	91.0 ± 4.00 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different

APPENDIX XX

TOTAL SPERM MORPHOLOGICAL ABNORMALITIES OF YANKASA RAMS PRE AND POST EXPERIMENTAL INFECTION WITH *Trypanosoma evansi*

WEEKS	INFECTED	CONTROL
1	21.0 ± 1.57	22.15 ± 1.20
2	22.97 ± 1.60	24.25 ± 1.61
3	22.22 ± 1.50	24.0 ± 2.31
4	24.22 ± 0.86	25.95 ± 2.07
5	22.64 ± 1.62	24.35 ± 1.98
6	20.86 ± 0.52	22.45 ± 2.96
7	28.55 ± 1.07	20.65 ± 1.02
8	31.08 ± 2.99[a]	25.60 ±1.82[b]
9	65.43 ± 5.96	23.15 ± 1.80
10	71.58 ± 7.15 [a]	25.3 ± 0.93 [b]
11	76.98 ± 5.88 [a]	26.8 ± 1.71 [b]
12	76.63 ± 4.52 [a]	22.05 ± 0.96 [b]
13	81.75 ± 6.08 [a]	22.4 ± 2.22 [b]
14	80.93 ± 5.67 [a]	21.35 ± 1.08 [b]
15	84.58 ± 5.02 [a]	24.45 ± 2.13 [b]
16	84.75 ± 6.16 [a]	25.0 ± 1.36 [b]
17	87.0 ± 3.99 [a]	26.2 ± 1.57 [b]
18	87.55 ± 4.23 [a]	26.4 ± 0.83 [b]
19	90.75 ± 2.73 [a]	26.7 ± 1.29 [b]
20	83.94 ± 4.61 [a]	25.35 ± 1.09 [b]

[a, b] Mean in the rows with different superscript alphabets between group A and B are statistically (p□ 0.05) different