EFFECTS OF EXPERIMENTAL *T. congolense* INFECTION ON THE OESTROUS CYCLE OF WEST AFRICAN DWARF EWES

BY

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DECLARATION

I hereby declare that the work reported in this thesis was carried out by me in the Department of Veterinary Surgery and Medicine, under the supervision of Professors E.O. Oyedipe; L.O. Eduvie; and D. Ogwu.

No part of this thesis has been presented in any previous application for a higher degree. All sources of information are specifically acknowledged by means of references.

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CERTIFICATION

This thesis, **"Effects of Experimental *T. congolense* Infection on the Oestrous Cycle of West African Dwarf Ewes"**, by Y. U. Abubakar meets the regulations governing the award of Master of Science (Theriogenology) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

TO MY TEACHERS
WHO PUT ME UP TO THIS

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ABSTRACT

Ten West African Dwarf (WAD) ewes (five infected and five control) were used to investigate the effects of experimental *T. congolense* infection on selected reproductive organs and on oestrous cycle parameters. Oestrus were synchronized at the onset of the experiment using progesterone sponges, following which the animals were observed for signs of oestrus twice daily (8-9 hours and 16-17 hours), using a vasectomized ram.

Five ewes in the infected group were infected with *T. congolense* (2295) following which clinical signs and oestrus behaviors were observed. Parasitaemia, peripheral blood changes and serum progesterone concentrations were measured. When the animals had three successive oestrous cycles 55 days after infection, three ewes from the infected group and two from the control group were randomly euthanized. The ovaries, brain, hypothalamus and adenohypophysis were observed for gross and histopathological lesions.

Infected ewes developed clinical trypanosomiasis which was characterized by many clinical manifestations including intermittent pyrexia. Elevated rectal temperatures of up to 40.3°C were recorded in the infected animals. The mean prepatent period recorded was 10.2 ± 1.2 days. A mean parasitaemia of 5 x 10^5 trypanosomes ml^{-1} of infected jugular blood with a peak parasitaemia of 5 x 10^6 trypanosomes ml^{-l} were obtained. There was a marked drop in the packed cell volume (PCV), haemoglobin (Hb) and red blood cell (RBC) counts of the infected animals compared

with normal values obtained in the control animals. The post-infection mean PCV, Hb and RBC count in the infected and control animals were 19.08 ± 0.11%, 6.39 ± 0.06 g dL^{-1}, 3.89 ± 0.06 million, and 25.57 ± 0.03%, 7.94 ± 0.03 g dL^{-1}, 6.68 ± 0.09 million, respectively. There was a significant difference ($P < 0.05$) between the infected and control values. Total leukocyte counts fell in all infected animals between days 0 and 24 after infection. Later, fluctuation leukocytosis was recorded. Total plasma protein decreased from the pre-infection mean of 8.30 ± 0.04 g dL^{-1} to the post-infection mean of 7.48 ± 0.02 g dL^{-1}. No significant difference ($P > 0.05$) occurred between these values.

Behavioural signs including seeking out the male, tail-wagging, bleating and restlessness were associated with most of the periods of oestrus in all animals both during the pre- and post-infection periods. Plasma progesterone profiles showed that the animals each completed five cycles. The mean cycle length in the infected and control animals was 16.4 ± 0.31 and 16.4 ± 0.11 days, respectively. The mean plateau progesterone concentration during the luteal phase was 6.7 ± 0.48 mg ml^{-1} in the control animals and 4.6 ± 0.14 mg ml^{-1} in the infected animals. There was no significant difference ($P > 0.05$) between these values.

Histopachological lesions observed in the infected ewes were slight focal necrosis of the ovaries, small number of ovarian follicles and slight focal necrosis in the adenohypophysis. There was no significant difference ($P > 0.05$) in all the parameters examined between the infected and control groups except in the

haematological indices. The results indicate that *T.congolense* infection did not significantly affect the oestrous cycle of WAD ewes.

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CHAPTER ONE

1.0 INTRODUCTION

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Trypanosomiasis has been recognised as one of the biggest constraints to increased livestock productivity in Africa (Mortelmans, 1984; ILRAD, 1987; Llewelyn et al., 1987; Jahnke et al., 1988; Reynolds and Ekwuruke, 1988). Direct economic losses result from mortality, abortion, infertility, weight loss, poor growth, low milk yield and reduced working capacity (Trail et al., 1985).

The pathogenesis and pathology of animal trypanosomiasis have been the subject of numerous investigations and several reviews are available (Losos and Ikede, 1972; Clarkson, 1976; Jennings, 1976; Murray, 1978; Tabel, 1978; Ikede et al., 1988; Esievo and Sarror, 1991; Sekoni, 1991; Mohammed, 1991).

Reports on work on the effects of trypanosomiasis on reproduction are relatively recent (Mutayoba et al., 1988). In domestic animals, reports available to date have described detailed reproductive anomalies in the male but there has been little in the female (Mutayoba et al., 1988). Extensive testicular lesions have been described following infection with *T.brucei* in rams (Ikede and Losos, 1972; 1975; Ikede, 1979) and horses (Ikede et al., 1973), *T.vivax*

infection in sheep and goats (Isoun and Anosa, 1974a; 1974b; Anosa and Isoun, 1980) and cattle (Isoun et al., 1975; Sekoni et al., 1988; Sekoni et al., 1990; Sekoni, 1994), and *T.congolense* infection in goats (Kaaya and Oduor-Okelo, 1980; Waindi et al., 1986).

Studies on the effects of trypanosomiasis on fertility in cows (Ige and Amodu, 1975; Masake, 1980; Ogwu, 1983; Ogwu et al., 1984; 1985; 1986; Llewelyn et al., 1985; Lorenzini et al., 1988) and in small ruminants (Isoun and Anosa, 1974; Ikede and Losos, 1975; Masake, 1980; Ogaa, 1983; Kanyar et al., 1983; O'Hara et al., 1985; Mutayoba et al., 1988; Adenowo, 1989; Bawa et al., 1991) have demonstrated its negative effects on both ovarian activity and on established pregnancy.

Various breeds of livestock have been recognised as having a degree of tolerance to trypanosomiasis enabling them to survive and produce in areas where other breeds would succumb (ILCA, 1979). West African Dwarf (WAD; Djallonke) sheep, a breed widely distributed across humid and subhumid West Africa, are included in the trypanotolerant group. However, Ikede and Losos (1972) reported an intrauterine infection of *T.vivax* in the blood of a newborn West African Dwarf lamb. Oyejide and Adeyemo (1989) reported alterations in the serum progesterone profile culminating in abortions and the delivery of a weak lamb following *T.brucei* infection at mid-pregnancy.

Recently, Bawa et al. (1991) in a series of experiments comparing the effects of trypanosome infection on the reproductive system of Yankasa and WAD ewes, observed abortions and mummified fetuses as some of the deleterious effects of trypanosome infection.

These studies therefore suggest that even in the trypanotolerant WAD sheep, trypanosomiasis can result in reproductive disorders.

However, there is no information available on the effects of trypanosome infection on clinical oestrus in correlations with progesterone profiles in WAD ewes.

This study was designed to:

1. Investigate the pathophysiology of the oestrous cycle of WAD ewes infected with *T.congolense* by determining the effects on the oestrous cycle and progesterone profiles.

2. Determine any gross and histopathological changes induced by the infection in the reproductive organs of WAD ewes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Although a lot of information is available with respect to the effects of trypanosomiasis in small ruminants in general, there still exists remarkable deficiency in our current knowledge of the effects of the disease on reproduction among the trypanotolerant breeds of sheep.

2.2 Factors Affecting the Susceptibility of Domestic Ruminants to Trypanosomiasis

For a given host species and a given stock of trypanosome, several factors interact to produce a particular disease syndrome. Among these are mainly age, stress, nutritional and genetic factors (Fiennes, 1970; Mohammed, 1991).

2.2.1 Age

Age has been found to be a significant factor that influences susceptibility to trypanosomiasis in domestic ruminants. In cattle, calves are more resistant to *Trypanosoma congolense* infection compared to adults (Fiennes, 1970). Wellde et al. (1981) reported spontaneous recovery of 10 out of 11 Hereford calves following experimental infection with *T.congolense*. Two of 11 calves within the range of 12 to 24 months also recovered

from *T.congolense* infection without treatment. At the same time, 20 adult Hereford cattle infected with the same parasite died or needed treatment. Similarly, Uzoigwe (1984) reported self-cure in *T.vivax* infected Zebu calves. Young lambs and kids aged between 2 and 64 days were observed to be more susceptible to *T.congolense* infection compared to adults (Griffin and Allonby, 1981). This degree of resistance depends on factors such as initial exposure of the dam and the new-born calf to low grade trypanosome challenge, continual exposure thereafter to the same antigenic types of the trypanosomes, and the level of husbandry (Chandler, 1952; Desowitz, 1959; Fiennes, 1970). Other factors include the transfer of trypanosomal antibodies from infected dams to the new-born calves via the colostrum and a low incidence of intercurrent disease (Murray and Trail, 1983). In view these observations, the influence of age factor in susc... among domestic ruminants varies from species to species.

2.2.2 Stress

When subjected to stress, the ability of domestic ruminants to withstand the effects of trypanosome infection is compromised. Such factors that can lead to exacerbation of trypanosomiasis include long distance trekking, poor management, intercurrent disease, overworking, pregnancy, parturition, and lactation (Murray et al., 1981; Murray and Trail, 1983; Murray et al., 1983).

2.2.3 Nutrition

Domestic ruminants on a poor nutritional plane succumb easily to trypanosome infection compared to those that are well fed (Maclennan, 1970a; Reynolds and Ekwuruke, 1988). These animals, irrespective of the breed, become less productive and may die in considerable numbers (ILCA, 1979; Anosa, 1983; Murray and Trail, 1983; Reynolds and Ekwureke, 1988). When exposed to high tsetse challenge, domestic ruminants on good nutritional plane may suffer chronic infections or even become refractory to the effects of trypanosome infection (Maclennan, 1970a; Reynolds and Ekwuruke, 1988).

2.2.4 Genetic

Genetically mediated resistance to the effects of trypanosome infection clearly exists, but the nature of the mechanisms involved and their genetic content are not yet understood. Efforts are being made to find markers that will identify trypanotolerant animals. It is hoped that ultimately such markers will identify the bovine genes that control resistance. ILRAD (1990) have reported the discovery of 2 markers of trypanotolerance and 1 marker of susceptibility in a cattle population under field challenge. The markers concerned are detectable variants of molecules on white blood cells of cattle.

2.3.0 Susceptibility and Trypanotolerance

A theory has been put forward that if animals of a species are not entirely refractory to trypanosomiasis, the severity of the disease that they experience is in a large part determined by the extent of the historical contact between animals of the species and the tsetse transmitted trypanosomes (Murray et al., 1982). Some West African breeds of livestock are remarkably trypanotolerant (ILCA, 1979). Tyrpanotolerance is the ability of infected animals to limit the effects of trypanosome infection and remain productive under low to medium tsetse challenge. Breeds that are especially trypanotolerant are the taurine Hamitic longhorn cattle represented today predominantly by the N'dama, and the West African Shorthorn such as Baoule and Muturu. These cattle are thought to have arrived in Africa as early as the fifth millennium BC. In contrast, breeds of zebu cattle are quite susceptible (Roberts and Gray, 1973). Zebu cattle were introduced to the continent more recently. Breeds of dairy cattle such as Ayrshire and Friesian are particularly susceptible to trypanosomiasis and as such require good management and chemotherapy if they are to live in the tsetse infested areas of Africa. N'dama cattle, whether subjected to natural or artificial challenge with trypanosomes become infected and develop a peak of parasitaemia similar to the first peak in zebu cattle. But, unlike zebu, they subsequently control their parasitaemia and do not develop severe anaemia. Control of anaemia is important since it is the severest clinical sign of trypanosomiasis in infected livestock. The ability to control anaemia is a heritable

characteristic and possession of this ability is correlated with trypanotolerance (Trail et al., 1989). N'dama, not only remain relatively productive while infected, but their reproductive performance is also maintained (Logan-Henfrey et al., 1992). The reproductive ability of zebu cattle is clearly compromised by trypanosome infection (Murray et al., 1982; Ogwu and Njoku, 1991).

Within the N'dama breed, individuals vary in their response to infection. Trypanotolerance does not correlate with a single characteristic. Data from East and West Africa suggest that a group of characteristics, including albumin, haemoglobin, isoenzyme, and lymphocytic antigen types, can be correlated with the expression of trypanotolerance (Trail et al., 1989; Logan-Henfrey et al., 1992). These characteristics, however, are probably breed characteristics rather than traits of trypanotolerance. The identification of, and selection for tolerance genes have not yet been accomplished (ILRAD, 1990; Logan-Henfrey et al., 1992).

While it has been convincingly demonstrated that the N'dama are trypanotolerant, the claims for trypanotolerance of some other breeds, including some East African zebu types, are still in dispute (Murray et al., 1982). These latter claims for trypanotolerance have not been assessed in cattle raised under tsetse-free conditions. Studies of trypanotolerance in cattle have been made almost exclusively in the field. In such studies, the relative importance of the contributions of innate and

induced resistance of cattle to trypanosomiasis is difficult to assess.

The degree of trypanotolerance in sheep and goats in various areas of Africa is not clear. It has been suggested that indigenous breeds, example the Djallonke sheep of West Africa (ILCA, 1979) and the Galla goats of East Africa (Griffin and Allonby, 1979b), are more trypanotolerant than imported breeds. In some areas of West Africa, small ruminants exist in areas of high challenge. They show high parasitaemia but develop mild or no disease. These animals serve as reservoirs of infection in such domestic and wild animals that can maintain trypanosome infections with few overt signs of disease (Logan-Henfrey et al., 1992).

2.3.1 Mechanisms of Trypanotolerance

The characteristics associated with trypanotolerance such as ability to control anaemia, ability to survive and be productive under moderate tsetse challenge, exhibition of less severe clinical signs, and lower mortality rates have been attributed to genetic, immunological, and physiological factors (ILCA, 1986).

a) Genetic Control

Breeding experiments to determine the heritability of trypanotolerance (Chandler,1952; Finelle, 1973; Griffin and

Allonby, 1979a) have revealed that trypanotolerance can be inherited as an incompletely dominant genetic trait. Efforts are being made to find markers that will identify trypanotolerant animals. Already, 2 markers of trypanotolerance and 1 marker or susceptibility in a cattle population under field challenge have been reported (ILRAD, 1990).

b. Immunological Control

It has been suggested that trypanotolerance is a result of superior immune response by the tolerant cattle (Desowitz, 1959). One of the first analyses of the immune responses of trypanotolerant cattle was undertaken by Chandler in 1958. He demonstrated that N'dama generated higher titres of neutralizing antibodies than zebu. His results were confirmed by Murray et al. (1986) who showed that, following a fly-transmitted *T.congolense* infection, two Baoule cows developed earlier and higher neutralizing antibody titres to parasites taken from the first wave of parasitaemia compared to two zebu cows. A study using N'dama and Boran cattle reared in a trypanosomiasis-free area of Kenya has shown that N'dama have a superior neutralizing anti-M VSG antibody response to *T.congolense* than Boran cattle (Paling et al., 1991b). In contrast, in a study in which Boule and zebu cattle were infected with *T.brucei*, Pinder et al. (1984) found no correlation between titres of VAT-specific antibodies and resistance to infection. N'dama and Boran

cattle produced similar amounts of VSG-specific antibodies following *T.congolense* infection.

N'dama cattle produced antibodies to three invariant trypanosome antigens of 110, 150, and 300 KDa (Shapiro and Murray, 1982). In contrast, a group of zebu responded to only one or two of the three antigens. However, the animals' history of exposure to infection in the field was not taken into consideration. Some studies of immune responses to invariant antigens by cattle infected with *T.congolense* have shown that N'dama cattle produced antibodies to an invariant antigen believed to be a protease of trypanosome origin. Most Boran cattle do not produce antigen (Authie and Williams, 1990 ; Logan-Henfrey, 1992).

Two studies have detected alterations in circulating leukocyte populations in Boran and N'dama cattle following primary and re-challenge infections with *T.congolense* (Ellis et al., 1987). Prior to, and throughout these studies the N'dama cattle had significantly higher numbers of lymphocytes in their circulation than Boran cattle. These studies revealed that N'dama maintained normal levels of CD4+,γ/δT cells, and normal granulocyte populations following infection, but Boran cattle did not. It is, however, not clear whether the maintenance of these populations contributed to the ability of N'dama to resist the disease.

There is a paucity of studies into the immune response of trypanotolerant small ruminants to trypanosome infection. However, results of work done in mice has revealed that trypanosomiasis resistant strains showed a more consistent antibody response to *T.congolense* compared to susceptible mice (Murray et al., 1981). Also, following *T.brucei* infection, resistant strains of mice developed higher antibody titres (Murray and Trail, 1983). The ability of trypanotolerant mice to control parasitaemia has been attributed to their superior innate immune response (Murray et al., 1981).

It has also been observed that immunodepression, a common feature in trypanosomiasis does not commonly occur in trypanotolerant animals (Griffin et al., 1980; Murray and Trail, 1983).

c. Physiological Control

Physiological factors associated with trypanotolerance include a more effective erythropoietic response to trypanosome infection (Murray et al., 1983), possession of a seven-fold increase in erythrocyte surface sialic acid compared to the susceptible breed (Esievo et al., 1986), possession of certain haemoglobin types (Bengham and Blumberg, 1958), superior adaptation to feed utilization, heat tolerance, and better capacity to conserve water by trypanotolerant animals (Murray et al., 1983).

Trypanotolerant N'dama cattle develop a less severe anaemia compared to the more susceptible zebu following trypanosome infection (Murray et al., 1977c; Murray and Morrison, 1979; Murray et al., 1983). This led to the hypothesis that N'dama cattle mount a more effective erythropoietic response to trypanosome challenge compared to zebu, and as such damaged erythrocytes are replaced at a much faster rate to prevent severe anaemia during trypanosomiasis (Murray et al., 1983). However, results of erythropoietic studies (Dargie et al., 1979) comparing *T.congolense* and *T.brucei* infections in N'dama and Zebu cattle revealed that anaemia in trypanosomiasis and the underlying processes are broadly related to the number of trypanosome parasites in the blood of the mammalian host. This report was further substantiated by the reports of the production of sialidase by *T.vivax* and loss of erythrocyte surface sialic acid and development of anaemia during peak parasitaemia (Esievo et al., 1982). Therefore, the early anaemia observed in infected animals is attributed to the ability of the circulating trypanosomes to produce sialidase which in turn cleaves off erythrocyte surface sialic acids, resulting in early removal from the peripheral circulation and thus rendering the erythrocyte more prone to phagocytosis by the reticuloendothelial system (Esievo and Saror, 1991).

It has been shown that the relative trypanotolerance of the N'dama breed of cattle may be attributed to the possession

of a 7-fold increase in erythrocyte surface sialic acids compared to the Zebu breed (Esievo et al., 1986). Esievo et al. (1990) have shown that erythrocytes of both the trypanosusceptible Zebu and the trypanotolerant N'dama breed have sialic acids which coincided with N-acetylneuraminic acid during polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS-PAGE). The N'dama erythrocytes showed another band or type of sialic acids, with estimated molecular weight of 30,000, trailing behind the N-acetylneuraminic acid; the additional band was absent in the Zebu. The existence of this additional type of sialic acid (Esievo et al., 1990) might account for the higher N'dama erythrocyte sialic acid concentrations (Esievo et al., 1986) and the relative trypanotolerance of the N'dama cattle.

It has also been proposed that, as N'dama cattle show almost 100 percent gene frequency for Haemoglobin (Hb) A, while Zebu have a mixture of Hb A and Hb B, animals could be selected by Hb type (Murray et al., 1983). However, the fact that certain exotic breeds such as the Friesian are predominantly Hb A (Bengham and Blumberg, 1958) and are highly susceptible to trypanosomiasis, makes it unlikely that Hb type will be of value as a marker.

Trypanotolerant N'dama cattle are known to exhibit a considerable temperature range from 34.4^{0}C at dawn to 41.1^{0}C by late afternoon. Furthermore, they can tolerate heat and

withstand high levels of humidity better than Zebu (Murray et al., 1983; Murray and Trail, 1983). These physiological adjustments are thought to be probably helpful in retaining water that would otherwise be lost by evaporation (Murray et al., 1983; Murray, 1987).

It has been suggested that the N'dama skin colour and smell may not be as attractive to tsetse flies as the Zebu's (Murray et al., 1983). Secondly, the skin of the N'dama might be tougher for the penetration of the proboscis of tsetse during feeding compared to that of Zebu (Murray et al., 1983). In addition to these, the phagocytic and complement systems might be so active that most of the parasites are destroyed at the site of inoculation in the N'dama. This speculation is thought to be connected with the fact that the N'dama breed has a significant higher levels of white blood cells, particularly eosinophils, compared to the Zebu (Murray et al., 1983; Murray, 1987).

2.4.0 Trypanotolerance and Livestock Production

There is now considerable interest in the exploitation of trypanotolerant livestock as an alternative strategy for the control of animal trypanosomiasis and improvement of livestock production in moderate tsetse challenge zones (Murray and Morrison, 1979; Murray et al., 1983). This interest has been enhanced by the observation made from the economic evaluations of the potentials of the trypanotolerant livestock (Murray and Morrison, 1979;

Griffin and Allonby, 1979b; Trail et al., 1983). Trypanotolerant livestock attain varying degree of productivity under light, medium and heavy tsetse challenge conditions which preclude the maintenance of non-trypanotolerant types. Under light tsetse challenge conditions, results suggest that the productivity of trypanotolerant livestock relative to other susceptible breeds may be much higher than previously assumed (Montsman, 1962; Trail et al., 1983).

2.4.1 Trypanotolerance in small ruminants

It has been well established that certain breeds of small ruminants survive in tsetse fly endemic areas without the aid of chemotherapy and are therefore considered trypanotolerant (Griffin, 1978; Murray et al., 1983). The susceptibility to *T.congolense* infection of different breeds of sheep and goats imported and indigenous to Kenya has been studied (Griffin and Allonby, 1979a; 1979c; 1979d). The breeds of these animals indigenous to Kenya and the neighbouring countries were observed to be generally more tolerant to trypanosomiasis than the exotic ones. When exposed to *T.congolense* challenge, infected indigenous Galla and East African goats and the Black head Persian and the Red Masai sheep exhibited low infection rates, low parasitaemia, slight anaemia, little body weight loss and low mortality rates. Most of these infected animals suffer from acute to subacute trypanosomiasis, leading to self-cure

(Griffin and Allonby, 1979a, 1979b, 1979c).

On the other hand, severe losses were recorded among the exotic Saanen goats and Karakul sheep. They developed acute, subacute, and chronic trypanosomiasis accompanied by high infection rates and parasitaemic peaks, severe anaemia and weight loss, resulting in high mortality rates (Griffin and Allonby, 1979a; 1979c; 1979d). Similarly, Toure et al. (1981) observed in Senegal that the WAD sheep was more resistant to experimental infection with *T.congolense* compared to the Sahelian Fulani sheep which were highly susceptible with high mortality. In another study comparing *T.vivax* infection in these two breeds of sheep, only a single death occurred in each. These observations suggest that the WAD sheep could be trypanotolerant. However, unlike calves (Wellde et al., 1981), lambs are generally susceptible to trypanosome infection (Griffin and Allonby, 1981).

In Nigeria, many workers (Anosa and Isoun, 1974; 1976; 1980; Ikede and Losos, 1975; Saror, 1980; Saror et al., 1981; Kalu, 1983; Maikaje, 1985; Ahmed, 1992) have investigated the effects of different trypanosome infections in the indigenous breeds of sheep and goats, however, trypanotolerance was not evaluated.

2.5.0 Clinical Manifestations of Trypanosomiasis

The major clinical signs of trypanosomiasis in domestic livestock include lethargy, fever, inappetence, anaemia, lachrymation, weight loss, decreased productivity, emaciation and death (Fiennes et al., 1946). Pyrexia is a well recognized feature of trypanosomiasis and is usually of low grade and intermittent in chronic infections (Fiennes, 1970; Valli et al., 1978). However, severe fever in excess of 41.7^{0}C is known to occur with some *T.vivax* infections in cattle (Hudson, 1944; Stephen, 1986).

Trypanosoma brucei brucei (Bruce, 1895) affects all domestic animals and causes mild to virulent infections in equidae, sheep, goats, and dogs, but unlike *T.b.gambiense* and *T.b.rhodesiense*, it is not infective for man (Hoare, 1970). *T.b.brucei* causes mild disease in ruminants but severe manifestations may occur in pigs (Hoare, 1972; Stephen, 1986; Agu and Bajeh, 1987), and cats (Losos and Ikede, 1972). Corneal opacity, conjunctivitis, and keratitis may be observed in dogs (Koch, 1904; Parkin, 1935), cattle (Ikede and Losos, 1972; Ilemobade and Schillhorn Van Veen, 1974), and horses (Yorke, 1911).

Trypanosoma equiperdum, the causative agent of dourine, a venereal disease, is a specific parasite of the equidae and is naturally transmitted through coitus (Hoare, 1972; Stephen, 1986).

Trypanosoma evansi is infective to all domestic animals causing a disease called Surra. The severity of the disease may, however, vary greatly for a given host depending on the virulence of the parasite strain and host susceptibility in different geographical regions (Hoare, 1972). For instance, in Indonesia, *T.evansi* infections in horses, unlike in cattle, are acute and result in 100% mortality (Dieleman, 1986). In India, cattle seldom show clinical signs (Verma et al., 1973).

Both *T.congolense* and *T.vivax* are primarily animal pathogene and cause moderate to severe clinical disease in a wide variety of domestic animals. Occasionally *T.vivax* infections in cattle run a severe course which is characterised by a haemorrhagic syndrome (Hudson, 1944; Wellde et al., 1983; Gardiner, 1989; Gardiner et al., 1989). Natural *T.vivax* infections in the pig and the dog are rare. Consequently, these animals are considered refractory to this trypanosome species (Stephen, 1986).

T.suis is infective to the pig and probably to wild suidae (Stephen, 1986). *T.simiae* causes a fulminating disease in pigs with high mortality rates (Hoare, 1936). Wild suidae such as the bush-pig and warthog are the reservoir hosts of *T.simiae*. Infections with the parasites can be induced experimentally in sheep, goats, and rabbits (Stephen, 1986).

Trypanosomiasis also affects the reproductive capacity of

male and female animals (Ikede et al., 1988; Sekoni et al., 1990; Ogwu, 1991; Edeghere et al., 1992). Anoestrus was observed in *T.vivax* infected zebu heifers (Ogwu et al., 1984). When the infection was initiated during the first trimester, abortion occurred in one out of four heifers. However, when the infection was induced in the second trimester, all four heifers delivered normally. Infection during the third trimester resulted in stillbirth and prematurely delivered calves which died soon after birth (Ogwu et al., 1985; Ogwu and Njoku, 1987). Some of the observed effects would appear to be direct since transplacental transmission of *T.vivax* is known to occur in goats (Rignear, 1932) and cattle (Ogwu et al., 1985). Infection with *T.vivax* or *T.congolense* in bulls causes a severe decrease in semen volume and concentration (Sekoni et al., 1988).

2.6.0 Immune Response

The host immune response during trypanosome infection is primarily humoral and is mediated by IgM and IgG (Morrison et al., 1981; Musoke et al., 1986) which are produced in response to trypanosome variant surface glycoprotein (VSG) and non-VSG antigens (Flynn et al., 1992; Williams et al., 1992. IgM may be the most important antibody type for control of parasitaemia and protection in laboratory animals (Campbell et al., 1978) and cattle (Luckins, 1976). In Zebu cattle exposed to infection by natural challenge, serum

levels of IgM and IgG increased nine-fold and two-fold, respectively (Luckins, 1972). Similar increases in serum IgM concentrations were observed in *T.vivax* infected cattle.

Based on studies carried out by De Gee (1980) and Vos et al. (1988a, 1988b), development of immunity in animals challenged with T.vivax appears to be different from that described for T.congolense and T.brucei (Wilson, 1971; Akol and Murray, 1983, 1985; Nantulya et al., 1984; Akol and Murray, 1985). In cattle infected through tsetse challenge with *T.brucei* or *T.congolense*, treatment with Diminazene aceturate administered 3-4 weeks of infection is followed by recovery and the animals are subsequently immune to homologous challenge. However, goats infected through tsetse challenge with *T.vivax* and treated with Diminazene aceturate 3-4 weeks after the challenge did not develop immunity to homologous or heterologous challenge regardless of whether the challenge and treatment protocol was repeated as many as three times. The different picture presented with *T.vivax* infections may be because only a few metacyclic trypanosomes are extruded by *T.vivax* infected flies, hindering inoculation of all metacyclic variable antigenic types (mVAT) in the serodeme. It would also appear that unlike *T.brucei* and *T.congolense*, tsetse transmitted *T.vivax* may not revert to a constant mVAT repertoire (De Gee, 1980). The extruded metacyclic population may undergo rapid antigenic variation that may hinder expression of all the mVATS and in sufficient numbers to allow adequate stimulation of

protective immune response.

2.7.0 Pathogenesis of Trypanosomiasis in Domestic Ruminants

The pathogenic effects of trypanosome infections in domestic ruminants depend on a number of factors affecting both the host and the parasites involved. Parasite factors include the species, the strain or virulence, the inoculum size, and geographical location (Maclennan, 1970a; Stephen, 1970). In natural infections among domestic ruminants, *T.vivax* is the most important trypanosome species in West Africa, but it takes the second place to *T.congolense* in East Africa (Maclennan, 1970a; Willet, 1970; Griffin, 1978; Leeflang, 1978; Anosa, 1983). The West African strains of *T.vivax* have been observed to be generally more pathogenic to domestic ruminants than the East African strains (Fairbain, 1953; Unsworth, 1953).

2.7.1 Virulence

The virulence of the disease caused by trypanosomes may vary within and between the same parasite species. In cattle, it was shown that the same strain of *T.vivax* could lead to acute or chronic infection (Krampitz, 1970). In another investigation conducted in cattle, it was observed that morphologically longer strains of *T.congolense* produced relatively more virulent infections than shorter strains (Godfrey, 1961). It would appear therefore that differences

in virulence may exist between parasites of the same species which occur in dissimilar geographical areas. This may be examplified by *T.vivax* (Mamman, 1994). The disease caused by *T.vivax* species in West African cattle are generally acute (Untsworth, 1953). In contrast, in East Africa, cattle infections with *T.vivax* are generally relatively mild (Hudson, 1994). Sekoni (1990), however, reported a more acute infection with *T.congolense* compared to infection with *T.vivax* in Zebu cattle. The severity of disease caused by a given species may also vary significantly with the host. For instance, *T.simiae* produces a fulminating disease in the pig, but produces only mild clinical signs in the sheep, and is non infective to cattle (Stephen, 1970).

2.7.2 Antigenic Variation

The surface coat of a trypanosome comprises a glycoprotein (Vickerman, 1969; ILRAD, 1990) and during infection, it forms an important barrier for the parasite against the host's immune response. As early as 1905, it was observed that, using *in vitro* studies, serum collected during the early phase of infection with *T.equiperdum* in a horse did not neutralize parasites isolated from the same host later in the course of the infection. With further observations indicating the ability of trypanosomes to survive and establish chronic infections in the presence of the host antibodies, it became clear that trypanosomes are able to change their surface coat. These observations have since

been validated in *in vitro* studies with *T.brucei* (Gray, 1970; 1972), *T.congolense* (Wilson, 1967; Uilenberg and Giret, 1972), and *T.vivax* (Clarkson and Awin, 1969; Jones and Clarkson, 1971). The phenomenon has also been shown to occur *in vitro* (Doyle et al., 1980).

The ability of a sub-population of trypanosomes to express an antigenically different surface glycoprotein is referred to as "antigenic variation" (England et al., 1982; Turner, 1984; ILRAD, 1990). The sub-population which expresses an antigenically different variable surface glycoproteins (VSG) survives the host immune response, re-establishes parasitaemia from which a subgroup with a further different VSG arises so that there is always a residual antigenically variant trypanosome sub-population which the host's immune response fails to eliminate, except when a variable antigenic type is re-expressed during the course of the infection (Nantulya et al., 1979; ILRAD, 1990). Serologically, 101 variant-specific surface antigens were shown to be expressed by a clone of *T.equiperdum* (Caphern et al., 1977). Furthermore, based on gene cloning and DNA hybridization studies conducted with *T.brucei* (Van den Ploeg et al., 1982), it was estimated that the number of VSG genes contained in a single trypanosome was 1,000 to 2,000.

Since genes encoding VSG may be very similar, and the genetic repertoire is rapidly expensible, the capacity of antigenic variation would appear to be infinite (Lamont et

al., 1986). Antigenic variation is one of the reasons that have frustrated efforts directed at developing VSG-based anti-trypanosomal vaccines (Morrison et al., 1985).

2.7.3 Intermittent Fever

Intermittent fever accompanies trypanosomiasis in domestic ruminants (Fiennes, 1970). Peaks of fever observed in *T.vivax* infected cattle Maxie et al., 1979; Obi and Anosa, 1980) were often associated with increases in the number of trypanosomes in circulation. Each febrile period is followed by parasite destruction and afebrile period, and the level of fever was not always proportional to that of the parasitaemic (Anosa and Isoun, 1974).

2.7.4 Intermittent Parasitaemia

The appearance of trypanosomes in the peripheral blood of a susceptible domestic ruminants from the time of inoculation is preceded by a prepatent period ranging from a few to many days. The length of this period depends on the infecting trypanosome, the dose, the route and method of inoculation, the host species, and its nutritional plane (Weitz, 1970; Maclennan, 1970a).

Variable prepatent periods have been observed in goats experimentally infected with *T.vivax* (10 days) and *T.congolense* (17 days) (Edwards et al., 1956a). Intravenous

infection of cattle produces a shorter prepatent period of four to six days (Saror, 1979; Rurangirwa et al., 1980) compared to 4 to 20 days following subcutaneous inoculation (Hudson, 1944; Dargie et al., 1979). In cyclically infected cattle, the prepatency period is between 9 and 20 days (Luckins, 1976; Mwongela et al., 1981). The prepatency period of 4 to 6 days has been reported in sheep following cyclical challenge using laboratory reared *Glossina palpalis* infected with *T.vivax* (Unsworth and Nesbit, 1952).

Following intraperitoneal *T.vivax* inoculation, a prepatency period of 3 to 10 days in splenectomized sheep and 8 to 10 days in intact sheep (Anosa and Isoun, 1980). However, following intravenous inoculation, prepatent periods of 2 to 3 days for *T.vivax* infected Yankasa sheep (Saror, 1980), two days for *T.vivax* infected Uda and Balami sheep (Maikaje et al., 1991; Ahmed, 1992), two days for *T.congolense* infected Welsh Mountain sheep (Mohammed, 1991) and 7 to 9 days for *T.congolense* infected West African Dwarf sheep (Kalu, 1983) were observed. The nature of parasitaemia, the number, heights and time intervals between parasitaemic peaks depend on the species, breed, and susceptibility of the host, the species and strain of the trypanosome and the severity of the disease (Maclennan, 1970a; Stephen, 1970). Trypanotolerant domestic ruminants are more capable of controlling parasitaemia to very low levels than their susceptible counterparts (Desowitz, 1959; Griffin and Allonby, 1979c; Murray et al., 1981). Domestic ruminants

with severe trypanosomiasis generally have higher parasitaemic peaks compared to those with asymptomatic infections (Anosa and Isoun, 1974; Maxie et al., 1979). In domestic ruminants, *T.vivax* produces much higher parasitaemic peaks than *T.congolense*, while *T.brucei* rarely causes heavy parasitaemia (Edwards et al., 1956a; Ikede, 1981; Murray et al., 1981). Anosa and Isoun (1974, 1980) observed that the highest levels of parasitaemia in *T.vivax* infections in domestic small ruminants occur during the early phases of the infection. The fall and rise of parasitaemic waves indicate the destruction of some members of each variant population (Weitz, 1970; Doyle, 1977). The number and height of parasitaemic peaks correlate with the number and population sizes of antigenic variants during an infection (Shwipiro and Murray, 1982).

2.7.5 Anaemia

Anaemia is the consistent feature of the trypanosomiases of livestock (Sekoni et al., 1990; Logan-Henfrey et al., 1992). The main features of this anaemia are low erythrocyte counts, low haemoglobin levels, low parked cell volumes, and short lives of the circulating erythrocytes (Esievo and Saror, 1991; Logan-Henfrey et al., 1992). Secondary changes are caused by anoxia which results from the anaemia. The anoxia may account for many tissue degenerative tissue lesions in the heart, skeletal muscles, liver, and kidneys (Logan-Henfrey et al., 1992). The anaemia may be either

acute or chronic. Acute anaemia is a result of haemolytic process and is largely due to extravascular haemolysis. The onset of anaemia and the drop in PCV are closely correlated with the onset of fever, the appearance, intensity, and duration of parasitaemia. By the second and up to the third week of infection there is a severe drop in erythrocyte counts and haemoglobin levels and an increase in the number of immature erythrocytes in the circulation. The immature erythrocytes are reticulocytes, macrocytes, and abnormally shaped or poikilocytic erythrocytes. Very early in the infection, the host's haemopoietic system responds to the stimuli induced by the erythrocyte destruction. At this time, erythrocytes are macrocytic and normochromic. Erythropoiesis increases during this phase of the disease but the myeloid granulocytic response may be poor contributing to a drop in the myeloid:erythroid ratio in the bone marrow. At this stage, the erythrocyte life span becomes short and iron utilization is abnormally high (Dargie et al., 1979a; 1979b; Preston et al., 1982b; Logan-Henfrey et al., 1992).

In animals with trypanosomiasis, erythrophagocytosis by resident macrophages within blood vessels and tissues occurs at many sites in the body, including the spleen, liver, lungs, hemal nodes, lymph nodes, and bone marrow (Logan-Henfrey et al., 1992; Anosa et al., 1992).

By the end of the acute phase of the disease, which is

characterised by progressive anaemia and fluctuating parasitaemia, the PCV of infected cattle may have dropped to 20% or lower, the acute phase is of 4 to 12 weeks duration. Following the acute phase, the disease may progress to the chronic phase, which is characterised by low-grade anaemia and low, transient, often undetectable parasitaemia. During this chronic phase the anaemia is normocytic normochromic. The PCV may continue to drop, resulting in the death of the animal, or it may fluctuate at a low level, or gradually improve as the animal recovers.

During the chronic phase of the disease, erythrocytes continue to be destroyed by the macrophage-phagocytic-system (MPS). The bone marrow becomes poorly responsive and the erythrocytes produced may be microcytic and hypochromic. The poor bone marrow function may be a result of defective iron metabolism caused by iron retention or trapping in the MPS (Dargie, 1980; Murray and Dexter, 1988). To date no detailed ferrokinetic or cytological study of the bone marrow response during the chronic phase of trypanosomiasis has been published (Logan-Henfrey et al., 1992).

Important changes in the blood , other than anaemia, occur in animals with trypanosomiasis. These involve leukocytes, thrombocytes, and plasma factors. Early in the infection there is pancytopenia with leukocyte counts reduced to about half that of normal. This leukopenia is caused by a reduction in the numbers of neutrophils and lymphocytes; the

numbers of monocytes and eosinophils are less severely affected. Even though there is a neutropenia, the neutrophil response in the bone marrow is poor. It is much weaker than the response of the erythroid, lymphoid and monocyte lineages (Logan et al., 1989; Anosa et al., 1990). Thrombocytopenia is a frequent finding in trypanosome infected animals and is characterised by a short thrombocyte life span (Wellde et al., 1978; Preston et al., 1982a). In response to the drop in numbers of circulating thrombocytes, there is an increase in the numbers of megakaryocytes in the bone marrow (Logan-Henfrey et al., 1992).

Mechanism of Anaemia: During the acute phase of trypanosomiasis, the anaemia is primarily due to extravascular hemolysis caused by a hyperplastic MPS. The macrophages remove large numbers of erythrocytes. Many mechanisms have been suggested to explain the excessive removal of erythrocytes by the MPS; for example, trypanosome infections may result physical or metabolic damage to erythrocytes; the erythrocyte may have surface-bound trypanosome antigen-antibody complexes; circulating anti-erythrocyte auto-antibodies may develop; or normal erythrocytes may be removed by the activated macrophages (Logan-Henfrey et al., 1992). It is well known that the physical alterations in the erythrocyte membrane can lead to the removal of the erythrocytes by the MPS (Esievo and Saror, 1991; Logan-Henfrey et al., 1992). The high temperature in febrile animals causes increased osmotic

fragility of erythrocytes, their decreased plasticity, and their membrane permeability to increase (Logan-Henfrey et al., 1992).

Disseminated intravascular coagulation (DIC) may occur in animals with extremely high parasitaemias. The condition may occur in pigs infected with *T.simiae* and cattle infected with some stocks of *T.vivax* that cause the haemorrhagic syndrome. DIC causes the deposition of fibrin thrombi in the microvasculature, and erythrocytes are damaged during the passage through these partially blocked capillaries and are subsequently removed by the MPS. Thrombosis may cause local ischemia and thereby tissue necrosis. Thrombosis may also cause severe thrombocytopenia (Logan-Henfrey).

Immunoglobulins (IgG and IgM) and complement are present on erythrocytes of trypanosome-infected cattle (Facer et al., 1982; Assoku and Gardiner, 1989; Logan-Henfrey et al., 1992). The autoantibody on erythrocytes is trypanosome specific in some cases (Facer et al., 1982). The trypanosome-specific antibody is specific for the VSG. While the VSG specific antibodies aid in the clearance of trypanosomes from the circulation, they may also have adverse immunopathological consequences. Immune complex deposition on erythrocytes may lead to their destruction. Such destruction, through the action of a type II hypersensitivity reaction, may contribute to anaemia (Logan-Henfrey et al., 1992). In this situation fragments of

disrupted trypanosomes, VSG, and immune complexes are bound to erythrocytes which results in the erythrocytes being recognized as foreign. They may be lysed by complement or, more likely, phagocytosed by MPS. The existence of immune mechanisms that act to destroy erythrocytes is supported by recent *in vitro* studies. These have shown that trypanosome VSG may be incorporated into erythrocyte membranes or stick to the erythrocyte surface. Trypanosome specific antibodies and complement may bind to the erythrocytes causing their removal by phagocytosis (Ritkin and Landsberger, 1990).

Autoantibodies to erythrocytes and thrombocytes occur in calves infected with *T.vivax* (Assoku and Gardiner, 1989). The reason why autoantibodies appear in the circulation of some trypanosome infected animals is a matter of speculation (Logan-Henfrey et al., 1992). Trypanosomes may damage erythrocyte membranes and thus expose normally hidden epitopes. These epitopes would then be recognized by those antibodies normally present in the circulation, which complex with damaged erythrocytes and facilitates their removal by the MPS. Another possibility is that there are common antigens in trypanosomes and erythrocytes. It is at least possible that spectrin, a structural protein found in many diverse organisms, including trypanosomes and mammalian red cells, could be such an antigen. This proposal is supported by the observation that monoclonal antibodies raised against ß-spectrin from human and porcine erythrocytes react with polypeptides from the paraflagellar

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rod of *T.b.brucei* (Woods et al., 1989).

Macrophages proliferate in the bone marrow early in the course of trypanosomiasis. It has been suggested that the trypanosomes somehow cause these macrophages to remove mature and immature erythrocytes and granulocytes in a non-specific manner (Anosa et al., 1992).

Evidence for the role of erythrocyte sialic acids in the pathogenesis of the anaemia in animal trypanosomiasis includes attachment of *T.congolense* to bovine erythrocytes through the sialic acid receptors on the erythrocytes leading to erythrocytic injuries (Banks, 1980), production of sialidase by *T.vivax* (Esievo, 1979), and loss of erythrocyte surface sialic acids and development of anaemia during peak parasitaemia (Esievo et al., 1982). Early anaemia observed in infected animals may be attributed to the ability of the circulating trypanosomes to produce sialidase, which in turn cleaves off erythrocyte surface sialic acids, resulting in their early removal from the peripheral circulation and thus rendering the erythrocytes more prone to phagocytosis by the reticuloendothelial system (Esievo et al., 1982). This would account for the reduction of erythrocyte life-span which is a characteristic feature of animal trypanosomiasis (Valli et al., 1978).

2.7.6 Gross Pathology

There are neither gross nor histopathological lesions that are pathognomonic for trypanosomiasis (Mohammed, 1991; Logan-Henfrey et al., 1992). Animals that die of acute trypanosomiasis may have very few post-mortem lesions. There may be normal deposits of body fat, but the lymph nodes, hemal nodes, spleen, and liver are often enlarged. There may be ascites, hydrothorax, hydropericardium, and oedema of the lungs. Red bone marrow may occur in the distal ends of long bones and may extend into the fatty marrow of the shafts. The bone marrow of the ribs and sternum will be red. Haemorrhage is not usually a prominent feature of trypanosomiasis; there may, however, be a few petechial and ecchymotic haemorrhages on the serosal surfaces of organs, particularly those of the heart (Mohammed, 1991).

A very acute haemorrhagic syndrome occurs in animals infected by some stocks of T.vivax which cause high parasitaemias in European breeds of cattle. Animals that die during the acute stage of infection with these *T.vivax* stocks often have extensive petechial and ecchymotic haemorrhages on mucosal and serosal surfaces, haematomas at various sites, and massive haemorrhage into the gastrointestinal tract. Icterus may occur in these animals. In cattle naturally infected with these stocks, sudden death with massive haemorrhage from the nasal sinuses or into the digestive tract may occur. This haemorrhagic syndrome has been shown to be due to severe thrombocytopenia caused by a consumptive coagulopathy (Wellde et al., 1983; Olubayo and

Mugera, 1987; Assoku and Gardiner, 1989; Gardiner et al., 1989).

Cattle chronically infected with trypanosomes are cachectic, have a rough hair, and pale mucous membranes. The skeletal muscles of the carcass may be wasted and pale, and occasionally wet (Mohammed, 1991; Logan-Henfrey et al., 1992). There is serous atrophy of fat throughout the body, the blood is watery, visceral organs may be pale, and there may be ascites, hydrothorax, and hydropericardium. haemorrhage is not a prominent feature of the chronic disease except, perhaps, on the subendocardial surface of the ventricles of the heart. The heart may be globosed, generally flabby, and with dilated ventricles. There is serous atrophy of fat in the coronary groove. Frequently, the lungs are oedematous and there may be intercurrent anterior bronchopneumonia. The lymph nodes may be enlarged and oedematous, normal in size, or smaller than normal. The liver may be enlarged and its surface may have a fine lobular pattern. The changes in the liver are as a result of chronic heart failure. The spleen may be either enlarged or normal in size. There are a few changes in the enteric tract. The bone marrow of the shafts of long bones may be occupied by fat that has undergone serous atrophy and lacks haemopoietic tissue. The marrow of the ribs and sternum is usually red (Lorgan-Henfrey et al., 1992).

2.7.7 Histopathology

Detailed studies of the histopathological changes in livestock infected with various trypanosomes have been carried out using *T.congolense* (Fiennes et al., Naylor, 1971; Losos et al., 1973; Valli and Mills, 1980; Dargie et al., 1979a), *T.vivax* (Masake, 1980; Masake and Morrison, 1981; Anosa and Isoun, 1983), and *T.brucei* (Ikede, 1974; Ikede and Losos, 1975; Moulton and Sollod, 1976; Ikede, 1979; Dargie et al., 1979b; Moulton, 1986). Tissue tropism differs among trypanosome species and this leads to differences in the histopathological lesions they induce.

Cattle infected with *T.b.brucei* develop low to intermittent parasitaemia and less severe anaemia than do those infected with *T.congolense* and *T.vivax*. *T.b.brucei* is present both intra- and extravascularly. In histological sections, it may be seen in extravascular sites. *T.b.brucei* infections often cause oedema and inflammation of connective tissue and damage to organs such as the eye, heart, and brain. As a result, animals infected with *T.b.brucei* often develop panophthalmitis, severe myocarditis and meningoenchephalitis. *T.congolense* and *T.vivax* are confined mainly to the blood and lymph. The most important pathological manifestation of infection with these parasites is anaemia. *T.vivax* has been reported to occur outside the vascular system, particularly in the chancre, efferent lymph, heart, bone marrow, and occasionally in the brain. *T.congolense* is mainly seen in the chancre, only

infrequently detected in the lymph, and is thereafter usually restricted to the blood (Luckins and Gray, 1978). *T.congolense* is frequently seen in the microvasculature of the brain and skeletal muscle, where individual trypanosomes attach themselves by their flagellar ends to endothelial cells or blood cells (Banks, 1978, 1979).

In animals with mixed infections of *T.congolense* and *T.b.brucei,* both parasites cross the blood brain barrier (Masake et al., 1984).

Concurrent with the development of parasitaemia, animals develop hyperplasia of the lymphoid system. The lymph nodes that drain the chancre become enlarged, and this is followed shortly thereafter by the development of a generalized lymphadenopathy and splenomegaly (Mohammed, 1991; Logan-Henfrey et al., 1992). The first histological change in the lymph node consists of paracortical hyperplasia due to the presence of many large lymphoblasts and macrophages; then numerous secondary follicles develop in the outer and deep cortex. The medullary cords become thickened with lymphocytes and plasma cells. The lymphoid sinuses may be filled with macrophages, and their may be evidence of erythrophagocytosis and haemosiderosis in the affected lymph nodes. These histologically detectable changes in the lymphoid system are evidence of continuing stimulation and of an immunological response. The changes in the lymph nodes are accompanied by elevation of levels of immunoglobulins in

the blood. As the disease becomes chronic, the lymph nodes may return to their normal size or may become smaller than normal. During the chronic stage of the disease, the lymphoid follicles become depleted, and eventually the lymph nodes atrophy and sclerose (Logan-Henfrey et al., 1992).

The generalized lymphadenopathy which characterizes the acute disease is accompanied, in young animals, by severe atrophy of the thymus, due to the depletion of cortical lymphocytes. In chronically infected young animals, the thymus may remain small. In animals beginning to recover, the cortical areas of the thymus become repopulated with lymphocytes.

In the early stages of the infection there are marked changes in both the white and red pulp of the spleen. The first change detected is hyperplasia of the lymphoblasts within the periarteriolar sheaths is followed by the development of secondary lymphoid follicles with prominent germinal centres. Concomitant with changes in the white pulp, the red pulp becomes hyperplastic. This sinus hyperplasia is due to a marked increase of fixed cells, i.e. macrophages, lymphocytes, and plasma cells. Erythrophagocytosis or haemosiderosis may be prominent.

Early in the infection, the bone marrow shows some expansion of the haemopoietic marrow into the spaces normally occupied by fatty marrow. There is active erythropoiesis but poor

granulopoiesis. The numbers of lymphoblasts, lymphocytes, and macrophages greatly increase in the bone marrow (Logan-Henfrey, 1989; Anosa et al., 1992). During the later stages of the disease, the bone marrow becomes hypocellular with dilation of the sinusoids, and ultimately, serous atrophy of the marrow tissue develops (Anosa et al., 1992).

Myocarditis occurs in animals infected with *T.b.brucei*, *T.vivax*, and *T.congolense*. There are widespread mononuclear cell infiltrates in the heart of infected animals. Myocardial fibres degenerate and the myocardium may occasionally necrose. There are deposits of haemosiderin in the heart tissue (Kimeto et al., 1990; Logan-Henfrey et al., 1992).

The liver may be enlarged and congested. Kupffer cell hyperplasia and periportal mononuclear cell infiltrations are common. In animals with severe anaemia, there is mild central lobular necrosis in the liver. In chronically infected animals in which there is heart failure, the liver changes are those caused by chronic passive congestion.

Very few renal lesions have been reported to occur in livestock infected with trypanosomes. The lesions that do occur consist of occasional large periarteriolar lymphoid cuffs at the cortico-medullary junction. In animals with severe anaemia, anoxia may cause tubular necrosis. There are a few reports of the occurrence of membrano-proliferative

glomerulonephritis in livestock (Logan-Henfrey et al., 1992).

Lesions in the endocrine glands of cattle infected with trypanosomes include focal infiltrates of mononuclear cells in the pituitary and adrenal glands (Ikede and Losos, 1975c). Lesions in the endocrine glands of goats suffering from chronic T.congolense infection include changes in the number of basophils and acidophils in the pituitary gland, atrophy and degeneration of the thyroid glands, and marked hypertrophy of the adrenals in some animals and atrophy in some others (Mutayoba et al., 1988a).

The lesions observed in the brain of *T.congolense* infected cows include marked gliosis, perivascular cuffing, localized cellular infiltration, and encephalomalacia. Subcapsular mononuclear cell infiltrations, degranulation of basophils, slight acidophilic proliferation, necrosis and sclerosis of the hypophysis occurred in the hypothalamus (Ogwu and Njoku, 1991).

The reproductive functions of goats, sheep, and cattle are disrupted by trypanosome infection. Abortions and lesions in the ovaries and testes occur (Isoun and Anosa, 1974; Ikede, 1977; Anosa and Isoun, 1980; Kaaya and Oduor-Okelo, 1980; Mutayoba et al., 1988). Low plasma progesterone levels and persistent corpora lutea occur in *T.congolense* infected goats and Boran cows (Llewelyn et al., 1987; Ogwu and Njoku,

1991) and in *T.vivax* infected Yankasa ewes (Adenowo, 1989). Mutayoba et al. (1988a, 1988b, 1988c) and detected a decline in estradiol-17ß and progesterone levels in *T.congolense* infected goats while Abebe et al. (1994) reported a decrease in cortison levels in *T.congolense* infected Boran cattle.

It is possible to detect various degenerative changes in the testes and ovaries of livestock infected with trypanosomes by histological examination (Ikede et al., 1988; Sekoni et al., 1990b, 1991). Atresia of the ovaries is characterized by marked fibrosis (Adenowo, 1989). There are abnormally low numbers of follicles and corpora lutea are lacking in goats infected with *T.congolense* (Mutayoba et al., 1988a) and sheep infected with *T.vivax* (Adenowo, 1989). In cows, ovarian follicular cystic degeneration, atrophy, and proliferation of the rete ovari have been reported (Ogwu and Njoku, 1991).

2.8.0 Pathogenesis and Pathology of *T.congolense* infection in sheep

The course of infection in animals with *T.congolense* shows great variation and is associated with the method of transmission, the strains of parasite used, and the host animal affected. Variations occur in the susceptibility of individual hosts, and different stocks may vary in pathogenicity (Hoare, 1972). The course of disease caused by *T.congolense* varies in severity and duration and can be acute, subacute, chronic, or symptomless (Hoare, 1972).

Some of the gross changes observed in infected animals include oedema and cachexia which differed between the various species of animals and strains of trypanosomes (Hoare, 1972).

One of the first reported investigations on the pathology of *T.congolense* in cattle was by Fiennes et al. (1946). Some pathological differences were encountered depending on whether the infection was acute or chronic (Fiennes, 1970). Godfrey (1960, 1961) showed differences in pathogenicity between the three morphological types of *T.congolense*. The dimorphon type was more lethal to cyclically infected cattle, sheep, and dogs followed by the intermediate type; the least pathogenic was the congolense type. Similar patterns were obtained in rats infected with bloodstream forms of the three morphological types.

Variations also occur in the development of a chancre, an early skin reaction that follows cyclical challenge (Roberts et al., 1969; Luckins and Gray, 1978). In some cases a distinct nodular skin swelling develops, while some strains of *T.congolense* do not result in chancre development (Luckins and Gray, 1969).

The prepatency period in animals infected with *Nannomonas* trypanosomes is variable. For *T.congolense*, it is about 15 days in cattle, 16 to 17 days in sheep and goats (Stephen,

1986; Mohammed, 1991), and 19 days in pigs (Lewis et al., 1943).

The only comparative study on the pathogenicity of the different morphologically defined kinds of *T.congolense* in domestic livestock was provided by Godfrey (1961). Prepatency periods following cyclical infection in sheep were 9 to 22 days in the *congolense* type, 19 to 25 days in the *intermediate* type, and 10 to 23 days in the *dimorphon* type. No pathognomonic gross lesions are associated with *T.congolense* infections in animals, although the carcass of chronically infected animals usually become anaemic and emaciated (Losos and Ikede, 1972; Stephen, 1986).

2.9.0 The Effects of Trypanosomiasis on Reproductive Parameters of Sheep

Oestrus (heat) is the period of sexual receptivity in domestic animals. It is determined clinically by behavioural manifestations of sexual activity and also by the endocrine events occurring during this stage of the reproductive cycle (Fradson, 1975; Pelletier et al., 1977). The mean duration of oestrus in WAD ewes is 17 days (Orji, 1976) while in the Yankasa it ranges between 16 to 21 days (Igono et al., 1982; Osinowo, 1982; Oyedipe et al., 1986a).

Endocrine profiles provide a clear understanding of the complex physiological events that occur during normal and abnormal functions (Edqvist and Stabenfeldt, 1980; Oyedipe

et al., 1986b). The development of radio-isotopic techniques for hormone analysis has been an important tool for studying reproductive functions in domestic animals. In this regard, progesterone assay has been found to be of greatest practical importance in assessing the reproductive status of the female (Perera and Abeyratne, 1979; Adeyemo, 1986; Choung et al., 1986). Thus, in the non-pregnant female, the concentration of progesterone in the peripheral circulation is usually an accurate index of the presence or absence of corpus luteum (CL) and reproductive status (Oyedipe et al., 1985; Eduvie et al., 1990).

Progesterone levels in the blood normally follow a cyclic pattern being low on the day of oestrus and high at dioestrus or mid-cycle (Kozaneck and Reklewska, 1982); Monty, 1984; Dobson, 1985; Oyedipe et al., 1986b; Eduvie et al., 1990). It has been shown, however, that under normal conditions, regression of the CL and subsequent decline in plasma progesterone occur at about 16 days after previous oestrus, that is 3 to 4 days before the onset of sexual receptivity (Roberts, 1971; Arthur, 1977; Oyedipe et al., 1986b). Closely following this decline in the level of plasma progesterone is a rise in the concentration of oestrogenic hormone. This acts via the hypothalamus to cause the behavioural oestrus. It also causes the release of an "ovulatory surge" of luteinizing hormone (LH) from the pituitary gland at the beginning of oestrus and progesterone increase at dioestrus.

The reproductive physiology of sheep is under the control of the hypothalamus and the pituitary. Hypothalamic gonadotropin releasing hormone causes release of both follicle-stimulating hormone (FSH) and LH from the anterior pituitary. The latter are responsible for follicular development and ovulation. Both FSH and LH are produced in the anterior pituitary gland (Arthur, 1977; Hansel and Convey, 1983; Junguira et al., 1985b). LH and FSH are released coincidentally at or near the onset of oestrus in ewes (Pant et al., 1977). Follicular development due to FHS, results in increase in circulating oestrogen.

The increase in oestradiol that occurs during the preovulatory period appears to be the stimulus that triggers the gonadotrophin surge. Following ovulation, the CL develops and secretes progesterone until the end of an infertile cycle. Pituitary hormones (gonadotrophins) play a major role in the reproductive process. In particular, Follicle stimulating growth hormone (GH) play an important part in the spermatogenic cycle in males and oestrous cycle in females. Consequently, absence of pituitary gonadotrophins as a result of hypophysectomy leads to a block in sperm maturation in rats (Clermont and Morgentaler, 1955) or to marked testicular degeneration and abortion in man and animals (Steinberger and Duckett, 1967; Apted, 1970; Ikede and Losos, 1975a). It has been recognized that trypanosomiasis influences the reproductive potential of

animals (Ogwu, 1983; Kanyari et al., 1983; O'Hara et al., 1985; Mutayoba et al., 1988).

2.9.1 Pathogenic Effects on Reproduction in Female Animals

Both direct and indirect detrimental effects of trypanosomiasis on reproduction in female animals have been reported. It is well established that most tissues and organs are affected during the course of an infection (Morrison et al., 1981).

The reproductive function of animals are controlled by a well coordinated and efficient neuro-endocrine system (Nalbandov, 1976). The hormones involved in reproduction originate in three principal structures, namely, hypothalamus, pituitary, and the gonads. Lesions caused by pathogenic trypanosomes in endocrine glands which produce hormones of reproduction involve the hypothalamus, pituitary gland, and the ovary of various animal species (Isoun and Anosa, 1974; Boyt, 1977; Kanyari et al., 1983; Ogwu, 1983; Ogwu et al., 1984; O'Hara et al., 1985; Mutayoba et al., 1988; Llewelyn et al., 1988; Adenowo, 1989; Ogwu and Njoku; 1991; Edeghere et al., 1992; Anosa et al., 1992).

Depletion of progesterone occurs in trypanosome infected cows (Llewelyn et al., 1988) and ewes (Adenowo, 1989). From the evidence of changes in clinical oestrus presented by animals, it is very certain that a disruption, or depression

in the production of releasing factors, gonadotrophic, and ovarian hormones occurs. The ovaries of such animals are usually static while the animals show various degrees of abnormal oestrus, ranging from complete anoestrus, silent oestrus, to temporary oestrus and other forms of irregular oestrus. The female genitalia of infected animals, in most cases, become flaccid. Animals treated with Berenil(R) 60 to 90 days after the onset of infection resume ovarian cyclical activities after 3 or 4 cycles (Luckins et al., 1986). However, shortened oestrus cycle and lowered progesterone levels have been reported in some animals (Llwelyn et al., 1988). Lack of conception in infected heifers has also been observed (Ogwu et al., 1984).

Death has been reported in some animals during pregnancy or shortly after difficult parturition of usually a weak or dead offspring, or following the expulsion of various grades of abnormal or dead fetuses (Ogwu et al., 1986; Bawa et al., 1991). Abortion is a common occurrence during the various stages of pregnancy in trypanosome infected animals (Boyt, 1971; Nuru, 1974; Ogwu et al., 1986; Fio-Ngaindiro, 1987; Bawa et al., 1991; Edeghere et al., 1992; Sekoni, 1994). Abortion and premature births occur in trypanotolerant breeds (Djabakou et al., 1985). Retained placenta following parturition has been reported in infected Zebu cows (Ogwu et al., 1986).

Pathogenic trypanosomes have been demonstrated in the

amniotic fluid of infected pregnant ewes (Isoun and Anosa, 1974), while fetal death, in-utero mummification and resorption are also known to occur (Bawa et al., 1991). Premature births, low birth weights, birth of weaklings, and neonatal death have been reported in ruminants (Ogwu et al., 1986; Reynolds and Ekwuruke, 1988; Bawa et al., 1991). Transplacental transmission of pathogenic trypanosomes to foetuses which develop high parasitaemias, weakness with low birth weights, anaemia, and other defects also occur (Ikede and Losos, 1972; Ogwu et al., 1986). Transplacental infections, in most instances, are accompanied by neonatal death. Transplacental transmission of pathogenic trypanosomes occur in many animals including camels, rats, mice, guinea pigs, domestic ruminants, buffaloes, horses, dogs, and man (Ogwu and Nuru, 1981).

Early treatment with trypanocidal drugs may alleviate some of the problems associated with trypanosomiasis in female animals (Llewelyn et al., 1988). Trypanotolerant breeds and well nourished female animals are more able to cope with various reproductive problems associated with trypanosomiasis compared to malnourished and trypanosensitive animals (Reynolds and Ekwuruke, 1988; Bawa et al., 1991).

2.9.2 Reproductive Disorders in Males

Male animals infected with the *brucei* group develop lesions

which are a combination of scrotal dermatitis, orchitis, and periorchitis. Soon after infection the parasites localize in the scrotal skin, hydrocoele, and invade the tunica vaginalis, testis, epididymis, and the spermatic cord provoking a non-purulent granulomatous inflammation in monkeys (Peruzzi, 1928), sheep (Ikede 1979), goats (Ngeranwa et al., 1991), bulls (Sekoni et al., 1990), rabbits (Van den Ingh and Van Dijk, 1975; Ikede and Akpavie, 1982), and mice (Anosa and Kaneko, 1984). Thrombosis of the pampiniform plexus has been reported in *T.brucei* infected dogs (Morrison et al., 1981). Seminiferous tubule degeneration, aspermatogonia, and in severe cases aspermia occur. Other factors such as fever may also be responsible for the testicular degeneration.

T.vivax and *T.congolense* are also associated with infertility in animals but the mechanisms appear different. Inflammatory changes in the genital organs are usually mild or absent but there is progressive and marked testicular degeneration that can lead to atrophy and aspermia (Isoun and Anosa, 1974; Isoun et al., 1975; Anosa and Isoun, 1980; Kaaya and Oduor-Okelo, 1980; Masake, 1980; Anosa, 1983; Sekoni et al., 1988; Sekoni et al., 1990).

The severity of testicular and epididymal lesions is reflected in poor quality of semen and the high percentage of abnormal spermatozoa present in the ejaculate of bulls (Isoun et al., 1975; Grundler, 1985; Sekoni et al., 1988)

and in rams (Isoun and Anosa, 1974; Anosa and Isoun, 1980; Akpavie et al., 1987) experimentally infected with *T.vivax*, *T.congolense* or *T.brucei*. It has also been shown in rabbits and in sheep that successful treatment with trypanocides will gradually lead to normal spermatogenesis over a period of several months if the original lesions have not been complicated by secondary bacterial infection (Ikede and Akpavie, 1982; Akpavie et al., 1987).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Animals

Ten cycling West African Dwarf (WAD) ewes and one vasectomised ram, two years old, were obtained from National Animal Production Research Institute, Shika; the ages of the animals were confirmed by the dental eruption pattern (Wilson and Durkin, 1984). The animals were accommodated in a flyproof housing, and allowed to acclimatize for four months prior to the commencement of the experiment. The animals were fed concentrates comprising of 68.25% crude protein and 1,575 kcal per animal per day. Local gamba hay, water, and mineral salt licks (Pfizer Ltd, Nigeria) were provided *ad libitum* throughout the course of the experiment.

Incidental bacterial infections were treated with Combiotic(R) (Pfizer Ltd, Nigeria) and spraying against ectoparasites was carried out weekly using 0.025% chlorfenvinphos (Pfizona(R); Pfizer Ltd, Nigeria). Prior to the commencement of the experiment, the animals were certified free of trypanosomes based on weekly haematocrit centrifugation tests (Woo, 1969).

3.2 Trypanosome stock

Stabilates of *Trypanosoma congolense* strain 2295 were used. This strain was isolated from a naturally infected cow in 1978 and then passaged once by syringe into mice. Stabilates were made and

preserved in 5% dimethylsulfoxide and stored in liquid nitrogen.

3.3 Clinical Examination

Clinical evaluations were conducted by observation and manipulation of the experimental animals. Appetite, gait, skin and coat conditions, vital parameters involving rectal temperature, respiratory rate, and the colour of the ocular mucous membrane, were recorded. Weight changes were monitored and recorded.

3.4 Experimental Design

Following the period of acclimatization, the ewes were divided into two groups (infected and control) of five based on stratified randomization of packed cell volumes. Also, prior to the commencement of the experiment the oestrous cycle of the ewes were synchronized using Veramix® (Upjohn Ltd, West Sussex, UK), an intravaginal progesterone sponge containing 60 mg of medroxyprogesterone acetate. The ewes were checked for standing heat between 8.00 and 9.00 hours and 16.00 and 17.00 hours using a vasectomized ram which is introduced at each time of the heat detection. This was continued throughout the period of the conduct of the experiment.

3.4.1 Oestrus Synchronization and Detection

Oestrus synchronization was performed in all the experimental

animals as described by Osinowo (1982). Oestrus was then monitored twice daily; the first between 8.00 and 9.00 hours, and the second between 16.00 and 17.00 hours. During each detection period, the behavioural responses of the ewes to the presence of the vasectomized ram was recorded. The oestrous cycle, duration of 'heat' (oestrus), and the number of mounts per oestrus ('heat' intensity) were determined. The length of the oestrus cycle was the period between two successive 'heats'; the duration of 'heat' is the period in hours of "ram acceptance" by the ewe, while 'heat' intensity was estimated by the number of mounts per oestrus (Roberts, 1971; Arthur, 1977).

3.4.2 Inoculation of Animals

A donor ewe was inoculated with the stabilated trypanosomes (Section 3.2) obtained from the Department of Veterinary Parasitology and Entomology of Ahmadu Bello University, Zaria. On the second day following detectable parasitaemia, blood from the donor ewe was sub-inoculated into each of five ewes in the infected group. The inoculum contained, approximately, 5.0×10^5 trypanosomes per ml of infected blood. The trypanosomes were enumerated using the buffy-coat parasitaemia scoring system (Paris et al., 1982).

3.4.3 Blood Sampling

Five milliliters of jugular blood was collected three times weekly (Mondays, Wednesdays, and Fridays), into test-tubes

containing disodium salt of ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. The blood was used for both haematological and parasitological analyses. At the same times, 2 ml of jugular blood was collected into sterile heparinized containers for blood biochemistry. Another 5 ml of jugular blood was collected into sterile test-tubes every other day. The blood was centrifuged and the serum separated and preserved at -20°C until analyzed for progesterone (P4).

3.5 Parasitological Examination

Parasitaemias were examined in all the animals by examining wet blood films under light microscopy using a x40 objective. The films were made by putting a drop of blood onto a clean glass slide under a cover slip (Paris et al., 1980). The microhaematocrit centrifugation technique (mHCT; Woo, 1970; Murray et al., 1977) was used to detect very low parasitaemias by finding the trypanosomes at the plasma/cell interface by phase contrast microscopy.

3.6 Haematological Examination

Blood samples were analyzed for the packed cell volume (PCV), haemoglobin (Hb) concentrations, total red blood cell (RBC), and total leukocyte (WBC) counts on the day of collection. The PCV was determined by the microhaematocrit centrifugation technique and the values were read using the Hawksley microhaematocrit reader (Gelman Hawksley Ltd., England). The haemoglobin (Hb)

concentration was measured by the cyanmethaemoglobin method using coulter haemoglobinometer (Coulter Electronics, Hearts, England). The RBC and WBC counts were estimated using a coulter haemocytometer (Coulter Electronics, Hearts, England).

The mean PCV, Hb, RBC, and WBC values in clinically normal WAD ewes were 28.0%, 8.64 g/dl, 7.7 x 10^6, and 14.887 x 10^3, respectively (Oduye, 1976).

Clinical signs of pale ocular mucous membrane with a PCV below the normal but still above 20% is scored as mild or moderate anaemia. However, a PCV below 20% is considered as severe while any PCV down to about 12% is more or less fatal (Kerr, 1989).

3.7 Blood Biochemistry

The plasma was separated from the blood samples collected for biochemical analyses. This was either analyzed the same day or else stored in a refrigerator overnight and analyzed the following day. Total plasma protein concentrations (g/dl) were determined for each blood sample using a refractometer (Coulter Electronics, Hearts, England) as described by Kerr (1989). The normal total plasma proteins ranges between 60 and 80 g/l (Kerr, 1989).

3.8 Serum Progesterone Measurement

Serum progesterone was measured using a solid phase

radioimmunoassay (RIA) kit (FAO/IAEA, RIA kit). Prior to the assay, antibody coated tubes were labelled for standards, quality control, and experimental samples. A 100 µl each of standard, quality control, and the experimental samples were pipetted into the bottom of the corresponding antibody coated tubes. One millilitre of ^{125}I labelled progesterone was then added within 5 minutes and vortexed for 30 seconds. The mixture was incubated for 4 hours at room temperature following which all the tubes, except the total count, were decanted. The tubes were tapped on tissue paper and the radioactivity bounded to the antibody coated tube was counted for 1 minute in a gamma counter (Neuclear Enterprises 1612 Gamma Counter).

Progesterone concentrations were estimated using a logit-log transformation performed with a RIA data software integrated in the gamma counter. The sensitivity of the assay defined as twice the standard deviations away from the zero standard was 0.06 ng/ml. The within and between assay coefficients of variation were 10% and 15%, respectively.

3.9 Post-Mortem Examination

Following the termination of the experiment on day 55 post-infection, 3 of the infected and two control ewes were euthanized and necropsied at the necropsy room of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. The necropsy was carried out in order to examine for possible gross lesions on the carcass, and to obtain tissue samples for histology.

The ewes were positioned on left lateral recumbency in order to facilitate the examination of abdominal organs. In all cases, a mid-ventral skin incision extending from the mandibular space to the pelvic area was made. The skin was reflected and the carcass condition evaluated. The peritoneum was incised and reflected in order to expose the abdominal viscera which were examined for gross lesions or abnormalities.

The chest cavity was opened by cutting through the costochondral junctions and the contents examined. The chest ventral neck muscles were cut and the tongue, trachea, oesophagus, lungs, and heart were removed and examined as a single unit. The lumen of the trachea and the oesophagus were cut open and their mucosal surfaces examined for gross abnormalities. The digestive tract was removed as a single entity and the rumen, small and large intestines were cut open and their mucosal and serosal surfaces examined. The kidneys, adrenal glands, and urinary bladder were examined as a unit. The regional lymph nodes, especially the inguinal and prefemoral were examined. The head was severed and the brain, hypothalamus, and pituitary gland were removed and examined for gross lesions.

3.10 Histology

Following post-mortem examinations, tissue samples were obtained from all the necropsied animals for histo-pathological evaluations. The samples included the ovary, brain, pituitary

gland, hypothalamus, lymph nodes, and spleen.

3.10.1 Preservation

Tissues were preserved in 40% formaldehyde for 48 hours. They were trimmed and loaded into labelled cassettes (Bright Co., Huntingdon, England) for processing.

3.10.2 Preparation of Tissue Sections

The tissue samples were manually processed in a 48 hour processing cycle in the Histotechniques Lab., Department of Veterinary Anatomy, Ahmadu Bello University, Zaria. The processing cycle involved treatment in neutral buffered formalin, followed by a series of dehydration treatments in 70%, 90%, and 100% ethyl alcohol. The tissues were cleared in three batches of Histoclear solution before finally receiving two treatments in paraffin wax. At the end of the cycle, tissues were removed and processed for 30 minutes in a vacuum impregnator containing molten paraffin wax in order to facilitate removal of air bubbles and the clearing agent. The processed samples were embedded in paraffin wax using Tissue-Tek (Ames Co. Ltd) embedder. Excess wax from the edges of the cassettes were removed and the blocks identified. Tissue sections were cut, 4 to 6 microns thick, using a Leitz base sledge 1400 microtome (Reidert-Jung Co., Germany). Sections were mounted on glass slides, allowed to dry, labelled and stained with H & Estain.

3.11 Statistical Analyses

The values of individual parameters (rectal temperature, PCV, Hb, RBC, WBC, plasma total proteins, body weights, and serum progesterone concentrations) for both the infected and control WAD ewes were respectively pooled together as pre- and post-infection values. Mean data on the various parameters for the infected and control groups of ewes were compared statistically using the Student's t-test (Steel and Torrie, 1960; Winer, 1971).

CHAPTER FOUR

4.0 RESULTS

4.1 Rectal Temperature

The pre-infection mean rectal temperature for the control ewes was 38.7^{0}C while in the infected ewes it was 38.5^{0}C. The infected ewes became febrile from day 13 post-infection. Fluctuations in the rectal temperature from normal values (considered to be 38.5^{0}C) up to 40.3^{0}C occurred in the infected ewes on day 49 post-infection (Fig. 1). The pattern of temperature elevations during the course of the infection coincided with the peaks of parasitaemia (Fig. 2). In control ewes, normal rectal temperatures were maintained throughout the course of the experiment (Fig. 1). The mean rectal temperature in the infected ewes was 39.4^{0}C which differed significantly ($P < 0.05$) from that of the control ewes.

4.2. Parasitaemia

All infected animals became parasitaemic 15 days following the inoculation of the trypanosomes. The mean prepatent period was 10.2 ± 1.2 days. The control ewes remained aparasitaemic throughout the course of the experiment. Parasitaemia was intermittent in all the infected ewes with an average aparasitaemic intervals of 2.0 days. A mean parasitaemia of 5 x 10^5 trypanosomes ml^{-1} of infected jugular blood was obtained while parasitaemic peaks of 5 x 10^6 trypanosomes ml^{-1} were recorded from

days 29 to 30 post-infection (Fig. 2). Thereafter, moderate to mild parasitaemias were maintained up to the time the experiment was terminated (Fig. 2).

4.3 Packed Cell Volume

The mean pre-infection PCV of the control and infected ewes were 26.1 ± 0.08% and 26.7 ± 0.07%, respectively. Following infection, a progressive decrease in PCV values occurred until it dropped to 16.6% on day 20 post-infection (Fig. 3). Thereafter, the mean PCV had a sudden rise to 23.0% and 20.2% on days 24 and 26, respectively. Another sharp drop to 15.6% occurred between days 28 and 29 post-infection. Thereafter, the mean PCV fluctuated between 20.2% and 16.2% up to the end of the experiment. The mean PCV in the control ewes remained normal relative to the pre-infection values throughout the course of the experiment. The mean post-infection PCV in the infected ewes (19.1 ± 0.11%) was significantly different ($P < 0.05$) from the mean post-infection PCV in the control ewes (25.7 ± 0.03).

4.4 Haemoglobin Concentrations

A progressive decrease in the Hb concentrations in the infected ewes occurred from a pre-infection mean (± standard error; SE) of 8.18 ± 0.04 g dl^{-1} to a post-infection mean of 6.39 ± 0.06 g dl^{-1}. These were found to differ significantly ($P < 0.05$). Also, the post-infection mean in the infected ewes was significantly different ($P < 0.05$) from the corresponding mean in the control

ewes (7.94 ± 0.03). The decrease in haemoglobin concentration was more severe on day 22 post-infection (5.26 g dl^{-1}) but later rose to 7.30 g dl^{-1} and 8.24 g dl^{-1} on days 24 and 26 post-infection, respectively. Thereafter, the mean Hb concentrations fluctuated with the lowest value of 5.08 g dl^{-1} on day 33 post-infection (Fig. 4). The mean Hb concentration of 7.84 g dl^{-1} was obtained in the control ewes and remained normal throughout the course of the experiment.

4.5 Red Blood Cell Counts

The pre-infection mean erythrocyte counts in the infected and control ewes were 6.83 ± 0.06 x 10^6 and 6.68 ± 0.09 x 10^6, respectively. This normal value was maintained throughout the course of the experiment in the control ewes while a progressive reduction in the RBC counts occurred post-infection in the infected animals (Fig. 5). The post-infection mean value of 3.89 ± 0.06 x 10^6 ml^{-1} in the infected group differed significantly ($P < 0.05$) from the mean erythrocyte counts in the control ewes. In the infected ewes, the RBC counts progressively decreased from the pre-infection mean value of 6.83 ± 0.06 x 10^6 down to 2.45 x 10^6 ml^{-1} on day 20 post-infection. It then rose to 6.50 x 10^6 ml^{-1} and 5.5 x 10^6 ml^{-1} on days 24 and 26 post-infection, respectively. The intermittent fluctuation of the erythrocyte levels continued up to the time the experiment was terminated with the lowest RBC counts of 2.30 x 10^6 ml^{-1} on day 47 post-infection (Fig. 5).

4.6 White Blood Cell Counts

The pre-infection mean WBC counts in the infected and control ewes were 125.5 ± 0.22 x 10^3 ml^{-1} and 122.2 ± 0.80 x 10^3 ml^{-1}, respectively. The post-infection means in the two groups were 126.5 ± 0.50 x 10^3 ml^{-1} and 118.8 ± 0.29 x 10^3 ml^{-1}, respectively. There was a significant difference ($P < 0.05$) between the post-infection leukocyte counts of the infected and the control ewes. A progressive drop in leukocyte counts occurred in the infected ewes from the pre-infection value of 125.5 ± 0.22 x 10^3 ml^{-1} to 104.0 x 10^3 ml^{-1} on day 17 post-infection. A sharp rise, 149 x 10^3 ml^{-1}, occurred on day 25 post-infection. A plateau was maintained up to day 39 post-infection following which the counts dropped to 113.8 x 10^3 ml^{-1} on day 42 post-infection. The leukocyte counts rose again towards the end of the experiment (Fig. 6). Fluctuations in the leukocyte counts in the control ewes were also observed during the post-infection phase but no statistically significant differences ($P > 0.05$) occurred between the mean pre-infection and mean post-infection counts in this group of animals.

4.7 Total Plasma Proteins

The mean total plasma proteins for the infected and control ewes during the pre-infection period were 8.35 ± 0.02 g dl^{-1} and 8.30 ± 0.04 g dl^{-1}. Following infection, the mean values in the infected ewes (7.48 ± 0.02 g dl^{-1}) was significantly ($P<0.05$) different from the mean values in the control ewes (8.04 ± 0.01 g dl^{-1}). The total plasma protein values in the infected ewes

showed fluctuations between the pre-infection value of 8.30 ± 0.04 g dl^{-1} to the lowest level of 6.72 ± 0.02 g dl^{-1} on day 35 post-infection (Fig. 7). Similarly, fluctuations occurred in the post-infection values in the control ewes but there was no significant difference ($P > 0.05$) between the pre-and post-infection means in this group (Fig. 7).

4.8.0 Oestrous Behaviour

In the control ewes, oestrus occurred in 30 out of 44 periods of basal progesterone, giving an oestrus detection rate of 68.2%. Behavioural signs including seeking out the male, tail-wagging, bleating, restlessness, oedematous/congested vulva, and mucus discharge from the vulva were associated with (90%) 27 out of 30 of the periods of oestrus. In 14 periods of basal progesterone, oestrus was not considered to have occurred, despite the fact that the ewes showed behavioural signs of oestrus in four (4) of these periods. Three out of 6 instances of undetected oestrus occurred in one ewe (No. 189).

Infected ewes showed behavioural signs of oestrus in 15 out of 20 periods of basal progesterone occurring before *Trypanosoma congolense* infection. After *T.congolense* infection, oestrus behaviours were observed in only 15 out of 25 periods of basal progesterone levels. Times of oestrus in relation to plasma progesterone profiles for individual ewes are given in Fig. 9.

Times of oestrus in relation to mean plasma progesterone profiles

for infected and control groups are given in Fig. 8.

4.8.1 Oestrous Cycles

The plasma progesterone profiles (Fig. 10) of the control ewes indicated that each completed 5 cycles giving a total of 25 cycles. The mean cycle length was 16.4 ± 0.11 days, with a range of 11 to 20 days. None of the ewes had cycles indicative of persistent corpora lutea.

The mean progesterone levels in the control ewes were below 1.0 ng ml^{-1} (basal level) from 1 to 2 days prior to oestrus (follicular phase) until 4 days after oestrus (early luteal phase). The mean plasma progesterone was 0.36 ± 0.02 ng ml^{-1} on the day of oestrus (day 0). A rising phase in progesterone concentration was noticeable from day 5 to day 9. This was followed by a peak mean value, "the plateaux phase" which ranged from 4.1 ± 0.26 ng ml^{-1} to 6.7 ± 0.48 ng ml^{-1}. This period lasted 5.96 ± 0.24 days. Thereafter, there was a rapid drop during the "declining phase" from day 14 as the ewes, again, moved into the follicular phase with basal progesterone levels of the next oestrous cycle. The mean duration of oestrus was 2.96 ± 0.026 days (Table 1) while the mean intensity of oestrus was 16.7 ± 0.34 mounts per oestrus.

The plasma progesterone profiles of the *T.congolense* infected ewes indicated that each of the 5 ewes completed 3 cycles giving a total of 15 cycles during the period prior to infection. The

pre-infection mean cycle length was 16.0 ± 0.09 days with a range of 14 to 17 days. The pattern of the profile was similar to that of the control ewes. The average basal level was 0.24 ± 0.02 ng ml^{-1} which rose to a peak of 4.16 ± 0.09 ng ml^{-1}. The "plateaux phase" lasted 6.5 ± 0.11 days and thereafter progressively declined corresponding to the successive follicular phase (Figs 8 and 9). The first post-infection oestrous cycle in each of the 5 infected ewes was 22 days, even though the progesterone profiles dropped to a basal level on day 10 post-infection; these were regarded as silent oestrus. The mean cycle length after infection averaged 16.4 ± 0.31 days with a range of 10 to 22 days. Also, the basal progesterone level averaged 0.31 ± 0.03 ng ml^{-1} while the peak level was 4.6 ± 0.02 ng ml^{-1}. The plateaux period lasted 4.3 ± 0.16 days. There were no significant differences ($P > 0.05$) between the pre-infection and post-infection oestrous cycle lengths, as well as between the pre-infection and post-infection peak values. There was also no significant difference ($P > 0.05$) between the post-infection oestrous cycle length of the control ewes (16.4 ± 0.11 days) and that of the infected ewes (16.4 ± 0.31 days).

The mean duration of oestrus after infection was 1.53 ± 0.10 days which differed significantly ($P < 0.05$) from the mean duration of oestrus in the control ewes. The mean intensity of oestrus post-infection (7.47 ± 0.93) differed significantly from that of the control ewes (16.7 ± 0.34)(Table 1).

4.9.0 Body Weight

All the infected ewes progressively lost weight during the course of the experiment. The average weight loss was 21.38% of the initial average weight, while the average weight of the control ewes increased by 8.69% over the same period.

4.10 Gross Pathological Observations

4.10.1 Ovaries

The mean ovarian weight in the control ewes was 1.45 ± 0.05 gm while it was 1.33 ± 0.06 in the infected ewes (Table 2). The mean number of follicles in the control ovaries was 3.25 ± 0.43 while in the infected ewes it was 2.0 ± 0.29. The average number of corpora lutea in the control ewes was 1.75 ± 0.31 while it was 1.5 ± 0.09 in the infected ewes. There were no significant differences ($P > 0.05$) in these parameters between the control and infected ewes. Petechial haemorrhage was observed in the left ovary of one infected ewe (No.186). No parasites were detected following impression smears conducted on the ovaries.

4.10.2 Brain

No detectable gross lesions were observed in both the infected and control ewes.

4.10.3 Hypothalamus

No detectable gross lesions were also observed in both the

infected and control ewes.

4.10.4 Adenohypophysis

No detectable gross lesions were seen in both the infected and control ewes.

4.11 Histopathological Observations

4.11.1 Ovaries

Histologically, the control ewes had normal ovaries containing variable numbers of primordial to tertiary follicles. In most cases the ovaries contained apparently functional corpora lutea. The ovaries of the infected ewes had fewer numbers of follicles, pyknosis of follicular cells, and few areas of necrosis (Plate 1).

4.11.2 Brain

There were no significant histological lesions in the brain of both the control and infected ewes.

4.11.3 Hypothalamus

There were no significant histological lesions in the hypothalamus of both the control and infected ewes (Plates 2 and 3).

4.11.4 Adenohypophysis

There were some slight areas of focal necrosis in the adenohypophyses of the infected ewes. The adenohypophyses in the control ewes were normal (Plates 4 and 5).

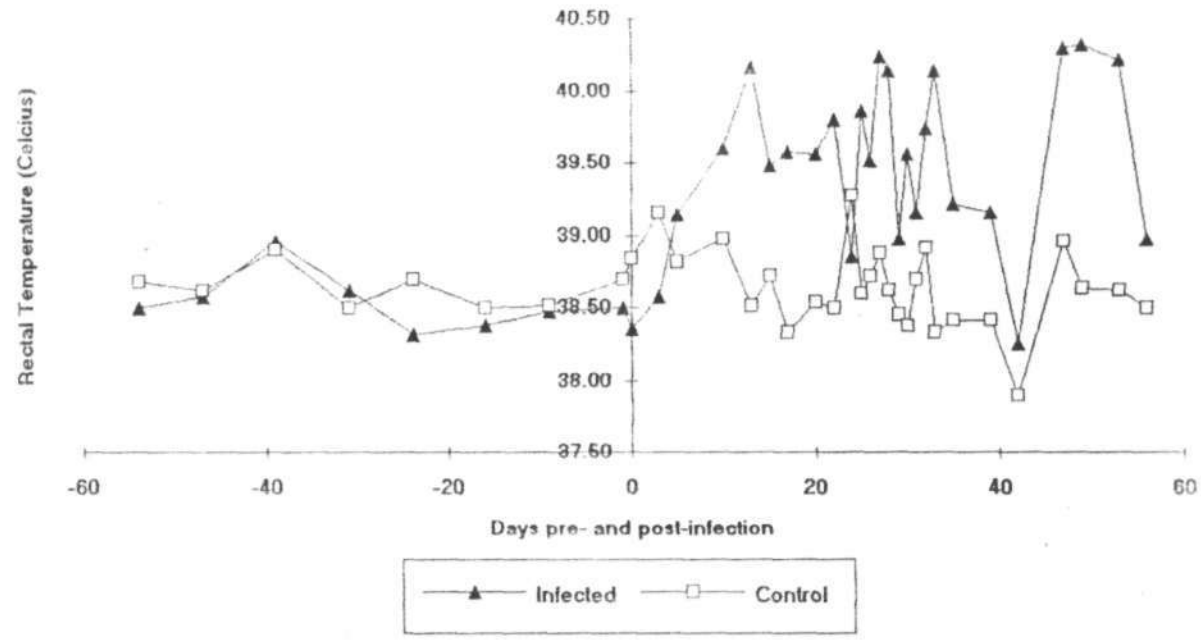


Fig. 1: Mean Rectal Temperature in *T.congolense* Infected and Control WAD Ewes

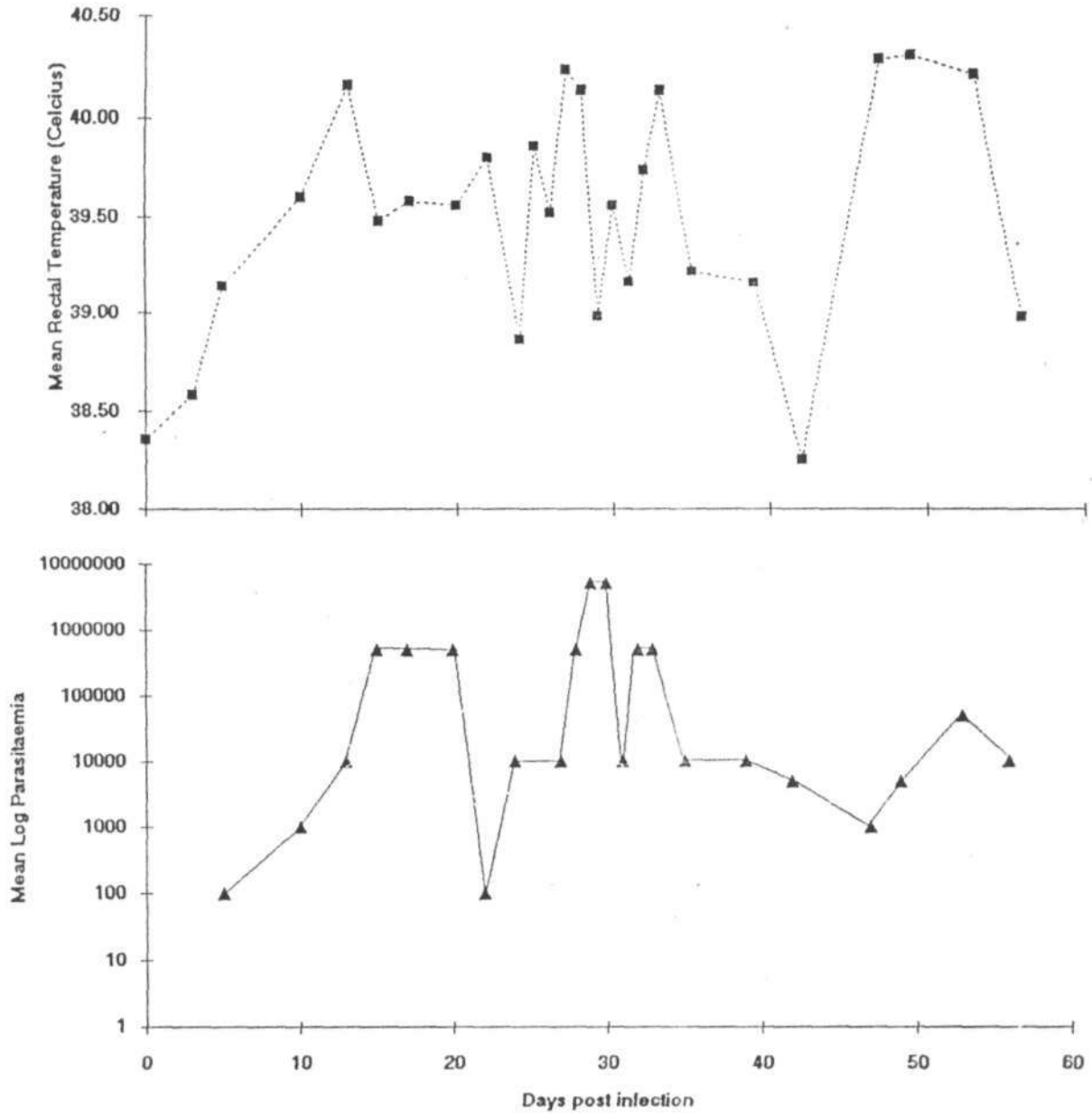


Fig. 2: Mean Rectal Temperature and Mean Log Parasitaemia in *T.congolense* Infected WAD Ewes Showing Temperature elevations Coinciding with Peaks of Parasitaemia.

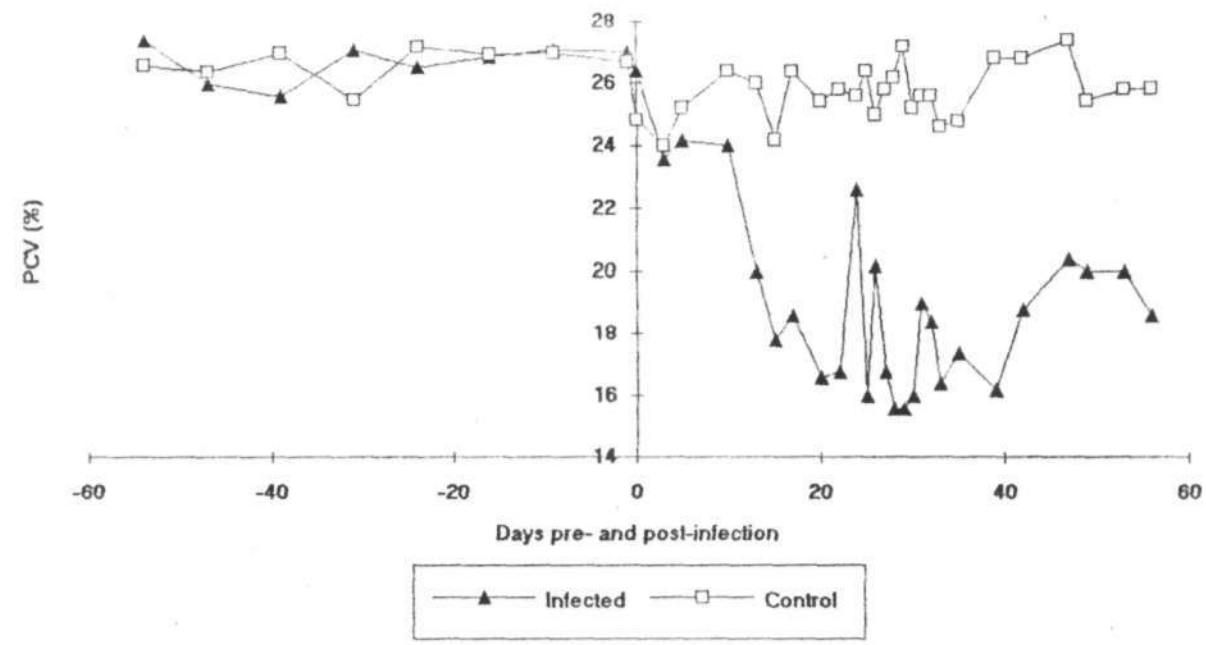


Fig. 3: Mean Packed Cell Volume in *T.congolense* Infected and Control WAD Ewes

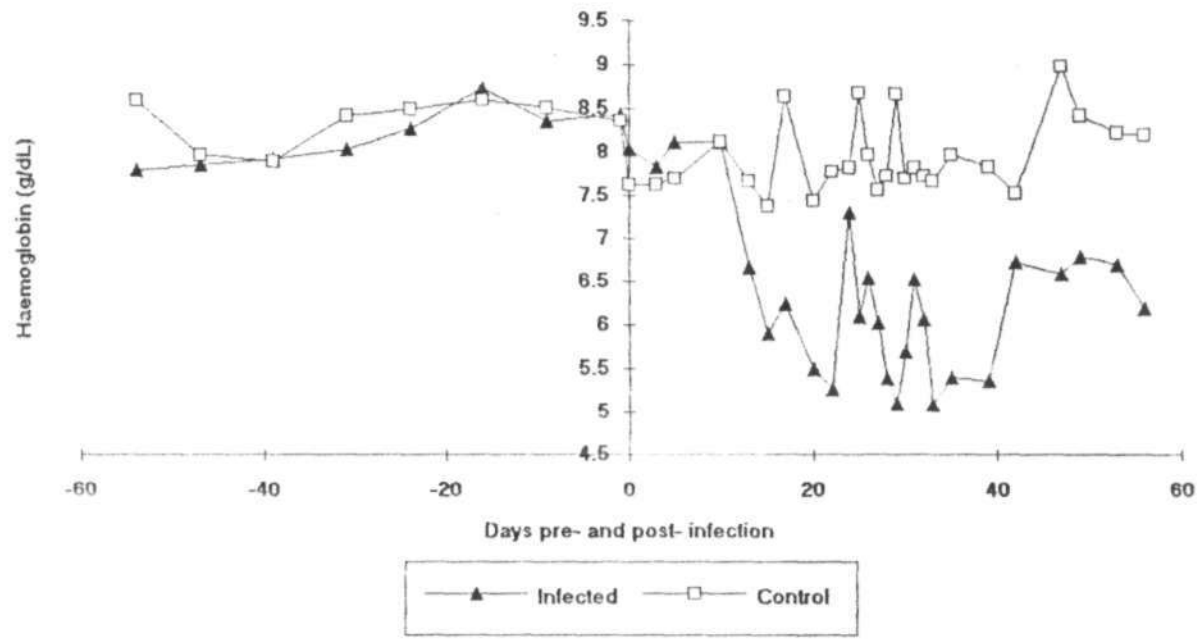


Fig. 4: Mean Haemoglobin Concentration in *T.congolense* Infected and Control WAD Ewes

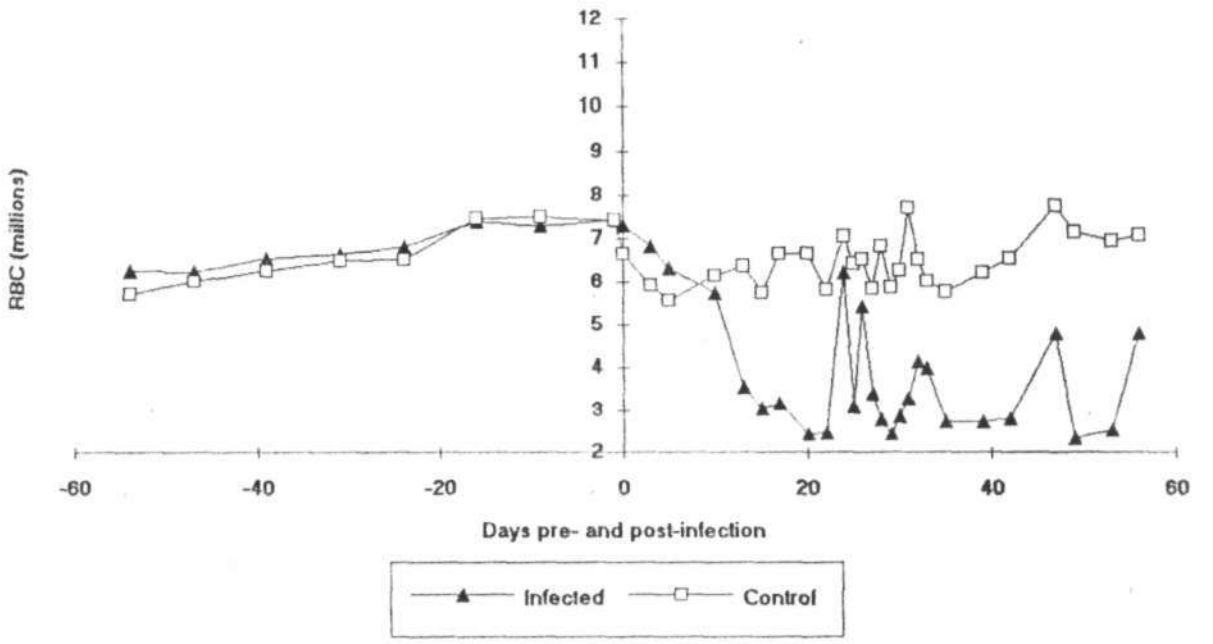


Fig. 5: Mean Erythrocyte Counts in *T.congolense* Infected and Control WAD Ewes

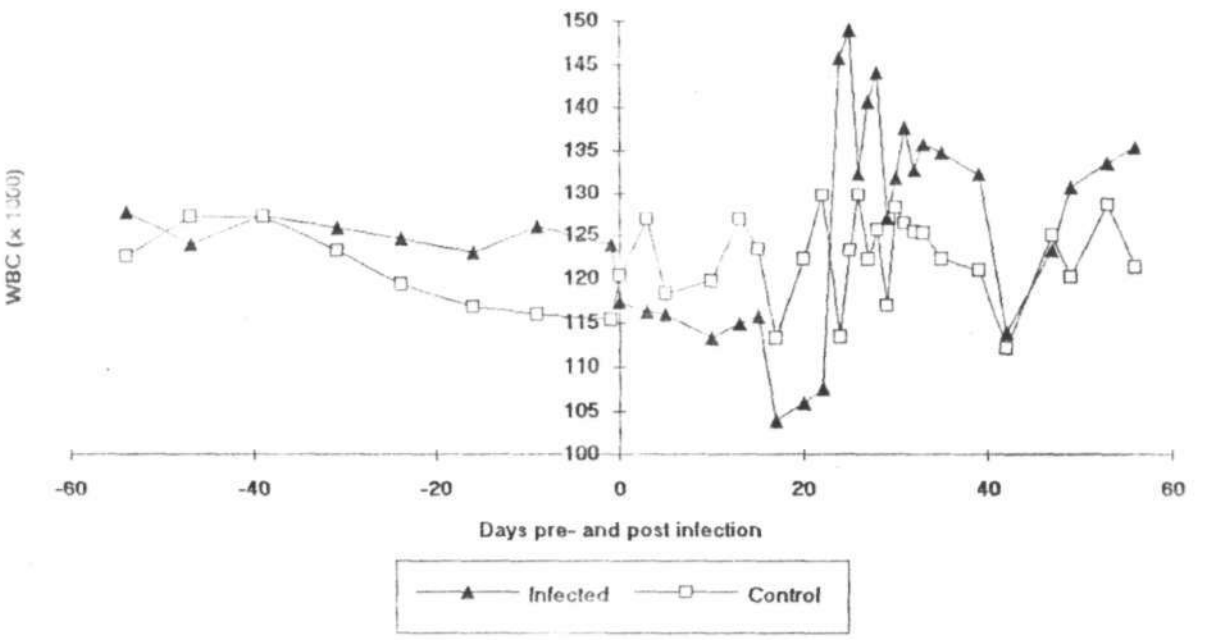


Fig. 6: Mean Leukocyte Counts in *T.congolense* Infected and Control WAD Ewes

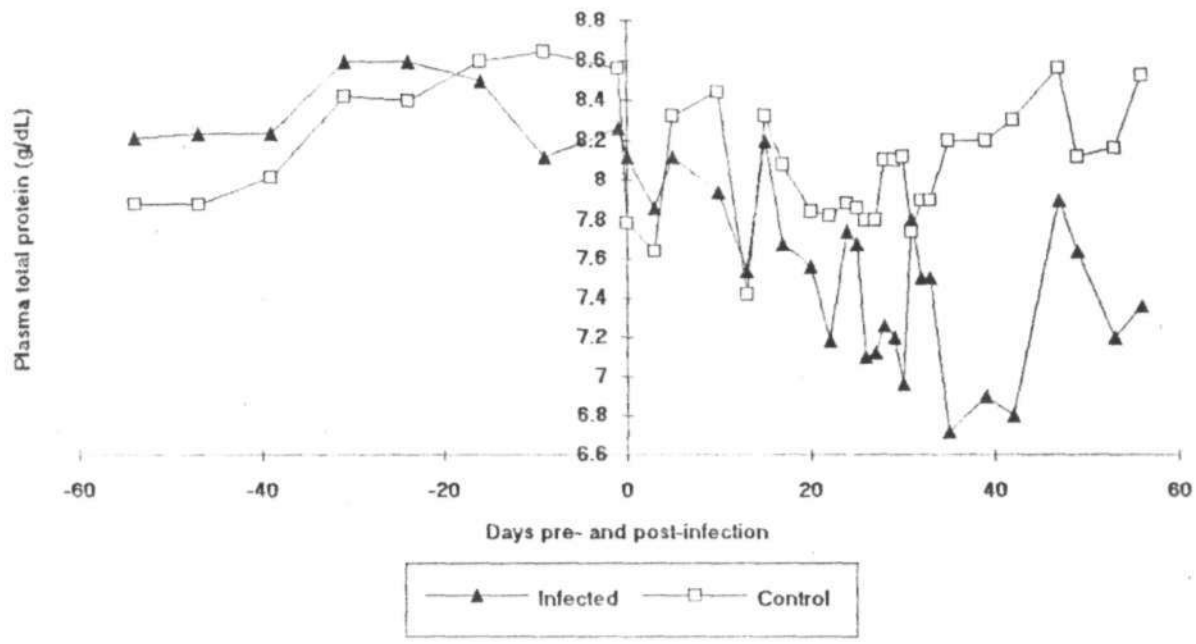


Fig. 7: Mean Plasma Total Protein in *T.congolense* Infected and Control WAD Ewes

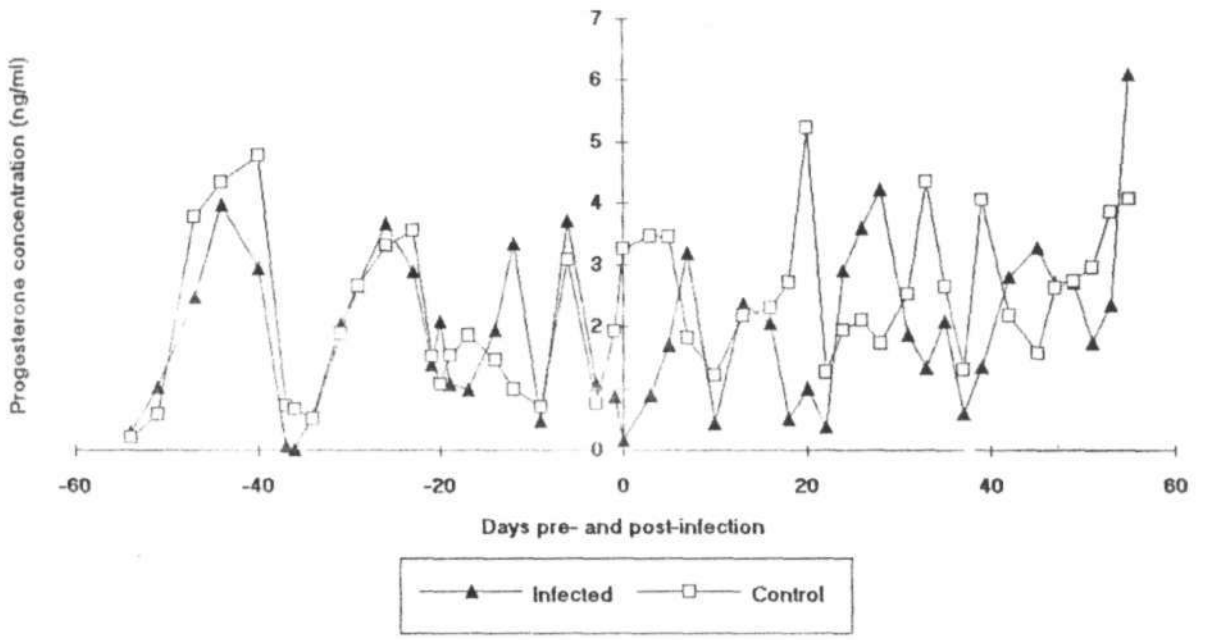


Fig. 8: Mean Progesterone Levels in *T.congolense* Infected and Control WAD Ewes

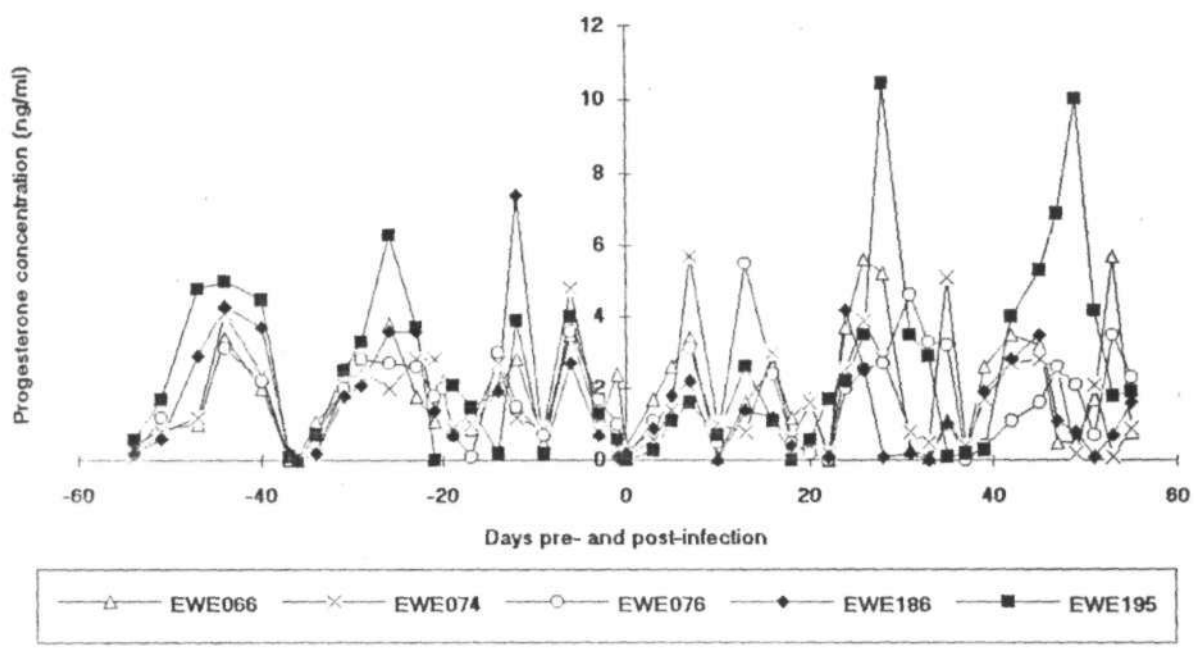


Fig. 9: Progesterone Concentrations in *T.congolense* Infected WAD Ewes

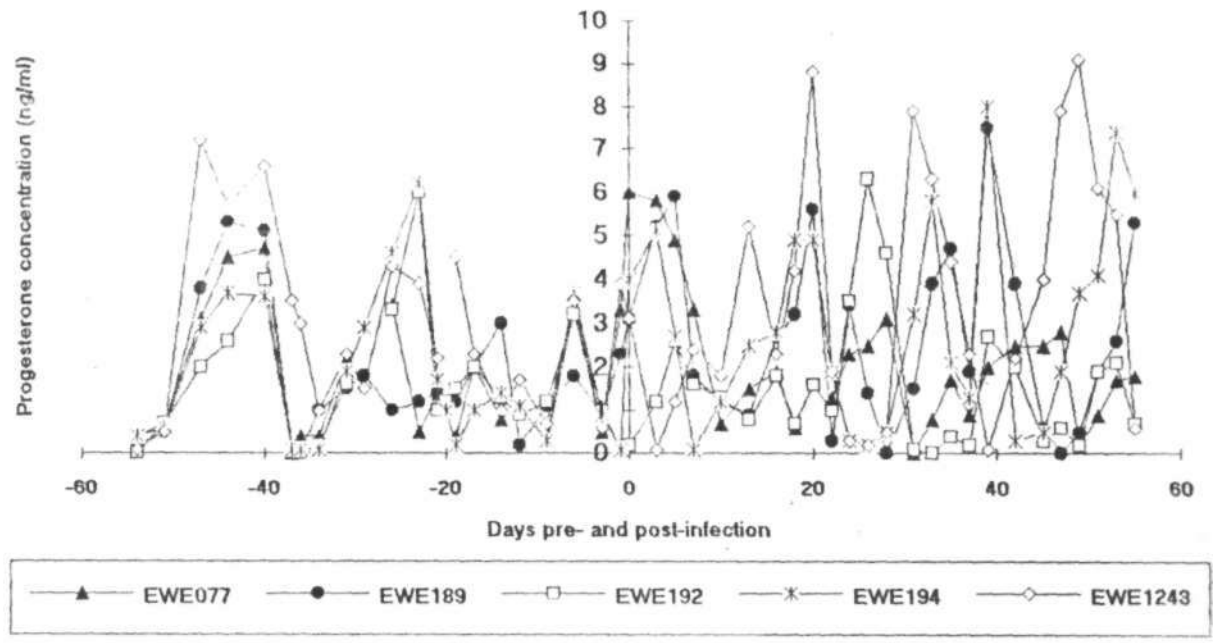


Fig. 10: Progesterone Concentrations in Control WAD Ewes

Table 1: Characteristics of oestrus *T.congolense* infected and control (WAD) ewes

Oestrous Parameter:	Control Ewes: Mean (± SEM*)	Infected Ewes: Mean (± SEM*)
Oestrous cycle (days)	16.4 ± 0.11	16.4 ± 0.31
Duration of oestrus (days)	2.96 ± 0.26	1.53 ± 0.10
Intensity of oestrus (mounts per oestrus)	16.73 ± 0.34	7.47 ± 0.93

* SEM = Standard error of the mean

Table 2: Mean Ovarian Weights, Number of Follicles and Number of Corpora Lutea in *T.congolense* infected and Control WAD Ewes

	Mean Ovarian Weights	Mean No. of Follicles	Mean No. of Corpora Lutea
Infected Ewes (n=3)	1.33 ± 0.06	2.00 ± 0.29	1.50 ± 0.09
Control Ewes (n=2)	1.45 ± 0.05	3.25 ± 0.43	1.75 ± 0.31

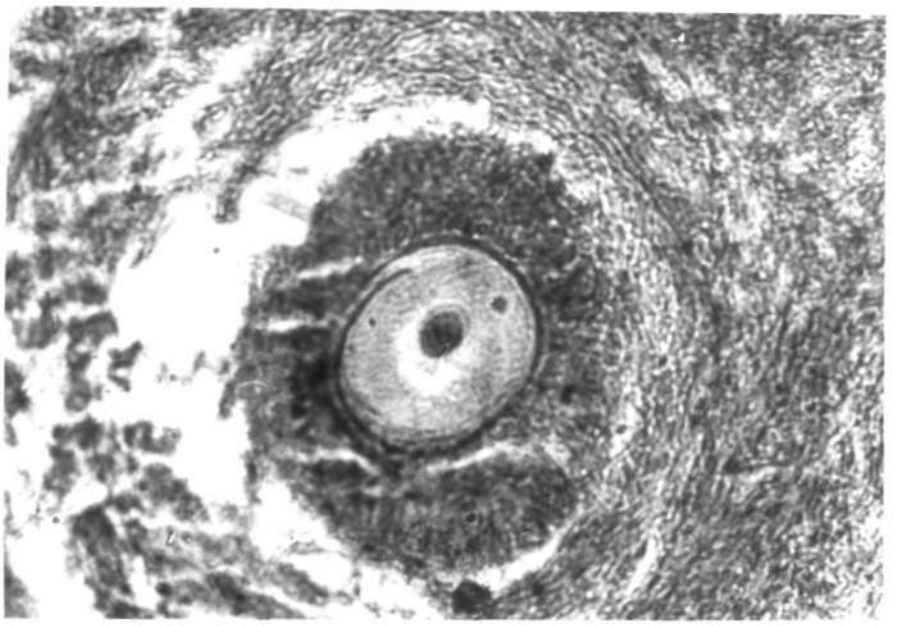


Plate 1: Ovary of an infected ewe showing Graafian follicles with focal areas of necrosis. H & E x 1000.

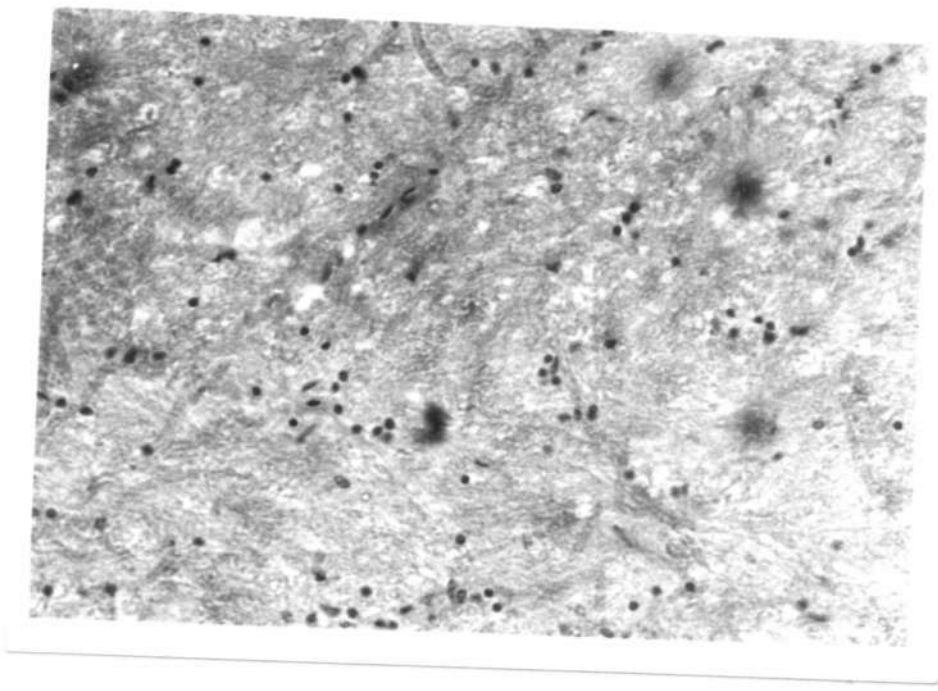


Plate 2: Hypothalamus of a control ewe showing normal nerve cells. H & E x 400.

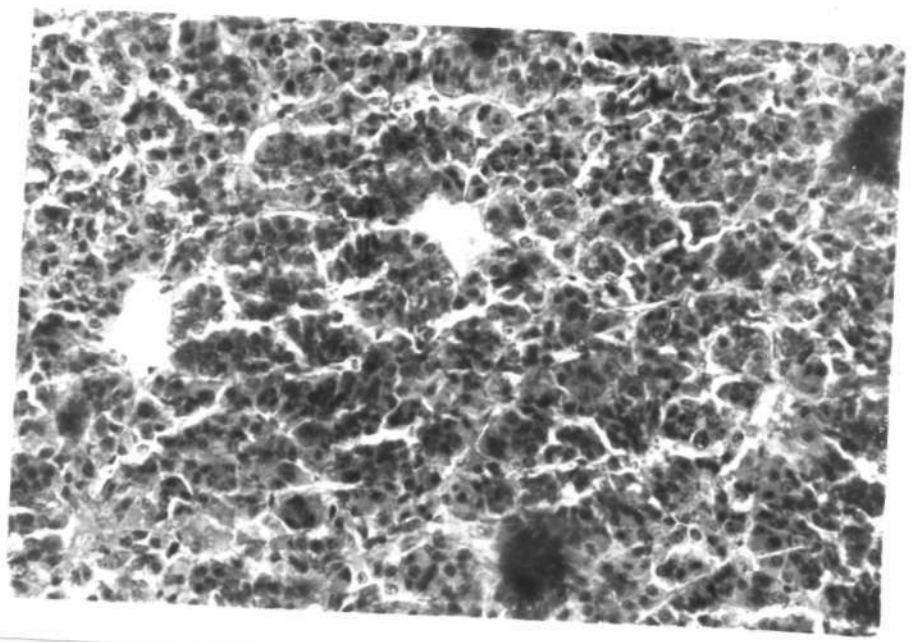


Plate 4: Adenohypophysis of a control ewe showing normal parenchymal cells. H & E x 400.

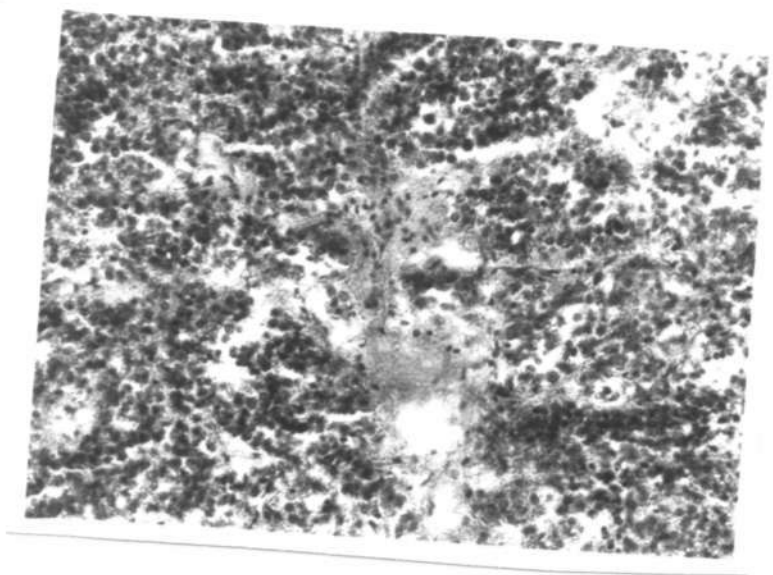


Plate 5: Adenohypophysis of an infected ewe showing focal necrosis of parenchymal cells. H & E x 400.

CHAPTER FIVE

5.0 DISCUSSION

In this study, clinical observations such as intermittent pyrexia, undulating parasitaemia, anaemia, and emaciation are in agreement with previous reports in *T.congolense* infected sheep and goats (Griffin and Allonby, 1979; Llwelyn et al., 1987) and bulls (Sekoni et al., 1990a), *T.vivax* infected sheep (Adenowo, 1989) and cattle (Ogwu et al., 1984).

Temperature fluctuations, which correlated with peaks of parasitemia occurred in all the infected ewes. This feature is commonly observed in animal trypanosomiasis (Murray and Dexter, 1988; Adenowo, 1989; Mohammed, 1991).

The prepatent period ranged between 5 to 13 days with a mean of 10.2 ± 1.2 days. This agrees with the findings of Kalu (1983) but longer than the report of Adenowo (1989) in *T.vivax* infected Yankasa sheep.

Following infection, there was a progressive drop in PCV, Hb concentrations, and RBC counts. The marked decrease in PCV, Hb concentrations, and RBC counts are indications of anaemia. None of such changes were significant in the control ewes. The normal PCV, Hb concentrations, and RBC counts in WAD ewes are 28%, 8.64 g/dl, and 7.7 x 10^6 ml^{-1}, respectively.

The sudden rise in PCV, Hb, and RBC counts on days 24, 25, and 47 post-infection was attributed to erythropoietic response to anaemia. This phenomenon was also observed by Sekoni et al. (1990) and Anosa et al. (1992). The occurrence of initial leukopenia following infection in this study has been earlier reported (Esievo and Saror, 1983; Anosa et al., 1992). Also, the recovery of the total leukocyte counts at the later part of the infection was reported by Esievo and Saror (1983), Anosa (1988), and Anosa et al., (1992).

The decrease in total plasma proteins in the infected ewes has been reported in *T.congolense* infected Welsh Mountain sheep (Mohammed, 1991), and bulls (Sekoni et al., 1990).

The weight loss in the infected ewes, and the slight weight increase in the control ewes encountered in this study are in agreement with previous studies in sheep (Adenowo, 1989).

In the present study, the infected ewes continued to show oestrus throughout the course of the experiment. The range and intensity of behavioural signs expressed during the periods of oestrus were comparably lower during the post-infection period in the infected ewes compared to the control ewes. Similar observations were made by Adenowo (1989) in *T.vivax* infected sheep, and by Llwelyn et al. (1987) in *T.congolense* infected goats. They indicated that standing to be mounted around the time of oestrus is influenced by the social hierarchy amongst the group of goats and or preference shown by the male goat towards individual female

goats.

The mean cycle length of 16.4 (range 10 to 22) days, and the duration of oestrus (24 to 36 hours) observed in this work, both in the control and infected ewes agree with that reported in apparently normal WAD ewes (Orji, 1976), but slightly lower compared to what was reported in apparently normal Yankasa ewes (Osinowo, 1982). The development of post-infection clinical oestrus as observed in this study, is in contrast with the report of its absence in *T.vivax* infected Yankasa ewes (Adenowo, 1989). It is, however, similar to the observation in 1 out of 4 goats infected with *T.congolense* (Llwelyn et al., 1987).

The plasma progesterone concentrations in the control ewes during the luteal phase of oestrous cycle ranged between 4 and 6 ng ml^{-1} which was similar to other reports (Osinowo, 1982; Mukasa-Mugerwa et al., 1990). The same range was obtained in the infected group and remained the same throughout the course of this study. This contrasts with the report of Adenowo (1989) where following T.vivax infection in sheep, the progesterone concentrations during the luteal phase decreased remaining within the range of 1-2 ng ml^{-1} in 2 ewes and became basal for about 40 days in another 2 ewes; while one ewe had basal levels throughout the course of that study.

Grossly, the mean ovarian weights in the infected ewes under this study were not significantly different compared to those of the

control ewes. It was also observed that the number of follicles in the infected ewes did not differ significantly from that of the control ewes. This observation is remarkably in contrast with the reports of Mutayoba et al. (1988) and Adenowo (1989) where the ovaries of the infected ewes atrophied and significant changes occurred. Histological changes observed in the ovaries of the infected ewes included mild degeneration of the ovarian stroma and fewer follicles. However, Adenowo (1989) reported ovarian lesions ranging from mild fibrosis of the ovarian stroma characterized by the presence of a few primary, secondary, atretic follicles, corpus luteum with no trace of tertiary and Graafian follicles, to extensive fibrosis with no evidence of corpus luteum and follicular development in *T.vivax* infected ewes. Likewise, Mutayoba et al. (1988) reported observing degeneration of ovarian stroma and primordial germ cells, in association with inhibition of follicular maturation, lack of corpus luteum formation and formation of polycysts in *T.congolense* infected ewes. However, in cows with *T.congolense* infection (Ogwu and Njoku, 1991), ovarian follicular cyst degeneration, and atrophy and proliferation of the rete ovari were observed, neither of which occurred in this study.

No significant gross lesions occurred in the brain, hypothalamus, and the adenohypophysis of the infected ewes under this study; gross lesions were however reported by Adenowo (1989). The absence of histopathological lesions in the hypothalamus as observed in this study is not in agreement with reports of generalised haemorrhages observed in *T.vivax* infected ewes

(Adenowo, 1989), marked gliosis, perivascular cuffing, localized cellular infiltrations, and encephalomalacia in *T.congolense* infected cows (Ogwu and Njoku, 1991). Although only mild focal necrosis was observed in the adenohypophysis of the infected ewes in this study, Adenowo (1989) reported the occurrence of pathological changes which included mononuclear cell infiltrations into the capsules and the parenchyma of the adenohypophysis, and necrosis of the acidophilic and basophilic cells with dark pyknotic nuclei and faded cytoplasm in *T.vivax* infected ewes. Similar changes were also reported in *T.congolense* infected goats (Mutayoba et al., 1988). In cows infected with *T.congolense* (Ogwu and Njoku, 1991), subcapsular mononuclear cell infiltrations, basophilic degranulation, slight acidophilic cell proliferation, necrosis, and sclerosis were observed in the adenohypophysis.

5.1 CONCLUSION AND SUGGESTIONS

In this study, infection of WAD ewes with *T.congolense* (strain 2295) for 56 days, showed normal ovarian activity with no marked pathological lesions in their reproductive organs in contrast to observations made following *T.vivax* infection in Yankasa ewes (Adenowo, 1989), and *T.congolense* infection in goats (Llwelyn et al., 1987) and cows (Lorenzini et al., 1988; Ogwu and Njoku, 1991). It therefore suggested that WAD ewes are capable of controlling the effects of trypanosomiasis on their reproductive cycle. WAD sheep are known to be trypanotolerant, a phenomenon that might have contributed to the relatively mild effects of

T.congolense infection on the ovarian activities of the ewes under this study. This finding is of great importance for the economic exploitation of this breed of sheep in tsetse infested areas. It is suggested that similar work be carried out in WAD sheep using other trypanosome species.

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APPENDIX 1: Clinical and mean haematological parameters in Control WAD ewes

Time of Infection (Days)	Temp. (^{0}C)	PCV (%)	Hb (g/dL)	RBC (Mil.)	WBC (x10 ^{3})	TProt. (g/dL)
-54	38.68	26.60	8.60	5.71	132.6	7.88
-47	38.62	26.40	7.98	6.01	127.2	7.88
-39	38.90	27.00	7.90	6.26	127.2	8.02
-31	38.50	25.48	8.42	6.50	123.2	8.42
-24	38.70	27.20	8.50	6.52	119.4	8.40
-16	38.50	25.96	8.60	7.48	116.8	8.60
- 9	38.52	26.98	8.52	7.50	116.0	8.64
- 1	38.70	26.72	8.36	7.42	115.4	8.56
0	38.84	24.82	7.62	6.64	120.1	7.78
3	39.16	24.00	7.62	5.93	126.9	7.64
5	38.82	25.20	7.70	5.57	118.3	8.32
10	38.98	26.40	8.12	6.15	119.7	8.44
13	38.52	26.00	7.66	6.37	126.9	7.42
15	38.72	24.20	7.38	5.75	123.3	8.32
17	38.34	26.40	8.64	6.65	113.2	8.08
20	38.54	25.40	7.44	6.64	122.2	7.84
22	38.50	25.80	7.78	5.83	110.9	7.82
24	39.28	25.60	7.82	7.05	113.4	7.88
25	38.60	26.40	8.68	6.42	123.8	7.86
26	38.72	25.00	7.98	6.52	129.8	7.80
27	38.88	25.80	7.56	5.85	122.2	7.80
28	38.62	26.20	7.72	6.82	125.6	8.10
29	38.46	27.20	8.66	5.88	117.0	8.10
30	38.38	25.20	7.70	6.27	128.2	8.12
31	38.70	25.60	7.82	7.71	106.4	7.74
32	38.92	25.60	7.72	6.53	105.4	7.90
33	38.34	24.60	7.66	6.01	105.2	7.90
35	38.42	24.80	7.98	5.76	122.2	8.20
39	38.42	26.80	7.84	6.23	121.0	8.20
42	37.90	26.80	7.52	6.54	112.1	8.30
47	38.96	27.40	8.98	7.74	105.0	8.56
49	38.64	25.40	8.42	7.14	120.2	8.12
53	38.62	25.80	8.22	6.94	128.6	8.16
56	38.50	25.84	8.20	7.08	121.4	8.52

APPENDIX 2: Clinical and mean haematological parameters in *T.congolense* infected WAD ewes

Time of Inf. (Days)	Temp. (^{0}C)	PCV (%)	T.Prot. (g/dL)	RBC (Mil)	Hb (g/dL)	WBC (x10^3)
-54	38.50	27.40	8.22	6.28	7.80	137.8
-47	38.58	26.00	8.24	6.24	7.86	134.0
-39	38.96	25.60	8.24	6.54	7.92	127.4
-31	38.62	27.08	8.60	6.64	8.04	125.8
-24	38.32	26.54	8.60	6.82	8.28	124.6
-16	38.38	26.90	8.50	7.40	8.74	123.0
- 9	38.48	27.08	8.12	7.30	8.36	126.0
- 1	38.50	27.04	8.26	7.42	8.44	123.8
0	38.36	26.38	8.12	7.30	8.04	117.4
3	38.58	23.60	7.86	6.83	7.84	116.2
5	39.14	24.20	8.12	6.32	8.12	116.0
10	39.60	24.00	7.94	5.74	8.12	113.2
13	40.16	20.00	7.50	3.54	6.66	114.9
15	39.48	17.80	8.20	3.05	5.90	115.7
17	39.58	18.60	7.68	3.16	6.24	104.0
20	39.56	16.60	7.56	2.45	5.50	106.0
22	39.80	16.80	7.18	2.46	5.26	107.6
24	38.86	22.60	7.74	6.22	7.30	145.8
25	39.86	16.00	7.68	3.10	6.10	149.0
26	39.52	20.20	7.10	5.42	6.54	132.2
27	40.24	16.80	7.12	3.38	6.02	140.6
28	40.14	15.60	7.26	2.78	5.38	144.0
29	38.98	15.60	7.20	2.45	5.10	127.0
30	39.56	16.00	6.96	2.86	5.70	131.8
31	39.16	19.00	7.80	3.30	6.52	137.6
32	39.74	18.40	7.50	4.13	6.06	132.8
33	40.14	16.40	7.50	3.99	5.08	135.8
35	39.22	17.40	6.72	2.74	5.40	134.8
39	39.16	16.20	6.90	2.73	5.36	132.2
42	38.26	18.80	6.80	2.82	6.72	113.8
47	40.30	20.40	7.90	4.80	7.58	123.2
49	40.32	20.00	7.64	2.34	6.78	130.8
53	40.22	20.00	7.20	2.53	6.70	133.6
56	38.98	18.60	7.36	4.79	6.18	135.4

APPENDIX 3: Mean progesterone concentrations in *T.congolense* infected and control WAD ewes

Time of Infection (Days)	Infected ewes	Control ewes
−54	0.300 (0.094)*	0.220 (0.080)
-51	1.040 (0.191)	0.600 (0.045)
-47	2.475 (0.885)	3.800 (0.897)
-44	3.890 (0.337)	4.360 (0.558)
-40	2.960 (0.486)	4.800 (0.521)
-37	0.080 (0.037)	0.740 (0.690)
-36	0.025 (0.025)	0.700 (0.580)
-34	0.580 (0.171)	0.540 (0.194)
-31	2.060 (0.121)	1.900 (0.158)
-29	2.675 (0.253)	2.667 (0.426)
-26	3.680 (0.730)	3.320 (0.632)
-23	2.900 (0.349)	3.560 (1.180)
-21	1.420 (0.457)	1.540 (0.199)
-20	2.100 (0.100)	1.100 (0.100)
-19	1.100 (0.337)	1.560 (0.774)
-17	1.000 (0.257)	1.860 (0.223)
-14	1.950 (0.486)	1.492 (0.391)
-12	3.360 (1.120)	1.000 (0.241)
- 9	0.500 (0.138)	0.720 (0.191)
- 6	3.720 (0.343)	3.100 (0.336)
- 3	1.100 (0.182)	0.780 (0.097)
- 1	0.900 (0.402)	1.940 (0.793)
0	0.164 (0.066)	3.276 (0.938)
3	0.920 (0.237)	3.480 (1.170)
5	1.725 (0.325)	3.460 (0.850)
7	3.200 (0.702)	1.840 (0.526)
10	0.460 (0.186)	1.240 (0.175)
13	2.380 (0.832)	2.180 (0.813)
16	2.060 (0.384)	2.300 (0.202)
18	0.540 (0.194)	2.720 (0.887)
20	1.025 (0.371)	5.225 (1.477)
22	0.400 (0.327)	1.280 (0.301)
24	2.920 (0.435)	1.960 (0.710)
26	3.600 (0.571)	2.120 (1.130)
28	4.240 (1.740)	1.740 (0.898)
31	1.880 (0.909)	2.540 (1.460)
33	1.360 (0.718)	4.360 (2.030)
35	2.100 (0.906)	2.660 (0.823)
37	0.620 (0.400)	1.320 (0.369)
39	1.380 (0.447)	4.060 (1.570)
42	2.820 (0.491)	2.180 (0.576)
45	3.280 (0.599)	1.580 (0.723)
47	2.720 (1.120)	2.640 (1.400)
49	2.720 (1.850)	2.740 (1.720)
51	1.760 (0.705)	2.980 (0.939)
53	2.360 (1.020)	3.860 (1.110)
55	6.100 (4.430)	4.080 (2.160)

* = Figures in parentheses are the Standard Error of Mean.

APPENDIX 4: Mean parasitaemia in *T.congolense* infected WAD ewes.

Day of infection	Estimated Number of Trypanosomes
5	1×10^2
10	1×10^3
13	1×10^4
15	5×10^5
20	5×10^5
22	1×10^2
24	1×10^4
27	1×10^4
28	5×10^5
29	5×10^6
30	5×10^6
31	1×10^4
32	5×10^5
33	5×10^5
35	1×10^4
39	1×10^4
42	5×10^3
45	5×10^3
47	1×10^3
49	5×10^3
53	5×10^4
56	1×10^4