THE EFFECTS OF *TRYPANOSOMA BRUCEI* INFECTION ON REPRODUCTION IN FEMALE PIGS

BY

ALLAM, LUSHAIKYAA

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A THESIS SUBMITTED TO THE POST-GRADUATE SCHOOL, AHMADU BELLO UNIVERSITY ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY MEDICINE,

DEPARTMENT OF VETERINARY SURGERY AND MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

FEBRUARY, 2004

DECLARATION

I hereby declare that this thesis has been written by me and it is a record of my own research work, carried out in the Department of Veterinary Surgery and Medicine under the direct supervision of Prof. D. Ogwu, Prof. R. I. S. Agbede and Dr. A. K. B. Sackey.

No part of this work 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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Date._____

CERTIFICATION

This thesis titled, The effects of *Trypanosoma brucei* on reproduction in female Pigs, by Allam, Lushaikyaa meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University Zaria and is approved for its contributions to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the Almighty God through his son Jesus Christ, my entire family and Veterinarians who are in Practice and Research all over the world.

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TABLE OF CONTENT

DECLARATION III

CERTIFICATION IV

ACKNOWLEDGEMENT VI

TABLE OF CONTENT VIII

LIST OF FIGURES XII

LISTS OF TABLES XIII

ABSTRACT XVI

CHAPTER 1 1

1.1 INTRODUCTION 1

1.2 REASONS OF THE STUDY AND JUSTIFICATIONS 4

1.3 OBJECTIVES 5

CHAPTER 2 6

2.1 LITERATURE REVIEW 6

2.1.1 PUBERTY IN GILTS 6

2.1.2 ESTRUS 7

2.2 INFERTILITY IN THE SOW 8

2.3 ABORTION IN PIGS 10

2.3.1 INFECTIOUS ABORTIONS IN PIGS 11

2.4 TRYPANOSOMES OF PIGS 12

2.4.1 DISEASE CAUSED BY *TRYPANOSOMA SIMIAE*.....13

2.4.2 DISEASE CAUSED BY *TRYPANOSOMA CONGOLENSE*.....14

2.4.3 DISEASE CAUSED BY *TRYPANOSOMA SUIS*.....15

2.4.4 DISEASE CAUSED BY *TRYPANOSOMA BRUCEI*.....15

2.5 EFFECTS OF TRYPANOSOMOSIS ON REPRODUCTION IN LIVESTOCK ..19

2.5.1 EFFECTS OF TRYPANOSOMOSIS ON REPRODUCTION IN FEMALE LIVESTOCK.....19

2.5.2. EFFECT OF TRYPANOSOMOSIS ON REPRODUCTION IN MALE LIVESTOCK.....21

2.6. DIAGNOSIS OF TRYPANOSOMOSIS.....23

2.7 CONTROL OF TRYPANOSOMOSIS.....24

2.7.1. CHEMOTHERAPY AND CHEMOPROPHYLAXIS.....24

2.7.2. BREEDING OF TRYPANOTOLERANT LIVESTOCK.....26

2.8.3. VECTOR CONTROL.....27

CHAPTER 3.....29

3.0 MATERIALS AND METHODS.....29

3.1 EXPERIMENTAL ANIMALS.....29

3.2 TRYPANOSOMES.....29

3.3 EXPERIMENTAL DESIGN.....30

3.3.1 INOCULATION OF ANIMALS.....30

3.3.2 CLINICAL EXAMINATION AND BLOOD SAMPLING FOR PARASITEMIA.....31

3.3.3 HEMATOCRIT CENTRIFUGE TEST FOR TRYPANOSOMES.....31

3.3.4 PACKED CELL VOLUME (PCV).....32

3.3.5 THIN BLOOD FILMS FOR TRYPANOSOME IDENTIFICATION.....32

3.3.6 WEIGHING OF ANIMALS.....32

3.4 GROSS PATHOLOGICAL EXAMINATION.....33

3.5 HISTOPATHOLOGICAL EXAMINATION.....33

3.6 REPRODUCTIVE EXAMINATION.....33

3.6.1 DETERMINATION OF ESTRUS.....33

3.6.2 DETERMINATION OF FERTILITY.....34

3.6.3 EVALUATION OF PREGNANCY.....34

CHAPTER 4.....35

4.1 CLINICAL SIGNS.....35

4.2 PARASITEMIA.....35

4.3 TEMPERATURE CHANGES.....36

4.4 PACKED CELL VOLUME (PCV).....37

4.5 WEIGHT GAIN.....37

4.6 EFFECTS ON REPRODUCTION.....41

4.6.1 ONSET OF ESTRUS.....43

4.6.2 DURATION OF ESTRUS.....43

4.6.3 MATING.....43

4.6.4 FERTILITY.....44

4.6.5 PREGNANCY.....44

4.7 GROSS PATHOLOGY 49

4.7.1 GENITALIA 49

4.7.2 FETUSES 49

4.7.3 PLACENTA 49

4.8 HISTOPATHOLOGICAL LESIONS 49

4.8.1 PITUITARY GLAND 49

4.8.2 OVARIES 49

4.8.3 FALLOPIAN TUBES 50

4.8.4 UTERUS 50

4.8.5 VAGINA 51

CHAPTER 5 ..... **ERROR! BOOKMARK NOT DEFINED.**

5.1 DISCUSION ..... **ERROR! BOOKMARK NOT DEFINED.**

5.2 CONCLUSION ..... **ERROR! BOOKMARK NOT DEFINED.**

REFERENCES ..... **ERROR! BOOKMARK NOT DEFINED.**

APPENDICES ..... **ERROR! BOOKMARK NOT DEFINED.**

LIST OF FIGURES

FIGURE4.1: MEAN PARASITIMIA OF INFECTED AND CONTROL ANIMALS……...37

FIGURE4.2: MEAN TEMPERATURE OF INFECTED AND CONTROL ANIMALS……..38

FIGURE4.3: MEAN PCV ON INFECTED AND CONTROL ANIMALS…….……39

FIGURE4.4: MEAN WEIGHT GAIN OF INFECTED AND CONTROL ANIMALS…….….40

LISTS OF TABLES

TABLE 4.1: AGE AT PUBERTY OF *T. BRUCEI* INFECTED AND CONTROL PIGS……..……...44

TABLE 4.2: DURATION OF ESTRUS FOR *T. BRUCEI* INFECTED AND CONTROL PIGS.....45

LIST OF PLATES

PLATE 4.1: EXTERNAL GENITALIA OF GILTS IN ESTRUS. 48

PLATE 4.2: EXTERNAL GENITALIA OF GILT NOT IN ESTRUS. 48

PLATE 4.3: GENITAL TOUCH RESPONSE OF GILT IN ESTRUS. 48

PLATE4.4: GENITAL TOUCH RESPONSE OF GILT NOT IN ESTRUS. 48

PLATE 4.5: BOAR MOUNTING GILT IN ESTRUS. 48

PLATE4. 6: INFECTED GILT IN ESTRUS FALLING DURING MOUNTING. 48

PLATE 4.7:INFECTED GILT IN ESTRUS FALLS BEFORE INTROMISSION COULD BE ACHIEVED. 48

PLATE4.8: INFECTED GILT IN ESTRUS UNABLE TO STAND. 48

PLATE 4.9: ANASARCA OF ABORTED FETUSES. 51

PLATE 4.10: PLACENTA SHOWING HEMORRHAGES. 58

PLATE 4.11: SEVERE CONGESTION OF THE ENTIRE GENITALIA. 59

PLATE 4.12: FOCAL AREAS OF NECROSIS IN THE PITUITARY GLAND. 60

PLATE 4.13: DIFFUSED AREAS OF NECROSIS AND FIBROSIS IN THE PITUITARY GLAND. 511

PLATE 4.14:INFLAMMATION AND NECROSIS OF OVARIES WITH DEGENERATION OF GRAAFIAN FOLLICLES 51

PLATE 4.15: RESORPTION OF FETAL TISSUES IN THE UTERUS. 52

PLATE 4.16: *T.BRUCE*I IN FETAL TISSUES IN THE UTERUS OF AN INFECTED PIG. 52

PLATE 4.17: ENDOMETRITIS WITH NECROSIS OF THE UTERINE GLANDS. 52

PLATE 4.18: VAGINA SHOWING INFLAMATION AND PHLEBITIS. 53

PLATE 4.19: NECROSIS OF THE STRATIFIED SQUAMOUS EPITHELIUM OF THE VAGINA. 54

LIST OF APPENDICES

APPENDIX 1: WEIGHTS OF INFECTED PIGS IN KGS AND NUMBER OF DAYS POST INFECTION AND MEANS FOR THE GROUP 84

APPENDIX 2: WEIGHTS OF CONTROL PIGS IN KGS AND NUMBER OF DAYS POST INFECTION AND MEANS FOR THE GROUP ..... **ERROR! BOOKMARK NOT DEFINED.**

APPENDIX 3:TEMPERATURE, PERCENTAGE PCV, LEVELS OF PARASITEMIA OF PIGS NO 1……....……....86

APPENDIX 4: TEMPERATURE, PERCENTAGE PCV, LEVELS OF PARASITEMIA OF PIGS NO 4…….89

APPENDIX 5 TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIGS NO 6……...90

APPENDIX 6 TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIGS NO 9……...…....92

APPENDIX 7 TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIGS NO 10……...94

APPENDIX 8:TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIGS NO 11……...96

APPENDIX 9:TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIG NO 13……..……...……..…...97

APPENDIX 10 TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIG NO 16……....99

APPENDIX 11 TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIG NO 15……..……..……..…...101

APPENDIX 12 TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIG NO 2…….……..…103

APPENDIX 13 TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIG NO 5……...……..….106

APPENDIX 14 TEMPERATURE, PERCENTAGE PCV, LEVEL OF PARASITEMIA OF PIG NO 17……...……..……...109

ABSTRACT

The aim of this research was to investigate the effects of *Trypanosoma brucei* infection on age of attainment of puberty, duration and intensity of estrus, fertility and pregnancy.

Twelve healthy crossbreed gilts were divided into two groups. Group one comprised eight gilts which were infected while group two had four which were uninfected control. The gilts in the infected group were each inoculated with 1.8×10^6 *Trypanosoma brucei*, when they were 153 days old.

Trypanosomosis took a short severe and acute course killing two gilts and later a debilitating disease causing wobbling of the hind legs of the remaining infected gilts. This was noticed between 42 and 49 days post infection and was characterized in some of the animals by anestrus, inability to mate and abortion. However, the disease appeared to have no effect on age at puberty and duration of estrus. Inflammation and necrosis of the reproductive organs, fetal tissues retained in the uterus of infected pigs under going necrosis, resorption and sometimes containing *T. brucei* were main features noticed histopathologically.

This study suggests that *Trypanosoma brucei* can seriously impair the reproductive performance of gilts, therefore adequate control measures are required if Nigeria is to boast of self sufficiency in pig production.

CHAPTER 1

1.1 INTRODUCTION

How can the problem of animal protein deficiency in Nigeria be solved within the shortest possible time based on the country's available resources? Many scientists believe that this could be achieved through pig resources. That is through the development of the piggery industry and by fully exploiting its potentials (Chidebelu and Amadi, 1990).

Nigeria has the second largest population of pigs in Africa, with a herd size of 4.41 million (Bukar *et al*., 1997). There has been a remarkable increase in this figure when compared to the population of 0.9 million pigs reported in 1986 by Federal Livestock Department of Nigeria (Chidebelu and Amadi, 1990).

Pigs are produced in all parts of the country except in the core North which has a predominantly Muslim population where much emphasis is being placed on increasing beef supplies particularly in the urban markets, but due to the long gestation period of cattle and the high rate of its take off, an increase in its number is invariably a slow process, animals with shorter gestation periods have to be considered to rapidly increase meat supply within a short time (Adebayo and Sorungbe, 2002).

It has been found that if proper feeding, good breeding systems, sound sanitation and proper housing are ensured, and then the disease aspect becomes the limiting factor in pig production (Ngatia and Kiptoon, 1989). One of such diseases is trypanosomosis, a complex disease that affects man and livestock and is responsible for major constraints to the agricultural and socio-economic development of tropical Africa (Ilemobade, 1981; Llewelyn *et al*., 1987; Ikede, 1989).

Trypanosomosis in man is caused by two species of trypanosomes: - *Trypanosoma rhodensiense* and *T. gambiense*. The disease in man is characterized by fever, severe headaches, lymphadenopathy, general debility, insomnia, impotence, abortion in pregnant women, convulsion, coma and death in the early stages. In the later stages of the disease the name sleeping sickness best describes the signs that are seen, in which the affected person becomes morose with a mask like expression, relaxed features, hanging of lower lip, puffiness, drooping of eye lids, a tendency to lapse into sleep, restlessness at night, slowed speech, swaying gait, twitching of muscles of the tongue, tremors of the glands, and reluctance to move. If untreated the condition of the patient worsens leading to coma and death (Losos, 1986).

The most commonly occurring pathogenic trypanosomes in animals are; *T. vivax, T. congolense* and *T. brucei*, which cause disease in cattle, buffaloes, sheep, goats, pigs, donkeys, horses, camels, dogs, cats and laboratory animals. *T. evansi* causes disease in dogs, buffalos, horses, sheep and goats. *T. equinum* causes disease in dogs and horses; *T. simiae* infects primarily pigs and sometimes camels, sheep, and goats while *T. equiperdum* causes disease in horses (Sekoni, 1994). The infections of these pathogenic trypanosomes cause disease which varies in severity depending on the virulence of the organisms and susceptibility of the host. Most forms of trypanosomosis do not have pathognomonic signs. The clinical signs exhibited by infected animals include - fever, anorexia, emaciation, anemia, agalactia, epiphora, pica appetite, abortion and death (Losos, 1986). The disease also affects the reproductive system of animals causing irregular estrus, depressed estrus, sperm abnormalities and premature deliveries (Ogwu and Nuru, 1981; Anosa, 1983; Murray *et al*., 1981; Mutayoba *et al*., 1988).

Reports on *T. brucei* infections in pigs have been contradictory. A mild chronic disease producing little or no pathological lesions has been documented (Stewart, 1947; Ilemobade and Balogun, 1981). Infected pigs have also been reported to recover without treatment (Barnett and Stewart,

1965), but an outbreak reported by Agu and Bajeh (1986), showed that the parasite was very pathogenic to pigs causing a fatal disease.

However little information on the effects of this parasite in pigs and particularly on the reproductive system exists when compared to other domestic animals. This may be due to lack of interest in this area of research, even though surveys in areas around Nsukka in eastern Nigeria have shown that the disease could limit reproductive performance of these animals (Onah, 1991).

1.2 REASONS OF THE STUDY AND JUSTIFICATIONS

The pig is a very important source of protein in most parts of the world. In addition to having a shorter gestation period, pigs have higher prolificacy than cattle, sheep or goats. Pigs are also of high nutritive value, have high dressing percentage and are non capital intensive, requiring minimal investments in addition to adapting easily to self feeding on farm residues coupled with high feed conversion efficiency (Eusebio, 1980).

Despite all these attributes of the pig, diseases like trypanosomosis have been a hindrance to its production.

1.3 OBJECTIVES

The objective of this work was to study the reproductive performance of pigs experimentally infected with *T. brucei*. In doing so attention was focused on the effects of the parasite on the following parameters:-

Age of attainment of puberty, duration of puberty, fertility and pregnancy.

CHAPTER 2

2.1 LITERATURE REVIEW

2.1.1 Puberty in Gilts

This is the age when reproduction becomes possible. It is usually considered to be the age at first estrus (Cupps *et al*., 1969). Ovulation is also known to occur at this time (Roberts, 1986). Puberty does not signify full or normal reproduction capacity which develops later, however its attainment is a slow and gradual process which depends upon the anterior pituitary (Roberts, 1986). Puberty occurs in gilts at 6 to 8 months of age. However gilts have reached puberty as early as 4 months and as late as 9 months (Cupps *et al*., 1969).

The age at which puberty is attained may be influenced by the level of nutrition, social environment, bodyweight, season of the year, breed of the pigs, infectious or non infectious diseases, parasitic diseases, management practices and genetic factors (Hafez *et al*., 1969; and Roberts, 1986). The age of the gilts is more important than weight and nutrition in determining the onset of puberty (Hughes and Valey, 1980; Hurtgen, 1981; Hurtgen, 1982).

Puberty is hastened in the gilts by cross breeding and by the presence of the male. However it is delayed in gilts penned individually as compared

to those penned in a group. Delay in attainment of puberty is also noticed in gilts that are on diet restricted in protein and those raised under stressful conditions (Hafez 1975; Zimmerman and Cunningham, 1975; Roberts, 1986).

2.1.2 Estrus

This is the phase in the estrus cycle in which the female will accept coitus (Cupps *et al*., 1969; Sorrensen, 1979). The period is well defined, and it is characterized by sexual desire and immobility in the presence of the male (Roberts, 1986). Estrus may begin at any time of the day (Cupps *et al*., 1969), its duration varies with individual animals. Various periods have been reported in pigs, these are, 59.3 hours (Cupps *et al*., 1969), 44 hours (Sorrensen, 1979), 24 to 96 hours (Roberts, 1986). However it is generally accepted that estrus begins with the time of the first acceptance of the male and ends with the last acceptance of the male (Roberts, 1986). It is influenced by factors such as parity, season, stress, boar effects, and when weaned piglets are removed from their mother (Weitze *et al*., 1994; Kemp and Soede, 1996; Soede and Kemp, 1997).

Noticeable changes of the vulva and behavioral patterns are seen during estrus and they vary with the time of estrus in individual animals. At the beginning of estrus the vulva swells, it becomes congested or red. The

animal at this time becomes easily disturbed. Other animals in the pen begin to mount it, but it usually will not stand to be mounted. The mammary glands at this stage begin to develop. Later, the vulva reddening starts to subside, mucus discharge is noticed, and the animal begins to mount other females and stands to be mounted. Other females are also known to emit high pitched grunts during this time which is said to be characteristic. Appetite decreases, ears become erect; lordoses response is noticed and rub marks are also seen on the back of the animal (Hafez *et al*., 1969; Sorrensen, 1979; Carr, 1996).

Estrus has also been shown to occur immediately after delivery of the conceptuses. This is referred to as post partum estrus. It frequently occurs within 1 to 3 days after parturition. If mated the sow fails to become pregnant (Dunne and Leman, 1975).

2.2 INFERTILITY IN THE SOW

Reproductive failure has been considered to be the main reason why sows and gilts are culled. However, only a small proportion of the animals that are culled are found to be permanently sterile (Arthur *et al*., 1983).

The incidence of infertility in sows may approach that seen in cattle, but because of their lower values and difficulties in diagnosis and treatment,

this has not been studied as much as infertility in cattle and horses (Roberts, 1986).The best index of accessing infertility in pigs is to determine the Percentage of females which farrow to the first service; this should be about 70-80% (Arthur *et al*., 1989).

The causes and type of reproductive failure are as a result of structural or functional abnormalities and infections (Arthur *et al*., 1983; Roberts, 1989). The structural defects of the genitalia of the female pig are congenital rather than acquired. They are seen more in gilts than in sows (Wilson *et al*., 1949). These lesions include double vagina, septae or strings in the vagina, hymeneal residue and hymeneal stricture (Teige, 1957).

Functional infertility is due to impairment of estrus, ovulation and gestation as a result of hereditary and environmental factors (Arthur *et al*., 1983). Lack of estrus is one of the chief components of female infertility in the pigs (Perry, 1956). Where management and nutrition are satisfactory, poor estrus manifestation in gilts is as a result of hereditary factors, persistent corpora lutea, pyometra and luteal cyst.

Ovulation is either absent or occurs at a low rate when gilts first show estrus at about 6 months of age (Arthur *et al*., 1983). Stress factors as well as diet affect ovulation. Supplementation of diet is known to increase the number of ovulations in the pigs, but if continued after estrus it predisposes

to embryonic death. Hereditary differences in ovulation rate occur between breeds and between families within breeds. (Arthur, 1983; Barbet *et al.,* 1979).

A considerable degree of embryonic mortality is regarded as a characteristic feature of normal porcine gestation and the death of 40% of fertilized ova is considered normal with mummification, abortion and maceration accounting for one third of the 40% while embryonic death makes up the remaining two third (Arthur,1983)

Specific infections of the genital tract that cause infertility in swine include brucellosis, leptospirosis, porcine parvovirus, porcine enterovirus, and pseudorabies (Floss and Tubbs, 1993).

2.3 ABORTION IN PIGS

Abortion is defined as the expulsion before full term of a conceptus incapable of an independent life. It differs from premature delivery which is the expulsion before full term of a fetus capable of independent life (Hubbert, 1971). Since abortion occurs before the gestation period is over, there is, therefore, fetal immaturity, and the newly aborted fetus is generally dead or dies shortly thereafter (Dunne and Leman, 1975).

Abortion may be spontaneous or induced, infectious or non infectious with the non spontaneous type being due to genetic, chromosomal, hormonal

or nutritional factors whilst spontaneous abortions may also occur in animals bred immediately after puberty or immediately after parturition (Roberts, 1986).

Other causes of abortion include infections due to bacteria, viruses, parasites, nutritional deficiencies, toxemias, poisoning, genetic factors, management and miscellaneous factors like starvation, mastitis, metritis and fatal stomach ulcer (Dunne and Leman, 1975).

2.3.1 Infectious Abortions in Pigs

Blood and Radostists (1989) reported that infectious abortions in pigs are due to; brucellosis, leptospirosis, parvovirus infections, Aujeszky disease, still birth, mummification, embryonic death and infertility virus SMEDI, and erysipelas. They described the various clinical pictures of each disease where brucellosis and leptospirosis cause abortion in the 3rd trimester. Also in brucellosis the herd history of infertility, orchitis in the boar and high neonatal mortality may give a general idea of the causative agent while in leptospirosis, hepatic necrosis in fetuses that have been aborted may help in the presumptive diagnosis. Distinguishing between the two diseases is usually by laboratory examination. Parvovirus infection can cause outbreaks of abortion involving all ages of sows with a concurrent occurrence of early embryonic death and infertility in association with still

birth, mummified fetuses and small litter size. Aujeszky disease in early pregnancy may result in embryonic death or abortion but there is early return to heat. The condition is usually accompanied by abundant vaginal discharge. The disease also causes abortion in the late pregnancy and birth of mummified fetuses. Parvovirus infections and SMEDI closely resemble pseudorabies in their reproductive inefficiency and they require laboratory and serological testing to differentiate them. Erysipelas causes abortion like other systemic bacterial infections that cause severe systemic reactions.

2.4 TRYPANOSOMES OF PIGS

Trypanosomes cause hemoparasitic diseases of great economic importance affecting animals and humans in regions of the African continent between latitudes of 14^{o}N and 20^{o}S, where the vector, tsetse fly abounds (Siegmund, 1973). These diseases are responsible for major constraints to the agricultural and socio-economic development of tropical Africa (Ilemobade, 1981; Llewelyn *et al*, 1987; Ikede, 1989).

Trypanosomosis of pigs is caused by *T. simiae*, *T. brucei*, *T. congolense* and *T. suis*. (Losos, 1986; Sekoni, 1994; Seifert, 1996). These trypanosomes are transmitted by *Glossina palpalis, G. tachinoides* (Riverine tsetse); *G. morsitans sub morsitans* and *G. longipalis*.(Savanna tsetse).

2.4.1 Disease Caused By *Trypanosoma simiae*

T. simiae is usually associated with deaths soon after parasitemia reaches its peak. It is difficult to observe a sick pig infected with this trypanosome, therefore it is described as the lightening destroyer of domestic pigs (Unsworth, 1952; Stephen, 1986). In Nigeria *T. simiae* is transmitted mostly by *Glossina morsitans* (Agu *et al*, 1983). The parasite has also been reported to be transmitted mechanically by other biting flies like *Stomoxys* and *Tabanids* to pigs in areas where tsetse flies are absent (Losos, 1986; Stephen, 1986).

The prepatent period of *T. simiae* in pigs is between 3 to 5 days (Ilemobade and Balogun, 1981). Stephen (1986), reported a prepatent period of up to 6 days, while Roberts and Gray, (1971) had earlier reported a prepatent period of between 4 to 5 days. The parasitemia seen in infected pigs is usually very high (Stephen, 1986) with the rise in parasitemia being very rapid and it keeps increasing until the animal becomes very ill (Stephen, 1986).

The major clinical signs, seen in pigs infected with *T. simiae* are fever, dullness, respiratory distress, reluctance to rise, unsteady gait, and cold extremities (Stephen, 1962). Other reported signs are salivation, frothing of the mouth, diarrhea and edema (Stephen, 1972). Poor carcass

weight, retarded growth, poor feed conversion and poor carcass traits due to infection with this parasite have been reported by Ilemobade and Balogun (1981). Hyperemia of the skin has also been reported in pigs infected with this parasite (Watson and Williamson, 1958).

The pathological lesions that have been reported in pigs infected with *T. simiae* include thrombosis, necrosis of tissues and organs and hemolysis of the blood (Losos, 1986). Thrombosis of medium sized blood vessels, large veins, arterioles and plugging of the capillaries of the liver, lungs, kidney cortex, lymph nodes, brain, adrenal cortex and intestinal mucosa have also been reported by Isoun (1968) and Mohammed (1992).

2.4.2 Disease Caused By *Trypanosoma congolense*.

Literature on *T. congolense* infections in pigs is scanty when compared to those on *T. simiae* and *T. brucei*. The prepatent period of this parasite in an experimental infection of pigs was between 7-9 days and this was followed by a pattern of parasitemia described as scanty and sporadic (Ilemobade and Balogun, 1981). Stephen and Gray (1960) had also observed the same pattern of parasitemia in pigs.

Pigs infected with *T. congolense* usually develop sub-clinical disease. The animals appear clinically normal. The disease is not considered a limiting factor for pig production unless such animals are subjected to

additional stress such as under nutrition and concurrent disease (Ilemobade and Balogun, 1981). However Stephen (1962) had reported poor weight gain, progressive anemia, muscular weakness, in-coordination and loss of body condition have been reported in pigs infected with this parasite.

2.4.3 Disease Caused By *Trypanosoma suis*

The infection of pigs with this parasite is considered to be the least important and most undefined form of trypanosomosis than the infection with the other trypanosomes infective to pigs (Stephen, 1986; Losos, 1986).

The prepatent period in an experimental infection was found to be between 6 to 9 days (Peel and Chardome, 1954).

Pigs infected with this parasite loose weight and appear sleepy with high parasitemia. However, their appetite is not affected by the disease (Stephen, 1986). The disease produced by this parasite is said to resemble chronic syndromes caused by *T. brucei* (Losos, 1986).

2.4.4 Disease Caused by *Trypanosoma brucei*.

T. brucei infection in pigs was considered as producing a mild chronic disease without associated pathological lesions and even when pathological

lesions were present, they were very little (Ilemobade and Balogun, 1981; Stewart, 1947). Cases of spontaneous recovery of infected pigs have been reported (Barnet and Stewart, 1965). However, there was an outbreak of trypanosomosis due to *T. brucei* which produced a fatal disease in pigs (Agu and Bajeh. 1986). The prepatent period of this infection in pigs has been reported to be 3 to 4 days (Agu and Bajeh, 1986), 3 to 5 days (Ilemobade and Balogun 1981) and 4 days (Omeke and Onuora, 1992).

Field and market surveys carried out in the endemic middle belt zone of Nigeria have shown that there is a significant prevalence of *T. brucei* infections in pigs. 66.5% of 1954 pigs surveyed had mixed infection with *T. congolense*, while 23.9% were infected with *T. brucei* alone. Another survey in three local government areas in Nsukka zone of Anambra state revealed 58.6% prevalence of *T. brucei* alone and 30.7% prevalence for mixed infection of *T. brucei* and *T. congolense* (Onah, 1991).

The level of parasitemia seen in pigs infected with *T. brucei* has been described as generally fluctuating during the course of the disease (Omeke and Ugwu, 1991; Omeke and Onuora, 1992). The parasite has been reported to sometimes disappear from peripheral circulation giving an indication of a self cure (Agu *et al.,* 1983). The invasion of the blood by actively dividing trypanosomes during this infection is associated with increased body

temperature. The initial parasitemia and fever noticed usually persist for several days before a trypanolytic crisis is said to occur, where the parasites become well reduced in the blood and the temperature of the animal returns to normal. The first trypanolytic crisis is usually followed by further intermittent periods of parasitemia associated with febrile attacks and remission of infection (Seifert, 1996).

The subsequent course and outcome of trypanosomosis varies considerably and is influenced by the breed, background and management of the stock concerned, the nature and severity of trypanosomes challenge, the pathogenicity of the infecting trypanosomes and the period of exposure to infection (Seifert, 1996).

The clinical signs exhibited by pigs infected with *T. brucei* have been reported to be intermittent fever, high and eventually fluctuating parasitemia, increasing degrees of anemia, progressive loss of weight and condition, anorexia, nervous signs of cycling, wobbling of hind legs dehydration, hyperemia, petechial hemorrhages, ascites and death (Onah and Uzoukwu, 1992; Otesile *et al.*, 1992; Omeke and Ugwu, 1991). Mild infections with little effect on packed cell volume, voluntary feed intake, mean live weight gain, feed conversion efficiency have also been noticed in pigs that were experimentally infected with the parasite (Ilemobade and Balogun, 1981).

The pathology caused by *T. brucei* is more widely distributed in the host when compared to other species of trypanosomes. The parasite infects blood plasma, intercellular fluid of the connecting tissue of various organs and the extra cellular fluid of the body cavity (Seifert, 1996). Gross pathological lesions associated with *T. brucei* infections in pigs are emaciated carcasses and atrophy of fat (Agu and Bajeh, 1986; Otesile *et al.*, 1992). Extensive hemorrhages involving mucus and serous surfaces, areas of emphysema in the lungs and gastro-enteritis, anaemic carcass, enlarged spleen and lymph nodes are other pathological lesions associated with this infection (Seifert, 1996).

Histologically, *T. brucei* has been reported to be responsible for mononuclear cellular infiltration, proliferation and distortion of follicles, tissue necrosis or fibrosis and in some cases invaded lymphoid tissues become hemorrhagic and are eventually destroyed. (Omeke and Ugwu, 1991) Interstitial myocarditis, meningo-encephalitis, interstitial nephritis with masses of extra vascular trypanosomes and widespread colonization of other organs and tissues by the trypanosomes has also been reported (Bungener and Mehlitz, 1984). Otesile *et al*, (1992) also reported congestion, edema and macrophage hyperplasia of lymph nodes in four pigs that were experimentally infected with *T. brucei*.

2.5 EFFECTS OF TRYPANOSOMOSIS ON REPRODUCTION IN LIVESTOCK

Reproduction in both male and female is under neuro-endocrine control. Diseases such as trypanosomosis that have pathological effects on the endocrine glands (especially the anterior pituitary gland and the gonads) are most likely to interfere with the endocrine control of reproduction and normal reproductive processes in both sexes (Sekoni, 1994).

2.5.1 Effects of Trypanosomosis on Reproduction in Female Livestock

Delayed puberty, depressed estrus and impaired fertility due to an infection in gilts by *T. brucei brucei* have been reported (Omeke and Rawlings, 1998). Anestrus was also identified as one of the problems sows infected with *T. brucei* and *T. congolense* in single or mixed infections had during a 12 month survey that was carried out in Nsukka zone of Anambra state (Onah, 1991).

Pregnant ewes have also been reported to have aborted their pregnancy when infected with *T. brucei*. These abortions occurred in the 1st, 2nd and 3rd trimesters. Some ewes were able to carry their pregnancy to term and delivered very weak lambs that later died. Other infected ewes died with their pregnancy, but one of the ewes delivered successfully (Edeghere *et al*., 1992; Adelakan and Adeyemo, 1989). *T. vivax* has been reported to be responsible for silent estrus, irregular estrus and temporary estrus that was

seen in ewes in an experimental infection. The infection also caused complete anestrus in the same animal specie (Adenowo, 1989; Elhassan, *et al*., 1994). *T. congolense* has been reported to cause irregular and short estrus cycles and anestrus in goats (Lunkins *et al*, 1986; Mutayoba *et al*, 1988). In cows both *T. vivax* and *T. congolense* have caused anestrus in infected cows (Ogwu *et al*., 1981; Llewellyn *et al*., 1988).

Trypanosomosis has also been shown to cause genital lesions like persistent corpora lutea in goats infected with *T. congolense* (Llewellyn *et al*., 1986; Lunkins *et al*., 1987), and cystic degeneration of ovaries due to *T. vivax* in goats (Isoun and Anosa., 1974; Mutayoba *et al*., 1985). Mutayoba *et al.* (1988) also reported atretic ovaries, reduced number of primordial and primary follicles, and absence of corpora lutea in goats infected with *T. congolense*. The genital lesions observed in the ewe is cystic degeneration due to *T. vivax* infection (Isoun and Anosa., 1974), while in the cow, flaccidity and atony of the uterine horns have been reported due to the same parasite (Ogwu *et al*., 1984). Massive endometrial mononuclear cellular infiltration, glandular atrophy, periglandular cellular infiltration, myometrial atrophy and fibroplasias in the uterus as a result of *T. vivax* infection in cows have also been documented (Ogwu *et al*., 1987).

Other reproductive anomalies associated with *T. vivax*, are infertility and sterility in West African Dwarf sheep and goats (Isoun and Anosa, 1974). Abortion during various stages of pregnancy, birth of weak offspring's, premature deliveries, birth of anemic offspring's, low birth weight, retained placenta after a difficult parturition and neonatal calf mortalities due to *T. vivax* and *T. congolense* infections in cattle have been reported (Ogwu *et al*., 1986, 1987).

2.5.2. Effect of Trypanosomosis on Reproduction in Male Livestock.

Studies carried out in rams and bucks have shown that infection with *T. vivax* causes scrotal enlargement, testicular and epididymal degeneration, and deterioration of semen characteristic (Isoun and Anosa, 1974; Anosa and Isoun, 1980; Agu *et al*., 1986; Sekoni, 1992). Also bucks infected with *T. evansi* developed orchitis, the quality and quantity of their semen deteriorated, some of the bucks became totally aspermic, in some, spermatozoan abnormalities and the number of dead spermatozoa increased significantly as the course of the disease progressed. The testis of these animals eventually became atrophied. Micro-thrombi were also seen in the testicular blood vessels. Calcium deposits obliterated most of the seminiferous vesicles, seminiferous sheets and epididymal ducts as a result of diffuse calcification that had set in (Ngeranwa *et al*., 1991).

Trypanosoma brucei infection in rabbits caused increased scrotal diameters, scrotal inflammation, and scab formation with alopecia, periochitis, epididymitis, severe testicular degeneration, abnormal spermatogenesis, aspermatogenesis, preputial hemorrhage and incomplete resolution of the genital organs (Ikede and Akpavie, 1982).

Studies carried out in bulls infected with *T. vivax* revealed testicular and epididymal degeneration as well as the production of poor quality semen (Isoun *et al.*, 1975). Deterioration of semen characteristics has also been reported in West African short horn bulls infected with *T. congolense*. (Grundler and Djabakou, 1985; Grundler *et al.*, 1988) Very severe degeneration of the bovine male genitalia, comprising the testes, epididymis, seminiferous tubules and the interstitial leydig cells were seen after an infection with *T. vivax* and *T. congolense*. There was also stoppage of spermatogenesis associated with lack of epididymal sperm reserve. The seminal vesicles, the sub urethral and prostrate glands of the bulls were also severely damaged (Sekoni *et al.*, 1990).

Genital lesions have also been reported in boars infected with *T. brucei* and *T. congolense* and these include degeneration of the testes involving the leydig cells, basement membrane, sertoli germ cells with loss of libido (Omeke and Onuora, 1992). Sub clinical trypanosomosis of pigs

due to *T. brucei* and *T. congolense* was found to have lowered the reproductive capacity of infected boars, their testes and epididymis weighed less than the control animals, and their testicular sperm reserves and epididymal sperm reserves were also small when compared to the infected control (Omeke, 1991).

2.6. DIAGNOSIS OF TRYPANOSOMOSIS

Due to the absence of pathognomonic signs, clinical examination is of little help in the diagnosis of this disease. Serological test can only indicate exposure to the trypanosomal antigens which may result from a current or past infection (Losos, 1986). Therefore, the procedure used for the detection of salivarian trypanosomal infections and diagnosis of disease in the field depends on the available laboratory facilities, the local epidemiological situation and whether a single animal or a herd of animals is involved. Additional considerations in selecting diagnostic methods also depend on the species of host and to the suspected species of trypanosomes (Losos, 1986).

According to Seifert (1996), definitive diagnosis of trypanosomosis depends on the detection of the parasites. This is accomplished through direct and indirect demonstration of the parasites through a blood smear in the form of a wet film with or without concentration as in the centrifugation in a Hematocrit Capillary Test (HCT), the Silicon Centrifugation Technique

(SCT), and the dark brown Buffy Coat Technique (DG) or by separating trypanosomes from blood by ion exchange chromatography. Stained blood smears with Romanowski or Giemsa stain as either thick or thin films, lymph nodes biopsy as in the case of *T. vivax* and transmission of blood to splenectomised calves or small laboratory animals are means through which diagnosis can be achieved

Recombinants plasmids which are used as probes to identify any organism containing DNA sequences can also be used to identify trypanosomes whatever their developmental stage and morphology and this method is highly sensitive and is not susceptible to antigenic variation and can also be used to distinguish *T. simiae* and *T. congolense* which usually present peculiar problems in identification (Majiwa, 1989).

2.7 CONTROL OF TRYPANOSOMOSIS

Chemotherapy, chemoprophylaxis, vector control and breeding of trypanotolerant livestock are the methods that are currently being used to control trypanosomosis (ILRAD, 1990).

2.7.1. Chemotherapy and Chemoprophylaxis

The trypanocides used in the treatment and protection are the most common single method employed for the control of animal trypanosomosis. Effective application of chemotherapy and chemoprophylaxis in the field

depends on several factors which include the species of the trypanosomes causing infection, severity of the challenge, species of the animal and lastly the occurrence of resistance strains (Losos, 1986).

New drugs for treatment or prophylaxis against trypanosomes have not been developed in recent times and some of the drugs that have been in use have either been withdrawn or are contra-indicated now because of resistance to them (Seifert, 1996). Rapid development of drug resistance is the one factor which complicates the use of trypanocides more than any other in the control of this disease in enzootic areas. The number of drugs available for field use is limited and therefore requires the most judicious use to prevent the development of resistant strains (Losos, 1986).

In Nigeria, chemotherapy and chemoprophylaxis of animal trypanosomosis depends on four drugs, diaminazene aceturate, isomethamedium chloride, homidium chloride and homidium bromide (Aliu, 1981; Ilemobade, 1988). The use of these drugs in pigs has not been without constraints. Seifert (1996) reported that treatment of *T. simiae* infections is ineffective. Omeke (1992), reported cure in boars treated with diaminazene aceturate against *T. brucei* and T congolense but listed short duration of action of the drugs, development of resistant strains, parasites relapse and finance as some of the constraints militating against this treatment.

Difluoromethylonithine (DFMO) and diaminazene aceturate used singly or combined at recommended doses, in pigs infected with *T. brucei*, produced varied period of aparasitemia, but relapse infection in all the treatment groups occurred (Jibike and Anika, 1991). The use of isomethamedium chloride in *T. brucei* infected pigs was able to clear the parasites from the blood within 72 hours and relapse infection did not occur over a period lasting 8 weeks (Otesile *et al*., 1992).

Recently a trypanocidal drug cymecarsan, has been found to be effective against *T. brucei* infection in animals. This drug belongs to a chemical group which has not previously been used against animal trypanosomosis (Raynaud *et al*., 1989). Perhaps, it might be the answer to the problem of *T. brucei* infection in pigs.

Otesile *et al*. (1992) suggested that rearing of pigs in fly proof pens will enhance control of this disease in pigs. Mally *et al*. (1982) recommended the use of antrycides plus suramine (40 mg/kg at 3 to 6 months intervals) and an annual curative therapy with antrycide chloride as a means of controlling trypanosomosis in pigs in endemic areas.

2.7.2. Breeding of Trypanotolerant Livestock

The emphasis of trypanotolerant livestock is more on cattle than other animal species. In this case, trypanotolerant cattle breeds have been reported

as showing lower mortalities and having lower reductions in calving rate than are shown by trypano-susceptible breeds (FAO, 2000). However in areas that are densely infested with tsetse flies or when a lower level of trypanosomosis challenge is combined with other stress factors such as ploughing and malnutrition, trypanotolerant breeds of livestock will need the protection of trypanocidal drugs in order to be productive (Jordan, 1995)

The selection of improved trypanotolerant breeds for use in livestock system under low parasite challenge requires more efficient tools for exploiting resistance traits. When the genetic basis for typanotolerance has been fully understood, it might become possible for this trait to be incorporated into other breeds of animals.

2.8.3. Vector Control

The use of special insecticide formulations applied to artificial attractive devices (insecticide-impregnated targets with or without available odour attractant) and some times applied on cattle have been found to be an efficient and sufficiently specific method to suppress tsetse target populations in most situations (Challier and Laveissiere, 1973; Vale, 1974, 1993; Kupper *et al*., 1982; Brand, 1988; Bauer *et al*., 1995). As with trypanocides, the widespread, unsupervised and insufficiently coordinated

use of insecticides on targets or animals will risk promoting the development of insecticide resistance (Guisance *et al*., 1980; Van der Vloedt *et al*., 1980)

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Twelve domestic cross breed female piglets from three different dams, aged eight weeks, were purchased from two piggeries in Zaria. On arrival, the piglets were housed in clean fly proof pens in the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. The piglets were ear notched for identification and were screened for endo- and ecto-parasites, as well as hemoparasitic infection. Baseline hematological data were also obtained and they were treated with Doramectin (Dectomax) at the dose rate of 200 ug/kg body weight sub-cutaneously.

The piglets were fed on compounded diet of 18% crude protein comprising maize, 36.8%; soya bean, 5%; ground nut, 23.5%; rice bran, 30%; beniseed, 2%; bone meal, 2%; premix, 0.2%; table salt, 0.5%, with water provided ad-libitum.

3.2 TRYPANOSOMES

Trypanosoma brucei obtained from The Nigerian Institute of Trypanosomiasis Research, Vom, which was originally isolated from a pure

natural infection in cattle in Federe, Kaduna state in 1995 was inoculated into two mice and transported to the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. At high parasitemia, determined by method described by Woo (1969), the infected blood of the mice was collected and inoculated into five rats. At high parasitemia, the rats were anesthesized in a jar containing chloroform and were bled through cardiac puncture. The infected blood of the rats was then pooled with Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant and stabilated. The stabilate was cryo preserved in 5% Dimethylsulfoxide (DMSO) and stored in liquid nitrogen.

3.3 EXPERIMENTAL DESIGN

After the period of acclimatization, which lasted for 97 days the pigs were randomly divided into two groups, eight infected ($N^{os.}$ 1, 4, 6, 9, 10, 11,13, 16) and four control (N^{os}. 2, 5, 15, 17).

3.3.1 Inoculation of Animals

The stabilate of *Trypanosoma brucei* was recovered from liquid nitrogen and allowed to thaw. Eight rats were inoculated with 0.2 ml of the stabilate each. At peak parasitemia, the rats were anesthesized in a jar containing chloroform. They were then bled via the heart and all the blood collected was pooled with EDTA as anti-coagulant in one beaker. The

number of trypanosomes per ml of blood was determined using hemocytometer. Two milliliters of the infected blood containing $1.8x10^6$ organisms were inoculated into each pig of the experimental group via the anterior vena cava. The pigs in the control group were not inoculated. All the pigs were 153 days old at this stage.

3.3.2 Clinical Examination and Blood Sampling For Parasitemia

Following inoculation, the pigs were clinically examined and their rectal temperatures noted daily. They were also bled daily until parasites were eventually seen in the blood of all the infected pigs. Subsequently, physical examination was conducted on all the pigs after which they were bled twice a week to determine the levels of parasitemia and packed cell volume. This continued throughout the period of the experiment.

3.3.3 Hematocrit Centrifuge Test for Trypanosomes

Microhematocrit capillary tubes, each containing approximately 70 µl of blood in anticoagulant were centrifuged at 2000 revolutions per minute for three minutes. The tubes were then placed on clean glass slides. Immersion oil was then put on the capillary tubes. The junction between the plasma and buffy coat was then examined under the microscope at x100 magnification. The degree of parasitemia was classified as describe by Woo (1960):-

+ = less than ten trypanosomes seen per field.

+ + = 10-20 trypanosomes seen per field.

+ + + = more than 20 trypanosomes seen per field.

+ + + + = massive number of trypanosomes seen per field.

3.3.4 Packed Cell Volume (PCV)

A microhematocrit capillary tube containing approximately 70 µl of blood in anti coagulant was centrifuged for three minutes at 2000 revolutions per minute (rpm). The PCV was read using Hawsley microhematocrit reader (Gelman, Hawwsley Ltd England).

3.3.5 Thin Blood Films for Trypanosome Identification.

About one micro liter of blood was placed on clean grease free glass slides and thin smears were made with the aid of another microscope slide. The slides were air dried and fixed in methanol for three minutes. They were then stained in 10% Giemsa, dried and examined under oil immersion using a microscope at x100 magnification.

3.3.6 Weighing of Animals

Both the infected and the control animals were weighed on the day of inoculation and then weighed once every two weeks through out the period of the experiment, using a weighing band.

3.4 GROSS PATHOLOGICAL EXAMINATION

Two of the infected pigs (N^{os} 11 and 4) died on day 70 and 74 post infection respectively. Six of the remaining infected pigs were sacrificed at the end of the experiment. Post mortem examinations were carried out on all the animals. The following tissues and organs were examined for gross lesions: pituitary gland, ovaries, fallopian tubes, uterus, cervix and vagina. Post mortem examination was also carried out on the fetuses that were aborted by pig. N^{o} 16 in the course of the experiment.

3.5 HISTOPATHOLOGICAL EXAMINATION

The samples of the pituitary gland, ovaries, fallopian tubes, uterus, cervix and vagina obtained from the dead pigs were put into appropriately labeled bijou bottles and fixed in 10% formalin. They were trimmed and processed in tissue maton embedded in paraffin and were then cut in to 5µm thickness on slides and stained with Hematoxyline and Eosin. The slides were examined under the microscope.

3.6 REPRODUCTIVE EXAMINATION

3.6.1 Determination of Estrus

From the one hundred eightieth day of age, the pigs in the infected and control groups were examined for the signs of estrus by observing the vulva and behavioral changes. Genital touch response was stimulated using a long stick and reaction was recorded.

3.6.2 Determination of Fertility

During the second estrus period two boars were purchased from two piggeries in Samaru village, Zaria. Their semen was analyzed and the boars were randomly allowed to mount each gilt as it came into estrus. Following service, the animals were observed for possible return to return to estrus again. Their abdominal regions and the development of the mammary glands were also observed closely.

3.6.3 Evaluation of Pregnancy

At the end of the breeding, all the animals that were bred successfully and did not return to estrus were considered pregnant. They were monitored closely during the period of gestation by observing their abdominal regions, the development of their mammary glands, discharges from the vulva as well as changes in their weights.

CHAPTER 4

RESULTS

4.1 CLINICAL SIGNS

All the inoculated pigs developed clinical trypanosomosis. The disease took a short, severe and acute course killing two pigs. Later a mild but debilitating chronic disease developed in the six remaining ones. In the acute phase of the disease, the clinical signs observed were; fever, slightly pale mucous membranes, anorexia, dullness, reduced weight gain, recumbency and death.

The chronic phase of the disease exhibited, weight loss, on-coordinated movements, emaciation, mucopurulent ocular discharges, hyperemia of the skin, lethargy and abortion. The abortion occurred in the third trimester. Other clinical signs noticed were reduction in feed intake weakness and wobbling of the hindquarters. The weakness and wobbly movements became more pronounced as the disease progressed, especially in the pregnant pig. Even after it had aborted, the pig progressively got weaker till the experiment was terminated.

4.2 PARASITEMIA

All the infected pigs developed parasitemia between the 2^{nd} and 3^{rd} day post infection (p.i.). Peak parasitemia was attained on days 11^{th} and 14^{th}

p.i. in six of the animals. In two of the pigs peak parasitemia was reached on day 21st and day 42nd p.i. respectively. There after, there were fluctuations in the levels of parasitemia. Two of the pigs died when the levels of the parasitemia were massive. By day 60 p.i. the parasites had started disappearing from the peripheral circulation of the infected animals. By day 119 p.i. none of the infected animals were positive for parasites in their peripheral circulation. Parasites were later seen in the blood of three of the infected pigs (N$^{o.}$ 1, 6 and 13) on days 95, 133 and 154 p.i. respectively. The parasites disappeared again on day 98, 140, 158 p.i. respectively. By day 224 p.i. the parasites were again observed in the blood of one of the infected pigs (N$^{o.}$ 1).This parasitemia continued till sampling was stopped on day 242 p.i. The levels of parasitemia are summarized in figure 4.1 and .shown in appendices 3 to 10.

4.3 TEMPERATURE CHANGES

The rectal temperatures of the infected pigs started rising from the second day p.i. An increase in temperature up to 40.3 ^{o}C was attained by one of the infected pigs (N$^{o.}$ 9), on day 39 p.i. The temperatures of the infected animals were significantly higher than those of the control ($P<0.05$). After the parasites had disappeared from peripheral circulation the rectal

temperatures returned to normal. The fluctuations in rectal temperatures are summarized in Figure 4.2 and shown in Appendices 3-10 .

4.4 PACKED CELL VOLUME (PCV)

There was a gradual decrease in the PCV of the infected pigs which started from day 14 p.i. in pig N^{o} 4. The lowest PCV value recorded was 24%., this was noticed in pig N^{o} 6. The mean of PCV values of the infected animals were significantly different from those of the control ($P<0.05$). A gradual increase in PCV was noticed in the infected animals following the disappearance of parasites from peripheral circulation. The PCV of two of the infected pigs (N$^{o.s}$ 9 and 13) rose to their pre-infective values by days 109 and 84 p.i. respectively while that of (N$^{o.}$ 16) exceeded its pre-infective value on day 126 p.i. The values of PCV of the control and the infected animals are summarized in Figure 4.3 and shown in Appendices 3 to 10.

4.5 WEIGHT GAIN

The infected animals from day 28 p.i were observed to be gaining less weight than the ones in the control group. The infected animals were gaining an average of 0.71kg every two weeks whereas the controls were gaining an average of 1.5 kg during the same period. By day 126 p.i the infected animals started losing weight while the control gained in weight steadily.

However slight drops in the weights of the control animals were noticed after parturition for about two weeks. The difference in the weights between the infected and the control was found to be statistically significant at $P<0.05$ (Figure 4.4., Appendices 1 and 2).

***Figure 4.1*: Mean Parasitemia of *T. brucei* Infected Pigs**

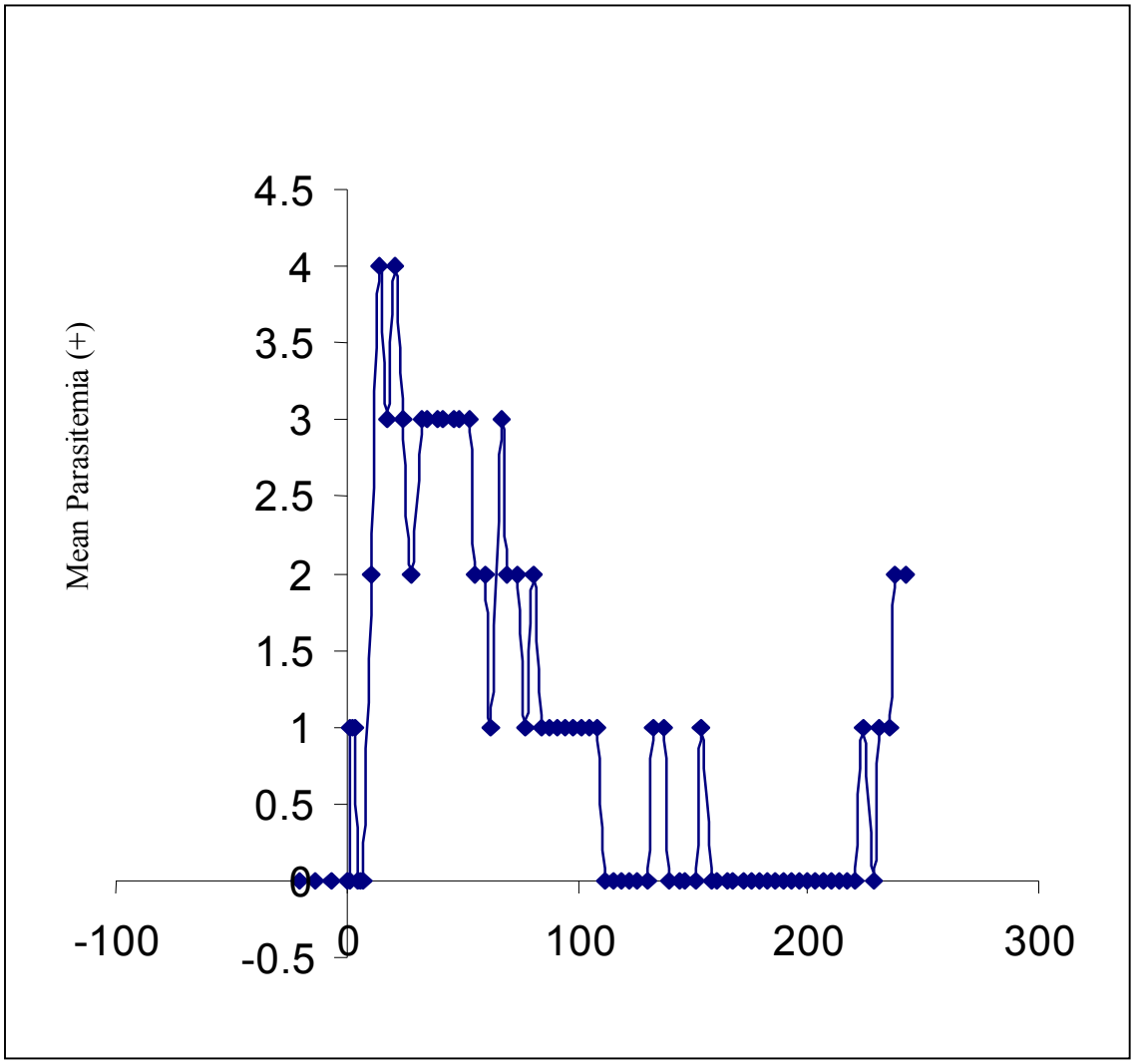


Figure 4.2: Mean Temperature (°C) of *T. brucei* Infected and Control Pigs

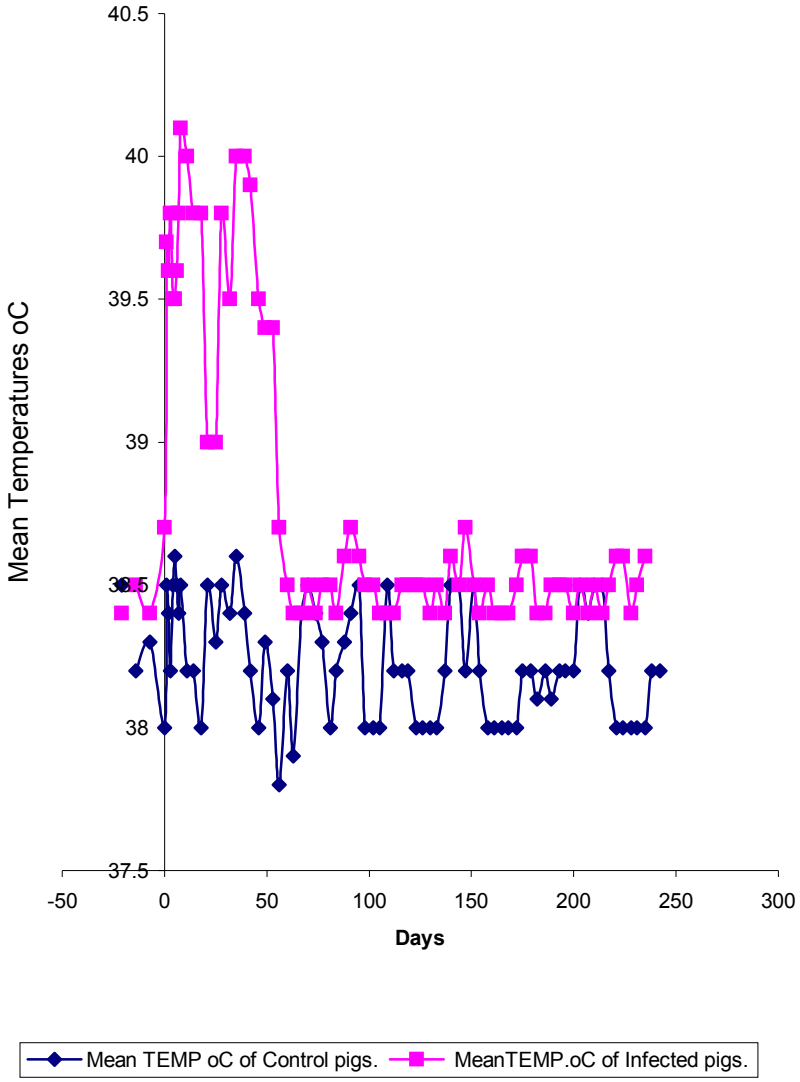


Figure 4.3: Mean PCV Values of *T. brucei* Infected and Control Pigs

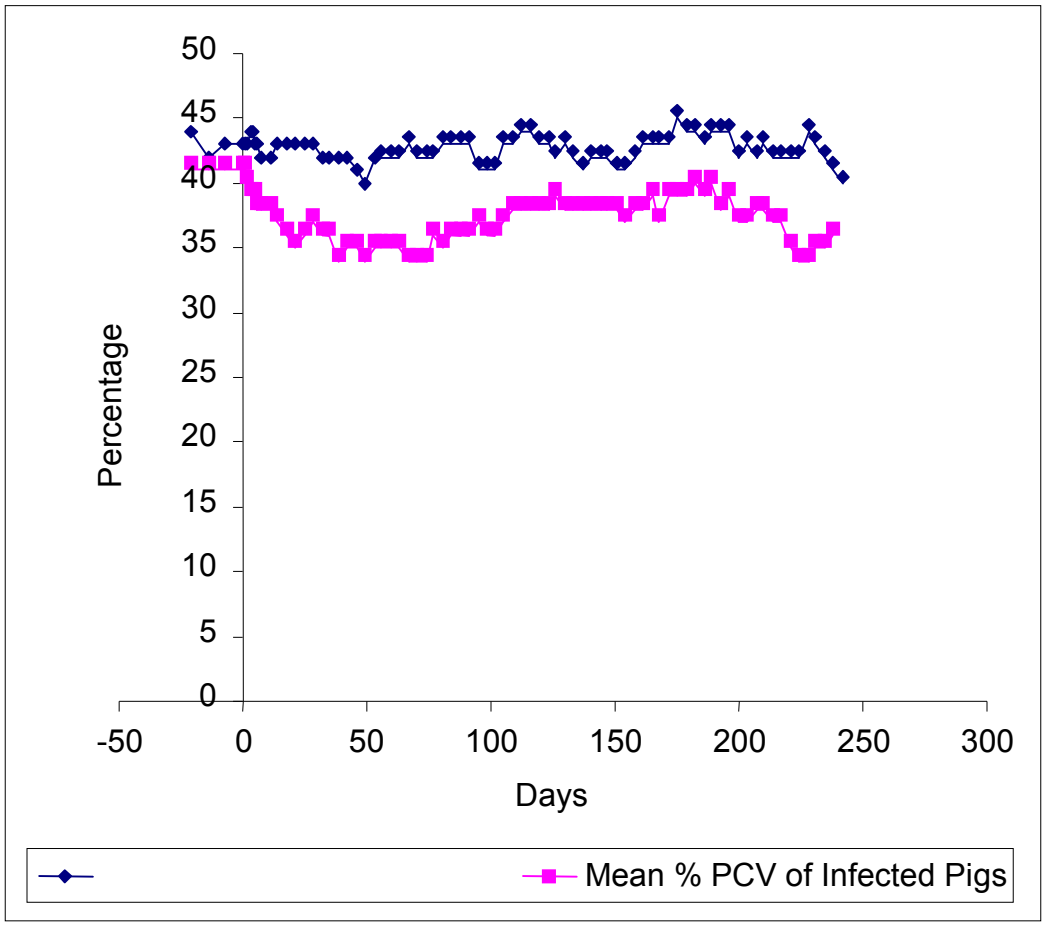
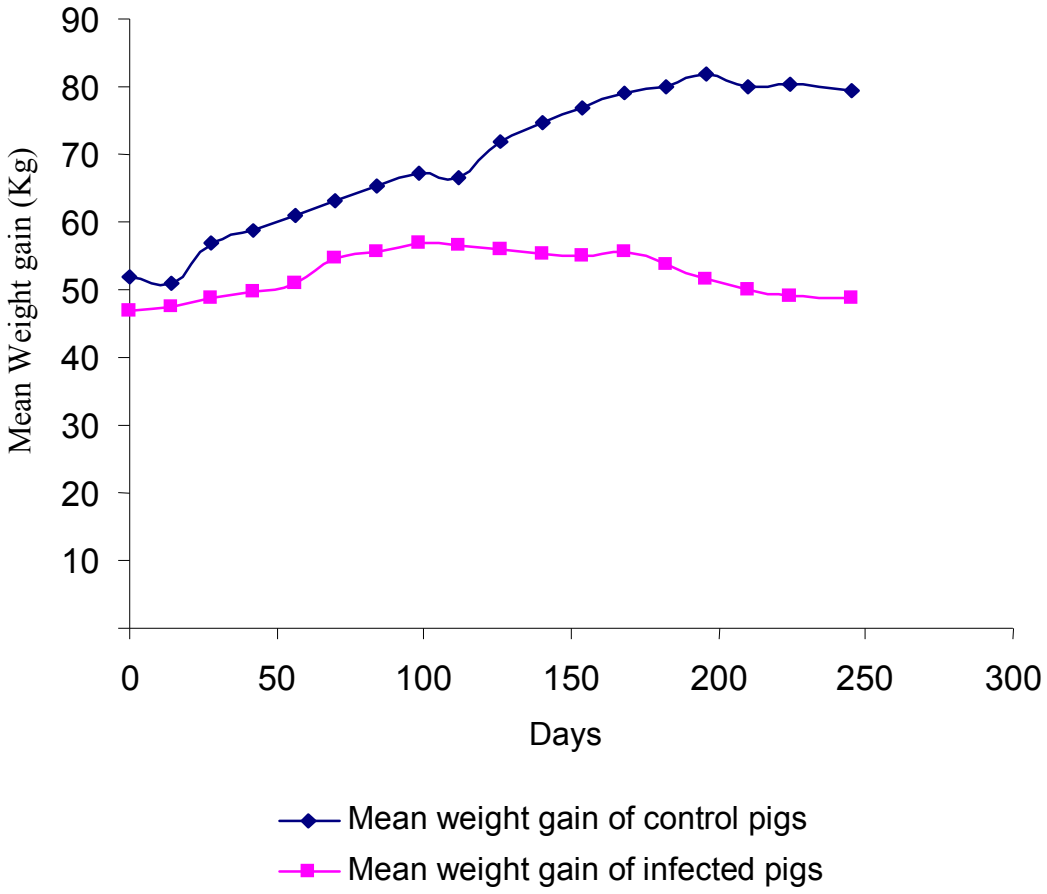


Figure 4.4: Mean Weight Gain of *T. brucei* Infected and Control Pigs



4.6 EFFECTS ON REPRODUCTION

4.6.1 Onset of Estrus

All the pigs attained puberty when they were between 230 and 236 days old. The external signs of estrus noticed were, pink, slightly edematous and relaxed vulva with a stringy clear mucoid discharge. The pigs in estrus responded to genital touch by standing still and raising their tails, while the pigs that were not in estrus responded by curving their tails downward and running away (Plates 4:1-4:4).

4.6.2 Duration of Estrus

There was no noticeable difference in the duration of estrus between the infected and control pigs. The duration of estrus in nine of the pigs was 36-72 hours. However, one pig in the control group (N$^{o.}$ 17) was in estrus for 96 hours (Table 4.1).

4.6.3 Mating

All the animals in the control group were bred successfully. However, mating between the boars and the gilts in the *T. brucei* infected group could not be achieved successfully. This was due to the weakness in the hind quarters that was noticed which made standing during mounting almost impossible because the gilts tended to fall down before the boar could

achieve intromission. However, two of the infected gilts were mated once but could not stand to be mounted again twenty four hours after the first breeding (Plates 4:5-4:8).

4.6.4 Fertility

All the animals in the control group conceived. In the infected group, the two animals (N^{os}.16 and 13) that stood to be bred conceived. Three others (N^{os}.1, 9, 10,) that could not stand to be bred did not conceive and returned to estrus, while the remaining one in the group (N^{o}.6) did not return to estrus till the experiment was stopped even though it also could not have been able to stand to be bred by the boars.

4.6.5 Pregnancy

All the animals in the control group carried their pregnancy to term. However, one of the infected animals (N^{o} 16) carried its pregnancy through the first and second trimesters without noticeable problems. On day 86 of pregnancy the pig started becoming sluggish and movement within the pen became increasingly difficult. It was seen lying down at frequent intervals and its feed intake had reduced. The weakness accompanied by wobbling of the hind quarters noticed earlier became more pronounced. On the 90th day of pregnancy the pig aborted. It continuously got weaker till it was sacrificed at the end of the experiment.

The second gilt (N$^{o.}$ 13) that was bred initially appeared to be pregnant. The mammary glands were seen to be developing but they eventually regressed during the second trimester and the pig was not seen to come into estrus again.

Table 4.1: Age at Puberty of *T. brucei* Infected and Control Pigs

PIG N$^{o.}$	AGE AT PUBERTY (DAYS)
1	232
2*	232
5*	232
6	233
9	231
10	232
13	230
15*	232
16	236
17*	234

Key:

*** Control Pigs**

Table 4.2: Duration of Estrus for *T. brucei* Infected and Control Pigs

PIG N$^{o.}$	DURATION OF ESTRUS (HOURS)
1	72
2*	48
5*	36
6	48
9	48
10	72
13	48
15*	48
16	72
17*	96

Key:

***Control Pigs**

Plate 4.1: External Genitalia of Gilts in Estrus Showing Pink and Edematous Vulva (V)

Plate 4.2: External Genitalia of Gilt Not in Estrus Showing Vulva with Normal Size and Colour (V)

Plate 4.3: Genital Touch Response of Gilt in Estrus. Tail is Raised and Gilt is Standing Still

Plate 4.4: Genital Touch Response of Gilt Not in Estrus. Tail is Curved Downwards

Plate 4.5: Boar Mounting a *T. brucei* Infected Gilt in Estrus

Plate 4.6: A *T. brucei* Infected Gilt in Estrus Falling During Mounting

Plate 4.7: A *T. brucei* Infected Gilt in Estrus Falls Before Intromission Could be Achieved

Plate 4.8: A *T. brucei* Infected Gilt In Estrus Unable to Stand for Mating

4.7 GROSS PATHOLOGY

4.7.1 Genitalia

The ovaries, fallopian tubes, uterus, cervix and vagina of the two infected pigs that died in the course of the infection were congested however, no gross pathological lesions were observed in the genitalia of the rest of the infected pigs that were sacrificed at the end of the experiment.

4.7.2 Fetuses

All the aborted fetuses had anasarca. Their liver had started undergoing necrosis

4.7.3 Placenta

The placentas of all the aborted fetuses were hemorrhagic.

4.8 HISTOPATHOLOGICAL LESIONS

4.8.1 Pituitary gland

Some of the infected pigs had focal necrosis in their pituitary gland while in the remaining diffused areas of necrosis which were interspersed with areas of fibrosis and mononuclear cellular infiltration were noticed.

4.8.2 Ovaries

Inflammatory cells predominantly neutrophils macrophages and lymphocytes were seen in the cortex, medulla and mesovarian. These were

interspersed by areas of necrosis and fibrosis in some cases. Graafian follicles undergoing degeneration was also a common finding.

4.8.3 Fallopian Tubes

Necrosis of the epithelial cells of the ampula and isthmus were observed. The tubes were also infiltrated by inflammatory cells which were sometimes interspersed with areas of fibrosis.

4.8.4 Uterus

There were areas of necrosis seen in the endometrium and myometrium of all the infected pigs. They were also infiltrated by mononuclear cells and proliferated fibrous connective tissues (Fig 4.18). Retained fetal tissues were present in the uterus of the two pigs that were bred (Nos.16 & 13) These fetal tissues in both cases were seen under going necrosis and were also infiltrated by numerous mononuclear cells predominantly macrophages, neutrophils, eosinophils and lymphocytes. Cartilage and bone specules of the fetus were also seen in the uterus the pigs. One of the infected pigs, (No.16) had *T.brucei* in the fetal tissues retained in its uterus (Fig 4.17). The fetal tissues retained in the other infected pig (No.13) were seen embedded into the necrotic maternal myometrium undergoing resorption. The necrosis noticed in the uterus of the pigs also affected the uterine glands.

4.8.5 Vagina

There was necrosis of the stratified squamous epithelium with mononuclear cellular infiltration predominantly neutrophils, macrophages and lymphocytes. These were noticed in the laminar propria and in the necrotized areas of tunica muscularis. Proliferation of fibrous connective tissues in the necrotized areas, arthritic blood vessels and phlebitis were also observed (Fig 4.19).

Plate 4.9: Aborted Fetuses Showing Prominent Areas of Anasarca (A)
ate 4.10: Placenta of Aborted Fetuses Showing Generalized Hemorrhages

4.11;Genitalia of a *T. brucei* Infected Gilt, Showing Congestion of Its Various Parts, Ovaries (O), Fallopian Tubes (F), Uterus (U) Cervix (C) and Vulva (V)

Plate 4.12: Anterior Part of the Pituitary Gland of *T. brucei* Infected Gilt Showing Focal Areas of Necrosis (N). H x E. x 400

Plate 4.13: Anterior Pituitary Gland of *T. brucei* Infected Gilt; Showing Diffused Areas of Necrosis (N). HxE, x 250

Plate 4.14: Ovary of *T. brucei* Infected Gilt Showing Mononuclear Cellular Infiltration (M), Areas of Necrosis (N), and Graafian Follicles undergoing Degeneration (G). HxE, x 400

te 4.15 Uterus of *T. brucei* Infected Pig Showing Retained Fetal Tissues Undergoing Resorption .Fetal Bone Spicules (B), Fetal Tissue (F), Myometrium (M), HxE, x160

Plate 4.16: Fetal Tissue Retained in the Uterus of *T. brucei* Infected Pig Showing Bone Spicules (B), *Trypanosoma brucei* (Tb) and Mononuclear Cellular Infiltration (M). HxE, x1000 (oil immersion)

Plate 4.17: Uterus of *T. brucei* Infected Pig Showing Necrosis of Uterine Glands (N) and Mononuclear Cellular Infiltration (M). HxE x 250

Plate 4.18: Vagina of *T. brucei* Infected Pig Showing Phlebitis (P) and Mononuclear Cellular Infiltration (M). HxE, x 250

Plate 4.19: Vagina of *T. brucei* Infected Gilt Showing Necrosis of Stratified Sqamous Epithelium (N), HxE, 1000 (oil immersion)