EFFECT OF SERA FROM SELECTED BIRDS AND MAMMALS ON *TRYPANOSOMA BRUCEI BRUCEI* PLIMMER & BRADFORD, 1899 AND *TRYPANOSOMA CONGOLENSE* BRODEN, 1894

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JULY, 2017

DECLARATION

I declare that the work in this thesis entitled Effect of Sera from selected Birds and Mammals on *Trypanosoma brucei brucei* Plimmer & Bradford, 1899 and *Trypanosoma congolense* Broden, 1894, has been carried out by me in the Department of Zoology. The information derived form the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "EFFECT OF SERA FROM SELECTED BIRDS AND MAMMALS ON *TRYPANOSOMA BRUCEI BRUCEI* PLIMMER & BRADFORD, 1899 AND *TRYPANOSOMA CONGOLENSE* BRODEN, 1894" by Felicite Djemna DJIEYEP meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D) in Zoology of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The natural anti-*Trypanosoma brucei brucei* and *Trypanosoma congolense* activities of sera from some uninfected birds and mammals and their curative and prophylactic potentials were investigated. Their xanthine oxidase content was also evaluated. All the samples were screened using thick blood film and micro-heamatocrit centrifugation technique, to ensure that the donors were not infected by *T.brucei brucei* and/or *T. congolense*. The *in vitro* trypanosomal activity of the sera was assessed by mixing each serum with each of the suspension of parasites in a supplemented RPMI 1640, and incubated at room temperature and under exogenous CO_2. Cessassion of parasite motility was taken as indication of the serum's anti-trypanosomal activity. Bird sera generally had anti-*Trypanosoma brucei brucei* and anti-*Trypanosoma congolense* activities *in vitro*, the best anti-*Trypanosoma brucei brucei* activity index (ATI) being found in broiler hen serum (0.83), with a survival time of 5.00±0.00 hours (compared to 30.00±0.00 hours in the control) while peacock serum had the highest anti-*Trypanosoma congolense* activity index *in vitro*, 0.88, with a mean survival time of just 8.67±3.21 hours (compared to 74.67±0.58 hours in the control). The selected mammalian sera had diverse effects on *T. brucei brucei* and *T. congolensein vitro*. For the selected rodents' sera, rabbit serum had some level of anti-*Trypanosoma brucei brucei* activitywith low index (ATI of 0.23 compared to 1.00 in the positive control). Similar observation was made with grass-cutter serum against *Trypanosoma congolense* (ATI of 0.25 compared to 1.00 in the positive control). Rabbit serum was supportive of the survival of *T. congolensein vitro*. Sera from the selected zoo primates i.e. the Red Patas monkeys and the Tantalus monkeys had a moderate anti-*Trypanosoma brucei brucei* activity (ATI of 0.27 and 0.34 respectively compared to 1.00 in the positive control) and a very high anti-*Trypanosoma congolense* activity *in vitro* (ATI of 0.91 and 0.90 for Red Pattas monkey and

Tantalus monkey respectively). Among the sera from selected small ruminants, only the West African dwarf goat serum had some level of anti-*Trypanosoma brucei brucei* activity (ATI of 0.29), meanwhile all these sera were supportive of the survival of *T. congolensein vitro*, without exception; sheep sera supported best the survival of the parasites. With exception of N'Dama serum with ATI of 0.38, sera from the selected large ruminants had a supportive effect for *T. brucei brucei*, but were all supportive of *T. congolensein vitro* without exception. Sera from the selected non-ruminant mammals, generally, showed some level of anti-*Trypanosoma brucei brucei* activity, but highly supported *T. congolense in vitro*, except the sera from camel and jackal, which manifested anti-*Trypanosoma congolense* activity, with ATI of 0.22 and 0.91 respectively. Sera from horse and large white pig had the highest supportive activity *in vitro*, and it was observed that their heat-inactivated forms could substitute the fetal bovine serum (FBS) in RPMI 1640 medium for the cultivation of *T. congolense*. All the sera from the birds and mammals contained xanthine oxidase (XO), except buzzard's serum. The XO content of most of the sera from the selected birds and mammals were associated with their *in vitro* trypanosomal activities. Broiler hen serum had the highest XO content, which coincided with its highest anti-*Trypanosoma brucei brucei* activity. The XO content of sera from Red Patas and Tantalus monkeys were significantly different ($P<0.05$) but showed no significantly different ($P>0.05$) anti-trypanosomal activity, and so, did not have relationship with their *in vitro* anti-trypanosomal activity. The trypanotolerance ability of the N'Dama cattle was not associated with its serum XO content. Xanthine oxidase activity was generally species-specific in mammals, and likely breed-specific in pig. Among the sera with *in vitro* anti-trypanosomal activity, jackal serum was slightly prophylactic against *T. congolense in vivo*. None of the *in vitro* effective sera were prophylactic against *T. brucei brucei*. Meanwhile, buzzard serum had a slight curative effect on

T. brucei brucei in vivo, while no serum exhibited curative effect against *T. congolense in vivo*. The sera of some of the birds and mammals have anti-*Trypanosoma brucei brucei* and/or anti-*Trypanosoma congolense* properties. All the selected bird and mammal sera had various concentrations of XO, except buzzard serum. The XO content of most of these sera was associated with their *in vitro* anti-trypanosomal activity. Jackal serum had prophylactic effect against *T. congolense in vivo* while buzzard serum had a slight curative effect on *T. brucei brucei in vivo*.

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DEFINITIONS, ABBREVIATIONS AND SYMBOLS

Abbreviations

AAT: Animal African trypanosomiasis

APOL1: Apolipoprotein L1

ATI: Anti-trypanosomal Activity Index

ATP: Adenosine Triphosphate

BF: Blood form

CDC: Center of Disease Control and prevention

DNA: Deoxiribonucleic acid

EDTA: Ethyl-diamine-tetra-acetic acid

ELISA: Enzyme-linked Immunosorbent assay

FBS: Fetal Bovine Serum

HAT: Human African trypanosomiasis

HCT: Haematocrit Centrifugation Technique

IMDM: Iscove's Modification of Dulbecco's MEM

MEM: Minimal Essential Medium

M-VATs: Metacyclic Variable Antigen Types

NAPRI: National Animal Production Research Institute

NITR: Nigerian Institute of Trypanosomiasis Research

PCR: Polymerase Chain Reaction

RFLP: Restriction Fragment Length Polymorphism

RPMI 1640: Rossvelt Park Memorial Institute 1640

SDM: Semi-defined Medium

SRA: Serum Resistant Associated protein/gene

T_c: Survival time of the parasites in the control medium

TLF: Trypanosome Lytic Factor

T_s: Survival time of the parasites in the serum

VAT: Variable Antigenic Type

VSG: Variant Surface Glycoprotein

XO: Xanthine Oxidase

CHAPTER ONE
INTRODUCTION

Trypanosomes are protozoan parasites that cause trypanosomiasis, called sleeping sickness in humans and nagana in livestock. They are obligate parasites with two hosts: an insect vector and a mammalian host. These parasites are transmitted by the bite of the blood-feeding tsetse flies *Glossina* spp. (Vickerman, 1985), which act as biological vectors; by fomites and mechanical vectors such as surgical instruments, needles, syringes and other various biting flies including horse flies (*Tabanidae*) and stable flies (*Stomoxys* spp.), respectively. (Eltahir1 *et al*., 2014). Twenty-three (23) species of tsetse are competent vectors in sub-Saharan Africa (between latitudes 14°N and 29°S), but *Glossina morsitans*, *G. palpalis* and *G. fusca* are the most important (OIE Terrestrial Manual, 2009). Trypanosomes are extracellular parasites that live in the blood and lymphoid tissues of mammalian hosts. African trypanosomiasis is caused by a variety of trypanosomatids including *Trypanosoma brucei brucei, Trypanosoma vivax, Trypanosoma congolense, Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense,* which are the most important species.

In cattle, sheep and goats, African trypanosomiasis is caused mostly by *T. congolense, T. vivax* or *T. brucei brucei;* in pigs by *T. simiae* and in camels by *T. evansi* (Molyneux and Ashford, 1983; Trail *et al*., 1989). The principal host species affected vary geographically, but buffalo, cattle, camels, and horses are particularly susceptible (Gutierrez *et al*., 2006).

Trypanosoma brucei brucei and *Trypanosoma congolense* display a complex life-cycle, with several distinct stages in both the insect vector and mammalian host. Vickerman

(1965) reported that during the course of a bloodstream parasitaemia, the trypanosomes proliferate initially as morphologically slender forms; as cell numbers increase in the blood, however, slender forms differentiate into morphologically stumpy forms. Stumpy forms later differentiate into the procyclic forms of the parasite, which multiplies in the tsetse fly's midgut (Tasker *et al*., 2000), then migrate to the salivary glands, where they attach as epimastigote proliferative forms. Eventually, they differentiate into non-proliferative infective metacyclic forms that are transmitted to a new mammalian host during a new blood meal (Barrett *et al*., 2003; Matthews *et al*., 2004; Matthews, 2005).

The symptoms of animal trypanosomiasis include: increased heat production and raised body temperature, intermittent fever, anaemia, lymphatic enlargement with hepatosplenomegally and a progressive cachexia (Dam *et al.*, 1996). The animals become lethargic and develop a dull starry coat, pale mucous membrane, subcutaneous oedema, ascites, occular discharge and emaciation (Losos *et al*., 1973). Some haematological changes (Omotainse and Anosa, 1995; Onah *et al*., 1996) and reproductive disorder (Sekoni, 1994) have also been observed in animals infected with trypanosomes.

Trypanosomiasis is one of the most important problems to man and his livestock, the other one being East coast fever (ILRAD, 1984). The African animal trypanosomiasis caused by several species, such as *Trypanosoma vivax*, *Trypanosoma congolense* and to a lesser extent, *Trypanosoma brucei brucei*, is one of the most devastating diseases that constrain agricultural development of more than a third of the African continent (ILRAD, 1990). Animal trypanosomiasis is a major constraint to livestock productivity in the tropics and has a significant impact on the life of millions of people globally (mainly in Africa, South America and south-east Asia) (Gutierrez *et al*., 2006). It causes about 3 million deaths in

cattle annually, causing annual production losses of about US$ 1.2 billion (Chamond *et al*., 2010). In many African countries, it is estimated that the disease costs up to $1,340 million to livestock producers and consumers every year (httpp://tryps.rockefeller.edu/Default.html). According to Sekoni (1994), *Trypanosoma*-induced reproductive disorders in animals are of significant economic importance, especially in sub-Saharan Africa where trypanosomiasis is endemic and causes losses of pasture.

Human African trypanosomiasis is a major public health problem in 35 sub-Saharan countries. Humans are infected by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense.* The trypanosomes that cause African animal trypanosomiasis are not considered to be pathogenic for humans; however, disease might be possible in people with certain genetic defects (Vanhollebeke *et al*., 2006).

Attempts to control trypanosomiasis are based mainly on the use of trypanocidal drugs and on vector control. Drugs such as isometamidium chloride, quinapyramine sulphate and chloride can be used as prophylaxis during transhumance or high seasonal parasitic pressure. Other drugs are diminazene aceturate and quinapyramine methylsulfate; the latter can be used as curative and sanative (OIE Terrestrial manual, 2009). However, because no new drugs have been developed in the last 50 years, drug resistance is increasing (Legros *et al*., 2002, Palomares *et al*., 2008).

Although there is a lot of research on trypanosomiasis, its control methods have been problematic due to the complex nature of the parasite. The entire parasite surface is predominantly covered by a surface antigen known as the variant surface glycoprotein

(VSG), and antigenic variation of this protein allows the parasites to escape the humoral immune defenses of the mammalian host (Pays and Nolan, 1998; Donelson *et al*., 1998). For these reasons, antigenic variation by the parasite in the bloodstreams of infected individuals prevents them from elimination by the host's immune system (Rudenko *et al*., 1988; Rudenko, 1999), and the development of vaccine using antibodies is not feasible (Kelly *et al*., 2001). In humans, the current line of treatment is problematic for many reasons: firstly, the drugs are toxic and require extensive hospitalization. Secondly, regular follow-ups to check for relapse are essential but difficult in many of the areas where trypanosomiasis is endemic (Bouteille *et al*., 2003). The disease therefore remains a major challenge to scientists, especially epidemiologists, and requires an urgent need for the development of new control strategies.

Researches have shown that there are some trypanoresistant wild mammals that present a high incidence of trypanosome infections but with low parasitemia (Ashcroft, 1959; Reduth *et al*., 1994; Thomson *et al*., 2009, 2014; Jirku *et al*., 2015; Cooper *et al*., 2016). The mechanisms that allow those trypanosomiasis-resistant wild animals to control blood trypanosomes are being investigated. In addition to a specific antibody, there are some non-specific factors that kill trypanosomes or limit their proliferation, contributing to host resistance (Black *et al.,* 1999). Reduth *et al*. (1994) and Muranjan *et al*. (1997) isolated a trypanocidal protein from Eland and Cape buffalo sera; the trypanocidal protein from the Cape buffalo was identified as xanthine oxidase – an enzyme that cathalyses the conversion of hypoxanthine to xanthine, and xanthine to uric acid. The activity of the trypanocidal protein from the Eland was inhibited by xanthine oxidase inhibitors. Black *et al*. (1999) also evaluated the constitutive anti-trypanosome activity of the sera of some wildlife

species and associated it with xanthine oxidase. These studies have shown that animals considered trypanotolerant are naturally endowed to overcome infection by trypanosomes. The sera from such animals can be exploited for passive induced immunity in susceptible animals towards the control of trypanosomiasis.

1.1 Statement of the Research Problem

Studies have shown that some animals are naturally endowed to resist trypanosome infection through innate factors such as xanthine oxidase. The rich and diverse nature of animals in the Nigerian environment may provide a rich pool of trypano-tolerant animal species, which is the subject of this investigation.

1.2 Justification

The control of human and animal trypanosomiasis is hinged on the use of a limited number of old drugs that are increasingly becoming less effective. Alternative means, such as the exploitation of natural factors that confer resistance in animals could be a new strategy/approach in developing additional weapon for tackling the menace of trypanosomiasis.

1.3 Aim of the Study

The aim of this work is to obtain sera from some uninfected birds and mammals in Nigeria and evaluate their curative and prophylactic potentials against *T. brucei brucei* and *T. Congolense, in vitro and in vivo*.

1.4 Objectives

The specific objectives of this study are to determine the:

i. Anti-trypanosomal activity of the sera from selected birds and mammals on *T. brucei brucei* and *T. congolense in vitro.*

ii. Concentration of xanthine oxidase in the sera of the selected birds and mammals.

iii. Presence of antibody against *T. brucei brucei* and *T. congolense* antigens in the sera from selected birds and mammals.

iv. Prophylactic effect of the sera of selected birds and mammals against *T. brucei brucei* and *T. congolense in vivo* in rats.

v. Curative effect of the sera of selected birds and mammals against *T. brucei brucei* and *T. congolense* infection *in vivo* in rats.

1.5 Hypotheses

i. The sera of the selected birds and mammals have no anti-trypanosomal activity against *T. brucei brucei* and *T. congolense in vitro*.

ii. The sera of the selected birds and mammals do not contain xanthine oxidase.

iii. The sera of the selected birds and mammals do not have antibody activity against *T. brucei brucei* and *T. congolense* antigens.

iv. The sera from selected birds and mammals do not have prophylactic effect against *T. brucei brucei* and *T. congolense* infection *in vivo.*

v. The sera from selected birds and mammals do not have curative effect against *T. brucei brucei* and *T. congolense* infection *in vivo*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification and Distribution of Trypanosomes and their vectors

Several trypanosome species infect mammals in Africa; of these the three most important are *T. brucei brucei*, *T. vivax* and *T. congolense*. Trypanosomes are divided into two groups: The salivarians and the non-salivarians (Stercorarian). Salivarians belong to the subgenera of *Duttonella, Trypanozoon, Pycnomonas* and *Nannomonas,* which are passed to the recipient in saliva of the tsetse fly. The *Trypanozoon* subgenus contains the species *Trypanosoma brucei*, *T. rhodesiense* and *T. equiperdum*. The subgenus *Duttonella* contains the species *T. vivax*. *Nannomonas* contains *T. congolense* (Jaffe *et al*., 1969). The genus *Trypanosoma* belongs to the class Kinetoplastida, a monophyletic (Hamilton *et al*., 2004) group of unicellular parasitic flagellates.

Trypanosomes are found where the most common vectors, the tsetse flies are prevalent. Therefore, the ecology of their vector and host is the ecology of the trypanosomes. The most common carrier of *Trypanosoma brucei* is the tsetse fly (*Glossina*), which is native to Africa. Tsetse flies are endemic in Africa between latitude 15° N and 29° S, from the southern edge of the Sahara Desert to Zimbabwe, Angola and Mozambique. Although the tsetse flies can be found over some 9 million squared kilometers of the African continent, the presence of their populations throughout the continent are far from continuous (www1.paho.org/hq/dmdocuments/2010/ZoonosesVol-3.pdf?ua=1). In general, the Sahara

and Somali Deserts limit the populations in the North, extending across the entire continent from Senegal in the west to Southern Somalia in the East. Tsetse populations are denser in West and Central Africa, and are found more sporadically to the East and down to the borders of the Kalahari and Namibian Deserts in Southern Africa. Trypanosomes, particularly *T. vivax,* can spread beyond the "Tsetse fly belt" by transmission through mechanical vectors. *Trypanosoma vivax* is also found in South and Central America and the Caribbean, areas free of the tsetse fly (OIE, 2009).

The habitats of the *Glossina* vary considerably, depending on the species of fly and its location, but climate and altitude - through their direct effects on vegetation, rainfall, and temperature - are still the primary determinants for their distribution. Unlike other insects, there are no seasonal interruptions in the life-cycle of *Glossina*. However, both adult longevity and puparial duration are related to temperature, and a significant seasonal decline in tsetse populations is normal, particularly in savannah habitats, during the dry season. The three main species of *Glossina* (*Glossina morsitans, G. palpalis* and *G. fusca*) are generally adapted to different habitats and ecozones (Medical ecology, 2004).

2.2 Morphology and Life-Cycle of Trypanosomes

2.2.1 Morphology

A sound knowledge of the basic features of the various trypanosomes enables the identification of each species, and therefore the exact cause of the disease. It helps in the diagnosis and control of trypanosomiasis. The trypanosome (trypomastigote) as seen in a stained preparation made from the blood of an infected animal consists of a single cell varying in length from 8 to over 50 μm. Haemoflagellates generally have several

morphological forms: amastigote, epimastigote, trypomastigote and promastigote. African trypanosomes show two of the morphological forms, the trypomastigote and the epimastigote. A typical trypomastigote has a small kinetoplast located at the posterior end,

a centrally located nucleus, an undulating membrane, and a flagellum running along the undulating membrane, leaving the body at the anterior end. The trypomastigote is found in the vertebrate host while the epimastigote resides in the salivary gland of the insect vector.

Among other basic morphological features, a distinct well-defined body, the kinetoplast, is seen near to the posterior end of the trypanosome and differs in size and position according to the species. It is adjacent to the parabasal body (from which the flagellum arises), and so close to it that it cannot easily be seen separately with the light microscope. The extent of the undulating membrane and the absence or presence of the free flagellum are also precious in specific identification of trypanosomes. Other morphological characters are the average length and the shape of the body (Uilenberg, 1998).

Figure 2.1 presents a diagrammatic illustration of the fundamental features of a trypanosome (trypomastigote) as seen in a stained preparation made from the blood of an infected animal.

2.2.2 Life-cycle.

As trypanosomes progress through their life-cycle, they undergo a series of morphological changes typical of trypanosomatids. Their life-cycle often consists of the trypomastigote form in the vertebrate host and the trypomastigote or epimastigote form in the gut of the invertebrate host. Intracellular life-cycle stages are normally found in the amastigote form.

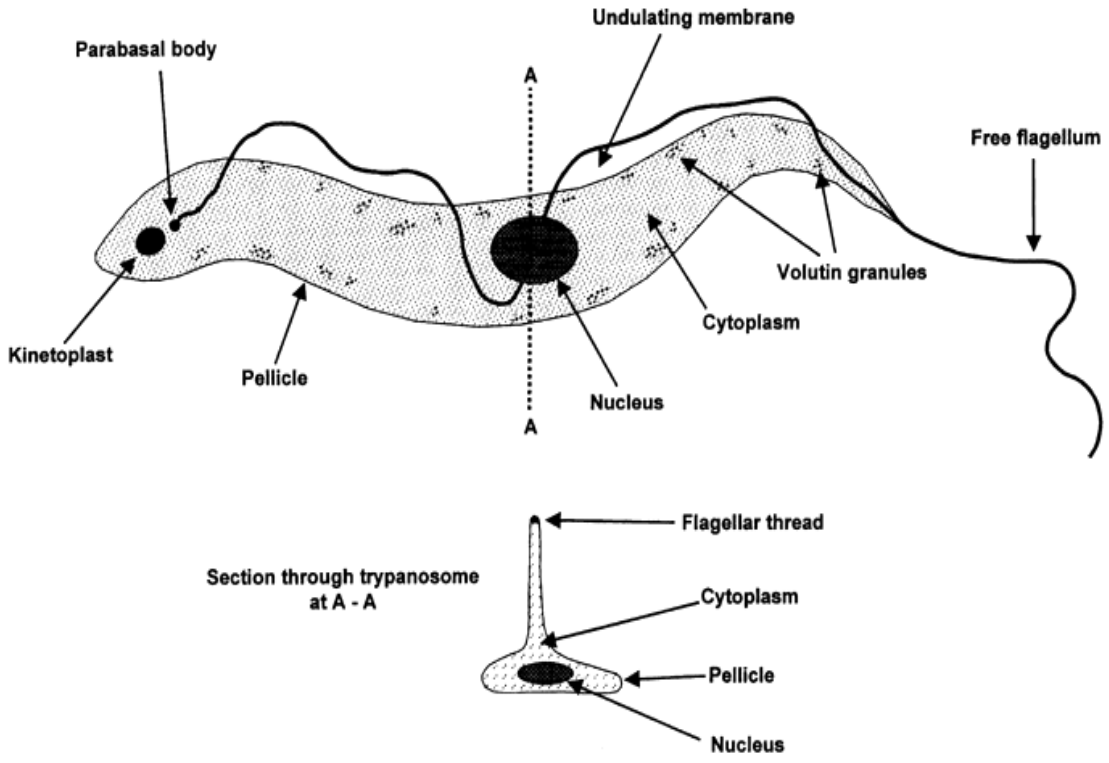


Figure 2.1: Schematic diagram of a trypanosome (trypomastigote) as seen in a stained preparation made from the blood of an infected animal (Uilenberg, 1998).

The trypomastigote morphology is unique to species in the genus *Trypanosoma*. Salivarian trypanosomes develop in the anterior gut of insects, most importantly the tsetse fly, and infective stages are inoculated into the host by the insect before it feeds.

The life-cycle is mostly the same for *Trypanosoma brucei* (including *T. brucei gambiense* and *T. brucei rhodesiense*), *Trypanosoma vivax* and *Trypanosoma congolense*. The life-cycle of *T. brucei* subspecies (Figure 2.2.) is similar to that of *T. congolense* except that after it has developed in the midgut of the insect vector, it goes forward to the proboscis not the salivary glands and *T. vivax* does not have a midgut stage but develops in the proboscis. *Trypanosoma vivax* has become wide spread in South America presumably because its simple life-cycle in the tsetse fly has made it easier to adapt to different vectors in South America (Bovine Trypanosomosis Consortium, 2013).

Reproduction in Trypanosomes

Once inside the bloodstream, trypanosomes grow into long and slender forms. Then, they multiply by binary fission (Figure 2.3); the daughter cells become short and stumpy (Chatterjee, 2009; CDC, 2016). The events of replication are as follows: the basal body duplicates and both remain associated with the kinetoplast. Each basal body forms a separate flagellum; kinetoplast DNA undergoes synthesis then the kinetoplast divides coupled with separation of the two basal bodies; nuclear DNA undergoes synthesis while a new flagellum extends from the younger, more posterior, basal body; the nucleus undergoes mitosis; cytokinesis progresses from the anterior to posterior; division completes with abscission (http://www.fao.org/3/a-i5459e.pdf). Sexual reproduction has also been demonstrated in trypanosomes with evidence for some form of genetic exchange among pathogenic species (Peacock *et al*., 2011; Peacock *et al*., 2014; Gibson *et al*., 2015).

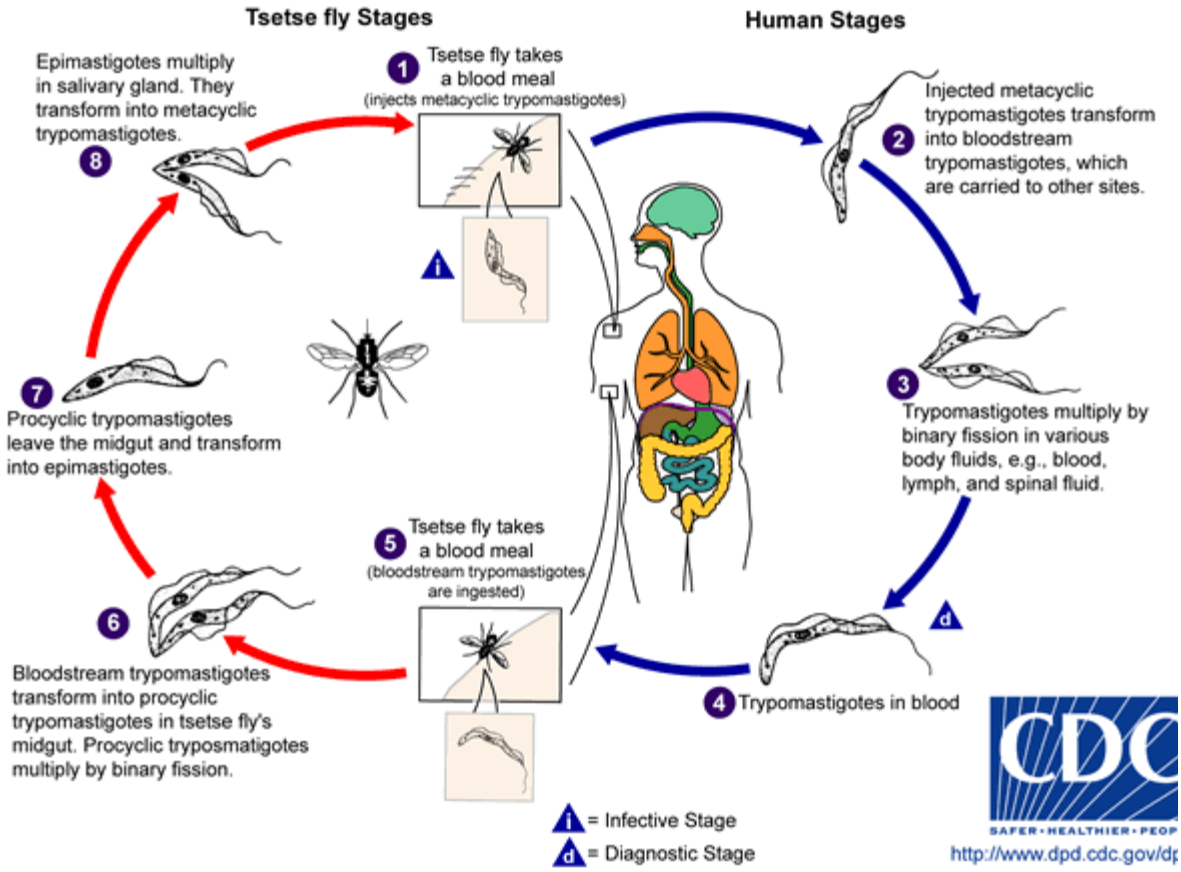


Figure 2.2: Life-cycle of *Trypanosoma brucei* (Source: Center of Disease Control and Prevention)

During a blood meal on the mammalian host, an infected tsetse fly (*Glossina*) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream ❶. Inside the host, they transform into bloodstream trypomastigotes ❷, are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission ❸. The entire life-cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host (❹, ❺). In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission ❻, leave the midgut, and transform into epimastigotes ❼. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission ❽. The entire life cycle of the fly takes about three weeks (Chatterjee, 2009).

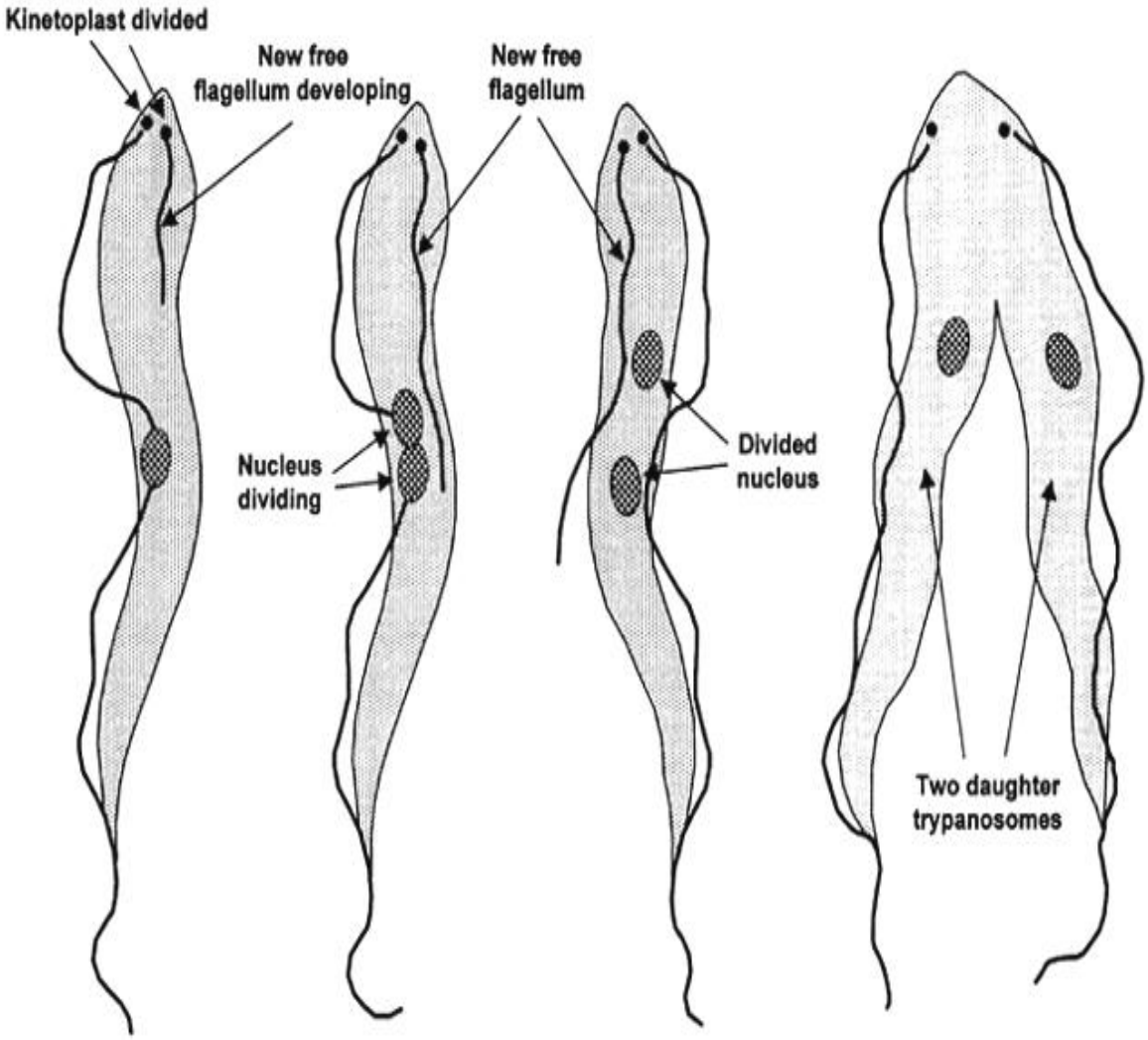


Figure 2.3: Schematic diagram of the binary fission of a trypanosome (Uilenberg, 1998)

2.3 Hosts of Trypanosomes

All domesticated vertebrates can be infected by trypanosomes as clinical cases have been described in cattle, water buffalo, sheep, goats, camels, horses, donkeys, alpacas, llamas, pigs, dogs, cats and other species. Wild or zoo species, including ruminants such as white-tailed deer, duikers, antelope and African buffalo, as well as wild Equidae, lions, leopards, warthogs, capybaras, elephants, nonhuman primates and various rodents are also known to be susceptible to infection (OIE, 2009).

The host preference of each trypanosome species may differ, but *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei brucei* have a wide host range among domesticated animals; *Trypanosoma godfreyi* and *Trypanosoma suis* occur in pigs; *Trypanosoma simiae* appears to be most important in pigs, but it has also been reported by PCR in camels, horses and cattle (OIE, 2009). The occurrence of African trypanosomes in domestic animals is presented in Table 2.1.

The three main species of tsetse flies responsible for transmission of trypanosomes are *Glossina morsitans*, which favors the open woodland of the savanna; *G. palpalis*, which prefers the shaded habitat immediately adjacent to rivers and lakes; and *G. fusca*, which favors the high, dense forest areas (http://www.vet.uga.edu/vpp/gray_book/ Handheld/ aat.htm).

Table 2.1: The occurrence of African trypanosomes in domestic animals (OIE, a field guide (Uilenberg, 1998))

Trypanosome species	Domestic animals affected	Reservoir hosts	Laboratory animals
T. congolense	Cattle, camels*, horses, dogs, sheep, goats, pigs	Several groups of wild mammals	Rats, mice, guinea pigs, rabbits
T. simiae	Pigs	Wart hog, bush pig	Rabbits, monkeys
T. godfreyi	Pigs	Wart hog	None are susceptible
T. vivax	Cattle, sheep, goats, domestic buffalo, horses	Several groups of wild mammals	Usually none are susceptible
T. uniforme	Cattle, sheep, goats	Several wild ruminants	None are susceptible
T. brucei brucei	Horses, camels*, dogs, sheep, goats, cattle, pigs	Several groups of wild mammals	Rats, mice, guinea pigs, rabbits
T. brucei gambiense, *T. brucei rhodesiense*	Human sleeping sickness; affect domestic animals as *T. brucei brucei***	Several groups of wild mammals (particularly *T. brucei rhodesiense*)	As for *T. brucei brucei* (after initial adaptation where *T. brucei gambiense* is concerned)
T. evansi	Camels, horses, dogs, domestic buffalo, cattle	Several wild mammals in Latin America	As for *T. brucei brucei*
T. equiperdum	Horses, donkeys, mules	None known	As for *T. brucei brucei* (after initial adaptation)
T. theileri and *T. ingens* (subgenus *Megatrypanum*)	Cattle, domestic buffalo*** (not pathogenic)	Several wild ruminants	None are susceptible

* Camels are highly susceptible to *T. congolense* and to *T. brucei*, but do not usually penetrate into tsetse country.

** In particular, the behaviour of *T. brucei rhodesiense* in domestic animals is quite similar to that of *T. b. brucei*, whereas *T. brucei gambiense* is on the average more chronic (as it is in humans).

*** Of the two only *T. theileri* has been reported from domestic buffalo.

The hosts of tsetse also vary. The summary of the main hosts of tsetse flies determined from analysis of blood meals (adapted from Moloo, 1993, in Medical ecology.org., 2004) is presented in Table 2.2.

The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota, Liperosia, Stomoxys*, and *Chrysops* flies have also been implicated. In Africa, both *T. vivax* and *T. brucei brucei* have spread beyond the "tsetse fly belts" (Roder *et al*., 1984), where transmission is principally by tabanid and hippoboscid flies (http://www.vet.uga.edu /vpp/gray book/ Handheld/aat.htm).

2.4 Infectivity and Infection of Trypanosomes

The ability for trypanosomes to invade and multiply in a host is refered to as its infectivity. This varies depending principally on the trypanosome species, strain and the species of the host. Within a given mammalian host, infectivity also depends on the heterogeneity of susceptibilities of individuals. It is measured by the number of organisms required to establish an infection and is therefore a quantifiable attribute of trypanosome populations. Under laboratory conditions, infectivity can be modified for a particular species of mammalian host by serial passages of the blood form of trypanosome in the particular species of host. Theoretically, all trypanosomes are potentially infective to humans. *Trypanosoma evansi*, which was known only in animal infections has been reported in humans (Dede, 2004).

Table 2.2: The main hosts of tsetse flies

Host	Morsitans Group	Palpalis Group	Fusca Group
Domestic animals including humans			
Human	3.6	13.00(3)	0.46
Cattle	3.56	4.2	2.38
Sheep and Goats	0.26	0.4	0.2
Donkey	0.02	0.05	0.02
Dogs	0.15	0.26	0.02
Domestic Pigs	0.03	14.00(2)	0.17
Sylvatic hosts			
Bushpig	5.60	2.20	28.0(1)
Warthog	31.60(1)	1.65	0.37
Total Suidae	**45.36**	**18.50**	**33.1**
Bushbuck	10.18(2)	25.12(1)	11.2(2)
Buffalo	7.50(3)	0.90	7.59(3)
Kudu	5.37		
Total Bovidae	**43.22**	**45.50**	**28.1**
Monitor Lizard	0.036	6.02	0.05
Hippopotamus		4.04	15.84*
Rhinocerus			14.20*

Numbers in parentheses show host ranking in order of importance for each subgenera.

* Ranking for anomalous species *G. brevipalpis* and *G. longipennis*

(Adopted from Moloo, 1993, in Medical ecology.org. 2004).

Taiwo *et al*. (1990) noted that the period immediately after the tsetse fly bite is important both in establishing infection and expression of individual metacyclic variable antigen types (M-VATS), as well as the induction of immunity in the host. Akinwale (1998) reported that the earliest sign of trypanosomiasis is an inflammatory reaction caused by the multiplication of trypanosomes in the skin, at the site of the tsetse fly bite, and that there is a slight increase in lymph flow and lymphocyte output; a dramatic rise in the relating number of lymphoblasts which coincide with the formation of chancre. This is a localized reaction in the skin of a susceptible host to the presence of proliferating trypanosomes (Barry and Emery, 1984). Morrison *et al.* (1985) observed that the development of chancre occurs within a few days after the susceptible host has been bitten by infected tsetse flies. They observed that the reaction reached a peak during the second week before declining to undetectable levels during the third week. These sites contain foci of trypanosomes in the collagenous connective tissue of the skin and persist for 1 to 3 weeks after infection (Akinwale, 1998).

The development of local skin reactions (chancres) at the site of inoculation of metacyclic trypanosomes, serves as the site for establishing infection and as the focus for multiplication and persistence of trypanosomes before they move into the blood stream (Akinwale, 1998). This chancre, which provides a meeting place for the first contact between the trypanosome antigens and the host immune system (Akol and Murray, 1982; 1983), is an established part of the pathology of trypanosomiasis (Taiwo *et al*., 1990).

Barry and Emery (1984) reported that the chancre could serve as a site for sensitization of the host's immune system as shown by the large numbers of lymphoblasts and plasma cells, some of which contain Russell bodies in the later stages of chancre development.

They also demonstrated that the development of protective immunity to a homologous tsetse-transmitted challenge depended on the timing of treatment in relation to the development of the chancre.

The incubation period for *T. congolense* varies from 4 to 24 days; for *T. vivax,* from 4 to 40 days; and for *T. brucei brucei*, from 5 to 10 days. After inoculation in the skin and replication at the chancre, trypanosomes then spread to the lymph nodes and blood and continue to replicate. *Trypanosoma congolense* localizes in the endothelial cells of small blood vessels and capillaries. *Trypanosoma brucei brucei* and *T. vivax* localize in tissue (http://www.vet.uga.edu/vpp/gray_book/Handheld/aat.htm).

2.5 Diagnosis of Trypanosomiasis

Clinical, parasitological, serological and molecular diagnoses are used to detect trypanosomiasis in animals. The clinical features of infection are not sufficiently specific, diagnosis of African Trypanosomiasis is therefore made through laboratory methods (CDC, 2012). This implies that under field conditions, in the absence of simple and portable diagnostic tools or access to laboratory facilities, veterinarians could rely on clinical diagnosis to screen and treat cases of bovine trypanosomosis presented by farmers but confirmatory diagnosis in diagnostic centres is essential (Magona *et al*., 2003).

Laboratory diagnosis (OIE, 2009)

A presumptive diagnosis can be made if pathogenic trypanosomes are observed in the blood, lymph nodes or other tissues by direct examination. The organisms must be distinguished from avirulent trypanosomes such as *T. theileri*. Parasites may be found in wet blood films and thick or thin stained blood smears. Wet unstained blood films are

examined by light microscopy (condenser aperture, phase-contrast or interference contrast), using 400x power to detect the motile trypanosomes. Thin smears fixed in methyl alcohol can be stained with Giemsa or May–Grünwald stain. Thick smears are air dried and stained with Giemsa. Other stains may also be used. Thick or thin films should be examined by light microscopy at high magnification. Presumptive species identification can be made based on the parasite's morphology in thin films. More than one species may be found. Thick films have the advantage of being able to detect parasites in low numbers; however, the morphology of the parasite is difficult to determine. Trypanosomes can be difficult to find in the blood, especially in animals with chronic disease or healthy carriers. Detection can be improved with parasite concentration techniques including mini anion–exchange chromatography, hematocrit centrifugation, the quantitative buffy coat method or the dark-ground/phase-contrast buffy coat technique. The latter three methods rely on the concentration of trypanosomes near the buffy coat after centrifugation.

Animal inoculation studies in rats or mice may occasionally be used to diagnose African animal trypanosomiasis. This technique is very sensitive and can detect low levels of parasites, but it is time consuming.

Most currently used tests do not identify trypanosomes to the species or subspecies level. *In vitro* cultivation, with species identification by techniques such as Restriction Fragment Length Polymorphism (RFLP), isoenzyme electrophoresis or DNA hybridization, are used to identify some trypanosome to species or subspecies level. Polymerase chain reaction (PCR) assays can identify parasites at the genus, species or subspecies level, although it may not identify all species or some genotypes. Antigen detection techniques are not currently in use (www.iiste.org/Journals/index.php/JNSR/article/download/25932/26710).

Serology can be helpful in diagnosis. Two serological tests, the indirect fluorescent antibody test and enzyme-linked immunosorbent assays (ELISA), are routinely used to identify seropositive cattle. Because reactions to previous infections can be detected, serology is useful only for a presumptive diagnosis. Cross-reactions can occur with other trypanosomes such as *T. theileri*, which is not pathogenic, and *T. evansi*, which causes surra (OIE, 2009).

2.6 Antigenic Variation and Immune Evasion: Unique Features of Trypanosomes

The surface of the trypanosome is covered by a dense coat of about 5 x 10^6 molecules of Variant Surface Glycoprotein (VSG) (Barry and McCulloch, 2001), one of the most remarkable features of trypanosomes which allows persistence of an infecting trypanosome population in the host as they regularly switch their surface coat and hence evade immune destruction. This mechanism is known as antigenic variation (Vanhamme *et al.*, 2001; Pays *et al*., 2004) and is one of the most spectacular adaptive mechanisms exhibited by the trypanosomes (Ottawa, 2010). A review article by Ottawa (2010) explained the mechanisms as follows: The bloodstream form of trypanosome is entirely covered with a monolayer made of 10^7 copies of the VSG, which is a major antigen of the parasite whose antigenicity is in continuous change. During the ascending phase of the parasitemia, the majority of parasites are of the same antigenic type (called homotype). The host immune system recognizes this homotype and makes antibodies against it. As the parasites of the major variable antigenic type (VAT) are eliminated the parasitemia goes in descending phase but at the same time, the parasites expressing the heterotype or the minor VATs are multiplying and one of them overgrows others. As a result, this one becomes the new homotype, leading to a new wave of parasitemia and resulting in a long-lasting chronic

infection. So expression of the VSG is central in the antigenic variation process and eventually for exhausting the host immune system to the benefit of the parasite. For the immune evasion, trypanosomes have also developed another method; macromolecular trafficking mechanism (Field *et al*., 2009) whereby the VSG-complexed with antibody are sorted and endocytosed (Engstler *et al*., 2007). This mechanism most probably protects the parasites from the complement-mediated killing and as such the trypanosomes escape from the host immune system. For the purpose of escaping from the host immune system, trypanosomes have more mechanisms such as capping of the surface-bound immune factors, restriction of the invariant receptors in the flagellar pocket, rendering them inaccessible to the host immune effectors (Field and Carrington, 2009).

2.7. Importance of Trypanosomiasis

Animal trypanosomiasis is a major constraint on ruminant livestock production in Africa, Asia and South America (Gutierrez *et al*., 2006). African animal trypanosomiasis (AAT) has a severe economic impact in the developing countries of Africa (Barret *et al*., 2007; Van den Bossche, 2001). Many untreated cases are fatal. Although acute cases can be seen, trypanosomiasis is often a chronic disease in susceptible animals (Batista *et al*., 2009). In Africa, trypanosomiasis appears as a persistent endemic disease; although seasonal changes can occur in the morbidity rate, the disease is never completely absent. The morbidity rate is high, and many untreated animals infected with *T. vivax*, *T. brucei brucei* or *T. congolense* eventually die. Coustou *et al*. (2010) reported that in terms of severity and consequences for productivity, *T. congolense* is the main causative agent of AAT. In some cattle infected with some strains, the mortality rate can reach 50-100% within months after

exposure, particularly when poor nutrition or other factors contribute to debilitation (Coustou *et al*., 2010).

Although AAT is found mainly in those regions of Africa where its biological vector, the tsetse fly, exists, one organism, *Trypanosome vivax*, has become established in South America, where it is transmitted by biting flies acting as mechanical vectors (Davila and Silva, 2000; Jones and Davila, 2001).

African animal trypanosomiasis is widely spread affecting 40 countries situated in regions that could potentially be the most productive. The challenge reduces the milk and meat production, the reproductive activity, and causes death. Estimates indicate increases of 16% and 14% in meat and milk production, respectively, following trypanosomiasis control (Jahnke *et al.*, 1988). It is also estimated that 50 million cattle and 70 million small ruminants are at risk, costing the continent between 1.5 and 5 billion US dollars per annum (Coustou *et al*., 2010; Chamond *et al*., 2010).

Although the trypanosomes that cause African animal trypanosomiasis are not considered to be pathogenic for humans, disease might be possible in people with certain genetic defects (Vanhollebeke *et al*., 2006).

2.8 Control of African Trypanosomiasis

Accurate epidemiological information obtained on the prevalence of animal trypanosomiasis in different livestock species is a requirement for the control of the disease in Africa, Asia and South America. Such information depends on the use of sensitive diagnostic tests. The control of trypanosomiasis can be approached from three different aspects: control the vector, vaccinate the host as a preventive measure, or treat the infected

host. All three approaches have their own benefits but at the same time suffer from some difficulties (Ottawa, 2010).

Vector/control methods currently available include the use of insecticide (through the sequential aerosol spraying technique, insecticide-treated targets or insecticide treated animals), the use of traps, and the sterile insect technique (Simarro *et al*., 2008). However more precise and possibly new methods of vector control are still needed (Ottawa, 2010).

2.9 Prevention and Treatment

The antigenic variation of the trypanosome parasites, as described above, has rendered the production of the vaccine almost impossible. Greca and Magez (2011) reported that to date, not a single experimental anti-trypanosome vaccination protocol has made it to a stage where preliminary promising results have been reported in a field setting. In endemic areas, control of African bovine trypanosomiasis mainly relies on chemotherapy and chemoprophylaxis using three trypanocidal compounds; isometamidium, homidium and diminazine. Suramin and quinapyramine are also in practice since long time in animal trypanosomiasis treatment. Reports showed that all of these drugs have been in widespread use for about 40 years and resistance has been reported in many parts (Peregrine, 1994; Eisler *et al*., 2001; Geerts *et al*., 2001; Delespaux *et al*., 2005). Resistance to a very widely used chemotherapeutic drug for treatment of livestock trypanosomiasis, diminazine aceturate (Berenil) (Van den Bossche *et al*., 2000), which has been effective against all three African animal trypanosomes, has also been reported in several foci (Bacchi, 1993, quoted by Ottawa, 2010), although many theories are being tested to explain the reason why drug resistance by *T. brucei* occurs (Delespaux and Harry, 2007).

The isometamidium drugs are also excellent chemotherapeutic agents as are the quaternary ammonium trypanocides Antrycide, Ethidium and Prothidium (Mare, 2004). Legros *et al*. (2002) and Kennedy (2004) reported that the chemotherapeutic drugs in current use for the treatment of trypanosomiasis are toxic and problems of resistance are increasing. All chemotherapeutic drugs have to be first of all tested *in vitro*, thus the need of cultivating the parasites.

2.10 Cultivation of Trypanosome Parasites

The development of *in vitro* techniques for cultivating African trypanosomes is essential for screening potential trypanocidal compounds, testing trypanocidal drugs as well as other substances for their anti-trypanosomal effects. Although some records show early attempts to maintain blood form (BF) *Trypanosoma brucei in vitro*, short-term propagation, in the presence of feeder cells (L-cells), was not achieved until 1967 (Le Page, 1967). Continuous propagation was not reported until 1977, when the Lister 427 strain (clone 052 from GAMC) was successfully propagated in RPMI 1640 (Rossvelt Park Memorial Institute 1640) medium with 25 mM HEPES and 20% heat-inactivated fetal bovine serum (FBS), in the presence of bovine primary fibroblast-like feeder cells (Hirumi *et al*., 1977; Doyle *et al*., 1980; Hirumi *et al*., 1980). Brun *et al*. (1979; 1981) introduced MEM (Minimal Essential Medium of Eagle) supplemented with MEM non-essential amino acids, 30 mM HEPES pH 7.5, and glutamine, and experimented with feeder cells and sera, to find conditions that allowed any *T. brucei* stock to be cultivated. Heat-inactivated 15% human or fresh (but not commercially supplied) rabbit serum supported much better growth than FBS. They reported that MEM gave better results than RPMI 1640 medium (Brun *et al.*, 1981). Further research (Duszenko *et al*., 1992) led to the rational elimination of feeder

cells from the MEM-based culture system and the demonstration that cysteine is an essential growth requirement but is actually toxic to trypanosomes at millimolar (mM) concentrations as he realized that by mistakenly adding cysteine instead of cystine the trypanosomes died immediately upon being placed in the freshly dissolved medium. Duszenko's subsequent research showed that trypanosomes took up cysteine, but not cystine, and proved that it was an essential amino acid, not just a reducing factor (Duszenko *et al*., 1992).

In 1989, Hirumi and Hirumi published the HMI-9 medium, based on Iscove's modification of Dulbecco's MEM (Modified Eagles Medium) (IMDM), which is now most widely used for culture of BF *T. brucei*, and introduced a copper chelator, bathocuproine sulfonate, whose use for the culture of *Pneumocystis carinii*, in the absence of feeder cells, had been published the previous year. Bathocuproine, at 0.05 mM, extended the life of the 1.5 mM cysteine-containing medium for at least 10 days (the maximum shelf life was not established). Addition of 2 mM pyruvate was also required to support the growth of BF during primary cultivation, but not once the cultures were established. This paper also reported growth in a serum-free version of HMI-9 (HMI-18), in which the Serum Plus™ component was increased from 10% to 20%. Serum Plus contains 20% serum (Cross, 2008).

About 20 different cell lines have been used as feeder layers to support growth of trypanosomes of the *T. brucei* group (Hill and Hirumi, 1983) and include bovine-derived cells (from heart, lung, peritoneum, pericardium, and aorta), blood cells (from white rabbit and bovine), and more-conventional cell types such as African green monkey kidney,

Chinese hamster lung, buffalo lung, and miscellaneous others such as mouse L-line and dog kidney cells. The cell types used represent mostly fibroblasts but also included epithelial and endothelial cells. In a study that focused on differentiation of bloodstream trypomastigotes into procyclic stages, typical of trypanosomes found in the tsetse fly gut, Simpson *et al*. (1985) used human fetal lung fibroblasts and human epithelial cells as feeder layers. They noted that trypanosomes growing in the fibroblast culture system were sequestered in intercellular spaces of the cell sheet, while those growing in cultures with epithelial layers were found in the supernatant fluid and were more readily observed and harvested.

Schuster and Sullivan (2002), in their reviewed article reported that with a better understanding of the relationship between the feeder layers and trypanosomes, it was possible to contrast growth in the presence and absence of tissue culture cells. That was how Baltz *et al*. (1985) cultured bloodstream forms of the *T. brucei brucei* complex with and without macrophage feeder layers, as well as *T. equiperdum* and *T. evansi*, in a semidefined medium of MEM in Earle's salts with rabbit or human serum at 10%. Additional supplements included pyruvate and a reducing agent (either 2-mercaptoethanol or thioglycerol), hypoxanthine, thymidine, and a feeder layer of mouse peritoneal macrophages. The presence of a reducing agent at 0.2 mM was found to be essential for trypanosome growth. They also noted differences in the sera used: commercial sera were less satisfactory than fresh sera, horse and pig sera were less satisfactory than rabbit serum, and human serum was toxic for the non-human trypanosome strains and even for a strain of *T. brucei rhodesiense*. Growth of 10 strains of trypanosomes in the semi-defined medium with freshly prepared rabbit serum ranged from $<1 \times 10^6$ to 4×10^6 cells/ml, with

generation times of 6 to 32 h. Baltz *et al*. (1985) characterized the steps in transition of trypanosomes from animal host to in vitro growth, recognizing an initiation phase with use of a feeder layer of macrophages, a period of adaptation with or without a feeder layer depending upon the trypanosomal strain, and finally, maintenance of the strain in the absence of the feeder layer.

Brun and Jenni (1987) postulated that the function of the feeder layer was to provide a reducing condition in the culture, a source of growth factors for trypanosome proliferation, detoxification of the added serum, and modulation of the gas phase in the culture.

The importance of a reducing agent for cultivation of bloodstream *T. brucei brucei* observed by Duszenko *et al*. (1985) led to the conclusion that reducing agents in the medium were converting cystine to cysteine and that cysteine was the growth factor provided to the trypanosomes by the tissue culture feeder cells; providing cysteine eliminated the need for the feeder cell layer. Optimal concentrations of cysteine and 2-mercaptoethanol were determined to be 10^{-3} and 10^{-6} M, respectively. The cysteine level was also critical; a cysteine concentration that was too high (2 to 3×10^{-4} M) was toxic to trypanosomes, presumably by undergoing auto-oxidation to generate hydrogen peroxide. The peroxide effect could be countered by addition of catalase, which is lacking in bloodstream trypanosomes, since Meshnick had shown, in 1978 (quoted in Cross, 2008) that *T. brucei* lacked catalase and glutathione reductase. However, the medium required replenishment of 2-mercaptoethanol twice daily in order to maintain a sufficient supply of cysteine.

Carruthers and Cross (1992) found that blood forms (BF) trypanosomes grew extremely well, with high plating efficiency on agarose plates containing HMI-9 medium.

Axenic cultures media are also used to culture trypanosomes for experimental purposes such as the test of antitrypanosomal activity of some substances including animal sera (Black *et al*., 1999), drug-sensitivity (Brun and Lun, 1994) and isolated compounds from plants (Bero *et al*., 2013). Black *et al*. (1999), used axenic culture medium and the trypanosomes were grown in BMEM supplemented with 10% heat-inactivated (56 C, 30 min) fetal bovine serum (FBS). Heat inactivation of sera used in medium preparation is to prevent complement-induced cell lysis (Schuster and Sullivan, 2002). For experimental cultures, washed trypanosomes were incubated for 24 or 48 hr at 37 C in a humid atmosphere of 8% CO2 in air, as Schuster and Sullivan (2002) reported that the use of 5% CO_2-95% air atmosphere is one of the fundamental features of *in vitro* cultivation of hemoflagellates in the presence of tissue culture cells, another one being the addition of penicillin-streptomycin or gentamicin to media to prevent or minimize contamination of cultures. Cross (2008) reported that some investigations in Paul Englund's lab led to a simpler and robust protocol provided by Shawn Motyka and Paul Englund, which showed that raising the serum concentration from 10% to 15% results in a "drastic improvement in cloning efficiency". The protocol was included in a July 2002 publication (Klingbeile *et al*., 2002). In the same line Bero and colleagues, evaluating the antitrypanosomal activity of some plant's essential oil and isolated compounds *in vitro* against *T. brucei brucei* (strain Lister 427) bloodstream forms and procyclic forms. The bloodstream forms (BF) were cultured *in vitro* at 37ºC with 5% CO_2 in HMI9 medium containing 10% heat-inactivated fetal bovine serum, while the procyclic forms, which correspond to the life

cycle stage living in the tsetse midgut, were cultured *in vitro* at 28ºC with 5% CO_2 in a semi-defined medium (SDM-79) supplemented with 15% heat-inactivated fetal bovine serum (Bero *et al*., 2013).

2.11 New Control Approaches against Trypanosomiasis

Because the damage due to African trypanosomiasis is considerable, the pathogenic parasites are constantly being studied. One of the targets in the fight against trypanosomiasis is glycolysis pathway (Verlinde *et al*., 2001), as the bloodstream form of *T. brucei brucei* is exclusively dependent on the breakdown of glucose by this process as its unique source of ATP production. Indeed, compounds which inhibit glycolysis have been shown to have antitrypanosomal activity (Fairlamb *et al*., 1977; Bakker *et al*., 1999; Beroa *et al*., 2012). On the other hand, in addition to specific antibodies, nonspecific factors that kill trypanosomes or limit their rate of population growth contributing to host resistance have been regarded as new control approach against trypanosomiasis (Muranjan *et al*., 1997; Black *et al*., 1999). In this regard, a trypanocidal protein has been isolated from Cape buffalo serum and identified as xanthine oxidase (Muranjan *et al*., 1997). The hydrogen peroxide (H_20_2) generated during catabolism of hypoxanthine and xanthine by the serum enzyme inhibits trypanosome glycolysis, leading to a precipitous decline in cellular ATP. Also, Black *et al*. (1999) reported that 3 distinct constitutive defense mechanisms against *T. brucei* are detected in sera from sub-Saharan mammals other than primates that show a high level of resistance to African trypanosomes and that these mechanisms may act together with parasite-specific antibody responses to control the severity of infection arising in the reservoir hosts.

2.12 Trypanoresistant Animals

The term trypanotolerance is used to describe genetic resistance to African trypanosomiasis. Trypanotolerance occurs in certain breeds of livestock, many species of wildlife, some strains of mice, and possibly humans (Murray *et.al.,* 1984). Roelants *et al.* (1987) observed that some, but not all, cattle from the *indicus* cattle like the zebu show high susceptibility, some breeds of cattle, notably the N'Dama, a West African *Bos taurus* breed show remarkable tolerance to nagana pathology (Roelants *et al.,* 1987)*;* Courtin Baoul*é* breed are naturally resistant to African trypanosomiasis, and that this resistance does not need repeated exposure to trypanosomes early in life but appears to be inherited and functional against many types of antigenically different trypanosomes. Naessens *et al.* (2003) demonstrated that trypanotolerance in cattle is a result of two independent mechanisms: the capacity to control parasitemia, which is independent of the hematopoietic system and the capacity to limit anemia, which is mediated by hemopoietic cells, and that the latter appears to be more important for survival and productivity.

Cape buffalo co-evolved with African trypanosomes, inhabit the humid and semihumid zones of Africa, and tolerate infection with African trypanosomes with few or no signs of disease (Dwinger *et al*.,1986, Grootenhuis *et al*., 1990, Olubayo *et al*., 1990, Reduth *et al.*, 1994). Following experimental infection, these same researchers reported that Cape buffalo rapidly clear most trypanosomes from the bloodstream and subsequently maintain low-level parasitemia. Eland serum has also been reported to contain trypanocidal protein (Reduth *et al.*, 1994), which is inhibited by agents that inhibit xanthine oxidase (Black *et al.,* 1999).

Trypanolytic factors are found only in a few species including humans, gorillas, mandrills, baboons and sooty mangabeys. This appears to be because haptoglobin-related protein and apolipoprotein L-1 are unique to primates (Lugli *et al*., 2004).

Just like Cape buffalo, restrained trypanosome parasitemia in some animals may result from the presence of growth-inhibitory serum components that are not antibodies and/or from the limited availability of an essential growth factor or growth nutrient. Murray *et al*. (1984) reported that since trypanotolerance appears to be related to the control of parasitemia, a capacity associated with an event that regulates parasite growth and determines how rapidly the immune response is triggered, identification of the factors that regulate this event and definition of their genetic basis may have important implications for the development of novel strategies for control of African trypanosomiasis.

2.12.1 Cape buffalo and Xanthine oxidase

Trypanosomiasis in Cape buffaloes, which are naturally selected to resist the disease, is characterized by the development of only one or a few waves of parasitemia, after which the infection becomes cryptic, being maintained by the presence of 1-20 mammal-infective organisms/ml of blood (Wang *et al*., 2002). This is in agreement with Muranjan *et al*. (1997) who reported that Plasma and serum from Cape buffalo (Syncerus caffer) kill bloodstream stages of all species of African trypanosomes in vitro, or prevents their replication, depending on the assay buffer constituents (Reduth *et al*., 1994). They went further to identify the trypanocidal material as xanthine oxidase. They explained that although all of the immune-purified trypanocidal proteins contained other materials in addition to xanthine oxidase, there was compelling evidence that the xanthine catabolic enzyme was responsible for the trypanocidal activity.

Identification of trypanocidal protein as xanthine: oxygen oxidoreductase clearly distinguishes it from human serum factors that selectively lyse *T. brucei brucei*. These factors are complexes of proteins that invariably include haptoglobin-related protein, are restricted to primates, and have peroxidase activity (Hajduk *et al*., 1989; Tomlinson *et al*., 1997).

2.12.2 Xanthine oxidase, a trypanocidal protein

The purified trypanocidal protein has a molecular mass of 150 kDa, and activity correlated with the presence of a 146-kDa polypeptide detected upon reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Amino acid sequences of three peptide fragments of the 146-kDa reduced polypeptide, ligand affinity and immunoaffinity chromatography of the native protein, and sensitivity to pharmacological inhibitors, identified the trypanocidal material as xanthine oxidase (EC 1.1.3.22). The trypanocidal activity of xanthine oxidase results from the inhibition of trypanosome glycolysis which is due to hydrogen peroxide (H_2O_2) produced during catabolism of extracellular xanthine and hypoxanthine by the purine catabolic enzyme (Muranjan *et al*., 1997).

2.12.3 Constitutive anti-trypanosomal properties of sera of some trypano-resistant animals

Some animals possess constitutive antitrypanosomal components in their serum, some of which can kill, inhibit or limit the growth or replication of trypanosomes. Black *et al*. (1999) reported that sera from warthog and springbok severely limited trypanosome replication but lacked xanthine oxidase. Sera from lion and leopards showed a concentration-dependent inhibition of the growth of *T. brucei* S427 clone 1 organisms, but not clone 22 organisms, though the sera lacked xanthine oxidase. Serum from Grant's

gazelle prevented replication of both *T. brucei* clones, and also lacked xanthine oxidase. These findings show that there are other trypanolytic factors in some animals' sera, different from xanthine oxidase, that can be exploited in the development of novel therapeutic against trypanosomes, as Black *et al*. (1999) concluded that 3 distinct constitutive defense mechanisms against *T. brucei* are detected in sera from sub-Saharan mammals other than primates that show a high level of resistance to African trypanosomes. These mechanisms may act together with parasite-specific antibody responses to control the severity of infection arising in the reservoir hosts.

Investigating the lytic activity of the sera from selected birds and mammals against *Trypanosoma brucei brucei* and *Trypanosoma congolense* might provide more control strategies against trypanosomiasis, the devastating desease.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical Statement

This research had ethical approval from the Committee on Animal Use and Care, Directorate of Academic Planning & Monitoring, Ahmadu Bello University, Zaria, with the Approval No: ABUCAUC/2017/007 (Appendix I). Local approvals were given by the Director of Kano Zoological Garden and the Assistant Director of Research, NAPRI, Ahmadu Bello University, Zaria. Blood samples were collected in accordance with best practice guidelines to minimise stress on donor animals. The laboratory animals used were kept in a clean aerated room and were given necessary care.

3.2 Donor Animals

Blood was collected from selected birds and mammals reared in some non-endemic areas for trypanosomes/Kano and Kaduna states of Nigeria. Some domesticated animals namely camel, donkey, horse, cattle, goat, sheep, pig, rabbit and chicken blood samples were obtained from the National Animal Production Research Institute (NAPRI) in Zaria, Kaduna State, Nigeria; while the following wild animals donors were provided by the Kano Zoological Garden, Kano State, Nigeria: red patas monkey, tantalus monkey, jackal, grass cutter, goose, crowned crane, hooded vulture, spooted eagle owl, buzzard, peacock, and white stork. The domesticated pigeon was also obtained from the Kano Zoological Garden. Guinea fowls were purchased from Samaru market, Zaria. The list of donor animals is

presented in Table 3.1. Pictures and some characteristics of the animals are presented in the Appendice XXXI - LII.

3.3 Experimental Animals

Wistar rats and mice were obtained from Bayero University, Kano, Kano State, Nigeria and reared at the animal room of the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria. They were given commercial feed (Grower, Vita brand) and water *ad libitum*.

3.4 Parasites

The parasites, *Trypanosoma brucei brucei* (Federe strain) and *Trypanosoma congolense* (Savannah strain) were obtained from the Nigerian Institute of Trypanosomiasis Research (NITR) Kaduna, Kaduna State, Nigeria.

3.5 Blood Collection from donor animals

Each blood sample was aseptically collected from the donor animal using a sterile seringe. 5-10ml of blood was collected, depending on the animal's size, through the wing vein for birds, the femoral vein for rodents and zoo primates or the jugular vein for larger animals. Each blood sample was divided into two. One part was kept as whole using EDTA (ethyl-diamine-tetra-acetic acid) while the other one was dispensed into sterile plain glass test tube to clot for 2-3 hours at room temperature, from which serum was collected after centrifugation at 1,000 g (Black *et al.*, 1999) and stored -20ºC until required for further analysis.

Table 3.1: List of donor animals.

Serial number	Donor's scientific and common names	Donor's local name (Hausa)	Place of collection
I	*Necrosyrtes monachus* (Hooded vulture)	Ungulu	Kano Zoo
Ii	*Bubo africanus* (Spotted eagle-owl)	Mujiya	Kano Zoo
Iii	*Buteo buteo* (Buzzard)	Shaho	Kano Zoo
Iv	*Alopochen aegyptica* (Goose)	Agwagwar Masar	Kano Zoo
V	*Balearica pavonina* (Crowned crane)	Gauraka	Kano Zoo
Vi	*Columba livia domestica* (Domestic pigeon)	Tantabara	Kano Zoo
Vii	*Pavo cristatus* (Peacock)	Dawisu	Kano Zoo
Viii	*Gallus gallus domesticus* (Chicken)	Kaza	NAPRI
Ix	*Numida meleagris* (Guinea fowl)	Zabua	Samaru market
X	*Ciconia ciconia* (White stork)	gàlàntòòyíí	Kano Zoo
Xi	*Haliaeetus vocifer* (African fish eagle)		Kano Zoo
Xii	*Thryonomys swinderianus* (Cane rat/grass-cutter)	Jauji	Kano Zoo
Xiii	*Oryctolagus cuniculus* (Rabbit)	Zomo	NAPRI
Xiv	*Capra aegagrus hircus* (Goat)	Akuya	NAPRI
Xv	*Ovis aries* (Sheep)	Tinkia	NAPRI
Xvi	*Bos Taurus* (Cattle)	Shanu	NAPRI
Xvii	*Equus africanus asinus* (Donkey)	Jaki	NAPRI
Xviii	*Camelus dromedarius* (Camel)	Rakumi	NAPRI
Xix	*Equus ferus caballus* (Horse)	Doki	NAPRI
Xx	*Sus scrofa domesticus* (Pig)	Alede	NAPRI
Xxi	*Canis aureus* (Jackal)	Dila	Kano Zoo
Xxii	*Erythrocebus patas* (Red Patas monkey)	Gata	Kano Zoo
Xxiii	*Chlorocebus tantalus* (Tantalus monkey)	Kirka	Kano Zoo

3.6 Screening Test for Trypanosomes

The blood of each donor animal was screened for trypanosomes to ensure that it is not infected. Screening was carried out using two of the standard detection techniques (thick films) for quick assessment and by haematocrit centrifugation technique (HCT) (Woo, 1989) which are described in details below.

3.6.1 Thick blood films (OIE Terrestrial manual, 2008)

A drop of blood (5–10 µl) was placed on a clean microscope slide and spread over an area of approximately 2 cm in diameter, using the corner of another slide. The film so made was air-dried without fixation, after which it was briefly dehaemoglobinised in distilled water and the slide was stood upright to drain-dry. The dried smear was kept away from dust, heat, flies and other insects. It was stained for 30 min with 4% diluted Giemsa stain in phosphate-buffered saline (pH 7.2). The stained smear was washed with buffered water and examined under ×40 and ×100 (oil immersion) objectives.

3.6.2 Microhaematocrit centrifugation technique (HCT) (Woo, 1989).

This technique is widely used for the diagnosis of animal trypanosomiasis and is based on the separation of the different components of blood according to their specific gravity. The procedure was done as follows:

Heparinised microhaematocrit tubes were filled up to three-quarter of their length with fresh blood by capillary attraction, and one end of each capillary tube was sealed with plastiseal. Thereafter, the sealed capillary tubes were placed in a microhaematocrit centrifuge with the sealed end pointing towards the outside. To ensure good balance, the tubes were loaded symmetrically. The rotary cover was screwed on, the centrifuge lid was

closed, and the tubes were centrifuged at 9000 *g* for 5 min. A tube carrier was made from a slide on which two pieces of glass 25 × 10 × 1.2 mm were fixed, 1.5 mm apart, to form a groove. The tube was placed in the groove, a cover-slip was placed on top and the interface was flooded with water. Alternatively, examination was done without flooding the interface with water, but in such case, the light condenser was placed in such a way that cells became refringent. The plasma/white blood cell interface (buffy coat) was then examined by slowly rotating the tube, and using the ×40 objective lens.

3.7 Assessment of the Anti-Trypanosomal activity of Sera samples *in vitro*

3.7.1 Detection of anti-trypanosomal activity of the test sera

Anti-trypanosomal activity was detected using microtitre plates as follows:

Trypanosomes from the buffy coat were suspended in RPMI 1640 (Rossvelt Park Memorial Institute 1640) medium supplemented with 2% glucose, 2mM sodium pyruvate (Appendix XXIV), 10% heat-inactivated (56º C, 30 min) fetal bovine serum (Black and Van-deweerd, 1989) and sodium bicarbonate (Tanner *et al*., 1980), and antibiotics (streptomycin 100µg/ml, penicillin 100U/ml). 50µl of each animal serum sample was introduced into one of the 96 wells of a microtitre plate and 50µl of *T. brucei brucei* or *T. congolense* suspension 8 per field (31.62×10^6/ ml of blood) was added to it, rocked gently to mix and incubated at room temperature. Control was without test serum. A drop of about 5µl of each mixture was examined microscopically on hourly basis using wet film method. Cessation in motility of parasites was taken as indication of serum activity against the parasites (Bulus *et al.*, 2008).

A formula was derived to determine the Anti-trypanosomal Activity Index (ATI) of each serum sample.

$$ATI = \frac{T_c - Ts}{T_c}$$

Where: T_c is the survival time of the parasites in the control medium

T_s is the survival time of the parasites in the sample

3.7.2 Assessment of the trypanosomal activity of some of the selected sera under some conditions

a. Under exogenous CO_2: this was done as described in 3.5.1, but using a CO_2 incubator with 9% CO_2, at 30ºC.

b. With addition of xanthine: some of the selected sera were tested for the association of their trypanosomal activity with xanthine oxidase, by adding 20µl of xanthine solution to each mixture prepared as decribed in session 3.5.1, making a final concentration of 250 µM xanthine (Black et al., 1999).

c. Heat inactivation: This was done using a water bath. The inactivation was achieved by incubating the sera for 30 min at 56ºC. The heat-inactivated form of sera from Horse and pig were tested as substitute to fetal bovine serum (FBS) supplement in RPMI 1640 culture medium, for *T. congolense*.

In each condition, the motility of *T. congolense* was examined as described in session 3.7.1.

3.7.3 Infectivity assessment of trypanosomes from sera exhibiting anti-trypanosomal activity

For each test serum where *in vitro* anti-trypanosomal activity was observed in 3.5.1 above, 80µl of the mixture was inoculated into mice and monitored by microscopy for trypanosomes on daily basis for 10 days in the case of *T. brucei brucei* and for 30 days in the case of *T. congolense*, to determine the nature of the observed anti-trypanosomal activity, whether inhibitory or cidal.

3.7.4 Detection of the xanthine oxidase content of the selected sera

Xanthine oxidase is a trypanocidal protein isolated from Cape buffalo serum (Muranjan *et al*., 1997). Hydrogen peroxide (H_20_2) generated during catabolism of hypoxanthine and xanthine by the serum enzyme inhibits trypanosome glycolysis, leading to a precipitous decline in cellular Adenosine triphosphate (ATP) (Black *et al*., 1999). The technique of Black *et al*. (1999) was adopted to detect the xanthine oxidase content of the test sera. Accordingly, aliquots (100 µl) of each serum was added to 900 µl of H_20_2-assay buffer (0.5 mM xanthine and 1 mM 2, 4, 6 tribromo-3-hydroxybenzoic acid in 0.1 mM 4-amino-antipyrine with a final concentration of 8 units horse-radish peroxidase per ml) (Appendix XXIV). The mixture was incubated at 25ºC for 30 min, immediately chilled in an ice bath, and absorbance read at 512 nm wave length was recorded after zeroing the spectrophotometer with an equivalent mixture lacking horseradish peroxidase. A serial dilution of the commercial cow's milk xanthine oxidase was done, absorbance was also recorded at 512 nm wave length and used to plot a standard curve. It is known that detection of H_20_2 produced in serum by this assay is not affected by other enzymes in

serum, including catalase (Le Tissier *et al*., 1994). The xanthine oxidase content of serum was determined by reading the serum value against the cow's milk xanthine oxidase standard curve.

3.8 Detection of Anti-Trypanosomal Antibodies in Serum Samples

This was done using a slightly modified Ouchterlony Double Diffusion test (Ouchterlony and Nilsson, 1978) as described in the user Manual antibody titration kit and in DNA analyst training Manual. 2g of agarose was mixed with 500ml phosphate buffer (pH 6.9) (Appendix XXIV). The mixture was heated to boiling in a conical flask of known weight (2-4 times the volume of the solution), until all the agarose was dissolved. Initial and final weights were noted; any difference in weight from the initial weight was made up by addition of distilled water (Henderson in Kirkhouse Trust, 2009). The agarose solution was poured into clean Petri dishes and allowed to solidify for 15-20 min (for complete polymerization). Part of the molten agarose solution was kept for sealing the wells. Molten agarose containing bubbles was gently swirled to remove them. Gel thickness has a profound effect on the resolution of smaller fragments; for optimal resolution horizontal gels were made 3-4mm thick. Inverted gels were stored in the refrigerator at 4°C (to retard dehydration). After solidification, holes were punched in the gel plates with a vacuum pipette; leaving 1.5 cm between the sample wells. After gel punching, the wells were sealed by adding 20 µl molten agarose into each well and they were allowed to solidify for 15-20 min, after ensuring that the distribution was uniform. The centre well was filled with 40 µl of the antigen (sonicated *T. brucei brucei* or *T. congolense*) suspension, and side wells with 30 µl of tested sera using a precision pipette. Wells appeared full, but care was

taken not to overfill the wells and cause spillage. The Petri dishes were covered and carefully placed in an incubator at 37°C for 48 hrs. Gel was run to allow the band of interest to migrate 40-60% down the length of the gel in case of any detectable antibody-antigen activity. Positive results are usually indicated by the bands of reaction indicating the presence of anti-trypanosome antibodies in the test sera (Hudson and Hay, 1989). The gel was stain with Coomassie blue and a destaining solution was used to remove the dye. These solutions were prepared as described in Appendix XXIV.

3.9 Anti-Trypanosomal Activity of the Effective Sera *In Vivo*

3.9.1 Acclimatization of the laboratory animals (Wistar rats)

8 weeks old Wistar rats, weighing 110-120g, male and female mixed, were kept 2 per cage for each treatment and allowed to acclimatize for two weeks. They were given commercial feed and water *ad libitum*.

3.9.2 Test on the prophylactic effect of test sera

Similar test was done for both *T. brucei brucei* and *T. congolense*.

200µl of each effective serum (serum with *in vitro* anti-trypanosomal activity) was administered intraperitoneally to each rat (two rats per treatment) an hour before the intraperitoneal inoculation of 100µl of *T. brucei brucei* or *T. congolense* suspension 8 per field (31.62×10^6/ml of blood). The control rats were inoculated with the same suspension of *T. brucei brucei* or *T. congolense* 8 per field (31.62×10^6/ml of blood) but without administration of any test serum. Rats were monitored daily till death for the presence of *T.*

brucei brucei or *T. congolense* by microscopic examination of tail blood on a slide/cover-slip preparation.

3.9.3 Test on the curative effect of test sera

Similar test was carried out for both *T. brucei brucei* and *T. congolense*. Rats were grouped and allowed to acclimatized as decribed in section 3.7.2. Rats were inoculated with the suspension of *T. brucei brucei* or *T. congolense* (8 per field; 31.62×10^6/ml of blood), and after the infection was established by microscopic examination of tail blood, 200µl of each effective serum was administered intraperitoneally to each rat. The negative control rats were inoculated with the same suspension of *T. brucei brucei* or *T. congolense* (8 per field; 31.62×10^6/ml of blood) but were not treated with test sera after infection had been established. The positive control rats were inoculated with the same suspension of *T. brucei brucei* or *T. congolense* (8 per field; 31.62×10^6/ml of blood), but given a standard anti-trypanosomal drug, Samorecide (Diminazene diaceturate), after the infection was established. The standard drug was administered at the dose of 0.5 ml/10Kg body weight, as recommended by the manufacturer. The rats were monitored daily till death for the presence of blood stream *T. brucei brucei* or *T. congolense* by microscopic examination of blood obtained from the tail on a slide/cover-slip preparation.

3.10 Data Management and Statistical Analysis

Data analysis was carried out using the software Statistical Package for Social Sciences (SPSS) version 20. One-way Analysis of variance (ANOVA) was used for sera inter-

species comparison of *T. brucei brucei* or *T. congolense* survival time and for the xanthine oxidase activity of the test sera. Student's t-test was used to compare the mean survival time of the two species of parasites, *T. brucei brucei* and *T. congolense* in the test sera, to compare the survival time of *T. congolense* in some of the test sera with and without exogenous $CO_{2,}$ with and without addition of xanthine, and to compare the prophylactic and the curative effects of the test sera. The significant difference was at the level of probability 0.5.

CHAPTER FOUR

RESULTS

4.1 Trypanosome Infection Status of Sera from Selected Birds and Mammals

All the blood samples from selected birds and mammals were negative for trypanosomes by thick blood film and haematocrit centrifugation technique (HCT).

4.2 Anti-trypanosomal Activity of Sera from the Selected Birds and Mammals on *Trypanosoma brucei brucei* and *Trypanosoma congolense in vitro*

4.2.1 Activity of sera of selected birds on *Trypanosoma brucei brucei*

Birds' sera, generally significantly reduced ($P<0.05$) the survival time of *T. brucei brucei*, when compared to the control (Table 4.1) The survival time of *T. brucei brucei* in the control medium was 30.00±0.00 hrs. Serum from broiler hen had a parasite survival time of 5.00±0.00 hrs, which was significantly lower ($P<0.05$) than that of all the sera from other birds, with the highest anti-trypanosomal activity index (ATI of 0.83) on *T. brucei brucei*. This was followed by sera from Guinea fowl, 9.33±1.15 hrs with ATI of 0.69; Parent stock brown hen, 10.00±2.00 hrs with ATI of 0.67; Shika brown hen, 11.33±1.15 hrs with ATI of 0.62; Spotted eagle owl, 11.33±1.15 hrs with 0.62 ATI and buzzard, 11.33±1.15 hrs and 0.62 ATI, all with ATI above 0.5 (50% of antitrypanosomal activity).

Table 4.1: *In vitro* activity of sera of selected birds on *Trypanosoma brucei brucei*

Source of serum	Mean survival time (hr)	Mean ATI

Gallus gallus domesticus (Broiler cock)	21.00±0.00^{de}	0.30
Gallus gallus domesticus (Broiler hen)	5.00±0.00^{a}	0.83
Gallus gallus domesticus (Shika brown cock)	19.67±4.04^{cde}	0.34
Gallus gallus domesticus (Shika brown hen)	11.33±1.15^{b}	0.62
Gallus gallus domesticus (Parent Stock White cock)	21.33±1.15^{e}	0.29
Gallus gallus domesticus (Parent Stock White hen)	16.67±2.89^{cd}	0.44
Gallus gallus domesticus (Parent Stock Brown Cock)	16.00±1.73^{c}	0.47
Gallus gallus domesticus (Parent Stock Brown Hen)	10.00±2.00^{b}	0.67
Numida meleagris (Guinea fowl)	9.33±1.15^{b}	0.69
Columba livia domestica (Domestic pigeon)	20.67±1.15^{de}	0.31
Plectropterus gambensis (Goose)	16.00±3.46^{c}	0.47
Balearica pavonina (Crowned crane)	18.67±4.16^{cde}	0.37
Pavo cristatus (Peacock)	18.33±3.78^{cde}	0.39
Bubo africanus (Spotted eagle owl)	11.33±1.15^{b}	0.62
Necrosyrtes monachus (Hooded vulture)	20.67±1.15^{de}	0.31
Buteo buteo (Buzzard)	11.33±1.15^{b}	0.62
RPMI 1640*	30.00±0.00^{f}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

4.2.2 Activity of sera of selected mammals on *Trypanosoma brucei brucei*

4.2.2.1 In vitro activity of sera from selected rodents on Trypanosoma brucei brucei

The results, illustrated in Table 4.2, showed that there was no significant difference ($P>0.05$) between the survival time of *T. brucei brucei* in cane rat/grass-cutter serum and the control. This serum had a high parasite survival time of 27.67±4.04 hrs with an ATI almost equal to zero (0.08). However, there was a significant difference ($P<0.05$) between the survival time of *T. brucei brucei* in rabbit serum and the control, where rabbit serum had a slight ATI of 0.23.

4.2.2.2 In vitro activity of sera from selected small ruminants on Trypanosoma brucei brucei

Sera from the selected small ruminants, except the West African Dwarf goat, did not exhibit any anti-trypanosomal activity but significantly increased ($P<0.05$) the survival time of the parasites. The survival times of *T. brucei brucei* in the sera of the selected small ruminants were significantly higher ($P<0.05$) than the control (30.00±0.00 hrs), except the West African Dwarf goat with a survival time of 21.33±1.15 hrs which was significantly lower ($P<0.05$) than that of the parasites in the control medium, indicating a slight ATI of 0.29 (Table 4.3). Sera from all selected breeds of sheep had similar supportive activity. Serum from Balami sheep had the highest supportive activity as the ATI was the highest on the negative side, - 0.5, with a mean survival time of 45.00±0.00 hrs.

Table 4.2: *In vitro* activity of sera of selected rodents on *Trypanosoma brucei brucei*

Source of serum	Mean survival time (hr)	Mean ATI
Thryonomys swinderianus (Cane rat/grass-cutter)	27.67 ± 4.04^{ab}	0.08
Oryctolagus cuniculus (Rabbit)	23.00 ± 1.00^{a}	0.23
RPMI 1640*	30.00 ± 0.00^{b}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.3: *In vitro* activity of sera of some small ruminants on *Trypanosoma brucei brucei*

Source of serum	Mean survival time (hr)	Mean ATI
Capra aegagrus hircus (West African dwarf goat)	21.33 ± 1.15^{a}	0.29
Capra aegagrus hircus (Red Sokoto goat)	41.33 ± 1.15^{cd}	-0.38
Capra aegagrus hircus (Sahel goat)	37.33 ± 6.43^{c}	-0.24
Ovis aries (Yankassa sheep)	43.00 ± 1.73^{d}	-0.43
Ovis aries (Uda sheep)	44.00 ± 1.73^{d}	-0.47
Ovis aries (Balami sheep)	45.00 ± 0.00^{d}	-0.50
RPMI 1640*	30.00 ± 0.00^{b}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

4.2.2.3 In vitro activity of sera from selected large ruminants on Trypanosoma brucei brucei

The results in Table 4.4 show that there were significant differences (P<0.05) in the interaction of sera from the selected large ruminants with *T. brucei brucei in vitro*, and the control, except of N'Dama serum which recorded a survival time of 18.67±1.15 hrs, with an anti-trypanosomal activity index of 0.38. *Trypanosoma brucei brucei* survival times in sera from White Fulani, Sokoto Gudali and Friesian cross/Bunaji were significantly higher (P<0.05) than the control, with negative ATI values, indicating a supportive activity on *T. brucei brucei*. The highest survival time, 59.67±6.81 hrs, was recorded from the serum of Friesian cross/Bunaji.

4.2.2.4 In vitro activity of sera from selected non-ruminant mammals on Trypanosoma brucei brucei

The survival time of *T. brucei brucei* in sera from non-ruminant mammals was significantly lower (P<0.05) than that of the control (30.00±0.00 hrs) (Table 4.5). The highest ATI (0.47) was recorded in serum of camel with *T. brucei brucei* survival time of 16.00±3.46 hrs. However, the ATI and *T. brucei brucei* survival times of sera from camel, 0.47 and 16.00±3.46 hrs; donkey, 0.40 and 18.00±3.46 hrs and jackal 0.38 and 18.50±0.71 hrs were not statistically different (P>0.05).

Table 4.4: *In vitro* activity of sera of some large ruminants on *Trypanosoma brucei brucei*

Source of serum	Mean survival time (hr)	Mean ATI
Bos taurus (N'Dama cattle)	18.67 ± 1.15^{a}	0.38
Bos taurus (Sokoto Gudali cattle)	51.33 ± 11.01^{b}	-0.71
Bos taurus (Friesian cross/Bunaji cattle)	59.67 ± 6.81^{b}	-0.99
Bos taurus (White Fulani cattle)	47.33 ± 4.62^{b}	-0.58
RPMI 1640*	30.00 ± 0.00^{a}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.5: *In vitro* activity of sera of some non-ruminant mammals on *Trypanosoma brucei brucei*

Source of serum	Mean survival time (hr)	Mean ATI
Equus africanus asinus (Donkey)	18.00 ± 3.46^{ab}	0.40
Equus ferus caballus (Horse)	20.67 ± 1.15^{bc}	0.31
Camelus dromedarius (Camel)	16.00 ± 3.46^{a}	0.47
Sus scrofa domesticus (Pettin Pig)	21.67 ± 1.53^{bc}	0.28
Sus scrofa domesticus (Large white Pig)	22.67 ± 0.58^{c}	0.24
Sus scrofa domesticus (Ducros Pig)	21.33 ± 1.15^{bc}	0.29
Canis aureus (Jackal)	18.50 ± 0.71^{abc}	0.38
RPMI 1640*	30.00 ± 0.00^{d}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

4.2.2.5 In vitro activity of sera from selected zoo primates on Trypanosoma brucei brucei

The effect of sera from red Patas monkey and Tantalus monkey on *T. brucei brucei* is presented in Table 4.6. There was no significant difference (P>0.05) in the parasites survival time of the sera of both primates (22.00±1.73 hrs for red patas monkey and 19.67±0.58 hrs for tantalus monkey); whereas, the survival times of the parasites in these sera were significantly lower (P<0.05) than that of the control (30.00±0.00 hrs), showing a slight anti-*Trypanosoma brucei brucei* activity with ATI values of 0.27 for red patas monkey and 0.34 for tantalus monkey.

4.2.3 Activity of sera of selected birds on *Trypanosoma congolense*

The survival time of *T. congolense* in sera of most of the selected birds was significantly lower (P<0.05) than that of the parasites in the control medium with each of these sera having a positive ATI, (Table 4.7). Peacock serum had the highest anti-*Trypanosoma congolense* activity index, 0.88, with a survival time of just 8.67±3.21 hrs which was significantly lower (P<0.05) than that of all the birds including the control (74.67±0.58 hrs). This was followed by sera from broiler hen, broiler cock and goose with ATI ≥ 0.50 and survival time not significantly different (P>0.05) from each other (ATI of 0.55, 0.54 and 0.50 and survival times of 33.67±1.53 hrs; 34.33±2.08 hrs and 37.33±2.31 hrs respectively). However, a reverse effect was observed with some of the selected avian sera such as sera from hooded vulture, spotted eagle owl and buzzard, whose parasites survival time of 86.00±3.46, 102.33±2.52 and 133.50±2.12 hrs respectively, were significantly higher (P<0.05) than the control, and ATI values negative, showing some supportive activities for *T. congolense in vitro*.

Table 4.6: *In vitro* activity of sera from selected zoo primates on *Trypanosoma brucei brucei*

Source of serum	Mean survival time (hr)	Mean ATI
Erythrocebus patas (Red Patas monkey)	22.00±1.73[a]	0.27
Chlorocebus tantalus (Tantalus monkey)	19.67±0.58[a]	0.34
RPMI 1640*	30.00±0.00[b]	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.7: *In vitro* activity of sera from selected birds on *Trypanosoma congolense*

Source of serum	Mean survival time (hr)	Mean ATI
Gallus gallus domesticus (Broiler cock)	34.33±2.08[b]	0.54
Gallus gallus domesticus (Broiler hen)	33.67±1.53[b]	0.55
Gallus gallus domesticus (Shika brown cock)	65.00±5.00[d]	0.13
Gallus gallus domesticus (Shika brown hen)	66.00±5.29[d]	0.12
Gallus gallus domesticus (Parent Stock White cock)	65.00±5.00[d]	0.13
Gallus gallus domesticus (Parent Stock White hen)	62.67±4.62[d]	0.16
Gallus gallus domesticus (Parent Stock Brown Cock)	64.33±4.04[d]	0.14
Gallus gallus domesticus (Parent Stock Brown Hen)	49.33±6.11[c]	0.34
Numida meleagris (Guinea fowl)	75.67±1.15[e]	-0.01
Columba livia domestica (Domestic pigeon)	49.67±0.58[c]	0.33
Plectropterus gambensis (Goose)	37.33±2.31[b]	0.50
Balearica pavonina (Crowned crane)	48.33±2.89[c]	0.35
Pavo cristatus (Peacock)	8.67±3.21[a]	0.88
Bubo africanus (Spotted eagle owl)	102.33±2.52[g]	-0.37
Necrosyrtes monachus (Hooded vulture)	86.00±3.46[f]	-0.15
Buteo buteo (Buzzard)	133.50±2.12[h]	-0.79
RPMI 1640*	74.67±0.58[e]	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

There was no significant difference (P>0.05) between the parasites survival time of guinea fowl serum (75.67±1.15 hrs) and the control survival time (74.67±0.58 hrs).

4.2.4 Activity of the selected mammal sera on *Trypanosoma congolense*

4.2.4.1 In vitro activity of sera from selected rodents on Trypanosoma congolense

Sera from the selected rodents showed different activities on *T. congolense in vitro* (Table 4.8). The serum of cane rat/grass-cutter had parasites survival time of 56.00±3.46 hrs, which is significantly lower (P<0.05) than the survival time of control (74.67±0.58 hrs), with ATI of 0.25, indicating a slight anti-T*rypanosoma congolense* activity. Rabbit serum survival time was 131.33±1.15 hrs, and was significantly higher (P<0.05) than the control survival time (74.67±0.58 hours), with a negative ATI (-0.76), indicating a lack of anti-trypanosomal activity and a very supportive activity.

4.2.4.2 In vitro activity of sera from selected small ruminants on Trypanosoma congolense

The results presented in Table 4.9 show the summary of the activity of sera from some small ruminants on *T. congolense in vitro.* All the sera did not exhibit any anti-trypanosomal activity against *T. congolense in vitro.* Their parasites survival times were all significantly higher (P<0.05) than the control (74.67±0.58 hrs), with ATI less than zero. The highest survival time was recorded with Yankassa sheep serum (171.33±2.31 hrs) with the highest negative ATI (-1.29), which showed a supportive activity significantly greater (P<0.05) than that of sera from other small ruminants. The West African Dwarf goat serum had the least survival time (100.00±8.72 hrs) among the selected small ruminants' sera, although significantly (P<0.05) higher than the control (74.67±0.58 hrs).

Table 4.8: *In vitro* activity of sera from selected rodents on *Trypanosoma congolense*

Source of serum	Mean survival time (hr)	Mean ATI
Thryonomys swinderianus (Cane rat/grass-cutter)	56.00 ± 3.46^{a}	0.25
Oryctolagus cuniculus (Rabbit)	131.33 ± 1.15^{c}	-0.76
RPMI 1640*	74.67 ± 0.58^{b}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.9: *In vitro* activity of sera from selected small ruminants on *Trypanosoma congolense*

Source of Serum	Mean survival time (hr)	Mean ATI
Capra aegagrus hircus (West African dwarf goat)	100.00±8.72[b]	-0.34
Capra aegagrus hircus (Red Sokoto goat)	151.33±4.16[c]	-1.03
Capra aegagrus hircus (Sahel goat)	150.00±2.00[c]	-1.01
Ovis aries (Yankassa sheep)	171.33±2.31[d]	-1.29
Ovis aries (Uda sheep)	152.67±3.05[c]	-1.04
Ovis aries (Balami sheep)	148.67±1.15[c]	-0.99
RPMI 1640*	74.67±0.58[a]	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

4.2.4.3 In vitro activity of sera from selected large ruminants on Trypanosoma congolense

The mean survival time of *T. congolense* in the sera of all the selected large ruminants was significantly higher ($P<0.05$) than that of the parasites in the control medium (74.67±0.58 hrs). All their ATI were negative, showing the lack of anti-*Trypanosoma congolense* activity. Sokoto Gudali serum had the highest supportive activity, significantly higher ($P<0.05$) than that of other large ruminants (ATI of -1.38), with a mean survival time of 178.00±2.00 hrs. N'Dama cattle serum had the least supportive activity (ATI of -0.42 and survival time of 106.00±3.46 hrs) (Table 4.10).

4.2.4.4 In vitro Activity of sera from selected non-ruminant mammals on Trypanosoma congolense

Table 4.11 presents the activity of sera from selected non-ruminant mammals on *T. congolense in vitro*. It was observed that serum from jackal had a very low parasites survival time of 6.50±2.12 hrs, which was significantly lower ($P<0.05$) than that of sera from all the other selected non-ruminant mammals, including the control, indicating a very high anti-*Trypanosoma congolense* activity *in vitro* with the best ATI of 0.91 (very close to 1.00). Camel serum had a parasite survival time of 58.00±3.46 hrs, which was significantly lower ($P<0.05$) than that of the control, with an ATI of 0.22, showing a slight anti-*Trypanosoma congolense* activity *in vitro*.

Table 4.10: *In vitro* activity of sera from selected large ruminants on *Trypanosoma congolense*

Source of serum	Mean survival time (hr)	Mean ATI
Bos taurus (N'Dama cattle)	106.00 ± 3.46^{b}	-0.42
Bos taurus (Sokoto Gudali cattle)	178.00 ± 2.00^{d}	-1.38
Bos taurus (Friesian cross/Bunaji cattle)	171.67 ± 1.15^{c}	-1.30
Bos taurus (White Fulani cattle)	171.33 ± 2.31^{c}	-1.29
RPMI 1640*	74.67 ± 0.58^{a}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.11: *In vitro* activity of sera from selected non-ruminant mammals on *Trypanosoma congolense*

Source of serum	Mean survival time (hrs)	Mean ATI
Equus africanus asinus (Donkey)	71.33 ± 7.23^{c}	0.04
Equus ferus caballus (Horse)	198.00 ± 0.00^{e}	-1.65
Camelus dromedarius (Camel)	58.00 ± 3.46^{b}	0.22
Sus scrofa domesticus (Pettin Pig)	181.33 ± 1.53^{d}	-1.43
Sus scrofa domesticus (Large white Pig)	183.67 ± 2.08^{d}	-1.46
Sus scrofa domesticus (Ducros Pig)	183.33 ± 1.53^{d}	-1.46
Canis aureus (Jackal)	6.50 ± 2.12^{a}	0.91
RPMI 1640*	74.67 ± 0.58^{c}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

A reverse effect was observed with sera from horse and the three breeds of pig (Pettin pig, Large white pig and Ducros pig). Serum from horse exhibited the highest supportive

activity (ATI of -1.65) on *T. congolense in vitro* with a mean survival time of 198.00±0.00 hours, significantly greater ($P<0.05$) than that of sera from other selected non-ruminant mammals. This was followed by the three breeds of pig. Donkey serum had a similar activity with the control as there was no significant difference ($P>0.05$) in their parasites survival times (71.33±7.23 hrs for donkey serum and 74.67±0.58 hrs for the control).

4.2.4.5 In vitro activity of sera from selected zoo primates on Trypanosoma congolense

Sera from the selected zoo primates exhibited very high anti-*Trypanosoma congolense* activity *in vitro* with very high index, close to one (ATI of 0.91 and 0.90 for red patas monkey and tantalus monkey respectively). This was shown by a very low survival time of *T. congolense*, 7.00±1.73 hrs in red patas monkey serum and 7.67±0.58 hrs in tantalus monkey serum (Table 4.12). These two values were not significantly different ($P>0.05$), but were significantly lower ($P<0.05$) than the control (74.00±1.00 hrs).

Table 4.12: *In vitro* activity of sera of selected zoo primates on *Trypanosoma congolense*

Source of serum	Mean survival time (hr)	Mean ATI
Erythrocebus patas (Red Patas monkey)	7.00±1.73[a]	0.91
Chlorocebus tantalus (Tantalus monkey)	7.67±0.58[a]	0.90
RPMI 1640*	74.67±1.00[b]	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different (P<0.05).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

4.2.5 Comparative *in vitro* effects of Sera on *Trypanosoma brucei brucei* and *Trypanosoma congolense*

4.2.5.1 Sera of the selected birds

The results confined in Table 4.13 illustrate the comparative effects of sera of the selected birds on *T. brucei brucei* and *T. congolense*. It shows that sera from the selected birds had more effect against *T. brucei brucei* than *T. congolense*. This is indicated by the fact that the survival times of *T. brucei brucei* in these sera were significantly lower ($P<0.05$) than that of *T. congolense*, with the exception of peacock serum which instead had a very high anti-*Trypanosoma congolense* activity. The survival time of *T. congolense* in peacock serum (8.67±3.21 hrs), was significantly lower ($P<0.05$) than that of *T. brucei brucei* (18.33±3.78 hrs).

4.2.5.2 Sera from the selected rodents

The survival times of *T. congolense* in sera of the selected rodents were significantly higher ($P<0.05$) than that of *T. brucei brucei* in the same sera (Table 4.14), indicating that the sera had more effect against *T. brucei brucei.*

4.2.5.3 Sera from the selected small ruminants

The sera from the selected small ruminants generally did not have anti-trypanosomal activity, rather, they caused increase in the survival times of *T. brucei brucei* and *T. congolense in vitro* except the West African dwarf goat serum which had an anti-*Trypanosoma brucei brucei* activity index of 0.29. The survival time of *T. congolense* was significantly higher ($P<0.05$) than that of *T. brucei brucei* in the sera of all the selected small ruminants (Table 4.15).

Table 4.13: Comparative *in vitro* effect of sera from selected birds on *Trypanosoma brucei brucei* and *Trypanosoma congolense*

Source of serum	Mean survival time of *T. brucei brucei*	Mean ATI on *T. brucei*	Mean survival time of *T. congolense*	Mean ATI on *T. congolense*

		brucei		
Gallus gallus domesticus (Broiler cock)	21.00±0.00[a]	0.30	34.33±2.08[b]	0.54
Gallus gallus domesticus (Broiler hen)	5.00±0.00[a]	0.83	33.67±1.53[b]	0.55
Gallus gallus domesticus (Shika brown cock)	19.67±4.04[a]	0.34	65.00±5.00[b]	0.13
Gallus gallus domesticus (Shika brown hen)	11.33±1.15[a]	0.62	66.00±5.29[b]	0.12
Gallus gallus domesticus (Parent Stock White cock)	21.33±1.15[a]	0.29	65.00±5.00[b]	0.13
Gallus gallus domesticus (Parent Stock White hen)	16.67±2.89[a]	0.44	62.67±4.62[b]	0.16
Gallus gallus domesticus (Parent Stock Brown cock)	16.00±1.73[a]	0.47	64.33±4.04[b]	0.14
Gallus gallus domesticus (Parent Stock Brown hen)	10.00±2.00[a]	0.67	49.33±6.11[b]	0.34
Numida meleagris (Guinea fowl)	9.33±1.15[a]	0.69	75.67±1.15[b]	-0.01
Columba livia domestica (Domestic pigeon)	20.67±1.15[a]	0.31	49.67±0.58[b]	0.33
Plectropterus gambensis (Goose)	16.00±3.46[a]	0.47	37.33±2.31[b]	0.50
Balearica pavonina (Crowned crane)	18.67±4.16[a]	0.38	48.33±2.89[b]	0.35
Pavo cristatus (Peacock)	18.33±3.78[b]	0.39	8.67±3.21[a]	0.88
Bubo africanus (Spotted eagle owl)	11.33±1.15[a]	0.62	102.33±2.52[b]	-0.37
Necrosyrtes monachus (Hooded vulture)	20.67±1.15[a]	0.31	86.00±3.46[b]	-0.15
Buteo buteo (Buzzard)	11.33±1.15[a]	0.62	133.50±2.12[b]	-0.78
RPMI 1640*	30.00±0.00[a]	0	74.67±0.58[b]	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$). ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.14: Comparative *in vitro* effect of sera from selected rodents on *Trypanosoma brucei brucei* and *Trypanosoma congolense*

Source of serum	Mean survival time of *T. brucei brucei*	Mean ATI on *T. brucei brucei*	Mean survival time of *T. congolense*	Mean ATI on *T. congolense*
Thryonomys swinderianus (Cane rat/grass-cutter)	27.67 ± 4.04^{a}	0.08	56.00 ± 3.46^{b}	0.25
Oryctolagus cuniculus (Rabbit)	23.00 ± 1.00^{a}	0.23	131.33 ± 1.15^{b}	-0.76
RPMI 1640*	30.00 ± 0.00^{a}	0	74.67 ± 0.58^{b}	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$). ATI: Anti-trypanosomal activity Index.

*Culture medium without serum (control).

Table 4.15: Comparative *in vitro* effect of sera of selected small ruminants on *Trypanosoma brucei brucei* and *Trypanosoma congolense*

Source of serum	Mean survival time of *T. brucei brucei*	Mean ATI on *T. brucei brucei*	Mean survival time of *T. congolense*	Mean ATI on *T. congolense*
Capra aegagrus hircus (West African dwarf goat)	21.33±1.15[a]	0.29	100.00±8.72[b]	-0.34
Capra aegagrus hircus (Red Sokoto goat)	41.33±1.15[a]	-0.38	151.33±4.16[b]	-1.03
Capra aegagrus hircus (Sahel goat)	37.33±6.43[a]	-0.24	150.00±2.00[b]	-1.01
Ovis aries (Yankassa sheep)	43.00±1.73[a]	-0.43	171.33±2.31[b]	-1.29
Ovis aries (Uda sheep)	44.00±1.73[a]	-0.47	152.67±3.05[b]	-1.04
Ovis aries (Balami sheep)	45.00±0.00[a]	-0.5	148.67±1.15[b]	-0.99
RPMI 1640*	30.00±0.00[a]	0	74.67±0.58[b]	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

4.2.5.4 Sera of selected large ruminants

Table 4.16 shows the comparative effects of sera from the selected large ruminants on *T. brucei brucei* and *T. congolense in vitro*. Only N'Dama serum had anti-trypanosomal activity against *T. brucei brucei*, but was not effective on *T. congolense in vitro*. Other sera were not effective for both *T. brucei brucei* and *T. congolense*. The survival times of *T. brucei brucei* in the sera of all the selected large ruminants were significantly lower ($P<0.05$) than that of *T. congolense*. The highest survival time of *T. brucei brucei* was 59.67±6.81 hrs in Friesian cross, meanwhile *T. congolense* survived for up to 178.00±2.00 hrs in Sokoto Gudali serum.

4.2.5.5 Sera of selected non-ruminant mammals

The results summarized in Table 4.17 show that sera from camel and jackal had both anti-*Trypanosoma brucei brucei* and anti-*Trypanosoma congolense* activities, while other sera only had anti-trypanosomal activity against *T. brucei brucei*. The survival time of *T. brucei brucei* in the sera of selected non-ruminant mammals was significantly lower ($P<0.05$) than that of *T. congolense*, with the exception of jackal serum, with survival times of 6.50±2.12 hrs for *T. congolense* and 18.50±0.71 hrs for *T. brucei brucei*. The highest mean survival time for *T. brucei brucei* was 22.67±0.58 hrs in Large white pig serum while that of *T. congolense* was 198.00±0.00 hrs in horse serum.

Table 4.16: Comparative *in vitro* effect of sera of selected large ruminants on *Trypanosoma brucei brucei* and *Trypanosoma congolense*

Source of serum	Mean survival time of *T. brucei*	Mean ATI on *T.brucei brucei*	Mean survival time of *T. congolense*	Mean ATI on *T. congolense*
Bos taurus (N'Dama cattle)	18.67±1.15[a]	0.38	106.00±3.46[b]	-0.42
Bos taurus (Sokoto Gudali cattle)	51.33±11.01[a]	-0.71	178.00±2.00[b]	-1.38
Bos taurus (Friesian cross/Bunaji cattle)	59.67±6.81[a]	-0.99	171.67±1.15[b]	-1.30
Bos taurus (White Fulani cattle)	47.33±4.62[a]	-0.58	171.33±2.31[b]	-1.29
RPMI 1640*	30.00±0.00[a]	0	74.67±0.58[b]	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different (P<0.05).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.17: Comparative *in vitro* effect of sera of selected non-ruminant mammals on *Trypanosoma brucei brucei* and *Trypanosoma congolense*

Source of serum	Mean survival time of *T. brucei brucei*	Mean ATI on *T. brucei brucei*	Mean survival time of *T. congolense*	Mean ATI on *T. congolense*
Equus africanus asinus (Donkey)	18.00±3.46[a]	0.40	71.33±7.23[b]	0.04
Equus ferus caballus (Horse)	20.67±1.15[a]	0.31	198.00±0.00[b]	-1.66
Camelus dromedarius (Camel)	16.00±3.46[a]	0.47	58.00±3.46[b]	0.22
Sus scrofa domesticus (Pettin Pig)	21.67±1.53[a]	0.28	181.33±1.53[b]	-1.43
Sus scrofa domesticus (Large white Pig)	22.67±0.58[a]	0.24	183.67±2.08[b]	-1.46
Sus scrofa domesticus (Ducros Pig)	21.33±1.15[a]	0.29	183.33±1.53[b]	-1.46
Canis aureus (Jackal)	18.50±0.71[b]	0.38	6.50±2.12[a]	0.91
RPMI 1640*	30.00±0.00[a]	0	74.67±0.58[b]	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

4.2.5.6 Sera of selected zoo primates

Table 4.18 presents the compared effect of sera from the selected zoo primates on *T. brucei brucei* and *T. congolense in vitro*. It is clear that these sera had a pronounced anti-*Trypanosoma congolense* activity, with very high ATI, 0.91 and 0.90 for red Patas monkey and tantalus monkey respectively. The survival times of *T. congolense* in these sera were significantly lower ($P<0.05$) than that of *T. brucei brucei* in the same sera.

4.2.6. *In vitro* effect of some non-effective sera on *Trypanosoma congolense* under different conditions

4.2.6.1 Some non-effective sera with and without exogenous CO_2

Table 4.19 shows that without exogenous CO_2, all the non-effective sera (sera without anti-trypanosomal activity *in vitro*) had survival times significantly higher ($P<0.05$) than that with exogenous CO_2, except the control. Horse serum had the highest *in vitro* supportive activity on *T. congolense*, with and without exogenous CO_2. Sera from the three breeds of pig were the next.

4.2.6.2 Some non-effective sera supplemented with xanthine with and without exogenous CO_2

Table 4.20 below shows the comparative effect of absence and presence of exogenous CO_2 on the survival time of the parasites in some non-effective sera supplemented with xanthine. It was observed that the selected non-effective sera supplemented with xanthine, under exogenous CO_2, had survival times significantly higher ($P<0.05$) than that without exogenous CO_2, except Sokoto Gudali serum and the control.

Table 4.18: Comparative *in vitro* effect of sera of selected zoo primates on *Trypanosoma brucei brucei* and *Trypanosoma congolense*

Source of serum	Mean survival time of *T. brucei brucei*	Mean ATI on *T. brucei brucei*	Mean survival time of *T. congolense*	Mean ATI on *T. congolense*
Erythrocebus patas (Red Patas monkey)	22.00 ± 1.73^{b}	0.27	7.00 ± 1.73^{a}	0.91
Chlorocebus tantalus (Tantalus monkey)	19.67 ± 0.58^{b}	0.34	7.67 ± 0.58^{a}	0.90
RPMI 1640*	30.00 ± 0.00^{a}	0	74.00 ± 1.00^{b}	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.19: Comparative *in vitro* effect of some non-effective sera on *Trypanosoma congolense* with and without exogenous CO_2

Source of serum	Mean survival time without exogenous CO_2	Mean ATI	Mean survival time under exogenous CO_2	Mean ATI
Equus ferus caballus (Horse)	198.00 ± 0.00^{b}	-1.64	131.00 ± 1.41^{a}	-0.02
Bos taurus (Sokoto Gudali cattle)	179.00 ± 1.41^{b}	-1.39	101.00 ± 1.41^{a}	0.21
Bos taurus (Friesian cross/Bunaji cattle)	172.00 ± 1.41^{b}	-1.29	103.00 ± 1.41^{a}	0.20
Bos taurus (White Fulani cattle)	172.00 ± 2.83^{b}	-1.29	129.00 ± 1.41^{a}	-0.01
Sus scrofa domesticus (Large white Pig)	184.00 ± 2.83^{b}	-1.45	130.00 ± 0.00^{a}	-0.02
Sus scrofa domesticus (Pettin Pig)	181.50 ± 2.12^{b}	-1.42	127.00 ± 1.41^{a}	0.01
Sus scrofa domesticus (Ducros Pig)	183.50 ± 2.12^{b}	-1.45	126.50 ± 2.12^{a}	0.01
Ovis aries (Yankassa sheep)	172.00 ± 2.83^{b}	-1.30	104.00 ± 2.83^{a}	0.19
RPMI 1640*	75.00 ± 0.00^{a}	0	128.00 ± 2.83^{b}	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

Table 4.20: Comparative *in vitro* effect of some non-effective sera supplemented with xanthine on *Trypanosoma congolense* with and without exogenous CO_2

Source of serum	Mean survival time without exogenous CO_2	Mean ATI without exogenous CO_2	Mean survival time with exogenous CO_2	Mean ATI with exogenous CO_2
Equus ferus caballus (Horse)	93.00±4.24[a]	-0.94	144.00±0.00[b]	-2
Bos taurus (Sokoto Gudali cattle)	92.00±5.66[a]	-0.92	96.00±0.00[a]	-1.00
Bos taurus (Friesian cross/Bunaji cattle)	72.00±0.00[a]	-0.50	141.00±4.24[b]	-1.94
Bos taurus (White Fulani cattle)	72.00±0.00[a]	-0.50	117.00±4.24[b]	-1.44
Sus scrofa domesticus (Large white Pig)	117.50±3.54[a]	-1.45	144.00±0.00[b]	-2.00
Sus scrofa domesticus (Pettin Pig)	96.00±0.00[a]	-1.00	142.00±2.83[b]	-1.96
Sus scrofa domesticus (Ducros Pig)	93.50±3.54[a]	-0.95	165.00±4.24[b]	-2.44
Ovis aries (Yankassa sheep)	69.00±4.24[a]	-0.44	144.00±0.00[b]	-2.00
RPMI 1640*	48.00±0.00[a]	0	48.00±0.00[a]	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Culture medium without serum (control).

4.2.6.2 Some non-effective sera with and without xanthine under exogenous CO_2

Results in Table 4.21 show that under CO_2 atmosphere, a part from Sokoto Gudali serum and the control, all the sera with addition of xanthine were more supportive of the survival of the parasites, with survival times significantly higher ($P<0.05$) than that of the same sera without addition of xanthine.

4.2.6.3 RPMI 1640 medium supplemented with heat inactivated form of selected non-effective sera with and without exogenous CO_2

Results of the comparative effects of sera from horse and Large white pig as substitute to FBS in RPMI 1640 medium, with and without exogenous CO_2 are presented in Table 4.22. The survival time of the parasites in the control medium was the same ($P>0.05$) with and without exogenous CO_2. Media supplemented with sera from horse and Large white pig were significantly supportive($P<0.05$) of the survival of the parasites under exogenous CO_2 than without exogenous CO_2.

Plate I shows *Trypanosoma brucei brucei* in a blood smear stained with Giemsa.

4.3 Infectivity of *Trypanosoma brucei brucei* and *Trypanosoma congolense* from sera exhibiting anti-trypanosomal activity

None of the exposed parasites was infective, except the control (parasites in RPMI 1640 medium without test serum) as none of the mice was found with parasites till the end of the experiment, except the control.

Table 4.21: Comparative *in vitro* effect of some non-effective sera with and without addition of xanthine on *Trypanosoma congolense* under exogenous CO_2

Source of serum	Mean survival time without xanthine under exogenous CO_2	Mean ATI without xanthine	Mean survival time with xanthine under exogenous CO_2	Mean ATI with xanthine
Equus ferus caballus (Horse)	131.00 ± 1.41^{a}	-0.02	144.00 ± 0.00^{b}	-2.00
Bos taurus (Sokoto Gudali cattle)	101.00 ± 1.41^{b}	0.21	96.00 ± 0.00^{a}	-1.00
Bos taurus (Friesian cross/Bunaji cattle)	103.00 ± 1.41^{a}	0.20	141.00 ± 4.24^{b}	-1.94
Bos taurus (White Fulani)	129.00 ± 1.41^{a}	-0.01	117.00 ± 4.24^{a}	-1.44
Sus scrofa domesticus (Large white Pig)	130.00 ± 0.00^{a}	-0.02	144.00 ± 0.00^{b}	-2.00
Sus scrofa domesticus (Pettin Pig)	127.00 ± 1.41^{a}	0.01	142.00 ± 2.83^{b}	-1.96
Sus scrofa domesticus (Ducros Pig)	126.50 ± 2.12^{a}	0.01	165.00 ± 4.24^{b}	-2.44
Ovis aries (Yankassa sheep)	104.00 ± 2.83^{a}	0.19	144.00 ± 0.00^{b}	-2.00
RPMI 1640*	128.00 ± 2.83^{b}	0	48.00 ± 0.00^{a}	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$). ATI: Anti-trypanosomal activity Index.

*Culture medium without serum (control).

Table 4.22: Comparative effect of selected compliment inactivated sera in RPMI 1640 medium on *Trypanosoma congolense* with and without exogenous CO_2

Source of serum	Mean survival time without exogenous CO_2	Mean ATI	Mean survival time with exogenous CO_2	Mean ATI
Equus ferus caballus (Horse)	121.00 ± 1.41^{a}	0.17	169.00 ± 1.41^{b}	-0.16
Sus scrofa domesticus (Large white Pig)	120.00 ± 0.00^{a}	0.18	144.50 ± 0.71^{b}	0.01
FBS*	145.50 ± 2.12^{a}	0	145.50 ± 2.12^{a}	0

Values are means ± standard deviation. Values with different superscripts across the rows are significantly different ($P<0.05$).
ATI: Anti-trypanosomal activity Index.
*Fetal bovine serum (control).

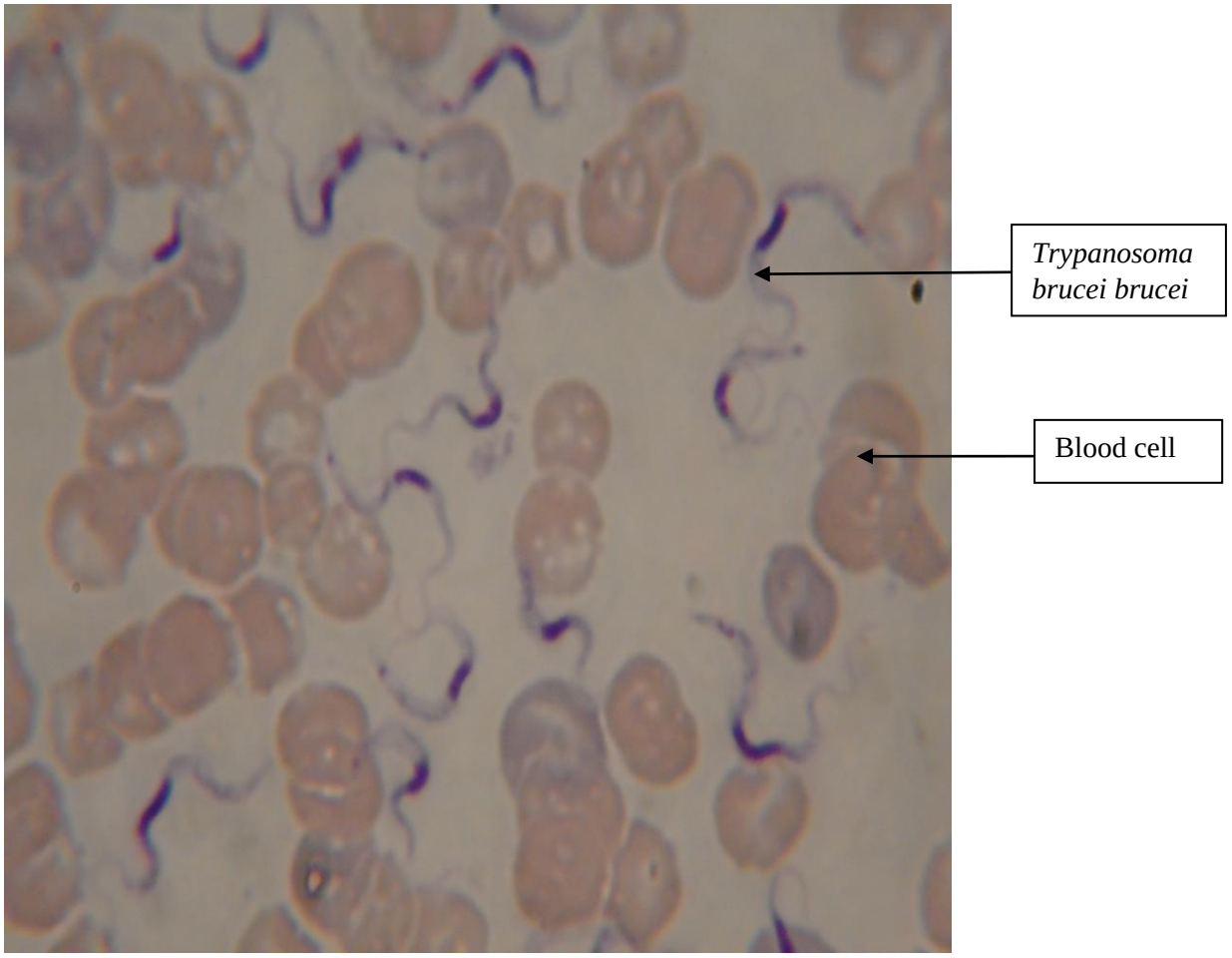


Plate I: *Trypanosoma brucei brucei* in thin blood smear stained with Giemsa (Magnification: times 1000).

4.4 Xanthine Oxidase Content of Sera from Selected Birds and Mammals

4.4.1 Xanthine oxidase content of sera of selected birds

Results on the xanthine oxidase content of sera from the selected birds are summarized in Table 4.23. It was observed that buzzard serum lacked xanthine oxidase. Parent stock brown hen had the lowest concentration of serum xanthine oxidase (0.13±0.30 µg/ml) while serum from broiler hen had the highest xanthine oxidase content (3.03±0.16 µg/ml), significantly different ($P<0.05$) from that of other selected birds. The serum from broiler cock had a serum xanthine oxidase content higher, but not statistically different ($P>0.05$) from that of Shika brown cock, domestic pigeon and parent stock white hen. The xanthine oxidase content of sera from parent stock white cock and hooded vulture were not significantly different ($P>0.05$). Also, sera from guinea fowl, Parent stock brown cock and Shika brown hen were not significantly different ($P>0.05$). Sera from peacock, spotted eagle owl and crown crane had significantly different ($P>0.05$) xanthine oxidase content.

4.4.2 Xanthine oxidase content of sera of selected rodents

Figure 4.1 presents the xanthine oxidase content of sera from selected rodents. The highest concentration of xanthine oxidase was recorded in grass-cutter serum (2.79±0.15 µg/ml). This was significantly higher ($P<0.05$) than that of other rodents. Rabbit Dutch serum followed and was higher than that of rabbit Chinchila and rabbit New-Zeland but not statistically ($P>0.05$). Sera from rabbit California and rabbit Grey also had different xanthine oxidase content but the difference was not statistical ($P>0.05$).

Table 4.23: Xanthine oxidase content of sera of selected birds

Source of serum	Concentration of the Xanthine oxidase (µg/ml)
Gallus gallus domesticus (Broiler cock)	2.60 ± 0.02^{gh}
Gallus gallus domesticus (Broiler hen)	3.03 ± 0.16^{h}
Gallus gallus domesticus (Shika brown cock)	2.42 ± 0.03^{fgh}
Gallus gallus domesticus (Shika brown hen)	1.59 ± 0.58^{def}
Gallus gallus domesticus (Parent Stock White cock)	1.99 ± 0.15^{efg}
Gallus gallus domesticus (Parent Stock White hen)	2.33 ± 0.40^{fgh}
Gallus gallus domesticus (Parent Stock Brown Cock)	1.62 ± 0.05^{def}
Gallus gallus domesticus (Parent Stock Brown Hen)	0.13 ± 0.30^{ab}
Numida meleagris (Guinea fowl)	1.63 ± 0.15^{def}
Columba livia domestica (Domestic pigeon)	2.38 ± 0.24^{fgh}
Plectropterus gambensis (Goose)	1.76 ± 0.03^{ef}
Balearica regulorum (Crowned crane)	0.68 ± 0.96^{abc}
Pavo cristatus (Peacock)	1.44 ± 0.07^{cde}
Bubo africanus (Spotted eagle owl)	0.85 ± 0.15^{bcd}
Necrosyrtes monachus (Hooded vulture)	1.91 ± 0.44^{efg}
Buteo buteo (Buzzard)	0.00 ± 0.00^{a}

Values are means ± standard deviation. Values with different superscripts down the column are significantly different ($P<0.05$).

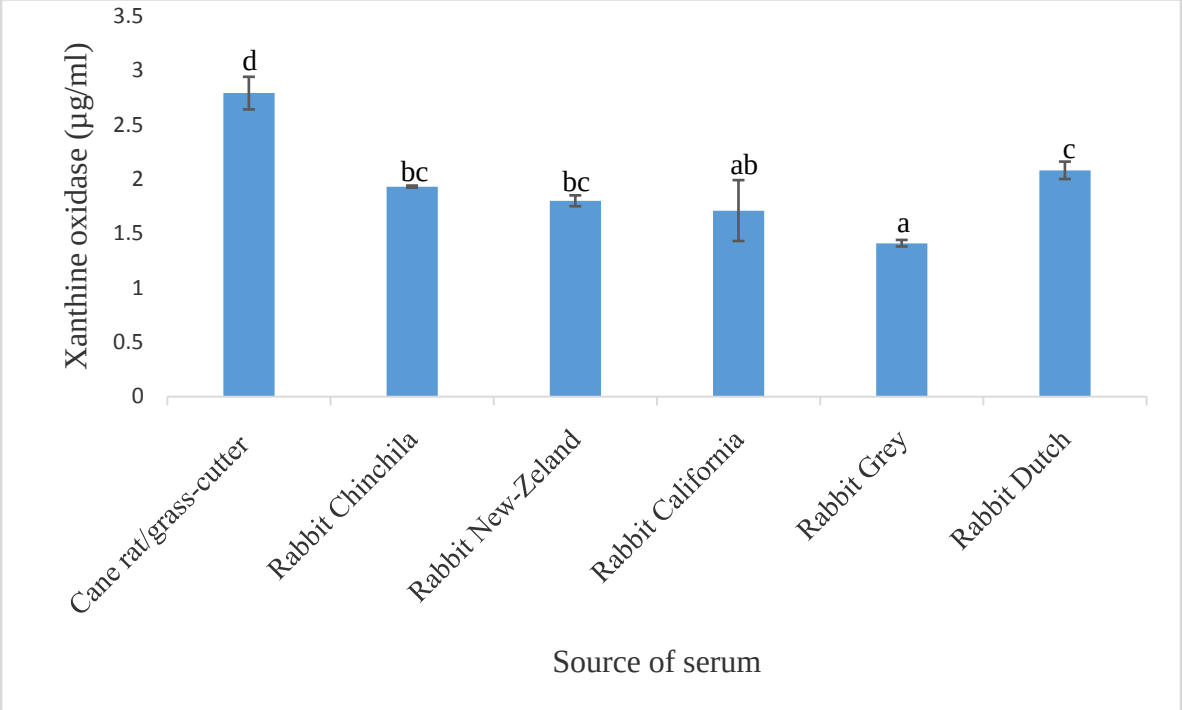


Figure 4.1: Xanthine oxidase content of sera of selected rodents
Values are mean concentrations. Values with different superscripts are significantly different ($P<0.05$).

4.4.3 Xanthine oxidase content of sera of selected small ruminants

It was observed as shown in Figure 4.2, that West African dwarf goat serum had the highest concentration of xanthine oxidase (2.06±0.09 µg/ml), followed by that of Uda

sheep, then Balami sheep, Sahel goat, Yankassa sheep and Red Sokoto goat which had the lowest concentration (0.70±0.00 µg/ml).

4.4.4 Xanthine oxidase content of sera of selected large ruminants

Result on xanthine oxidase content of sera of the selected large ruminants is illustrated in Figure 4.3. It is clear that sera from all the selected large ruminants had the same range of xanthine oxidase activity, as their concentrations were not significantly different ($P>0.05$).

4.4.5 Xanthine oxidase content of sera of selected non-ruminant mammals

Figure 4.4 summarises the xanthine oxidase content of sera from some non-ruminant mammals. Serum from camel had the least concentration (0.93±0.21 µg/ml). Next to it was the serum from pig Pettin. The xanthine oxidase content of sera from donkey, horse and pig large white were not significantly different ($P>0.05$) form each other, but were significantly different ($P<0.05$) from that of jackal.

4.4.6 Xanthine oxidase content of sera of selected zoo primates

The xanthine oxidase content of sera of the selected zoo primates is presented in Figure 4.5. It was observed that concentration of xanthine oxidase in the serum of red patas monkey was significantly higher ($P<0.05$) than that of Tantalus monkey (2.26±0.10 and 1.02±0.33 µg/ml respective

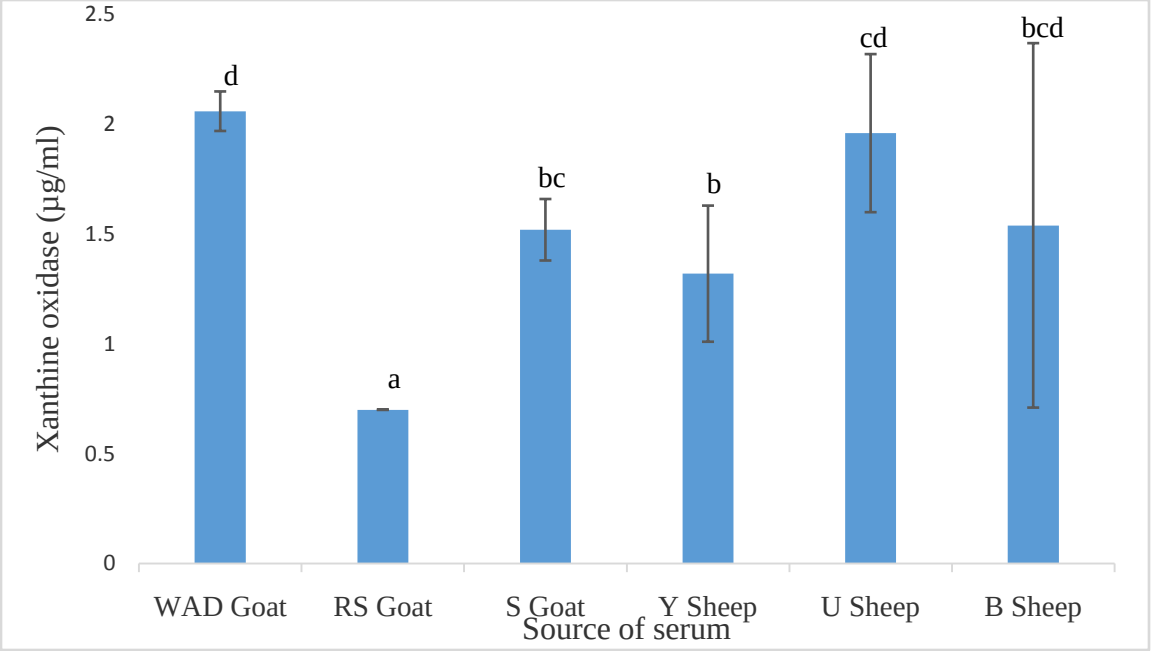


Figure 4.2: Xanthine oxidase content of sera from selected small ruminants

Values are mean concentrations. Values with different superscripts are significantly different ($P<0.05$).

Key: WAD: West African Dwarf, RS: Red Sokoto, S: Sahel, Y: Yankassa, U: Uda, B: Balami

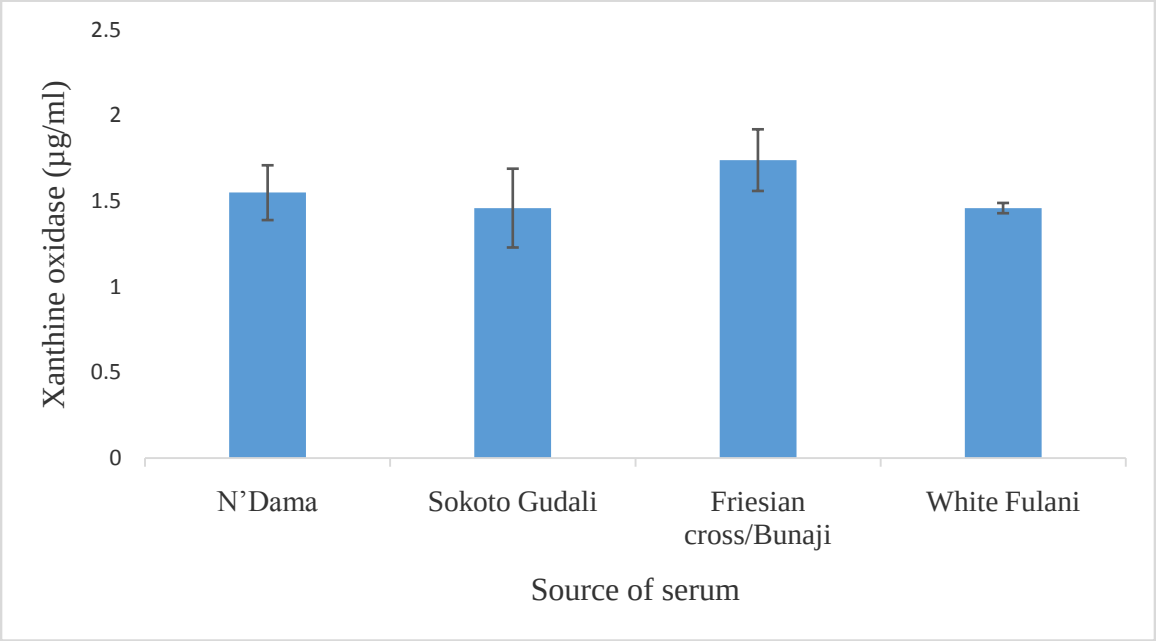


Figure 4.3: Xanthine oxidase content of sera of selected large ruminants
Values are mean concentrations.

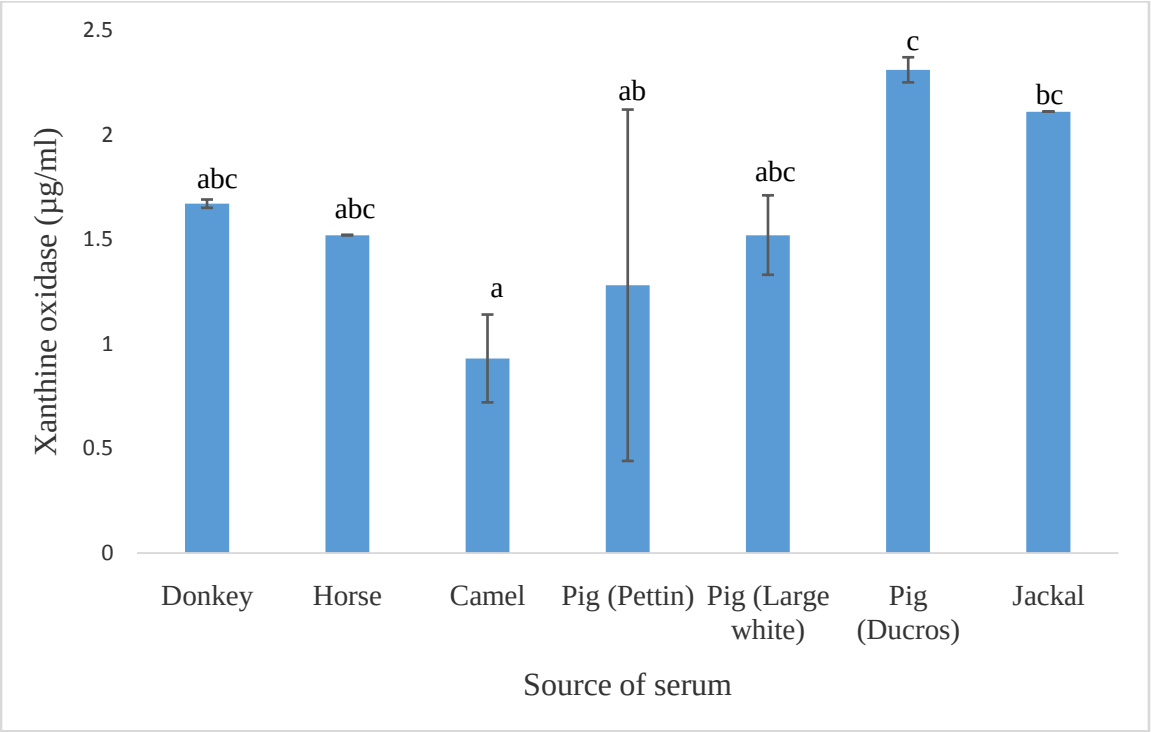


Figure 4.4: Xanthine oxidase content of sera of some non-ruminant mammals
Values are mean concentrations. Values with different superscripts are significantly different ($P<0.05$).

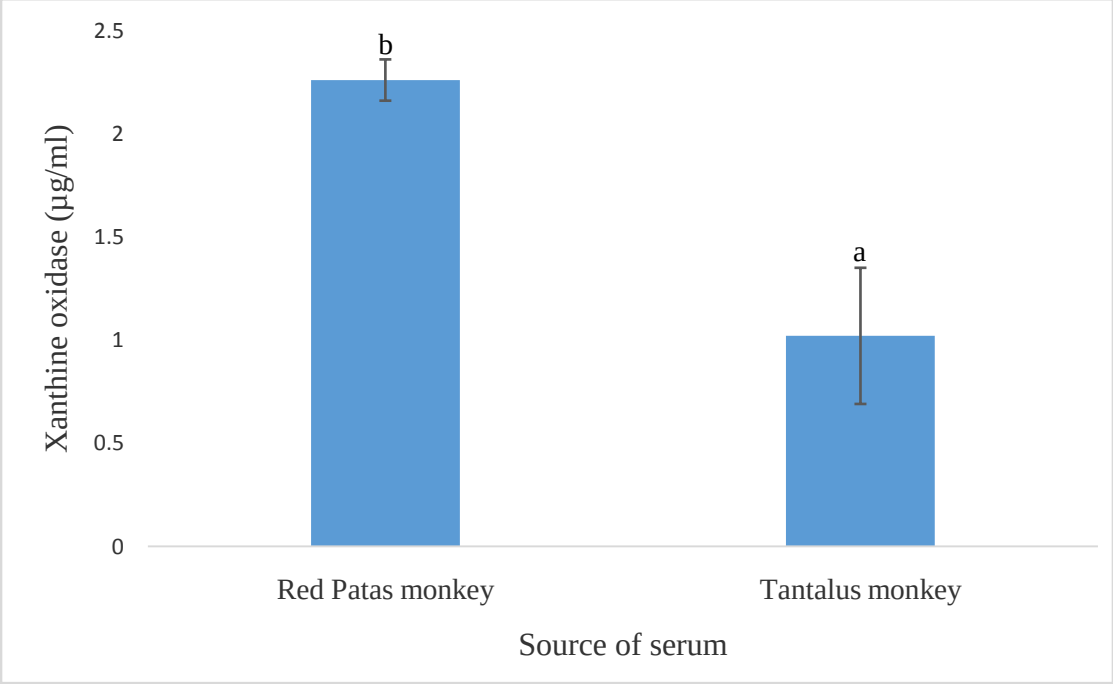


Figure 4.5: Xanthine oxidase content of sera of selected zoo primates
Values are mean concentrations. Values with different superscripts are significantly different ($P<0.05$).

4.5. Assay for antibody against *Trypanosoma brucei brucei* and *Trypanosoma congolense* antigens in sera from selected birds and mammals

4.5.1. Antibody activity of effective sera from selected birds and mammals against *Trypanosoma brucei brucei* antigens

No antibody activity against *T. brucei brucei* antigens was detected in the *in vitro* effective sera (sera with *in vitro* anti-trypanosome activity) of the selected birds and mammals as shown by Plate II in Appendix XXIV.

4.5.2. Antibody activity of effective sera from selected birds and mammals against *Trypanosoma congolense* antigens

No antibody activity against *T. congolense* antigens was detected in the *in vitro* effective sera of the selected birds and mammals as shown by Plate III in Appendix XXV.

4.6 Prophylactic Effect of the Effective Sera *In Vivo*

4.6.1 Prophylactic effect of the effective sera on *Trypanosoma brucei brucei*

The *in vivo* prophylactic activity of the effective sera on *T. brucei brucei* is presented in Figure 4.6. The survival times of the Wistar rats to which the effective sera were administered before infection, were not significantly different ($P>0.05$) from each other. None of the test sera prolonged the survival of the rats as all the treated rats were infected and died at the same time as the control rats.

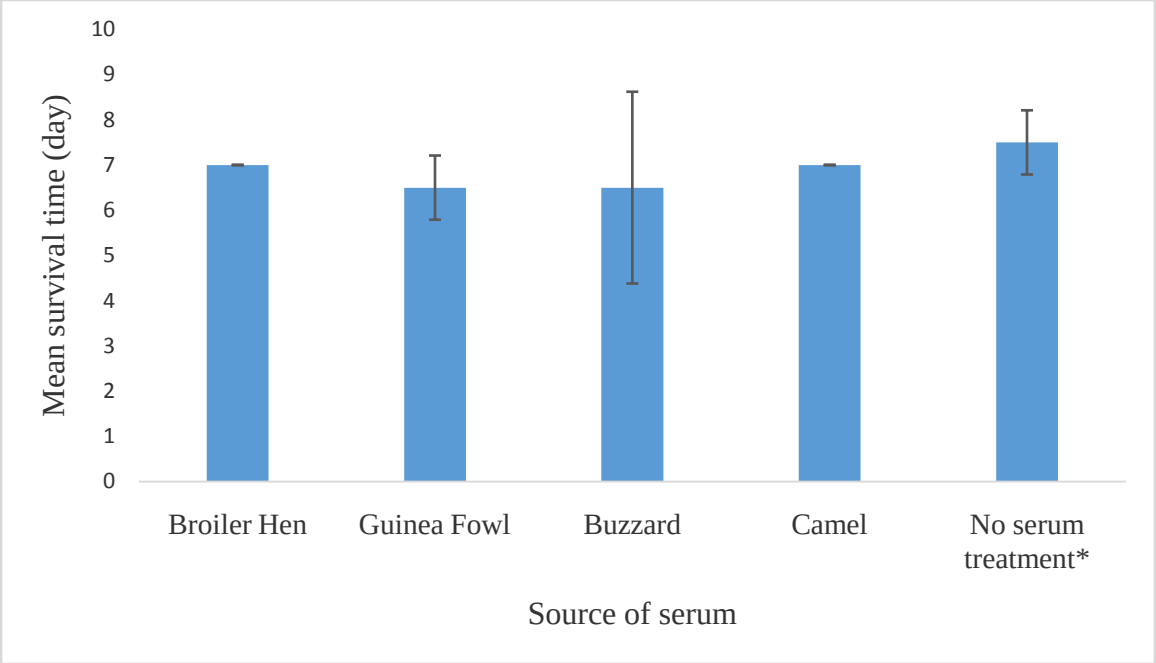


Figure 4.6: Prophylactic effect of the effective sera on *Trypanosoma brucei brucei in vivo* in rats
Values are mean survival time.
*Control.

4.6.2 Prophylactic effect of the effective sera on *Trypanosoma congolense*

Figure 4.7 shows the *in vivo* prophylactic activity of the effective sera on *T. congolense*. Wistar rats inoculated with jackal serum before inoculated with *T. congolense* survived longer than all the other treated rats (28.00±1.41 days). Rats inoculated with sera from tantalus monkey and peacock had the same range of survival time, not significantly different from the control. Red patas monkey serum did not show any *in vivo*

prophylactic activity on *T. congolense* as the survival time of the rats treated with this serum was 18.33±1.53 days while the control rats had a survival time of 26.00±7.07 days.

4.7 Curative Effect of the Effective Sera *in Vivo*

4.7.1 Curative effect of the effective sera on *Trypanosoma brucei brucei*

Results in Figure 4.8 show the *in vivo* curative effect of the effective sera on *T. brucei brucei* infection. None of the test sera could clear the infection and prevent the wistar rats from dying. However, Buzzard serum treated *T. brucei brucei* infected wistar rats had a survival time (10.50±0.71 days) significantly higher ($P<0.05$) than the control (8.50±0.71 days), and those treated with sera from other animals, showing some curative activity against *T. brucei brucei in vivo*. The survival times of the rats treated with sera from guinea fowl and camel were not significantly different ($P>0.05$) from the control. Broiler hen serum recorded the least survival time (8.00±0.00 days).

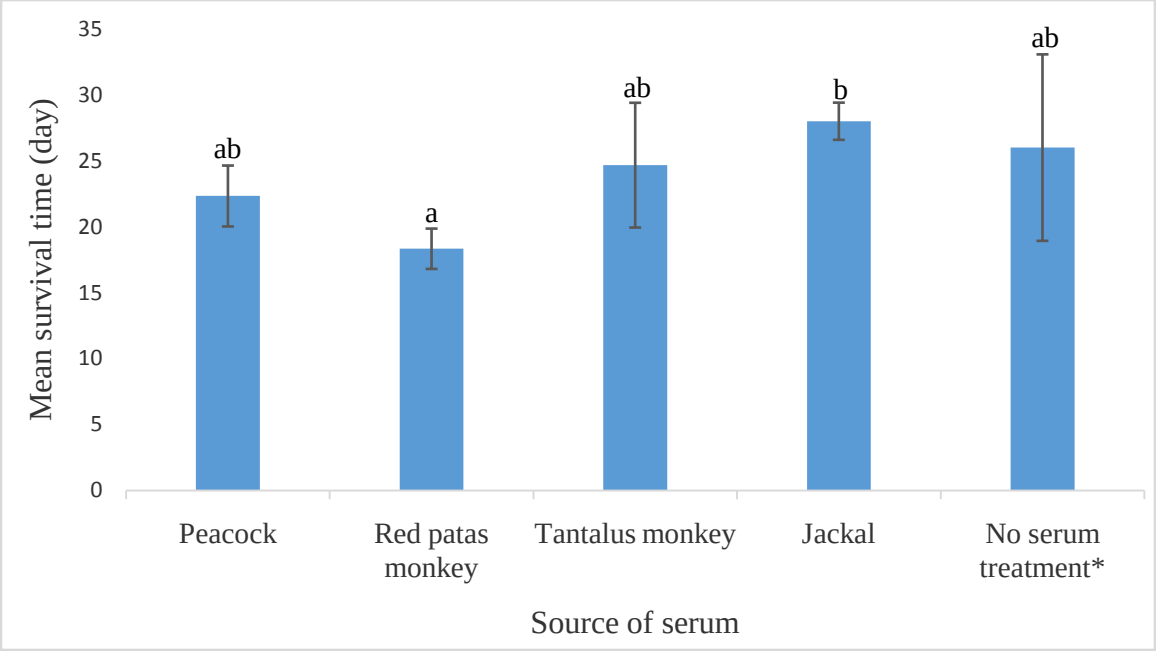


Figure 4.7: Prophylactic effect of the effective sera on *Trypanosoma congolense in vivo* in rats

Values are mean survival time. Values with different superscripts are significantly different ($P<0.05$).

*Control.

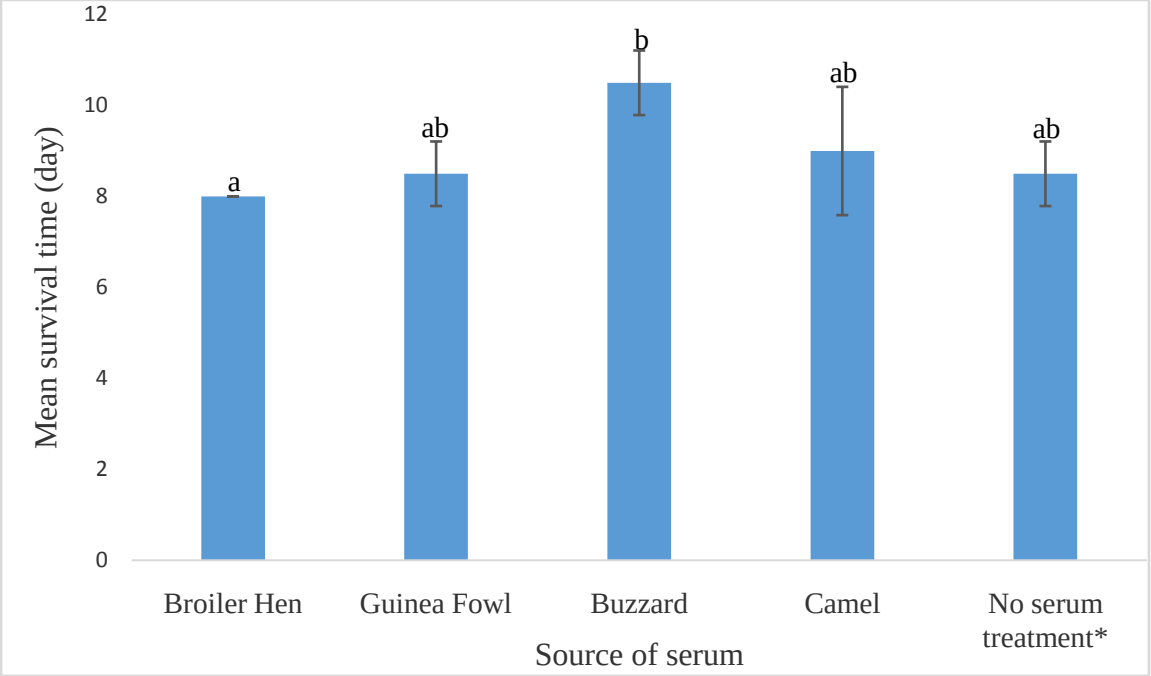


Figure 4.8: Curative effect of the effective sera on *Trypanosoma brucei brucei in vivo* in rats
Values are mean survival time. Values with different superscripts are significantly different (P<0.05).
*Control.

4.7.2 Curative effect of the effective sera on *Trypanosoma congolense*

Results on the *in vivo* curative effect of the effective sera on *T. congolense* are summarized in Figure 4.9. None of the sera exhibited any curative activity against *T. congolense in vivo*. All the Wistar rats treated with sera had the same range of survival time which was significantly lower ($P<0.05$) than that of the control group (36.50±2.12 days). Rats treated with Jackal serum had the highest survival time of 26.00±4.24 days, although not statistically different ($P>0.05$) from that of other sera.

4.8 Comparative Prophylactic and Curative Effects of the Effective Sera *in Vivo*

4.8.1 Comparative prophylactic and curative effects of the effective sera on *Trypanosoma brucei brucei*

Figure 4.10 presents the compared prophylactic and curative effect of the effective sera on *T. brucei brucei in vivo*. There was no significant difference ($P>0.05$) between the *in vivo* effect of the sera administered before infection (prophylactic) and after infection (curative). The survival time of the experimental animals treated before or after infection with *T. brucei brucei* were not statistically different ($P>0.05$).

4.8.2 Comparative prophylactic and curative effects of the effective sera on *Trypanosoma congolense*

The compared prophylactic and curative effect of the effective sera on *T. congolense in vivo* is presented in Figure 4.11. It was observed that the effect of the sera administered before infection (prophylactic) was not significantly different ($P>0.05$) from that of the same sera administered after infection (curative). In both cases the mean survival times of the Wistar rats were statistically the same ($P>0.05$) for each of the sera.

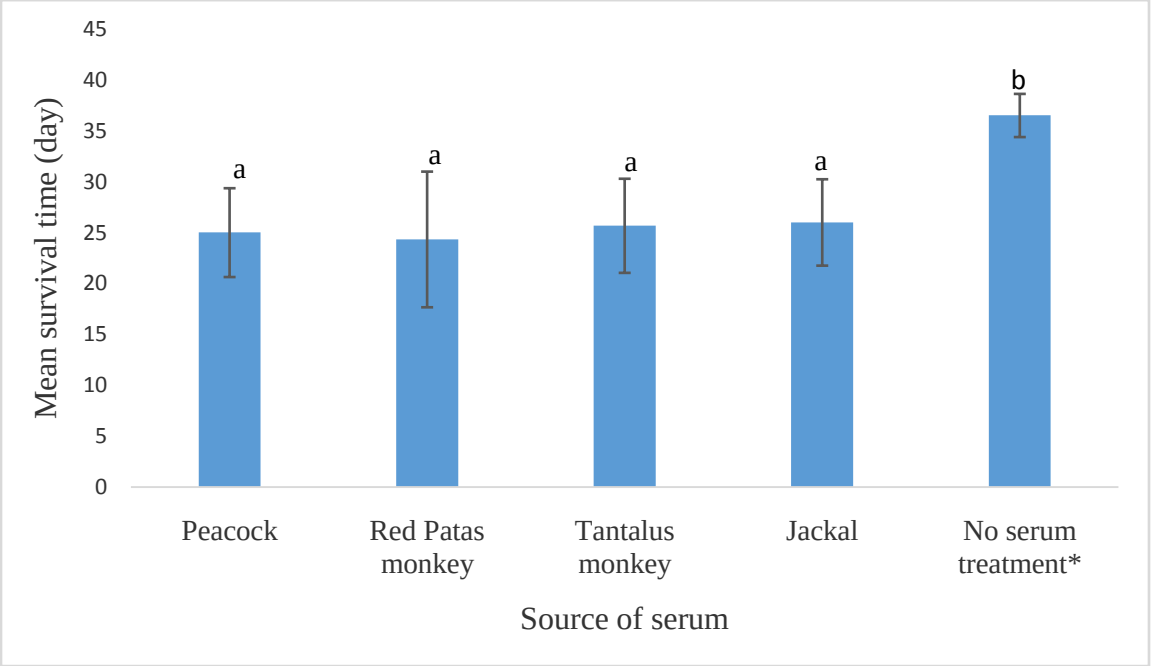


Figure 4.9: Curative effect of the effective sera on *Trypanosoma congolense in vivo* in rats

Values are mean survival time. Values with different superscripts are significantly different ($P<0.05$).

*Contol.

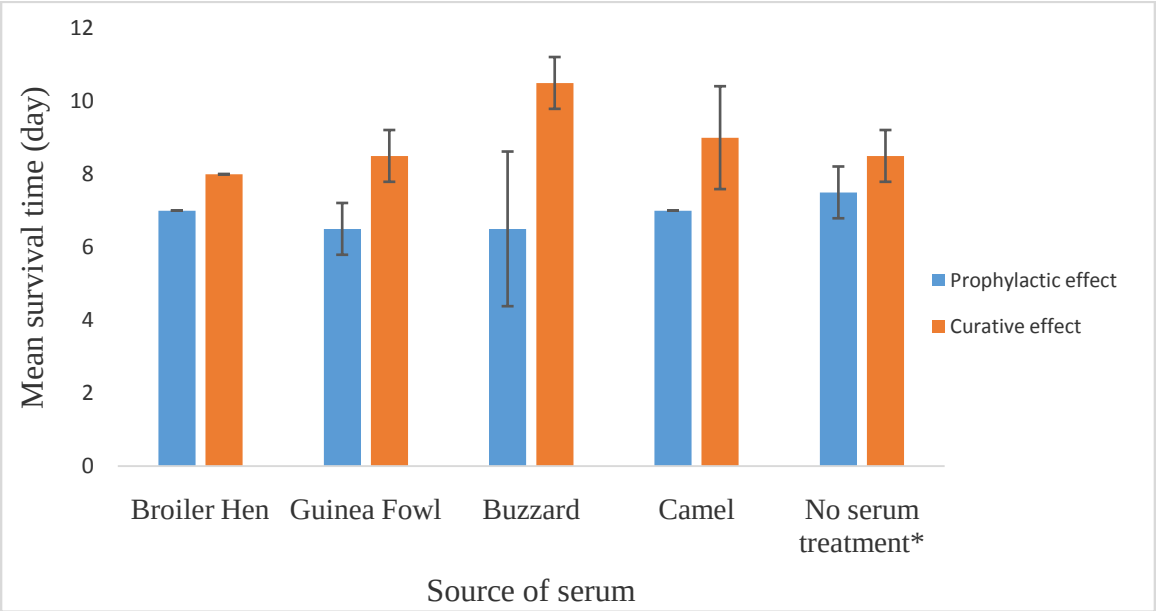


Figure 4.10: Comparative prophylactic and curative activities of the effective sera on *Trypanosoma brucei brucei in vivo* in rats
Values are mean survival time.
*Control.

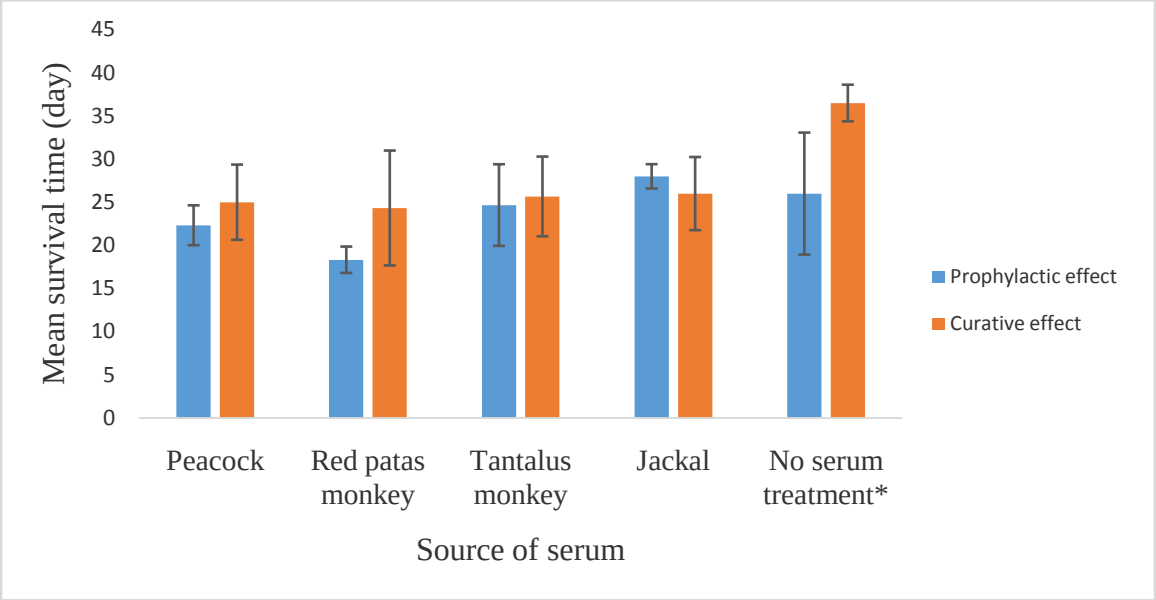


Figure 4.11: Comparative prophylactic and curative activities of the effective sera on *Trypanosoma congolense in vivo* in rats
Values are mean survival time.
*Control.

CHAPTER FIVE

DISCUSSION

The aim of the present work was to evaluate the natural interaction of *Trypanosoma brucei brucei* and *Trypanosoma congolense* with sera from uninfected birds and

mammals; the curative and prophylactic potentials of the sera were also assessed with a view to developing more control strategies against trypanosomiasis. In this study, the simple technique of trypanosome motility as indicator of parasite viability was used (Bulus *et al*., 2008), since it has long been established that parasite motility could be used as a measure of viability among most zooflagelate parasites (Peter *et al*., 1976). Atawodi *et al*. (2003) reported that the technique correlated well with other *in vitro* methods. It was found in this study that some of the sera manifested anti-trypanosomal activity, while other sera supported their survival.

Sera from the selected birds examined during this research generally exhibited anti-*Trypanosoma brucei brucei* activity *in vitro*, with serum from the broiler hen showing the most pronounced activity. Most of the sera from the birds showed anti-*Trypanosoma congolense* activity as well; the serum of peacock exhibited the highest anti-T*rypanosoma congolense* activity, with an index of 0.88. These findings may be associated with a mechanism that has been postulated to be responsible for the natural resistance of birds to *Trypanosoma cruzi* infection, and described as being antibody-independent, by Kierszenbaum *et al*. (1981). The researchers emphasized the lack of requirement of antibodies, and the important role of complement in the natural resistance against *T. cruzi* infection exhibited by birds and the lytic activity displayed by their sera on virulent forms of *T. cruzi*. The latter claim supports the fact that the *in vitro* anti-trypanosomal activity of the avian sera was higher on the most virulent species, *T. brucei brucei*. The *in vitro Trypanosoma congolense* supportive activity observed in sera of hooded vulture, spotted eagle owl and buzzard implies that these birds have not developed any protective mechanism against *T. congolense*.

Although the serum from the white stork had anti-*Trypanosoma congolense* activity, this could not be analysed statistically and presented because the serum was obtained from a single bird. The result on the serum from the African fish eagle, which did not demonstrate any anti-trypanosomal activity is not being presented for the same reason.

The mild anti-T*rypanosoma brucei brucei* activity observed in rabbit serum concurs with the findings of Ferrante (1985) who reported trypanolytic activity on *Trypanosoma lewisi* in rabbit serum. The slight anti-*Trypanosoma congolense* activity (0.25 ATI) of grass-cutter serum probably indicates the occurrence of some anti-*Trypanosoma congolense* materials. The observation that rabbit serum supported the survival of *T. congolense* can be attributed to the fact that the rabbit is a laboratory animal used to maintain these parasites *in vivo*, and the fact that the heat-inactivated form of rabbit serum has been used as supplement in trypanosome culture media (Brun *et al*., 1981; Black *et al*., 1999).

The fact that sera from the zoo primates, Red Patas monkey and Tantalus monkey showed a slight anti-*Trypanosoma brucei brucei* activity (ATI of 0.27 and 0.34 respectively) and a very high anti-*Trypanosoma congolense* activity *in vitro* (ATI of 0.91 and 0.90 respectively) with a very low mean survival time of the parasites of about 7.00±1.73 hours for Red patas monkey and 7.67±0.58 hours for Tantalus monkey, suggest that these primates possess an innate immunity to *T. congolense*, and to a lesser extent to *T. brucei brucei*. Humans and several other primates are known to be resistant to infection by several animal-specific trypanosome species including *T. brucei brucei*, *T. congolense*, *T. vivax* and *T. evansi* (Namangala, 2011). The results obtained here suggest that sera from red Pattas monkey as well as Tantalus monkey might possess some trypanolytic factors against *T. congolense*, and to a lesser extent against *T. brucei brucei*. This claim can be

supported by the fact that the innate immunity of humans and several other primates to the trypanosomes is due to the expression of a unique subset of high-density lipoproteins (HDLs) referred to as the trypanosome lytic factors (TLFs) in their blood (Namangala, 2011). Being that the trypanosome lytic factor is primarily composed of Apolipoprotein L1 (APOL1) and a haptoglobin-related protein (Raper and Friedman,2013); considering the findings of Jirku *et al*. (2015) who demonstrated that mandrill serum was able to efficiently lyse *T. brucei brucei* and *T. brucei rhodesiense*, and to some extent T. *brucei gambiense*, while the chimpanzee serum failed to lyse any of these subspecies because of the secondary loss of the APOL1 gene; the anti-trypanosomal activity of red pattas monkey and tantalus monkey sera observed in this study could be attributable to Apolipoprotein L1 (APOL1). These findings also concure with some previous ones among which the identification of an Apolipoprotein L1 (APOL1) in a subset of Old World monkeys (Lugli *et al*., 2004; Poelvoorde *et al*., 2004; Thomson *et al*., 2009, 2014); the demonstration of the *in vitro* lytic ability of serum and purified recombinant protein of an Apolipoprotein L1 (APOL1) ortholog from the West African Guinea baboon (Papio papio), which caused lysis of all subspecies of *T. brucei* including *T. b. gambiense* (Cooper *et al*., 2016).

This study has revealed that sera from the selected small ruminants are mostly supportive of the survival of *T. brucei brucei* and *T. congolense in vitro*; sera from sheep were found to be more supportive since the highest survival times was recorded for *T. brucei brucei* in Balami sheep serum and for *T. congolense* in Yankassa sheep serum. Ng'ayo *et al*. (2005) reported that sheep were more succeptible to infection by trypanosomes than goats. The fact that the least supportive activity for both species of trypanosomes was

recorded in the serum of West African Dwarf goat in this study could be attributed to the trypanotolerance of these goats. This seems to suggest some relative genetic purity of this goat because Geerts *et al*. (2009) reported that the introgression of genes of trypanosusceptible breeds into the West African Dwarf goat populations, which is being practised, could lead to their loss of trypanotolerance.

The supportive activity for *T. brucei brucei* observed with sera from the large ruminants *in vitro,* with the exception of serum from N'Dama cattle, may be associated with the succeptibility of these ruminants to *T. brucei brucei* and the reputable trypanotolerance of the N'Dama cattle that has been demonstrated by many findings (Hornby, 1921; Murray, 1974; Morrison *et al*., 1981; Morrison and Murray, 1985; ILRAD, 1986; Paling *et al*., 1991). The least supportive activity for *T. congolense,* shown by N'Dama serum as against sera from other large ruminants that exhibited some high level of support implies that they have different degrees of susceptibility for this species of trypanosome. The highest activity recorded with Friesian cross serum and Sokoto Gudali serum suggests that these two breeds of cattle are the most susceptible among the large ruminants to *T. brucei brucei* and *T. congolense* respectively.

The relatively low anti-*Trypanosoma brucei brucei* activity in sera from the non-ruminant mammals such as camel, donkey, horse, domestic pig and jackal suggests limited susceptibility levels in these animals and probably some anti-*Trypanosoma brucei brucei* factors. This was not the case with *T. congolense in vitro* as all the sera, except that of camel and jackal, elicited supportive activity. Serum from the donkey had no effect while sera from the horse and the three breeds of pig exhibited a very high

supportive activity. These findings suggest that these mammals, especially the horse and pig might possess some Serum Resistance Associated (SRA) proteins (Van Xong *et al*., 1998; De Greef *et al*., 1989), which inhibit lytic activity against *T. congolense*. This phenomenon has been reported for some humans of African ancestry whose serum exhibits lytic activity against *T. brucei rhodesiense* but not *T. brucei gambiense* (Genovese *et al*., 2010). Ng'ayo *et al*. (2005) also reported the presence of Serum Resistant Associated gene in a pig. This could be what makes these mammals, especially the horse and the pig highly susceptible to *T. congolense* infection, therefore reservoirs for this species of trypanosome.

In this study, jackal serum had a high anti-*Trypanosoma congolense* activity (ATI of 0.91) since the survival time of the parasites was just 6.50±2.12 hours. It is likely that jackal serum possesses some trypanolytic factors. However, it is unlikely that the jackal anti-trypanosome material bears any relationship to that of humans and ground-dwellimg primates of Africa (Seed *et al*., 1990 and Black *et al*., 1999), since the gene encoding haptoglobin-related protein required for the lytic activity is restricted to humans, gorillas, orangutans, old world monkeys and chimpanzees; the protein cannot be expressed by chimpanzees (McEvoy and Maeda, 1988).

The observation that *T. brucei brucei* and *T. congolense* exposed to the effective sera *in vitro* were not able to cause infection in mice until the end of the experiment suggests that the effect *in vitro* was trypanocidal and not trypanostatic.

Apart from the peacock, red Patas monkey, Tantalus monkey and jackal sera, all the selected sera supported the survival of *T. congolense* better than *T. brucei brucei.* This implies that most of the selected animals lacked innate immunity against *T. congolense*

and had not yet developed mechanism for resisting infection with this species of trypanosome.

On subjecting some supportive sera to different experimental conditions (exogenous CO_2, addition of xanthine and heat inactivation), the observation that *T. congolense* survived longer *in vitro* in supportive sera without exogenous CO_2 than under exogenous CO_2 seems to contradict the fact that CO_2 is a requirement for the cultivation of trypanosomes (Schuster and Sullivan, 2002). However, the ameliorating effect of the exogenous CO_2 on sera supplemented with xanthine, wich supported the survival of the parasite longer, emphasises the necessity of CO_2 incubator in culturing trypanosomes; since the pH of the medium is dependent on the delicate balance of dissolved carbon dioxide (CO_2) and bicarbonate (HCO_3–), because changes in the atmospheric CO_2 can alter the pH of the medium. Therefore, it is necessary to use exogenous CO_2 when using media buffered with a CO_2-bicarbonate based buffer, especially if the cells are cultured in open dishes or transformed cell lines are cultured at high concentrations (Thermo Fisher Scientific, 2015).

The increase in survival time of trypanosomes after the addition of xanthine in most sera except serum from Sokoto Gudali (addition of xanthine reduced survival time), confirms that the trypanosomal activity in these particular mammalian sera is not associated with xanthine oxidase; it however suggests that the observation in Sokoto Gudali may have some association with the xanthine oxidase activity. This result confirms a previous claim (in this study) that the trypanosomal activity of these particular mammalian sera is not associated with xanthine oxidase; and suggest that the *in vitro* trypanosomal activity of Sokoto Gudali serum could have some association with the xanthine oxidase activity. The

generation of hydrogen peroxide (H_2O_2) in excess of neutralizing the pyruvate of the medium most likely accounts for the reduction of the survival time of the parasites in Sokoto Gudali's serum supplemented with xanthine since catalase was not added (Black *et al*., 1999). The heat-inactivated sera from the horse and pig could be used as substitutes for the already known foetal bovine and rabbit sera in *T. congolense* culture (using RPMI 1640 medium), since they were sufficiently supportive of the trypanosomes *in vitro.*

The aim of evaluating the xanthine oxidase in the test sera was to relate it to the *in vitro* trypanosomal activities observed. Muranjan *et al*., (1997) found that the trypanocidal activity of Cape buffalo serum *in vitro* resulted in the inhibition of glycolysis in the trypanosomes as a result of the hydrogen peroxide produced during the catabolism of extracellular xanthine and hypoxanthine by xanthine oxidase. Also, Wang *et al*. (2002) reported that the control of the acute phase of parasitemia in Cape buffaloes correlates with a decline in blood catalase activity and the generation of trypanocidal hydrogen peroxide (H_2O_2) in serum during the catabolism of endogenous purine by xanthine oxidase.

The fact that the broiler hen serum had the highest xanthine oxidase content coincided with the highest anti-*Trypanosoma brucei brucei* activity observed with this serum. Although buzzard serum lacked xanthine oxidase, it showed a considerable level of anti-*Trypanosoma brucei brucei* activity meaning that the xanthine oxidase activity did not account for the anti-*Trypanosoma brucei brucei* activity observed. This result suggests that buzzard serum might have some nonspecific factors other than xanthine oxidase with anti-*Trypanosoma brucei brucei* effect. However, being the best supportive serum for *T.*

congolense among the sera from the birds suggests that differences in parasites anti-xanthine oxidase may also exist. Sera from the parent stock brown hen, crowned crane and goose had very low xanthine oxidase content, the lowest being that of the parent stock brown hen; meanwhile, these sera had moderate anti-*Trypanosoma brucei brucei* and anti-*Trypanosoma congolense* activity.

Xanthine oxidase content of the grass-cutter serum being higher than that of the other rodents could be associated with its slight anti-*Trypanosoma congolense* effect, but not with its support of *T. brucei brucei*. The slight differences observed with the xanthine oxidase content of sera from the five breeds of the rabbit used in this study suggest that xanthine oxidase activity could be breed-specific in rabbits.

The difference in the xanthine oxidase content of sera from red patas monkey and Tantalus monkey indicates that the similar anti-trypanosomal activity observed is not associated to the activity of this enzyme. This finding gives more support to our suggestion on the possible trypanolytic factor previously mentioned.

Just like in birds, in ruminants, the serum with the lowest supportive activity, from the West African dwart goat, having the highest concentration of xanthine oxidase suggests some association of the trypanosomal activity with the xanthine oxidase activity. The low concentration of the enzyme in Red Sokoto goat serum seems to be in order with the lack of anti-trypanosomal effect observed.

That sera from all the selected large ruminants including the N'Dama serum had similar xanthine oxidase activity confirmed the claim that the trypanotolerance ability of N'Dama cattle is rather genetic and not catabolic. D'leteren *et al.* (1998) reported that

trypanotolerance is not only a breed characteristic but is also a heritable trait within the N'Dama population. This confirmed the findings of Trail *et al*. (1989) who examined phenotypes that appeared more characteristic of the N'Dama in comparison with those of East African Zebu cattle for associations with trypanotolerance traits.

The fact that sera from selected non-ruminant mammals had different concentrations of xanthine oxidase suggests that serum xanthine oxidase activity is very species-specific. The low xanthine oxidase activity observed in camel serum is somehow in contrast with the anti-trypanosomal activity observed. The high xanthine oxidase activity of sera from horse and pig does not reflect the high supportive effect observed for *T. congolense*. These results show that the trypanosomal activity of these mammals is not associated with the xanthine oxidase activity. On the contrary, the xanthine oxidase content of jackal serum, which was comparatively high seems to tally with the anti-trypanosomal activity observed *in vitro* with the serum, although this may not adequately explain the activity observed. Jackal serum might contain non-specific factors other than xanthine oxidase. The fact that sera from the breeds of pig used in this study had varying xanthine oxidase content suggests breed-specific enzyme activity.

It seems that the antitrypanosomal activity of sera associated with xanthine oxidase depends on its level in the plasma. Black *et al*. (1999) had obtained 37µg/ml, 35µg/ml and 5µg/ml from the serum of cape buffalo, eland and greater kudu respectively, and reported that such animals with a high xanthine oxidase serum content could impose a significant growth constraint on *T. brucei brucei*. They also explained that the H_2O_2 generated during purine catabolism could accumulate in the plasma of normal Cape buffalo, giraffe, and greater kudu to a level that can affect trypanosomes.

The observation that none of the *in vitro* effective sera showed antibody activity against *T. brucei brucei* and *T. congolense* antigens could be explained by the fact that the sera were obtained from birds and mammals that had probably not previously been exposed to trypanosome infection. These results are also suggestive of the nonspecific nature of the anti-trypanosomal materials present in the effective sera.

That the administration of effective sera *in vivo* before infection with *T. brucei brucei* did not affect the survival of the Wistar rats could be as a result of physiological factors, and probably the dose of serum administered.

The rats inoculated with sera from peacock and tantalus monkey before infection with *T. congolense* having the same range of survival time with the control rats indicates the lack of prophylactic activity of these sera, and could be explained as previously with *T. brucei brucei*. The fact that the Red Patas monkey serum instead supported the multiplication/ growth of trypanosomes, causing the death of the treated rats before the control showed the lack of prophylactic effect of the serum. On the contrary, jackal serum showed a prophylactic effect on *T. congolense in vivo*, as the rats treated with this serum survived longer than the control. This observation suggests that an increased dose of jackal serum could provide better prophylaxis against *T. congolense*.

The infected rats treated with sera from guinea fowl and broiler hen having the same survival capacity with the control rats, indicates the lack of curative effect, which could be as a result of physiological factors or the dose of serum used. The dose of sera administered was probably not sufficient to cure the rats infected with *T. brucei brucei*, as a long course of treatment is usually required for African sleeping sickness (Kaminsky and Brun, 1998) and Chagas disease and leishmaniasis (Croft, 1997). The observation

that Wistar rats treated with camel serum had the same survival time with the control rats showed the lack of curative activity. Buzzard serum exhibited a curative effect against *T. bruei brucei in vivo* by significantly increasing the survival time of the rats; this suggests ethat an increase dose of buzzard serum could produce better curative effect against *T. brucei brucei.*

All the *in vitro* effective sera used as treatment for Wistar rats experimentally infected with *T. congolense* lacked curative properties, as none could cure or keep the animals longer than the control. Nevertheless, just like in the prophylactic test, jackal serum had the higest survival time, although lower than the control.

When comparing the effect of the administration of the effective sera before infection (prophylactic effect) and after infection (curative effect), there appears to be no significant difference. This could be due to the fact that the test sera administered to confer to the rats an artificial passive immunity, were being used by the rats just as received, to fight the parasites.

The longer survival time of *T. congolense* than *T. brucei brucei* in general, including the control, both *in vitro* and *in vivo* is not surprising. Considerable understanding of the relationship between susceptibility, parasite growth and immune response has been provided by the work of Black *et al.* (1985) on the mouse model system, indicating that mice varied in their susceptibility to *T. brucei* (Black *et al*., 1983), *T. congolense* (Morrison *et al*., 1978) and *T. vivax* (Mahan *et al*., 1986).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study revealed that:

- The sera of the selected birds generally have anti-trypanosomal activity on both *T. brucei brucei* (78.38% of the selected animals) and *T. congolense* (37.84% of the selected animals) *in vitro*, except the spotted eagle owl, hooded vulture and buzzard which do not have anti-trypanosomal activity on *T. congolense.* The best anti-*Trypanosoma brucei brucei* activity is found in broiler hen serum while peacock serum has the highest anti-*Trypanosoma congolense* activity *in vitro*. Among the sera from the selected mammals, sera from rabbit, Red Patas monkey, Tantalus monkey, West African Dwarf goat, N'Dama cattle, donkey, horse, camel, pig, jackal, have anti-trypanosomal activity on *T. brucei brucei in vitro*, while sera from cane rat/grass-cutter, Red Patas monkey, Tantalus monkey, camel and jackal have anti-trypanosomal activity on *T. congolense in vitro*.

- The sera of the selected birds and mammals contain xanthine oxidase (XO), except serum from buzzard.

- The sera of the selected birds and mammals do not contain antibodies reactive to *T. brucei brucei* and *T. congolense* antigens.

- Only the serum from jackal exhibited prophylactic effect against *T. congolense in vivo*.

- Only the serum from buzzard exhibited curative potential against *T. brucei brucei* infection *in vivo*.

6.2 Recommendations

- Further investigations need to be carried out on sera from broiler hen, guinea fowl, peacock, Red patas monkey and Tantalus monkey, for their *in vivo* activity.
- Serum from buzzard could be used to develop new therapy against *T. brucei brucei* while serum from jackal, could be exploited for therapeutic control of trypanosome infection by *T. congolense.*
- Heat inactivated form of horse serum and pig serum are recommended as substitute to foetal bovine serum (FBS) in RPMI 1640 medium for the cultivation of *T. congolense*.
- Further studies should be carried out to screen sera from other animals on other classes of blood based protozoan parasites.
- Further studies need to be carried out on the sera of birds, mammals and other vertebrate groups, to detect nonspecific factors other than xanthine oxidase, with anti-trypanosomal activity.

REFERENCES

Akinwale, O.P. (1998). Comparative studies on the susceptibility of Red Sokoto, Sahel and West African Dwarf goats to experimental *Trypanosoma vivax* infection. An unpublished PhD Thesis submitted to the Department of Biological Sciences, Ahmadu Bello University, Zaria, 139 pp.

Akol, G.W.O. and Murray, M. (1982). Early events following challenge of cattle with tsetse infected with *Trypanosoma congolense*. Development of the local skin reaction. *Veterinary Record*, **110**: 295-302.

Akol, G.W.O. and Murray, M. (1983). *Trypanosoma congolense*: Susceptibility of cattle to cyclical challenge. *Experimental Parasitology*, **55**: 386-393.

Algers, B. and Uvnäs-Moberg, K. (2007). Maternal behavior in pigs. *Hormones and Behavior*, **52**(1):78–85. ISSN 0018-506X. PMID 17482189. doi:10.1016/j.yhbeh.2007.03.022.

Ali, S. and Ripley, S.D. (2001). *Handbook of the Birds of India and Pakistan (paperback).* India: Oxford University Press. Volume 1 (2nd edition). Pp 99–101.

Allan, D. (2000). *A Photographic Guide to Birds of Prey of Southern, Central and East Africa.* Struik Publishers, Cape Town. P. 7.

Amistadt, A. (2014). What is a peacock's diet? Demand Media. (February, 2014) http://animals.pawnation.com/peacocks-diet-10104.html.

Animal diversity web. *Canis aureus golden jackal*. http://animaldiversity.org/accounts/Canis_aureus/#geographic_range

Atawodi, S.E., Bulus, T., Ibrahim, S., Ameh, D.A., Nok, A.J., Mamman, M., Galadima, M. (2003). *In vitro* trypanocidal effect of methanolic extract of some Nigerian savannah plants. *African Journal of Biotechnology*, **2**(9):317–321.

Bacchi, C.J. (1993). Resistance to clinical drugs in African trypanosomes. *Parasitology Today*, **9**(5):190–193.

Bakker, B.M., Michels, P.A., Opperdoes, F.R. and Westerhoff, H.V. (1999). What controls glycolysis in bloodstream form of *Trypanosoma brucei*? *Journal of Biological Chemistry*, **274**:14551–14559.

Baltz, T., Baltz, D., Giroud, C. and Crockett, J. (1985). Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense*, and *T. gambiense*. *EMBO Journal*, **4**:1273-1277.

Barrett, M. P., Burchmore, R. J., Stich, A., Lazzari, J. O., Frasch, A. C., Cazzulo, J. J. and Krishna, S. (2003). The trypanosomiases. *Lancet*, **362**:1469-1480.

Barrett, M.P., Boykin, D.W., Brun, R. and Tidwell, R.R. (2007). Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *British Journal of Pharmacology*, **152**:1155–1171.

Barry, J.D. and Emery, D.C. (1984). Parasite development and host responses during the establishment of *T. brucei* infection transmitted by tsetsefly. *Parasitology*, **88**: 67-84.

Barry, J.D. and McCulloch, R. (2001). Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. *Advances in Parasitology*, **49**: 1–70.

Batista, J.S., Oliveira, A.F., Rodrigues, C.M., Damasceno, C.A., Oliveira, I.R., Alves, H.M., Paiva, E.S., Brito, P.D., Medeiros, J.M., Rodrigues, A.C. and Teixeira, M.M. (2009). Infection by *Trypanosoma vivax* in goats and sheep in the Brazilian semiarid region: From acute disease outbreak to chronic cryptic infection. *Veterinary Parasitology*. [Epub ahead of print]

Belknap, M. (2004). *Horsewords: The Equine Dictionary* (Second ed.). North Pomfret, VT: Trafalgar Square Publishing. Yale University Press, pp. 400–401.

Bero, J., Beaufay, C., Hannaert, V., Hérent, M.-F., Michels, P.A. and Quetin-Leclercq, J. (2013). Antitrypanosomal compounds from the essential oil and extracts of *Keetia leucantha* leaves with inhibitor activity on Trypanosoma brucei glyceraldehyde-3-phosphate dehydrogenase. *Phytomedicine*, **20**:270 – 274.

Bird Life International (2012). "*Balearica pavonina*". IUCN Red List of Threatened Species. *Version 2013.2*. International Union for Conservation of Nature. *Retrieved 26 November 2013*, from http://research.omicsgroup.org/ index.php/Black_crowned_crane.

Bird Life International (2012). *"Bubo africanus"*. IUCN Red List of Threatened Species. Version 2013.2. *International Union for Conservation of Nature*. *Retrieved 26 November 2013*, from http://zipcodezoo.com/index.php/Bubo_africanus.

Bird Life International (2013). "*Balearica regulorum*". IUCN Red List of Threatened Species. *Version 2013.2*. International Union for Conservation of Nature. *Retrieved 26 November 2013*, from http://eol.org /pages/ 915338/ hierarchy_entries/24919546/details.

Bishop, I. (1984). Macdonald, D., ed. *The Encyclopedia of Mammals*. New York: Facts on File. p. 703. ISBN 0-87196-871-1.

Black, S.J., Sendashonga, C.N., Lalor, P.A., Whitelaw, D.D., Jack, R.M., Morrison, W.I. and Murray, M. (1983). Regulation of the growth and differentiation of *Trypanosoma (Trypanozoon) brucei brucei* in resistant (C57B1/6) and susceptible (C3H/He) mice. *Parasites and Immunology*, **5**: 465-478.

Black, S.J., Sendashonga, C.N., O'Brien, C.O., Borowy, N.R., Naessens, M., Webster, P. and Murray, M. (1985). Regulation of parasitaemia in mice infected with *Trypanosoma brucei*. *Current Topics in Microbiology* and *Immunology*, **117**: 93-118.

Black, S.J. and Vandeweerd, V. (1989). Serum lipoproteins are required for multiplication of Trypanosoma brucei under axenic conditions. *Molecular and Biochemical Parasitology*, **37**:65-72.

Black, S.J., Wang, Q., Makadzange, T., Li, Y., Van Praagh, A., Loomis, M. and Seed, J.R. (1999). Anti-trypanosoma brucei activity of nonprimate zoo sera. *Journal of Parasitology*, **85**(1):48-53.

Blechman, A. (2007). *Pigeons-The fascinating saga of the world's most revered and reviled bird.* Grove Press. 256 pp.

Bornstein, S. (2010). Important ectoparasites of Alpaca *(Vicugna pacos)*. *Acta Veterinaria Scandinavica*, **52** (Suppl 1):S17.

Bouteille, B., Oukem, O., Bisser, S. and Dumas, M. (2003). Treatment perspectives for human African trypanosomiasis. *Fundamental & Clinical Pharmacology*, **17**: 171-181.

Bovine Trypanosomiasis Consortium (2013). African bovine trypanosomiasis. http://www.genomics.liv.ac.uk/tryps/trypsmovie.html

Britannica Online Encyclopedia. *Peacock (bird)*. Inc… **9**: 53 Blue Nile (river in Africa).

Brown, L. and Amadon, D. (1986). *Eagles, Hawks and Falcons of the World*. The Wellfleet Press, ISBN 978-1555214722.

Brun, R. and Jenni, L. (1987). Salivarian trypanosomes: bloodstream forms (Trypomastigotes). *In* A. E. R. Taylor and J. R. Baker (eds.), *In vitro* methods for parasite cultivation. *London Academic Press*, pp. 94-117.

Brun, R. and Lun, Z.R. (1994). Drug-sensitivity of Chinese *Trypanosoma-evansi* and *Trypanosoma-equiperdum* isolates. *Veterinary Parasitology*, **52**: 37–46.

Brun, R., Jenni, L., Schonenberger, M., Schell, K.F. (1981). *In vitro* cultivation of bloodstream forms of *Trypanosoma brucei, T. rhodesiense,* and *T. gambiense*. *Journal of Protozoology*, **28**:470-479.

Brun, R., Jenni, L., Tanner, M., Schonenberger, M., Schell, K.F. (1979). Cultivation of vertebrate infective forms derived from metacyclic forms of pleomorphic *Trypanosoma brucei* stocks. *Acta Tropica*, **36**:387-390.

Budiansky, S. (1997). *The Nature of Horses*. New York: NY: Free Press, p**.** 31.

Budiansky, S. (1999). *The Covenant of the Wild: Why animals chose domestication*. Yale University Press, New Haven. 212 pp.

Bulus, T., Atawodi, S.E. and Mamman, M. (2008). *In vitro* Antitrypanosomal Activity and Phytochemical Screening of Aqueous and Methanol Extracts of *Terminalia avicennioides. Nigerian Journal of Biochemistry and Molecular Biology*, **23**(1): 7 – 11.

Carruthers, V.B. and Cross, G.A.M. (1992). High-efficiency clonal growth of bloodstream- and insect-form *Trypanosoma brucei* on agarose plates. *Proceeding of the National Academy of Science*, **89**: 8818-8821.

CDC (Centers for Disease Control and Prevention). (2012). Parasites - African Trypanosomiasis (also known as Sleeping Sickness). Diagnosis. Page last updated: August 29, 2012.

Centers for Disease Control and Prevention (CDC). (2016). Parasites - African Trypanosomiasis (also known as Sleeping Sickness). *Retrieved 29 January 2016.* https://www.cdc.gov/parasites/sleepingsickness/

Century Dictionary and Cyclopedia. *Retrieved 28 November 2012*, from*"llama"*. wordnik. http://si-referral.duniakicau.web.id/IT/en/2807-2696/camel_6431_si-referral-duniakicau.html

Chamond, N., Cosson, A., Blom-Potar, M.C., Jouvion, G., D'Archivio, S., Medina, M., Droin-Bergère, S., Huerre, M., Goyard, S., Minoprio, P. (2010). *Trypanosoma vivax* Infections: Pushing Ahead with Mouse Models for the Study of *Nagana*. I. Parasitological, Hematological and Pathological Parameters. *PloS Neglected Tropical Diseases*, **4**(8):e792.

Chatterjee, K.D. (2009). *Parasitology (Protozoology and Helminthology) in relation to clinical medicine* (13 ed.). New Delhi: CBC Publishers, pp. 56–57.

Clutton-Brock, J. (1987). *A Natural History of Domesticated Mammals*. Cambridge University Press, Cambridge pp.73-74.

Cooper, A., Capewell, P., Clucas, C., Veitch, N., Weir, W., Thomson, R., Raper, J., MacLeod, A. (2016). A Primate APOL1 Variant That Kills *Trypanosoma brucei gambiense*. *PLOS Neglected Tropical Diseases*, 5;**10**(8): e0004903. doi: 10.1371/journal.pntd.0004903. eCollection 2016.

Courtin, D., Berthier, D., Thevenon, S., Dayo, G.K., Garcia, A., Bucheton, B. (2008). "Host genetics in African trypanosomiasis". *Infection Genetics and Evolution*, **8** (3):229–238.

Coustou, V., Guegan, F., Plazolles, N. and Baltz, T. (2010). Complete *In Vitro* Life-Cycle of *Trypanosoma congolense*: Development of Genetic tools. *PLoS Neglected Tropical Diseases*, 4(3): e618. https://doi.org/10.1371/journal.pntd.0000618.

Cramp, S. (1977). *Handbook of the Birds of Europe, the Middle East and North Africa, the Birds of the Western Palearctic.* Vol. **1**: Ostrich to Ducks. Oxford University Press. ISBN 0-19-857358-8. P. 332

Croft, S.L. (1997). The current status of antiparasitic chemotherapy. *Parasitology*, **114**: S3–S15.

Cross G.A.M. (2008). The cysteine requirement and cloning procyclic forms. A personal commentary on the history and current art of culturing *Trypanosoma brucei*. *Trypanosoma brucei commentary written in May 2002 and updated September 4, 2008.* http://tryps.rockefeller.edu.

Dam, J.T.P. Van, Schrama, J.W., Hel, W., van der, Verstegen, M.W.A. and Zwart, D., (1996). Heat production, body temperature and body posture in West African dwarf goats infected with Trypanosoma vivax. *Veterinary Quarterly*, **18**:55-59.

Daniels, M.J., Ball, N., Hutchings, M.R. and Greig, A. (2001). "The grazing response of cattle to pasture contaminated with rabbit faeces and the implications for the transmission of paratuberculosis". *The Veterinary Journal*, **161**(3):306–313.

Dávila, A.M. and Silva, R.A. (2000). Animal trypanosomiasis in South America. Current status, partnership, and information technology. *Annals of New York Academy of Science*, **916**:199-212.

Dede, M.P. (2004). Experimental studies on non-cyclical transmission of *Trypanosoma brucei brucei*. Unpublished PhD Thesis, submitted to the Department of Biological Sciences, Ahmadu Bello University, Zaria. 162 pp.

De Greef, C., Imberechts, H., Matthyssens, G., Van Meirvenne, N., Hamers, R. (1989). A gene expressed only in serum-resistant variants of *Trypanosoma brucei rhodesiense*. *Molecular and Biochemical Parasitology*, **36**: 169–176.

Deane Lewis (2015). Spotted Eagle Owl ~ *Bubo africanus.* http://www.wow.com/wiki/Spotted_eagle-owl

DeFilippis, C. (2006). *The Everything Horse Care Book.* Avon, MA: Adams Media. p**.** 4.

Delespaux, V. and Harry P. (2007). "Drugs and drug resistance in African trypanosomiasis". *Drug Resistance Updates*, **10** (1-2):30-50.

Delespaux, V., Geysen, D., Majiwa, P.A.O. and Geerts, S. (2005). "Identification of a genetic marker for isometamidium chloride resistance in *Trypanosoma congolense*," *International Journal for Parasitology*, **35** (2): 235–243.

Del Hoyo, J., Elliot, A. and Sargatal, J. (1994). *Handbook of the Birds of the World* Volume **2**: New World Vultures to Guineafowl. Lynx Edicions. ISBN 84-87334-15-6.

Deneice A. (2016). Goat Breed Facts Sahelian. Second opinion doctor. http://www.second-opinion-doc.com/goat-breed-facts-sahelian.html

Dinets V. (2015). The Canis tangle: a systematic overview and taxonomic recommendations. Vavilovskii Zhurnal Genetiki i Selektsii – Vavilov *Journal of Genetics and Breeding*, **19** (3):286-291.

D'leteren, G.D.M., A u t h i é, E., Wissocq, N. and Murray, M. (1998). Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. *Revue scientifique et technique (Office international des Epizooties)*, **17** (1):154-175.

Dohner, J. V. (2001). *The Encyclopedia of Historic and Endangered Livestock and Poultry Breeds.* Yale University Press. Pp. 560.

Donelson, J. E., Hill, K. L. and El-Sayed, N. M. (1998). Multiple mechanisms of immune evasion by African trypanosomes. *Molecular Biochemical Parasitology,* **91**:51-66.

Doyle, J.J., Hirumi, H., Hirumi, K., Lupton, E.N. and Cross, G.A.M. (1980). Antigenic variation in clones of animal-infective *Trypanosoma brucei* derived and maintained *in vitro*. *Parasitology*, **80**:359-369.

Dumas, M. and Boa, F.Y. (1988) Human African Trypanosomiasis. *Hand Book of Clinical Neurology: Microbial Disease*, **8**(52): 339.

Duszenko, M., Ferguson, M.A.J., Lamont, G.S., Rifkin, M.R., Cross, G.A.M. (1985). Cysteine eliminates the feeder cell requirement for cultivation of *Trypanosoma brucei* bloodstream forms *in vitro*. *Journal of Experimental Medicine,* **162**:1256-63.

Duszenko, M., Muhlstadt, K. and Broder, A. (1992) Cysteine is an essential growth factor for *Trypanosoma brucei* bloodstream forms. *Molecular and Biochemical Parasitology,* **50**:269-74.

Dwinger, R. H., Grootenhuis, J.G., Murray, M., Moloo, S.K. and Gettinby, G. (1986). Susceptibility of buffaloes, cattle and goats to infection with different stocks of *Trypanosoma vivax* transmitted by *Glossina morsitans centralis*. *Research in Veterinary Science,* **41**:307–315.

Edwards, E.H. (1994). The Encyclopedia of the Horse. Dorling Kindersley publishers Ltd. London, UK. Pp 400. In Ihedioha, J.I. and Agina, O.A. (2013). Serum biochemistry profile of nigerian horses (*Equus caballus*, Linnaeus 1758). *Animal Research International,* 10 (3).

Eisler, M., Brandt, J., Bauer, B. (2001). "Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle." *Veterinary Parasitology*, **97** (3): 171–182.

Elliott, A. (1992). *"Family Ciconiidae (Storks)"*. In del Hoyo, J., Elliott, A., Sargatal, J. *Handbook of the Birds of the World*. Vol. 1: Ostrich to Ducks. Barcelona: Lynx Edicions. ISBN 84-87334-10-5. Pp. 460-461.

Elliott, A. (1992). *"Family Ciconiidae (Storks)"*. In del Hoyo, J., Elliott, A., Sargatal, J. *Handbook of the Birds of the World*. Vol. 1: Ostrich to Ducks. Barcelona: Lynx Edicions. ISBN 84-87334-10-5. p. 437.

Eltahir1, H.A., Nadia, M. Osman, A/Rahman1, A.H., Hassan1, M.A. and Mohamed-Ahmed2, M.M. (2014). Response of Tabanids (Diptera: Tabanidae) to Synthetic Attractants at Khartoum Industrial Complex Farming Area, Jabra, Khartoum, Khartoum State, Sudan. *Sudan Journal of Veterinary Research,* **29**:33-38.

Engstler, M., Pfohl, T., Herminghaus, S. (2007). "Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes," *Cell,* **131**(3):505–515

Fairlamb, A.H., Opperdoes, F.R., Borst, P. (1977). New approach to screening drugs for activity against African trypanosomes. *Nature,* **265**:270–271.

Fedewa, J.L. (2000). *Camelus bactrianus*. Animal Diversity Web. University of Michigan Museum of Zoology. *Retrieved 4 December 2012,* from http://animaldiversity.ummz.umich.edu/accounts/camelus_bactrianus

Ferguson-Lees, J. and Christie, D.A. (2001). Raptors of the World. *Christopher Helm Publishers Ltd London; New ed.*, Pp. 320.

Ferrante, A. (1985). Trypanolytic activity, agglutinins, and opsonins in sera from animals refractory to Trypanosoma lewisi. *Infection and Immunity*, **49**(2):378-382.

Field, M.C. and Carrington, M. (2009). "The trypanosome flagellar pocket," *Nature Reviews Microbiology*, **7**(11):775–786.

Field, M.C., Lumb, J.H., Adung'a, V.O., Jones, N.G. and Engstler, M. (2009). Macromolecular trafficking and immune evasion in African trypanosomes, *International Review of Cell and Molecular Biology*, **278**: 1–67.

Food and Agricultural Organization (FAO). (2016). African animal trypanosomes. *Retrieved 28 January 2016*. http://www.fao.org/3/a-i5459e.pdf

Forbes, T.D.A. and Hodgson, J. (1985). The reaction of grazing sheep and cattle to the presence of dung from the same or the other species. *Grass and Forage Science*, **2**: 177–182.

French, J. (1997). Social Behaviour. In: Elizabeth Svendsen (ed.), *The professional handbook of the donkey*, 3rd edition. London: Whittet Books, pp.127–165.

Geerts, S., Holmes, P.H., Diall, O. and Eisler, M.C. (2001). African bovine trypanosomiasis: the problem of drug resistance. *Trends in Parasitology*, **17**(1):25–28.

Geerts, S., Osaer, S., Goossens, B. and Faye, D. (2009). Trypano-tolerance in small ruminants of sub-Saharan Africa. *Trends in Parasitology*, **25**(3):132-138.

Genovese, G., Friedman, D.J., Ross, M.D., Lecordier, L., Uzureau, P., Freedman, B.I. (2010). Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science. American Association for the Advancement of Science*, **329**:841–845.

Gentry, A.; Clutton-Brock, J. and Groves, C. P. (2004). The naming of wild animal species and their domestic derivatives. *Journal of Archaeological Science*, **31**(5): 645–651.

Gerard, P.W.A. (2008) "Farmer Jerry". "Frequently asked questions about chickens & eggs". Ferry Landing Farm & Apiary. *Retrieved August 13, 2008*.

Gibson, W., Peacock, L., Ferris, V., Fischer, K., Livingstone, J., Thomas, J. and Bailey, M. (2015) Genetic recombination between human and animal parasites creates novel strains of human pathogen. *PLoS Negleted Tropical Disease*, **9**(3): e0003665. doi:10.1371/journal.pntd.0003665.

Giffin, M.D., James M. and Tom Gore, D.V.M. (1998). *Horse Owner's Veterinary Handbook* (*Second edition).* New York: Howell Book House, Pp. 18.

Grande, J.M., Negro, J.J. and María Torres, J. (2004). The evolution of bird plumage colouration; A role for feather-degrading bacteria? *Ardeola,* **51**(2):375–83. doi:10.1007/s00114-008-0462-0.

Greca, F.L. and Magez, S. (2011). Vaccination against trypanosomiasis. *Human Vaccine,* **7**(11): 1225–1233.

Groenen, M.A.M.; Archibald, A.L.; Uenishi, H.; Tuggle, C.K.; Takeuchi, Y.; Rothschild, M.F.; Rogel-Gaillard, C.; Park, C.; Milan, D.; Megens, H.J.; Li, S.; Larkin, D.M.; Kim, H.; Frantz, L.A.F.; Caccamo, M.; Ahn, H.; Aken, B.L.; Anselmo, A.; Anthon, C.; Auvil, L.; Badaoui, B.; Beattie, C.W.; Bendixen, C.; Berman, D.; Blecha, F.; Blomberg, J.; Bolund, L.; Bosse, M.; Botti, S. (2012). Analyses of pig genomes provide insight into porcine demography and evolution. *Nature,* **491**(7424):393 -398.

Grootenhuis, J.G., Dwinger, R.H., Dolan, R.B., Moloo, S.K. and Murray, M. (1990). Susceptibility of African buffalo and Boran cattle to *Trypanosoma congolense* transmitted by *Glossina morsitans centralis*. *Veterinary Parasitology,* **35**:219–231.

Groves, C.P. (2005). *Mammal Species of the World: A Taxonomic and Geographic Reference* (3rd edition). Wilson, D.E.; Reeder, D.M., eds. Baltimore: Johns Hopkins University Press, p. 160.

Grubb, P. (2005). Order Perissodactyla. In Wilson, D.E.; Reeder, D.M. *Mammal Species of the World: A Taxonomic and Geographic Reference (*3rd edition*).* Johns Hopkins University Press, pp. 630–631.

Grzimek, B., Thoney, D.A., Schlager, N., Trumpey, J.E. and Hutchins, M. (2004). *Grzimek's Animal Life Encyclopedia.* Detroit: Thomson-Gale. ISBN 0787657778.

Gutierrez, C., Corbera, J. A., Morales, M. and Büscher, P. (2006), Trypanosomosis in Goats. *Annals of the New York Academy of Sciences,* **1081**: 300–310.

Hajduk, S.L., Moore, D.R., Vasudevacharya, J., Siqueira, H., Torri, A.F., Tytler, E.M. and Esko J.D. (1989). Lysis of *Trypanosoma brucei* by a toxic subspecies of human high density lipoprotein. *Journal of Biological Chemistry,* **264**:5210–5217.

Hamilton, P.B., Stevens, J.R., Gaunt, M.W., Gidley, J., Gibson, W.C. (2004). Trypanosomes are monophyletic: evidence from genes for glyceraldehyde

phosphate dehydrogenase and small subunit ribosomal RNA. *International Journal of Parasitology,* **34**(12):1393–404.

Hardiman, J. (2007). How 90 years of poultry breeding has shaped today's industry. *Poultry International, Retrieved July 1, 2012*, from www.atbuftejoste.com/ index.php/joste/article/view/183

Hill, G.C., and Hirumi, H. (1983). African trypansomes. In *J. B. Jensen (ed.), In vitro* cultivation of protozoan parasites. CRC Press, Inc., Boca Raton, Fla. Pp 193-219.

Hiromi, S., Hie, L.K., Kaori, K., Sayaka, S., Hideo, G., Masaru, T., Naoyuki, T., Yoko, S. and Fumihito, A. (2010). The Origin and Genetic Variation of Domestic Chickens with Special Reference to Junglefowls *Gallus g. gallus* and *G. varius*. *PloS One*, 5(5): e10639. doi: 10.1371/journal.pone.0010639.

Hirst, K.K. (2008). The History of the Domestication of Goat. Accessed August 18, 2012. http://archaeology.about.com/od/do. *Sokoto Journal of Veterinary Sciences,***10**(1).

Hirumi, H., Doyle J.J., Hirumi K. (1977). African trypanosomes: cultivation of animal-infective *Trypanosoma brucei in vitro*. *Science,* **196**:992-4.

Hirumi, H., Hirumi, K., Doyle, J.J., Cross, G.A.M. (1980). *In vitro* cloning of animal-infective bloodstream forms of *Trypanosoma brucei*. *Parasitology,* 80:371-82.

Hirurni, H. and Hirurni, K. (1989). Continuous Cultivation of *Trypanosoma brucei* Blood Stream Forms in a Medium Containing a Low Concentration of Serum Protein Without Feeder Cell Layers. *Journal of Parasitology.*, **75**(6):985-989.

Hoffmann, M. (2008). *Thryonomys swinderianus*. IUCN Red List of Threatened Species. *Version 2013.1*. *International Union for Conservation of Nature*. *Retrieved 2013-09-12.*
http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T21847A9323360.en

Hornby, H.E. (1921). Trypanosomes and trypanosomiasis of cattle. *Journal of Comparative Pathology,* **34**:211-240.

Hudson, L. and Hay, F.C. (1989). *Practical Immunology*. 3rd edition. Blackwell Scientific Publications. pp. 229-236.

Huffman, B. (2006). *The ultimate ungulate page* (http://www.ultimateungulate.com) .*UltimateUngulate.com*. Retrieved December 26, 2006.

ILRAD. (1984). Anaemia in Trypanosomiasis: Haemorrhagic *Trypanosoma vivax* may provide a clue. In: Annual Report, International Laboratory for Research in Animal Diseases (ILRAD), **2**: 1 – 3.

ILRAD. (1986). Annual Report of the International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

ILRAD. (1990). Annual Report of the International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

"Info on Chicken Care". *ideas4pets. 2003. Retrieved August 13, 2016.* http://www.ideas-4-pets.com/pages-infopages/pages_id-24/index.html.

International Commission on Zoological Nomenclature (2003). "Usage of 17 specific names based on wild species which are pre-dated by or contemporary with those based on domestic animals (Lepidoptera, Osteichthyes, Mammalia): conserved. Opinion 2027 (Case 3010)". *Bulletin of Zoological Nomenclature,* **60** (1): 81–84.

IUCN Red List 2012. www.iucnredlist.org/details/22695185/0

Jaffe, J.J., McCormack, Jr J.J. and Gutteridge, W.E. (1969). Dihydrofolate reductases within the genus *Trypanosoma*. *Experimental Parasitology*. **25**:311–318.

Jahnke, H.E, Tacher, G., Keil, P. and Rojat, D. (1988). Livestock production in tropical Africa, with special reference to the tsetse-affected zone: *Livestock production in tsetse affected areas of Africa*. *Proceedings of a meeting held 23-27 November 1987*.ILCA/ILRAD, Nairobi, Kenya.

Jirků, M., Votýpka, J., Petrželková, K.J., Jirků-Pomajbíková, K., Kriegová, E., Vodička, R., Lankester, F., Leendertz, S.A.J., Wittig, R.M., Boesch, C., Modrý, D., Ayala, F.J., Leendertz, F.H. and Lukeš, J. (2015). Wild chimpanzees are infected by *Trypanosoma brucei*. *International Journal of Parasitology: Parasites Wildl*, **4**(3):277–282.

Jobling, J.A. (2010). *The Helm Dictionary of Scientific Bird Names*. London: Christopher Helm. p. 81. ISBN 978-1-4081-2501-4.

Johst, K., Brandl, R. and Pfeifer, R. (2001). Foraging in a patchy and dynamic landscape: Human land use and the White Stork. *Ecological Applications*, **11**:60–9.

Jones, T.W. and Dávila, A.M. (2001). *Trypanosoma vivax*-out of Africa. *Trends Parasitology*, **17**(2):99-101.

Kaminsky, R. and Brun, R. (1998). *In Vitro* and *In Vivo* Activities of Trybizine Hydrochloride against Various Pathogenic Trypanosome Species. *Antimicrobial Agents and Chemother*apy, **42**(11):2858–2862.

Kelly, J.M., Quack, G. and Miles, M.M. (2001). *In Vitro* and *In Vivo* Activities of Aminoadamantane and Aminoalkylcyclohexane Derivatives against *Trypanosoma brucei*. *American Society for Microbiology*, **45**(5):1360-1366.

Kennedy, P.G.E. (2004). Human African trypanosomiasis of the CNS: current issues and challenges. *Journal of Clinical Investigations*, **113**(4):496-504.

Kierszenbaum, F., Gottlieb, C. A. and Budzko, D.B. (1981). Antibody-Independent, Natural Resistance of Birds to *Trypanosoma cruzi* Infection. *The Journal of Parasitology*, **67**(5):656-660.

Kingdon, J. and Gippoliti, S. (2008). *Chlorocebus tantalus*. In: IUCN 2008. IUCN Red List of Threatened Species. *Retrieved 4 January 2009*. www.iucnredlist.org /pdflink.4259825

Kingdon, J., Butynski, T. M. and De Jong, Y. (2008). *Erythrocebus patas*. In: IUCN 2008. IUCN Red List of Threatened Species. *Retrieved* 4 January 2009. http://eol.org/pages/311175/hierarchy_entries/24932308/details

Klingbeil, M.M., Motyka, S.A. and Englund, P.T. (2002). Multiple mitochondrial DNA polymerases in *Trypanosoma brucei*. *Molecular Cell*, **10**:175-186.

Kreling, K. (2005). The Horse's Teeth. Horses' Teeth and Their Problems: Prevention, Recognition, and Treatment. *Guilford, CT: Globe Pequot*, pp. 12–13.

Lamprecht, J. (1987). "Female reproductive strategies in bar-headed geese (Anser indicus): Why are geese monogamous?". *Behavioral Ecology and Sociobiologydon*, **21**(5):297–305.

Legros, D., Ollivier, G., Gastellu-Etchegorry, M., Paquet, C., Burri, C., Jannin, J. and Büscher, P. (2002). Treatment of human African trypanosomiasis - present situation and needs for research and development. *Lancet Infectious Diseases*, **2**(7):437-440.

Le Page, R.W. (1967). Short term cultivation of *Trypanosoma brucei in vitro* at 37ºC. *Nature*, **216**:1141-2.

Le Tissier, P. R., Peters, J. and Skidmore, C. J. (1994). Development of an assay method for purine catabolic enzymes in the mouse and its adaptation for use on an autoanalyzer. *Analytical Biochemistry*, **222**:168-175.

Lever, C. (2005). *Naturalised Birds of the World*. *London, United Kingdom*: T & A D Poyser. pp. 24–26.

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., Zody, M.C., Mauceli, E., Xie, X., Breen, M., Wayne, R.K., Ostrander, E.A. *et al.* (2005). Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature*, **438**(7069):803–819.

Learning About Goats.Texas Department of Agriculture. Pp. 1-8. Retrived 27th March, 2017. http://www.tsgra.com/admin/resources/secogoats.pdf

Losos, G.J., Paris, J., Wilson, A.J. and Dar, F.K. (1973). Pathology of the disease in cattle caused by *T. congolense*. *Bulletin of Epizootic diseases of Africa*, **12**:239 – 248.

Lugli, E.B., Pouliot, M., Portela, M.D.P.M., Loomis, M.R., Raper, J. (2004). Characterization of primate trypanosome lytic factors. *Molecular and Biochemical Parasitology*, **138**(1):9–20. pmid:1550091.

Madge and McGowan. (2002). *Pheasants, Partridges and Grouse*. Princeton University Press, Princeton, New Jersey, and Oxford. 488 pp.

Magona, J.W., Mayende, J.S.P., Olaho-Mukani, W., Coleman, P.G., Jonsson, N.N., Welburn, S.C. and Eisler, M.C. (2003). A comparative study on the clinical, parasitological and molecular diagnosis of bovine trypanosomosis in Uganda. *Onderstepoort Journal of Veterinary Research*, **70**(3):213-218.

Mahan, S.M., Hendershot, L. and Black, S.J. (1986). Control of trypanodestructive antibody responses and parasitaemia in mice infected with *Trypanosoma* (*Duttonella) vivax. Infection and Immunity*, **54**:213-221.

Maré, C.J. (2004). African animal trypanosomiasis. http://www.vet.uga.edu/vpp/gray

Matthews, K. R. (2005). The developmental cell biology of *Trypanosoma brucei*. *Journal of Cell Sciences*, **118**:283-290.

Matthews, K. R., J. R. Ellis, and A. Paterou. (2004). Molecular regulation of the life cycle of African trypanosomes. *Trends Parasitology*, **20**:40-47.

Matthews, J. (2008) The Value of Grasscutters, *World Ark*, (January–February, 2008), pp. 23–24.

McEvoy, S.M., and Maeda, N. (1988). Complex events in the evolution of the haptoglobin gene cluster in primates. *Journal of Biochemistry*, **263**:15740-15747.

Medical ecology.(2004). Medicalecology.org. http://dna.kdna.ucla.edu/168-2011/african-tryps/pdf%20files%20of%20papers/d african_trypano.htm

Mendoza-Palomares, C., Biteau, N., Giroud, C., Coustou, V., Coetzer, T., Authié, E., Boulangé, A. and Baltz, T. **(**2008). Molecular and Biochemical Characterization of a Cathepsin B-Like Protease Family Unique to *Trypanosoma congolense. American society for Microbiology*, **7**(4):684-697.

Merriam-webster.com (2012). Piglet - Definition and More from the Free Merriam-Webster Dictionary. Merriam-webster.com. 31 August 2012. Retrieved 15 September 2013. http://eol.org/pages/42318/details; https://en.wikipedia.org/wiki/Pig

Micro-livestock Micro-livestock: Appropedia (1991). - Little-known Small Animals with a Promising Economic Future 7. http://www.appropedia.org/Micro-livestock. Assessed 27th March, 2017.

Moloo S.K. (1993) The distribution of Glossina species in Africa and their natural hosts. *Insect Science and its Application*, **14**:511-527.

Molyneux, D.H. and Ashford, R.W. (1983). *The biology of trypanosome and leishmania. Parasites of man and domestic animals*. Taylor and Francis limited. London, Pp. 29-151.

Morrison, W.I. and Murray, M. (1985). The role of humoral immune responses in determining susceptibility of A/J and C57B1/6 mice to infection with *Trypanosoma congolense*. *Parasite Immunology*, **7**:63-80.

Morrison, W.I., Murray, M. and. McIntyre, W.I.M. (1981). Bovine trypanosomiasis. In: Diseases of Cattle in the Tropics. Economic and Zoonotic Relevance. M. Ristic and I. McIntyre eds. *Current Topics in Veterinary Medicine and Animal Science*, Vol. 6. The Hague: Martinus Nyhoff. pp. 469-497.

Morrison, W.I., Roelants, G.E., Mayor-Withey, K.S. and Murray M. (1978). Susceptibility of inbred strains of mice to *Trypanosoma congolense*: correlation with changes in spleen lymphocyte populations. *Clinical and Experimental Immunology*, **32**:25-40.

Muranjan, M., Wang, Q., Li, Y., Hamilton, E., Otieno-Omondi, F.P., Wang, J., Van Praagh, A., Grootenhuis, J.G., And Black, S.J. (1997) The Trypanocidal Cape Buffalo Serum Protein Is Xanthine Oxidase. *American Society for Microbiology. Infection and Immunity*, **65**(9):3806–3814.

Murray, M. (1974). The pathology of African trypanosomiasis. In: Progress in Immunology II. L. Brent and J. Holborow, eds. Amsterdam: *North Holland Publishing Company*, pp. 181-192.

Murray, M., Trail, J.C., Davis, C.E., Black, S.J. (1984). Genetic resistance to African Trypanosomiasis. PubMed Commons. *Journal of Infectious Diseases*, **149**(3):311-319.

Nadia, M. Osman, Ghada, Kaila, J., Eltahir, H.A. and Rahman, A.H.A. (2011). Serum Biochemical Changes in Nubian Goats, Nilotic Dwarf Goats and Garag Ewes Experimentally Infected with a Mechanically Transmitted Trypanosoma vivax Stock. *International Journal of Biological Chemistry*, **5**:136-142.

Naessens, J., Leak, S.G., Kennedy, D.J., Kemp, S.J., Teale, A.J. (2003). Responses of bovine chimaeras combining trypanosomosis resistant and susceptible genotypes to experimental infection with Trypanosoma congolense. *Veterinay Parasitology*, **111**:125–142.

Namangala, B. (2011). How the African trypanosomes evade host immune killing. *Parasite Immunology*, **33**(8):430-437. doi:10.1111/j.1365-3024.2011.01280.x.

National Geographic (2012). Bactrian Camel: Camelus bactrianus. Retrieved 28 November 2012, from https://www.revolvy.com/main/index.php?s=Camel%20meat&item_type=topic.

Ng'ayo, M.O., Njiru, K.Z., Kenya, U.E., Muluvi, M.G., Osir, O.E. and Masiga, K.D. (2005). Detection of trypanosomes in small ruminants and pigs in western Kenya: important reservoirs in the epidemiology of sleeping sickness? *Kinetoplastid Biology and Disease*, **4**: 5.

Oak Tree Veterinary Centre (2012). Information for Rabbit Owners — Oak Tree Veterinary Centre. Oaktreevet.co.uk. Archived from the original on 2016-06-23. Retrieved 30 August 2015, from https://blog.nus.edu.sg/ lsm2251student /2010/ 04/14/who-eats-its-poop

Onah, D.N., Hopkins, J. and Luckins, A.G. (1996). Haematological changes in sheep experimentally infected with *Trypanosoma evansi*. *Parasitology Research*, **82**:659 – 663.

OIE *Terrestrial Manual* (2008). Trypanosomosis. https://www.oie.int/doc/ged/ *D7709.PDF*.

OIE *Terrestrial Manual* (2009). Trypanosomosis. *www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/*

Olubayo, R.O., Grootenhuis, J.G. and Rurangirwa, F.R. (1990). Susceptibility of African buffalo and Boran cattle to intravenous infection with *Trypanosoma congolense* (IL1180) bloodstream forms. *Tropical Medical Parasitology*, **41**:181–184.

Omotainse, S.O. and Anosa, V.O. (1995). Leucocyte and thrombocyte responses in dogs experimentally infected with *Trypanosoma brucei*. *Revue d'Elevage et de Medecine veterinaire des Pays tropicaux*, **48**:254 – 258.

Ouchterlony, O. and Nilsson, L.A. (1978). Immunodiffusion and immunoelectrophoresis. In: *Handbook of experimental immunology*, DM Weir (ed), 3rd ed., Oxford: Blackwell Scientific Publication, 19.16.-19.28.

Ottawa, O.N. (2010). Immunobiology of African Trypanosomes: Need of Alternative Interventions. Review Article. *Journal of Biomedicine and Biotechnology*, Volume 2010 (2010), Article ID 389153, 24 pp.

Paling, R.W., Moloo, S.K., Scott, J.R., McOdimba, F.A., Logan-Henfrey, L.L., Murray, M., Williams, D.J. (1991) Susceptibility of N'Dama and Boran cattle to tsetse-transmitted primary and rechallenge infections with a homologous serodeme of *Trypanosoma congolense*. *Parasite Immunology*,**13**(4):413-425.

Pays, E., and Nolan, D. P. (1998). Expression and function of surface proteins in *Trypanosoma brucei*. *Molecular Biochemical Parasitology*. **91**:3–36.

Pays, E., Vanhamme, L., Perez-Morga, D. (2004). Antigenic variation in *Trypanosoma brucei*: facts, challenges and mysteries. *Current Opinion in Microbiology*,**7**:369– 374.

Peacock, L., Ferris, V., Sharma, R., Sunter, J., Bailey, M., Carrington, M. and Gibson, W. (2011). Identification of the meiotic life cycle stage of *Trypanosoma brucei* in the tsetse fly. Proceedings of the National Academy of Sciences of the United State of America, **108**(9):3671–6. doi:10.1073/ pnas.1019423108. Epub 2011 Feb 14. PubMed PMID: 21321215; PubMed Central PMCID: PMC3048101.

Peacock, L., Ferris, V., Bailey, M. and Gibson, W. (2014) Mating compatibility in the parasitic protist *Trypanosoma brucei*. *Parasites & Vectors*, **7**:78.

Peregrine, A.S. (1994). Chemotherapy and delivery systems: haemoparasites, *Veterinary Parasitology*, **54**(1–3):223–248.

Peter, D., Honigberg, B.M. and Fern, A.M. (1976). An improved method of cryopreservation of *Trypanosoma (Nannomonas) Congolense* brooden in liquid nitrogen. *Journal of Parasitology*, **62**(1):136-137.

Pigeon Control Resource Centre (PCRC). (2009). Goodview man finds racing pigeon. Winona Post. Retrieved 2017-03-27, from http://www.pigeoncontrolresourcecentre.org/html/pigeon-and-bird-control-products.htmlPorter.

Poelvoorde, P., Vanhamme, L., Van Den Abbeele, J., Switzer, W.M., Pays, E. (2004). Distribution of apolipoprotein L-I and trypanosome lytic activity among primate sera. *Molecular and Biochemical Parasitology*, **134**:155–157. pmid:14747153.

Price, S.D., Spector, D.L., Gail, R. and Burn, B.B. (1998). *The Whole Horse Catalog: Revised and Updated* (Revised edition). New York: Fireside. ISBN 0-684-83995-4. Pp.158-159.

Pugh, D.G. (2001). *Sheep and Goat Medicine*. Elsevier Health Sciences. ISBN 0-7216-9052-1. 468 pp.

Raper, J., and Friedman, D.J. (2013). Parasitology: molecular one-upmanship. *Nature*, **501**:322–323.

Rasmussen, P.C. and Anderton, J.C. (2005). *Birds of South Asia: The Ripley Guide. Volume 2*. Washington, DC: Smithsonian Institution and Barcelona: Lynx edicions. p. 63. ISBN 84-87334-66-0.

Reduth, D., Grootenhuis, J.G., Olubayo, R.O., Muranjan, M., Otieno-Omondi, F.P., Morgan, G.A., Brun, R., Williams, D.J.L. and Black, S.J. (1994). African buffalo serum contains novel trypanocidal protein. *Journal of Eukaryotic Microbiology*, **41**:95–103.

Roberts, M.B.V. (1986). *Biology: A Functional Approach*. Nelson Thornes, pp. 234–235, 241.

Roder, P.L., Scott, J.M., and Pegram, R.G. (1984). Acute *Trypanosoma vivax* infection of Ethiopian cattle in the apparent absence of tsetse. *Tropical Animal Health and Production*, **16**:141-147.

Roelants, G.E., Fumoux, F., Pinder, M., Queval, R., Bassinga, A. and Authié, E. (1987). Identification and selection of cattle naturally resistant to African trypanosomiasis. PubMed Commons. *Acta Tropica*, **44**(1):55-66.

Rudenko, G. (1999). Genes involved in phenotypic and antigenic variation in African trypanosomes and malaria. *Current Opinion in Microbioly*, **2**:651–656.

Rudenko, G., Cross, G.A.M. and Borst, P. (1998). Changing the end: variation orchestrated at the telomeres of African trypanosomes. *Trends Microbiology*, **6**:113–116.

Saunders Comprehensive Veterinary Dictionary (2007). Definition of the word Salivarian. 3rd ed. Elsevier, Inc. http://medical-dictionary. thefreedictionary.com/salivarian. Assessed 27th march, 2017.

Schmidt-Nielsen, K. (1964). Desert Animals: Physiological Problems of Heat and Water. New York: Oxford University Press. *Cited in "Coat of fur on the camel"*. Temperature and Water Relations in Dromedary Camels (*Camelus dromedarius*). Davidson College. Archived from *the original* on February 25, 2017.

Schuster, F.L. and Sullivan, J.J. (2002). Cultivation of clinically significant Hemoflagellates. *Clinical Microbiology Review*, **15**(3):374-389.

Science and Nature. *Animals: Feral Pigeon*. (Web article). BBC.CO.UK. Retrieved 2017-01-05.

Seed, J.R., Sechelski, J.B. and Loomis, M.R. (1990). A survey for a trypanolytic factor in primate sera. *Journal of Parasitology*, **37**:393-400.

Sekoni, V.O. (1994). Reproductive disorders caused by animal trypanosomiasis: a review. *Theriogenology*, **42**: 557 – 570.

Simarro, P.P., Jannin, J. and Cattand, P. (2008). Eliminating human African trypanosomiasis: where do we stand and what comes next? *PLoS Medicine*, **5**(2):e55.

Simpson, A.M., Hughes, D. and Simpson, L. (1985). *Trypanosoma brucei*: differentiation of *in vitro*-grown bloodstream trypanomastigotes into procyclic forms. *Journal of Protozoology*, **32**:672-677, 685.

Sinclair, I. (1994). *Field Guide to the Birds of Southern Africa*. Cape Town: Struik Publisher, 368pp.

Sinclair, I., Hockley, P., Tarboton, W., Ryan, P. (2011). *SASOL birds of Southern Africa*. Struik Publisher, 464 pp.

Taiwo, V.O., Nantulya, V.M., Moloo, S.K. and Ikede, B.O. (1990). Role of the chancre in induction of immunity to tsetse-transmitted *Trypanosoma congolense* in goats. *Veterinary Immunology and Immunopathology*, **26**: 59-70.

Tasker, M., Wilson, J., Sarkar, M., Hendriks, E. and Matthews, K. (2000). A Novel Selection Regime for Differentiation Defects Demonstrates an Essential Role for the Stumpy Form in the Life Cycle of the African Trypanosome. *Molecular Biology of the Cell*, **11**(5):1905-17.

Thermo Fisher Scientific. (2015). pH and CO2 levels. © 2015 Thermo Fisher Scientific Inc. All Rights Reserved. https://www.thermofisher.com/us/en/home/life-science/lab-equipment/ph-ion-conductivity-oxygen-measurement.html.

Thomson, R., Genovese, G., Canon, C., Kovacsics, D., Higgins, M.K, Carrington, M. (2014). Evolution of the primate trypanolytic factor APOL1. *Proceedings of National Academic Sciences, USA. National Academic Sciences,* **111**:2130–2139.

Thomson, R., Molina-Portela, P., Mott, H., Carrington, M., Raper, J. (2009). Hydrodynamic gene delivery of baboon trypanosome lytic factor eliminates both animal and human-infective African trypanosomes. *Proceeding of National Academic Sciences USA.***106**:19509–19514.

Tomlinson, S., M. Muranjan, V. Nussenzweig, and J. Raper. (1997). Haptoglobin-related protein and apolipoprotein AI are components of the two trypanolytic factors in human serum. *Molecular and Biochemical Parasitology,* **86**:117–120.

Trail, J.C., D'Ieteren, G.D., Teale, A.J. (1989) Trypanotolerance and the value of conserving livestock genetic resources. *Genome,* **31**(2):805-812.

Uilenberg, G. (1998). A field guide for the diagnosis, treatment and prevention of african animal trypanosomosis. (adapted from the original edition by W.P. Boyt). Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00100 Rome, Italy. ISBN 92-5-104238-1. P. 34.

Van den Bossche, P., Doran, M. and Connor, R.J. (2000). An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta Tropica*, **75**(2):247–258.

Van Xong, H., Vanhamme, L., Chamekh, M., Chimfwembe, C.E., Van Den Abbeele, J., Pays, A. (1998). A VSG Expression Site–Associated Gene Confers Resistance to Human Serum in *Trypanosoma rhodesiense*. *Cell,* **95**:839–846.

Vanhamme, L., Pays, E., McCulloch, R., Barry J.D. (2001). An update on antigenic variation in African trypanosomes. *Trends Parasitology*, **17**:338–343.

Vanhollebeke, B., Truc, P., Poelvoorde, P., Pays, A., Joshi, P.P., Katti, R., Jannin, J.G. and Pays, E. (2006). Human *Trypanosoma evansi* infection linked to a lack of apolipoprotein L-I. *New Englan Journal of Medicine,* **355**(26):2752-6.

Verlinde, C.L., Hannaert, V., Blonski, C., Willson, M., Perie, J.J., Fothergill-Gilmore, L.A., Opperdoes, F.R., Gelb, M.H., Hol, W.G., Michels, P.A. (2001). Glycolysis as a target for the design of new anti-trypanosome drugs. *Drug Resistance Updates,* **4**:50–65.

Vickerman, K. (1965). Polymorphism and mitochondrial activity in sleeping sickness. *Nature*, **208**:762-766.

Vickerman, K. (1985). Developmental cycles and biology of pathogenic trypanosomes. *British Medical Bulletin*, **41**:105-114.

Walker, M. (2009). *Wild camels 'genetically unique'*. Earth News (BBC). *Retrieved 4 December 2012*. https://en.wikipedia.org/wiki/Camel.

Wanda Embar. 2008-2015. Animal facts-Donkeys. http://www.veganpeace.com/animal_facts/Donkeys.htm

Wang, J., Van Praagh, A., Hamilton, E., Wang, Q., Zou, B., Muranjan, M., Murphy, N.B., Black, S.J. (2002). Serum xanthine oxidase: origin, regulation, and contribution to control of trypanosome parasitemia. *Antioxidants & Redox Signaling*, **4**(1):161-78.

Why study pigeons? To understand why there are so many colors of feral pigeons. *Cornell Lab of Ornithology*. Retrieved 2015-01-06. http://www.birds.cornell.edu/programs/urbanbirds/about/ubs_PIWWhyStudyEN.html

Wink, M., Heidrich, P. and Fentzloff, C. (1996). A mtDNA phylogeny of sea eagles (genus *Haliaeetus*) based on nucleotide sequences of the cytochrome b-gene. *Biochemical Systematics and Ecology*, **24**:783-791.

Wong, G.K. (2004). A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. *Nature*, **432**:717–722.

Woo, P.T.K. (1989). The haematocrit centrifuge for the detection of trypanosomes in blood. *Canadian Journal of Zoology*, **47**:921-923.

Woods, C.A. and Kilpatrick, C.W. (2005). *"Infraorder Hystricognathi"*. In Wilson, D.E.; Reeder, D.M. Mammal Species of the World: A Taxonomic and Geographic Reference (3rd ed.). Johns Hopkins University Press. p. 1545. ISBN 978-0-8018-8221-0. OCLC.

World Heritage Encyclopedia. *Hooded vulture.* WHEBN0000187168. http://nationalpubliclibrary.com/articles/Hooded_vulture

World Organization for Animal Health (OIE). (2009). African Animal Trypanosomiasis. Pp 1-5.

Yorzinski, J.L. and Annop, K. R. (2012). Peacock Copulation Calls Attract Distant Females. *Behaviour*, **150**:61–74.

http://tryps.rockefeller.edu/Default.html (African trypanosomiasis).

http://www.foxnews.com/health/2014/05/09/why-pigs-are-so-valuable-for-medical-research.html

http://www.vet.uga.edu/vpp/gray_book/Handheld/aat.htm

https://www.revolvy.com/main/index.php?s=Rabbits&item_type=topic. Rabbit Habitats. Retrieved 27 March 2017.

http://eol.org/pages/326380/details

https://a-z-animals.com/animals/jackal/

https://a-z-animals.com/animals/patas-monkey/

https://anahitaqatuzyan.wordpress.com/2017/04/12/pecoke-and-bigest-butterfly-in-the-world/

https://anahitaqatuzyan.wordpress.com/2017/04/12/pecoke-and-bigest-butterfly-in-the-world/

http://www.newworldencyclopedia.org/entry/Goose

http://www.newworldencyclopedia.org/entry/Goose

https://www.beautyofbirds.com/commonbuzzards.html

http://www.ideas-4-pets.com/pages-infopages/pages_id-24/index.html.

http://www.ideas-4-pets.com/pages-infopages/pages_id-24/index.html.
http://wwtrade.org/m/index.php/Chicken

http://animaldiversity.org/accounts/Canis_aureus/#geographic_range

http://nationalpubliclibrary.com/articles/Hooded_vulture

https://media1.britannica.com/eb-media/82/154482-004-5ADDEFD9.jpg

http://eol.org/pages/38902/details

http://si-referral.duniakicau.web.id/IT/en/2807-2696/camel_6431_si-referral-duniakicau.html

https://media1.britannica.com/eb-media/82/154482-004-5ADDEFD9.jpg

http://eol.org/pages/38902/details

http://si-referral.duniakicau.web.id/IT/en/2807-2696/camel_6431_si-referral-duniakicau.html

http://www.fao.org/3/a-i5459e.pdf

http://www.worldanimal.foundation/advocate/donkeys

https://www.pinterest.com/pin/432908582912666023

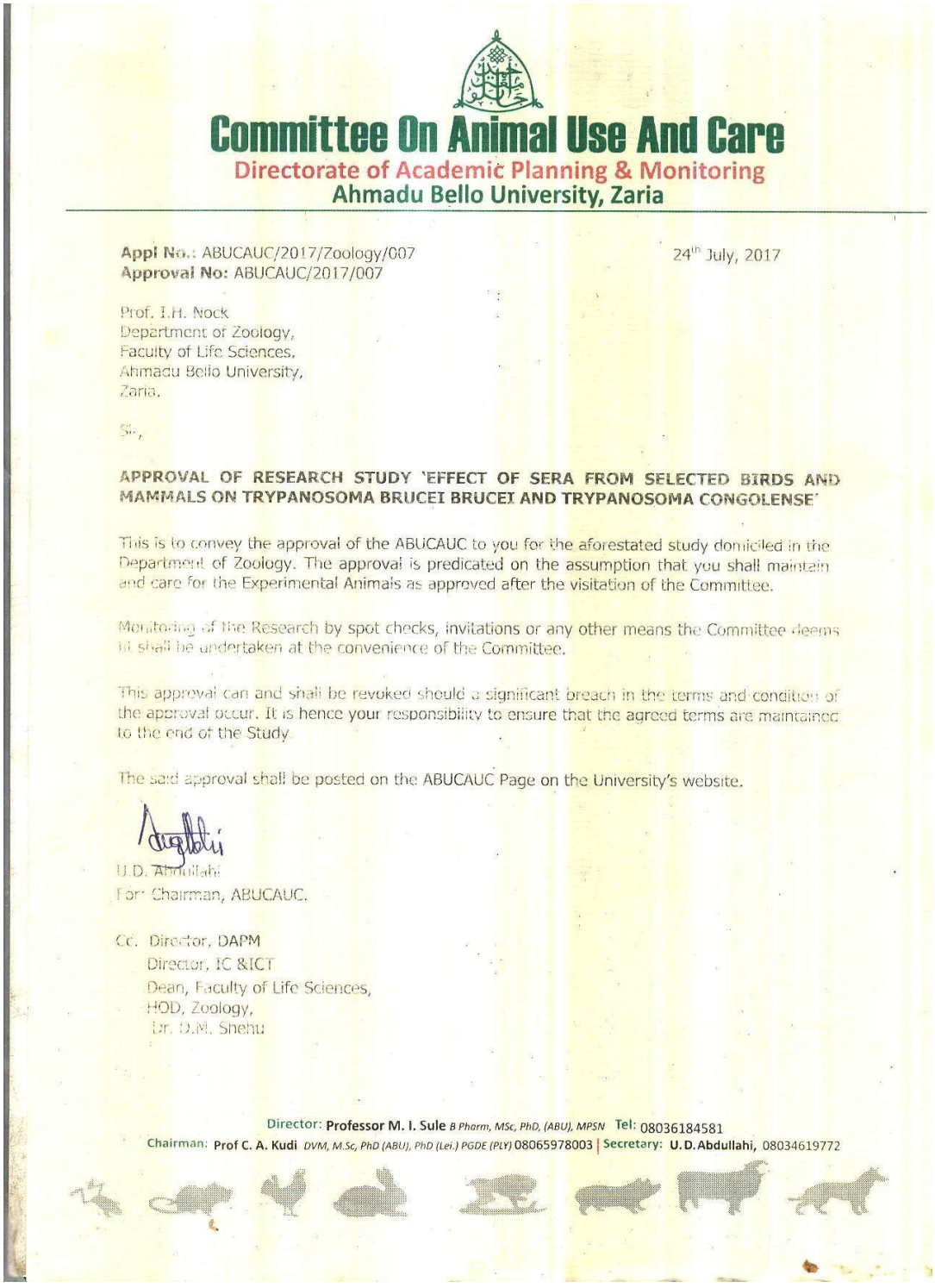
http://eol.org/pages/327977/details

www.iiste.org/Journals/index.php/JNSR/article/download/25932/26710

www1.paho.org/hq/dmdocuments/2010/ZoonosesVol-3.pdf?ua=1

APPENDICES

Appendix I: Ethical approval



Committee On Animal Use And Care
Directorate of Academic Planning & Monitoring
Ahmadu Bello University, Zaria

Appl No.: ABUCAUC/2017/Zoology/007
Approval No: ABUCAUC/2017/007

24th July, 2017

Prof. I.H. Nock
Department of Zoology,
Faculty of Life Sciences,
Ahmadu Bello University,
Zaria.

Sir,

APPROVAL OF RESEARCH STUDY 'EFFECT OF SERA FROM SELECTED BIRDS AND MAMMALS ON TRYPANOSOMA BRUCEI BRUCEI AND TRYPANOSOMA CONGOLENSE'

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Zoology. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.

U.D. Abdullahi
For: Chairman, ABUCAUC.

Cc. Director, DAPM
Director, IC &ICT
Dean, Faculty of Life Sciences,
HOD, Zoology,
Dr. D.M. Shehu

Director: **Professor M. I. Sule** *B Pharm, MSc, PhD, (ABU), MPSN* Tel: 08036184581
Chairman: **Prof C. A. Kudi** *DVM, M.Sc, PhD (ABU), PhD (Lei.) PGDE (PLY)* 08065978003 | Secretary: **U. D. Abdullahi,** 08034619772

Appendix II: One-way analysis of variance comparing the survival time of *Trypanosoma brucei brucei* in the sera of the selected birds *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Broiler cock	3	21.0000	.00000	.00000	21.0000	21.0000	21.00	21.00
Broiler hen	3	5.0000	.00000	.00000	5.0000	5.0000	5.00	5.00
Shika brown commercial cock	3	19.6667	4.04145	2.33333	9.6271	29.7062	15.00	22.00
Shika brown commercial hen	3	11.3333	1.15470	.66667	8.4649	14.2018	10.00	12.00
Parent Stock White cock	3	21.3333	1.15470	.66667	18.4649	24.2018	20.00	22.00
Parent Stock White hen	3	16.6667	2.88675	1.66667	9.4956	23.8378	15.00	20.00
Parent Stock Brown Cock	3	16.0000	1.73205	1.00000	11.6973	20.3027	15.00	18.00
Parent Stock Brown Hen	3	10.0000	2.00000	1.15470	5.0317	14.9683	8.00	12.00
Guinea fowl	3	9.3333	1.15470	.66667	6.4649	12.2018	8.00	10.00
Domestic pigeon	3	20.6667	1.15470	.66667	17.7982	23.5351	20.00	22.00
Goose	3	16.0000	3.46410	2.00000	7.3947	24.6053	14.00	20.00
Crown crane	3	18.6667	4.16333	2.40370	8.3244	29.0090	14.00	22.00
Peacock	3	18.3333	3.78594	2.18581	8.9285	27.7381	14.00	21.00
Spotted eagle owl	3	11.3333	1.15470	.66667	8.4649	14.2018	10.00	12.00
Hooded vulture	3	20.6667	1.15470	.66667	17.7982	23.5351	20.00	22.00
Buzzard	3	11.3333	1.15470	.66667	8.4649	14.2018	10.00	12.00
Control	2	30.0000	.00000	.00000	30.0000	30.0000	30.00	30.00
Total	50	16.0400	5.94519	.84078	14.3504	17.7296	5.00	30.00

Appendix III: One-way analysis of variance comparing the survival time of *Trypanosoma congolense* in the sera of the selected birds *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Broiler cock	3	34.3333	2.08167	1.20185	29.1622	39.5045	32.00	36.00
Broiler hen	3	33.6667	1.52753	.88192	29.8721	37.4612	32.00	35.00
Shika brown commercial cock	3	65.0000	5.00000	2.88675	52.5793	77.4207	60.00	70.00
Shika brown commercial hen	3	66.0000	5.29150	3.05505	52.8552	79.1448	60.00	70.00
Parent Stock White cock	3	65.0000	5.00000	2.88675	52.5793	77.4207	60.00	70.00
Parent Stock White hen	3	62.6667	4.61880	2.66667	51.1929	74.1404	60.00	68.00
Parent Stock Brown Cock	3	64.3333	4.04145	2.33333	54.2938	74.3729	60.00	68.00
Parent Stock Brown Hen	3	49.3333	6.11010	3.52767	34.1550	64.5117	44.00	56.00
Guinea fowl	3	75.6667	1.15470	.66667	72.7982	78.5351	75.00	77.00
Domestic pigeon	3	49.6667	.57735	.33333	48.2324	51.1009	49.00	50.00
Goose	3	37.3333	2.30940	1.33333	31.5965	43.0702	36.00	40.00
Crown crane	3	48.3333	2.88675	1.66667	41.1622	55.5044	45.00	50.00
Peacock	3	8.6667	3.21455	1.85592	.6813	16.6521	5.00	11.00
Spotted eagle owl	3	102.3333	2.51661	1.45297	96.0817	108.5849	100.00	105.00
Hooded vulture	3	86.0000	3.46410	2.00000	77.3947	94.6053	84.00	90.00
Buzzard	2	133.5000	2.12132	1.50000	114.4407	152.5593	132.00	135.00
Control	3	74.6667	.57735	.33333	73.2324	76.1009	74.00	75.00
Total	50	60.7200	26.68259	3.77349	53.1369	68.3031	5.00	135.00

Appendix IV: One-way analysis of variance comparing the survival time of *Trypanosoma brucei brucei* in the sera of the selected rodents *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Cane rat (grass-cutter)	3	27.6667	4.04145	2.33333	17.6271	37.7062	23.00	30.00
Rabbit	3	23.0000	1.00000	.57735	20.5159	25.4841	22.00	24.00
Control	2	30.0000	.00000	.00000	30.0000	30.0000	30.00	30.00
Total	8	26.5000	3.77964	1.33631	23.3401	29.6599	22.00	30.00

Appendix V: One-way analysis of variance comparing the survival time of *Trypanosoma congolense* in the sera of the selected rodents *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Cane rat (grass-cutter)	3	56.0000	3.46410	2.00000	47.3947	64.6053	54.00	60.00
Rabbit	3	131.3333	1.15470	.66667	128.4649	134.2018	130.00	132.00
Control	3	74.6667	.57735	.33333	73.2324	76.1009	74.00	75.00
Total	9	87.3333	34.02573	11.34191	61.1788	113.4878	54.00	132.00

Appendix VI: One-way analysis of variance comparing the survival time of *Trypanosoma brucei brucei* in the sera of the selected small ruminants *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
West African dwarf goat	3	21.3333	1.15470	.66667	18.4649	24.2018	20.00	22.00
Red Sokoto goat	3	41.3333	1.15470	.66667	38.4649	44.2018	40.00	42.00
Sahel goat	3	37.3333	6.42910	3.71184	21.3626	53.3041	30.00	42.00
Yankassa sheep	3	43.0000	1.73205	1.00000	38.6973	47.3027	42.00	45.00
Uda sheep	3	44.0000	1.73205	1.00000	39.6973	48.3027	42.00	45.00
Balami sheep	3	45.0000	.00000	.00000	45.0000	45.0000	45.00	45.00
Control	2	30.0000	.00000	.00000	30.0000	30.0000	30.00	30.00
Total	20	37.8000	8.66086	1.93663	33.7466	41.8534	20.00	45.00

Appendix VII: One-way analysis of variance comparing the survival time of *Trypanosoma congolense* in the sera of the selected small ruminants *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
West African dwarf goat	3	100.0000	8.71780	5.03322	78.3438	121.6562	90.00	106.00
Red Sokoto goat	3	151.3333	4.16333	2.40370	140.9910	161.6756	148.00	156.00
Sahel goat	3	150.0000	2.00000	1.15470	145.0317	154.9683	148.00	152.00
Yankassa sheep	3	171.3333	2.30940	1.33333	165.5965	177.0702	170.00	174.00
Uda sheep	3	152.6667	3.05505	1.76383	145.0775	160.2558	150.00	156.00
Balami sheep	3	148.6667	1.15470	.66667	145.7982	151.5351	148.00	150.00
Control	3	74.6667	.57735	.33333	73.2324	76.1009	74.00	75.00
Total	21	135.5238	32.97669	7.19610	120.5130	150.5346	74.00	174.00

Appendix VIII: One-way analysis of variance comparing the survival time of *Trypanosoma brucei brucei* in the sera of the selected large ruminants *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ndama	3	18.6667	1.15470	.66667	15.7982	21.5351	18.00	20.00
Sokoto Gudali	3	51.3333	11.01514	6.35959	23.9702	78.6965	40.00	62.00
Frasian cross (Bunaji)	3	59.6667	6.80686	3.92994	42.7575	76.5758	52.00	65.00
White Fulani	3	47.3333	4.61880	2.66667	35.8596	58.8071	42.00	50.00
Control	2	30.0000	.00000	.00000	30.0000	30.0000	30.00	30.00
Total	14	42.2143	16.60481	4.43782	32.6270	51.8016	18.00	65.00

Appendix IX: One-way analysis of variance comparing the survival time of *Trypanosoma congolense* in the sera of the selected large ruminants *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ndama	3	106.0000	3.46410	2.00000	97.3947	114.6053	104.00	110.00
Sokoto Gudali	3	178.0000	2.00000	1.15470	173.0317	182.9683	176.00	180.00
Frasian cross (Bunaji)	3	171.6667	1.15470	.66667	168.7982	174.5351	171.00	173.00
White Fulani	3	171.3333	2.30940	1.33333	165.5965	177.0702	170.00	174.00
Control	3	74.6667	.57735	.33333	73.2324	76.1009	74.00	75.00
Total	15	140.3333	43.59172	11.25533	116.1930	164.4736	74.00	180.00

Appendix X: One-way analysis of variance comparing the survival time of *Trypanosoma brucei brucei* in the sera of the selected non-ruminant mammals *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Donkey	3	18.0000	3.46410	2.00000	9.3947	26.6053	14.00	20.00
Horse	3	20.6667	1.15470	.66667	17.7982	23.5351	20.00	22.00
Camel	3	16.0000	3.46410	2.00000	7.3947	24.6053	14.00	20.00
Pig Pettin	3	21.6667	1.52753	.88192	17.8721	25.4612	20.00	23.00
Pig Large white	3	22.6667	.57735	.33333	21.2324	24.1009	22.00	23.00
Pig Ducros	3	21.3333	1.15470	.66667	18.4649	24.2018	20.00	22.00
Jackal	2	18.5000	.70711	.50000	12.1469	24.8531	18.00	19.00
Control	2	30.0000	.00000	.00000	30.0000	30.0000	30.00	30.00
Total	22	20.8182	4.05482	.86449	19.0204	22.6160	14.00	30.00

Appendix XI: One-way analysis of variance comparing the survival time of *Trypanosoma congolense* in the sera of the selected non-ruminant mammals *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Donkey	3	71.3333	7.23418	4.17665	53.3626	89.3040	63.00	76.00
Horse	3	198.0000	.00000	.00000	198.0000	198.0000	198.00	198.00
Camel	3	58.0000	3.46410	2.00000	49.3947	66.6053	54.00	60.00
Pig Pettin	3	181.3333	1.52753	.88192	177.5388	185.1279	180.00	183.00
Pig Large white	3	183.6667	2.08167	1.20185	178.4955	188.8378	182.00	186.00
Pig Ducros	3	183.3333	1.52753	.88192	179.5388	187.1279	182.00	185.00
Jackal	2	6.5000	2.12132	1.50000	-12.5593	25.5593	5.00	8.00
Control	3	74.6667	.57735	.33333	73.2324	76.1009	74.00	75.00
Total	23	124.5217	68.74847	14.33505	94.7927	154.2508	5.00	198.00

Appendix XII: One-way analysis of variance comparing the survival time of *Trypanosoma brucei brucei* in the sera of the selected zoo primates *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Red Pattas monkey	3	22.0000	1.73205	1.00000	17.6973	26.3027	20.00	23.00
Tantalus monkey	3	19.6667	.57735	.33333	18.2324	21.1009	19.00	20.00
Control	2	30.0000	.00000	.00000	30.0000	30.0000	30.00	30.00
Total	8	23.1250	4.48609	1.58607	19.3745	26.8755	19.00	30.00

Appendix XIII: One-way analysis of variance comparing the survival time of *Trypanosoma congolense* in the sera of the selected zoo primates *in vitro*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Red Pattas monkey	3	7.0000	1.73205	1.00000	2.6973	11.3027	5.00	8.00
Tantalus monkey	3	7.6667	.57735	.33333	6.2324	9.1009	7.00	8.00
Control	3	74.0000	1.00000	.57735	71.5159	76.4841	73.00	75.00
Total	9	29.5556	33.35083	11.11694	3.9198	55.1913	5.00	75.00

Appendix XIV: One-way analysis of variance comparing the concentration of Xanthine oxidase in the sera of the selected birds

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum

					Lower Bound	Upper Bound		
Broiler cock	2	2.6041	.02304	.01629	2.3970	2.8111	2.59	2.62
Broiler hen	2	3.0320	.15865	.11218	1.6065	4.4574	2.92	3.14
Shika brown commercial cock	2	2.4194	.03120	.02206	2.1392	2.6997	2.40	2.44
Shika brown commercial hen	2	1.5944	.58218	.41167	-3.6363	6.8251	1.18	2.01
Parent Stock White cock	2	1.9929	.14681	.10381	.6739	3.3120	1.89	2.10
Parent Stock White hen	2	2.3305	.39896	.28211	-1.2540	5.9150	2.05	2.61
Parent Stock Brown Cock	2	1.6182	.05382	.03806	1.1347	2.1017	1.58	1.66
Parent Stock Brown Hen	2	.1328	.30378	.21480	-2.5966	2.8621	-.08	.35
Guinea fowl	2	1.6335	.15481	.10947	.2426	3.0244	1.52	1.74
Domestic pigeon	2	2.3809	.24393	.17248	.1894	4.5725	2.21	2.55
Goose	2	1.7587	.03396	.02402	1.4535	2.0639	1.73	1.78
Crown crane	2	.6795	.96102	.67954	-7.9549	9.3139	.00	1.36
Peacock	2	1.4360	.07170	.05070	.7917	2.0802	1.39	1.49
Spotted eagle owl	2	.8480	.15308	.10824	-.5274	2.2234	.74	.96
Hooded vulture	2	1.9063	.43757	.30941	-2.0251	5.8377	1.60	2.22
Buzzard	2	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	32	1.6479	.88715	.15683	1.3281	1.9678	-.08	3.14

Appendix XV: One-way analysis of variance comparing the concentration of Xanthine oxidase in the sera of the selected rodents

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Cane rat/Grass-cutter	2	2.7869	.14828	.10485	1.4546	4.1192	2.68	2.89
Rabbit Chinchila	2	1.9268	.00939	.00664	1.8425	2.0111	1.92	1.93
New-Zeland	2	1.8016	.05367	.03795	1.3194	2.2838	1.76	1.84
Rabbit California	2	1.7054	.27762	.19631	-.7889	4.1997	1.51	1.90
Rabbit Grey	2	1.4083	.02781	.01967	1.1584	1.6582	1.39	1.43
Rabbit Dutch	2	2.0759	.08416	.05951	1.3197	2.8320	2.02	2.14
Total	12	1.9508	.45666	.13183	1.6607	2.2410	1.39	2.89

Appendix XVI: One-way analysis of variance comparing the concentration of Xanthine oxidase in the sera of the selected small ruminants

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
West African dwarf goat	2	2.0616	.08517	.06022	1.2964	2.8268	2.00	2.12
Red Sokoto goat	2	.6989	.00399	.00282	.6630	.7348	.70	.70
Sahel goat	2	1.5187	.14176	.10024	.2450	2.7923	1.42	1.62
Yankassa sheep	2	1.3249	.31137	.22017	-1.4726	4.1224	1.10	1.55
Uda sheep	2	1.9622	.35798	.25313	-1.2541	5.1785	1.71	2.22
Balami sheep	2	1.5356	.08342	.05898	.7861	2.2850	1.48	1.59
Total	12	1.5170	.49195	.14201	1.2044	1.8295	.70	2.22

Appendix XVII: One-way analysis of variance comparing the concentration of Xanthine oxidase in the sera of the selected large ruminants

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ndama	2	1.5450	.16328	.11545	.0781	3.0120	1.43	1.66
Sokoto Gudali	2	1.4616	.22730	.16072	-.5806	3.5037	1.30	1.62
Frasian cross	2	1.7358	.17669	.12494	.1483	3.3233	1.61	1.86
White Fulani	2	1.4643	.03363	.02378	1.1622	1.7665	1.44	1.49
Total	8	1.5517	.17324	.06125	1.4069	1.6965	1.30	1.86

Appendix XVIII: One-way analysis of variance comparing the concentration of Xanthine oxidase in the sera of the selected non-ruminant mammals

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Donkey	2	1.6681	.02284	.01615	1.4629	1.8733	1.65	1.68
Horse	2	1.5166	.00096	.00068	1.5080	1.5252	1.52	1.52
Camel	2	.9322	.20780	.14694	-.9348	2.7992	.79	1.08
Pig Pettin	2	1.2797	.84157	.59508	-6.2816	8.8409	.68	1.87
Pig Large white	2	1.5152	.19345	.13679	-.2229	3.2533	1.38	1.65
Pig Ducros	2	2.3133	.06427	.04545	1.7358	2.8907	2.27	2.36
Jackal	2	1.0542	1.49090	1.05423	-12.3410	14.4495	.00	2.11
Total	14	1.4685	.65175	.17419	1.0922	1.8448	.00	2.36

Appendix XIX: T-test analysis comparing the concentration of Xanthine oxidase in the sera of the selected zoo primates

	N	Mean	Std. Deviation	Std. Error Mean
Red Pattas monkey	2	2.2568	.10476	.07407
Tantalus monkey	2	1.0204	.33259	.23518

Appendix XX: One-way analysis of variance comparing the prophylactic effect of the effective sera on *Trypanosoma brucei brucei in vivo* in rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Broiler Hen	2	7.0000	.00000	.00000	7.0000	7.0000	7.00	7.00
Guinea Fowl	2	6.5000	.70711	.50000	.1469	12.8531	6.00	7.00
Buzzard	2	6.5000	2.12132	1.50000	-12.5593	25.5593	5.00	8.00
Camel	2	7.0000	.00000	.00000	7.0000	7.0000	7.00	7.00
Control	2	7.5000	.70711	.50000	1.1469	13.8531	7.00	8.00
Total	10	6.9000	.87560	.27689	6.2736	7.5264	5.00	8.00

Appendix XXI: One-way analysis of variance comparing the prophylactic effect of the effective sera on *Trypanosoma congolense in vivo* in rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum

					Lower Bound	Upper Bound		
Peacock	3	22.3333	2.30940	1.33333	16.5965	28.0702	21.00	25.00
Red pattas monkey	3	18.3333	1.52753	.88192	14.5388	22.1279	17.00	20.00
Tantalus monkey	3	24.6667	4.72582	2.72845	12.9271	36.4062	21.00	30.00
Jackal	2	28.0000	1.41421	1.00000	15.2938	40.7062	27.00	29.00
Control	2	26.0000	7.07107	5.00000	-37.5310	89.5310	21.00	31.00
Total	13	23.3846	4.59236	1.27369	20.6095	26.1597	17.00	31.00

Appendix XXII: One-way analysis of variance comparing the curative effect of the effective sera on *Trypanosoma brucei brucei in vivo* in rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Broiler Hen	2	8.0000	.00000	.00000	8.0000	8.0000	8.00	8.00
Guinea Fowl	2	8.5000	.70711	.50000	2.1469	14.8531	8.00	9.00
Buzzard	2	10.5000	.70711	.50000	4.1469	16.8531	10.00	11.00
Camel	2	9.0000	1.41421	1.00000	-3.7062	21.7062	8.00	10.00
Control	2	8.5000	.70711	.50000	2.1469	14.8531	8.00	9.00
Total	10	8.9000	1.10050	.34801	8.1127	9.6873	8.00	11.00

Appendix XXIII: One-way analysis of variance comparing the curative effect of the effective sera on *Trypanosoma congolense in vivo* in rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		

Peacock	3	25.0000	4.35890	2.51661	14.1719	35.8281	22.00	30.00
Red pattas monkey	3	24.3333	6.65833	3.84419	7.7931	40.8735	17.00	30.00
Tantalus monkey	3	25.6667	4.61880	2.66667	14.1929	37.1404	23.00	31.00
Jackal	2	26.0000	4.24264	3.00000	-12.1186	64.1186	23.00	29.00
Control	2	36.5000	2.12132	1.50000	17.4407	55.5593	35.00	38.00
Total	13	26.9231	5.86603	1.62694	23.3783	30.4679	17.00	38.00

Appendix XXIV: Preparation of stock solutions

XXIV.a Phosphate buffer (pH 6.9)

The following were weighed into a 500ml conical flask:

Anhydrous NaH_2PO_4, 2.7 g. (.01m)

Anhydrous Na_2HPO_4, 3.9 g. (.01m)

NaCl 0.2 g. (.002m)

Distilled water was added to the mixture to make a total volume of 500 ml. The resulting solution is expected to have a pH of 6.9-7.

PBS tablets from Sigma were also used. Each tablet was dissolved in 100ml of distilled water. This gave a solution containing 0.02M phosphate buffer, 0.0054M KCl, 0.274M NaCl and a pH of 7.4 at 25ºC. To obtain 500ml of PBS solution, 5 PBS tablets were used.

XXIV.b Coomassie Blue Staining solution

Coomassie brilliant blue (1.25g) was weighed and dissolved in 50ml glacial acetic acid and 185ml of distilled water. The solution was stored at room temperature.

XXIV.c Destain Solution for destaining Coomassie Blue Stained gels

50ml of glacial acetic acid was mixed with 185ml of distilled water and stored at room temperature.

XXIV.d Xanthine solution

1.5211g of xanthine powder was dissolved in 10ml of distilled water, to make a stock solution of 1M. 0.5mM xanthine solution was prepared from the stock.

XXIV.e Solution of 2,4,6 tribromo-3-hydroxybenzoic acid

To prepare a stock solution of 2,4,6 tribromo-3-hydroxybenzoic acid 1M, 0.37481g of the acid was dissolved in 1ml of distilled water. 1mM was prepared from the stock solution.

XXIV.f Solution of 4-amino-antipyrine

1M stock solution of 4-amino antipyrine was prepared by dissolving 0.20324g of the powder in 1ml of distilled water. 0.1mM was then prepared from the stock.

XXIV.g Horse-radish peroxidase

300units Horse-radish peroxidase was dissolved in 1ml of distilled water, to make a stock of 300U/ml. The stock was used to obtain a buffer with a final concentration of 8 units Horse-radish peroxidase (0.02667mg).

XXIV.h Commercial cow's milk xanthine oxidase

The stock of cow milk xanthine oxidase solution was obtained by mixing 100mg of it with 1ml distilled water. The stock of 100mg /ml was then used to prepare the required concentrations of the solution.

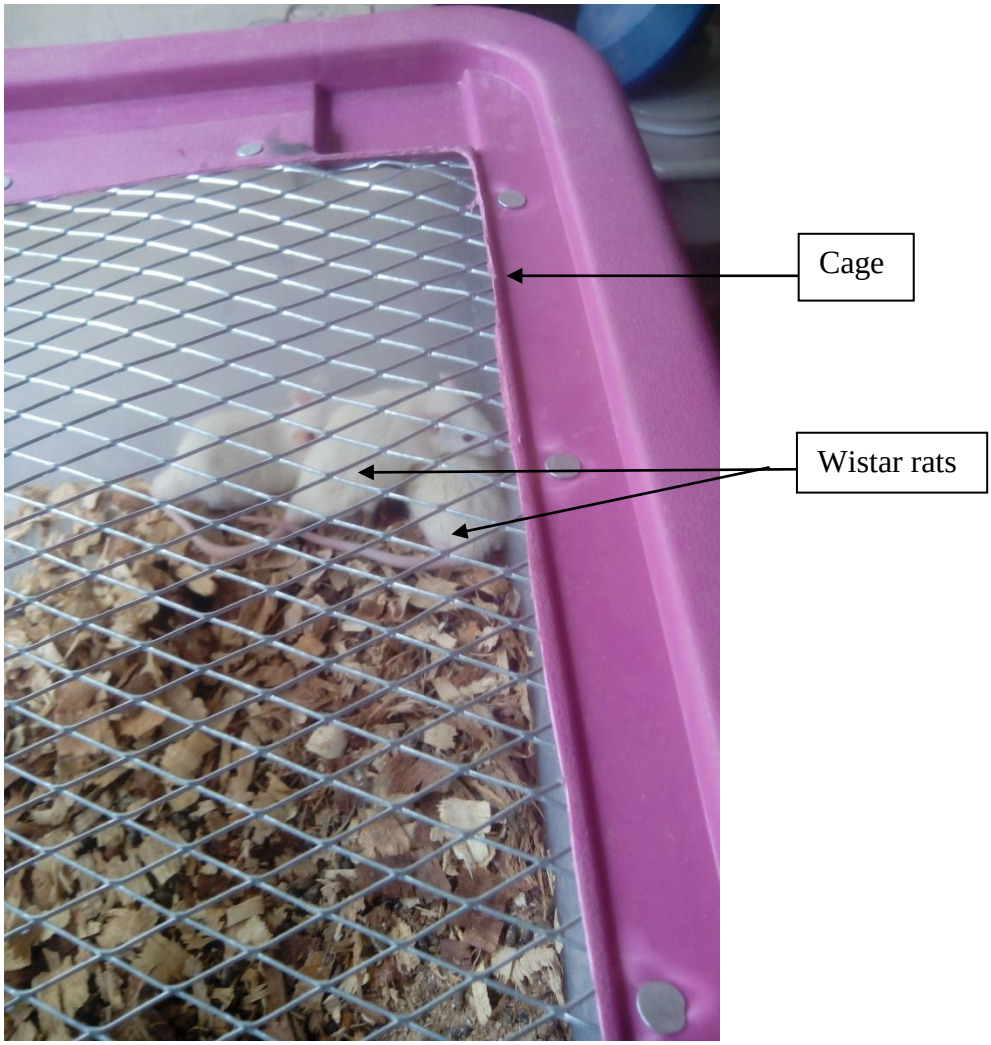
XXIV.i Sodium pyruvate

0.11005g of sodium pyruvate powder was dissolved in 1ml of distilled water to make a stock solution of 1M, which was used to obtain the required concentration in the culture medium.

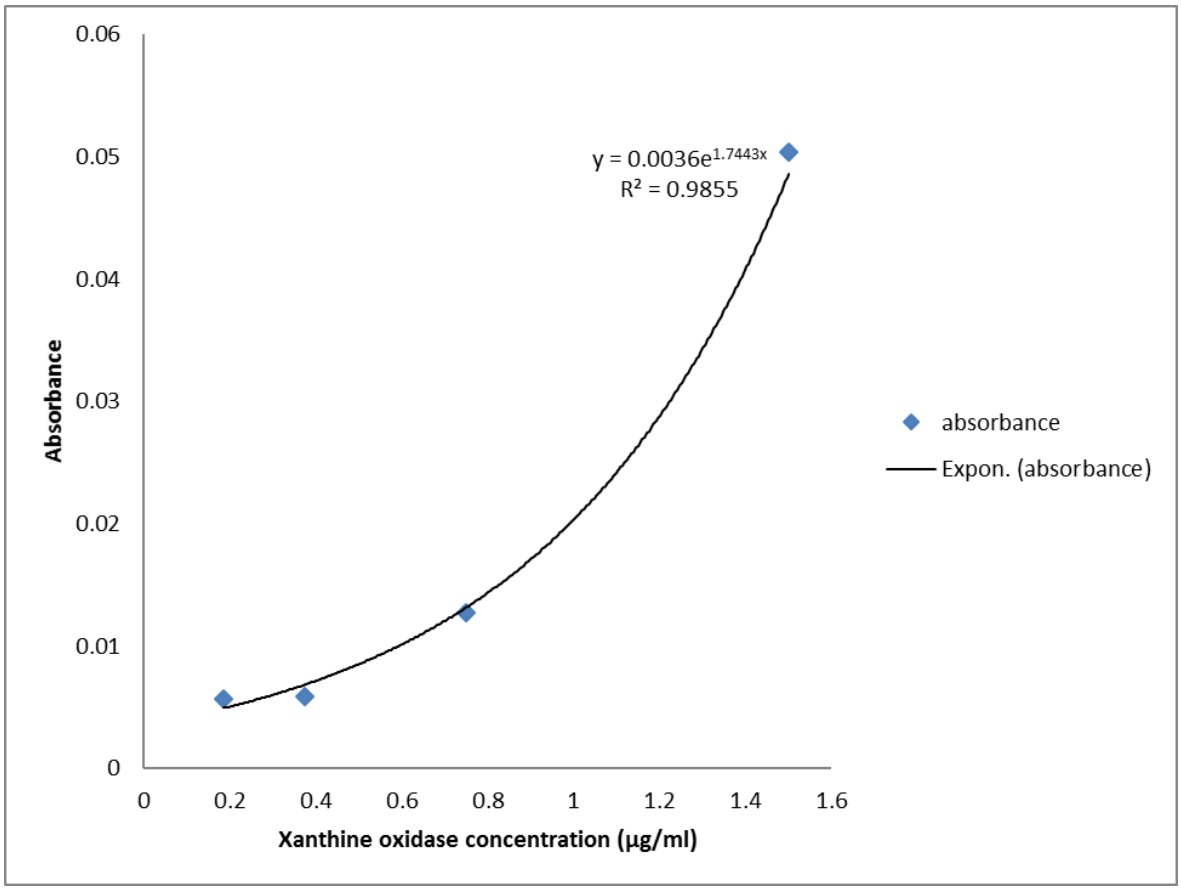
Appendix XXV: A CO_2 Incubator for sustaining the parasites



Appendix XXVI: Wistar rats in a cage



Appendix XXVII: Xanthine oxidase standard curve


0.06
0.05
0.04
0.03
0.02
0.01
0
Absorbance
y = 0.0036e1.7443x
R² = 0.9855
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Xanthine oxidase concentration (µg/ml)
absorbance
Expon. (absorbance)

cli

Appendix XXVIII: Some characteristics of the Hooded vulture, *Necrosyrtes monachus* (Gloger, 1841 in IUCN Red List 2012))

Hooded vulture is one of the smaller vulture of the Old World. It is native to Sub-Saharan Africa. Often associated with human settlements, but is also found in open grassland, forest edge, wooded savanna, desert and along coasts (Ferguson-Lees and Christie 2001). The hooded vulture is a typical vulture, with a bald head that is usually white, but flushes red when agitated (Sinclair *et al*., 2011). Like other vultures it is a scavenger, feeding mostly from carcasses of dead animals and waste which it finds by soaring over savannah and around human habitation, including waste tips and abattoirs. It often moves in flocks, and is very abundant (World Heritage Encyclopedia).



Appendix XXVIII continued: *Necrosyrtes monachus* (Hooded vulture)

Appendix XXIX: Some characteristics of the Spotted eagle-owl, *Bubo africanus* (Temminck, 1821)

The spotted eagle-owl is a medium-sized species of owl, one of the smallest of the eagle owls. It is mostly nocturnal, but sometimes active before sunset. It inhabits open or semi-open woodland with shrubs and bushes, mostly with sparse ground cover, savanna with thorny shrubs and scattered trees, and rocky hillsides with groups of trees and bushes. It is also fund in semi-deserts such as the Kalahari. Dense rainforest is avoided (Deane Lewis, 2015).

The spotted eagle-owl feeds on large insects and other arthropods, small mammals, birds and reptiles. It has been known to feed on carrion. It normally hunts from a perch, gliding down on to prey, but sometimes dashes at roosting birds. It also catches flying insects, bats and possibly nightjars in flight. This owl drinks regularly when water is available (Deane Lewis, 2015).



Appendix XXIX continued: *Bubo africanus* (Spotted eagle-owl)

Appendix XXX: Some characteristics of the African fish eagle, *Haliaeetus vocifer* (Daudin, 1800)

The African fish eagle occurs throughout much of Sub-Saharan Africa (Ferguson-Lees and Christie, 2001). Like other sea eagles, the African fish eagle has structures on its toes called spiricules that allows it

to grasp fish and other slippery prey. The African fish eagle is a generalist species, requiring only open water with sufficient prey and a good perch. This is evident by the number of habitats types that this species may be found in, including grassland, swamps, marshes, tropical rainforest, fynbos and even desert bordering coastlines (BBC Nature, 1970). The African fish eagle is associated with a diverse range of aquatic habitats from rivers, lakes and dams to swamps and mangrove lagoons (Sinclair, 1994; Allan, 2000; Ferguson-Lees and Christie, 2001).

With its distinctive plumage and evocative cry, the African fish eagle is probably the most Familiar bird of prey in Africa (Allan, 2000; Ferguson-Lees and Christie, 2001). The African fish eagle is known to peculate other bird's species (such as goliath herons) of their catch. It also feeds on waterfowl such as ducks, small turtles and terrapins, baby crocodiles, greater flamingos and lesser flamingos, lizards, frogs and carrion. Occasionnally, it may even carry off mammalian prey, such as hyraxes and monkeys ("The African fish eagle", 2012). Adult African fish eagles are normally seen in pairs, but on large, productive lakes, nests and roosts may be only a few hundred metres apart, and many birds can be found together in one area (Ferguson-Lees and Christie, 2001).



Appendix XXX continued: *Haliaeetus vocifer* (African fish eagle) (Source: Ferguson-Lees and Christie, 2001).

Appendix XXXI: Some characteristics of the Buzzard, *Buteo buteo* (Linnaeus, 1758))

Although buzzard is the common name of several related species of bird of prey, the common buzzard (*Buteo buteo*) is a medium-to-large bird of prey whose range covers most of Europe and extends into Asia. They are raptors with a robust body and broad wings. Buteo is the Latin name

of the common buzzard (Jobling, 2010). The *Buteo* hawks include the red-tailed hawk of Nord America, the common buzzard of Eurasia, and the roadside hawk of tropical Central and South America. Most Northern Hemisphere species are at least partially migratory (Ferguson-Lees, and Christie, 2001). Buzzard subspecies fall into two groups: the western Buteo group which is mainly resident or short-distance migrants and the eastern vulpinus group which includes migrant breeders (https://www.beautyofbirds.com/commonbuzzards.html). It has been reported that all *Buteo* species are to some extent opportunistic when it comes to hunting, and prey on almost any type of small animal as it becomes available to them, including snakes and lizards, and can often be seen walking over recently ploughed fields looking for worms and insects. However, most have a strong preference for small mammals, mostly rodents; rodents of almost every family in the world are somewhere preyed upon by *Buteo* species (Ferguson-Lees, and Christie, 2001; Del Hoyo et al., 1994; Brown, L. and Amadon, D.,1986). It was reported that buzzards do not normally form flocks, but several may be seen together on migration or in good habitat; they inhabit a wide range of habitats across the world, but tend to prefer some access to both clearings, which provide ideal hunting grounds, and trees, which can provide nesting locations and security (Del Hoyo et al., 1994; Brown and Amadon, 1986).



Appendix XXXI continued: *Buteo buteo* (common Buzzard)

Appendix XXXII: Some characteristics of the Goose

Geese (plural of goose) are waterfowls belonging to the tribe *Anserini* of the family *Anatidae*. Goose is the common name for any member of a variety of species of wild or domesticated large waterbirds in the family *Anatidae*, and especially the "true geese" in the subfamily *Anserini* comprising the three genera: *Anser* (the grey geese, including the graylag goose, and domestic

geese), *Branta* (the black geese, such as the Canada goose) and *Chen* (the white geese, often included in *Anser*). A number of other birds, mostly related to the shelducks, have "goose" as part of their names. More distantly related members of the family *Anatidae* are swans, most of which are larger than true geese, and ducks, which are smaller than geese and have a more pointed bill (http://www.newworldencyclopedia.org/entry/Goose). The spur-winged goose, *Plectropterus gambensis*, is most closely related to the shelducks, but distinct enough to warrant its own subfamily, the *Plectropterinae*. The blue-winged goose, *Cyanochen cyanopterus*, and the Cape Barren goose, *Cereopsis novaehollandiae*, have disputed affinities. They belong to separate ancient lineages that may ally either to the *Tadorninae*, *Anserinae*, or closer to the dabbling ducks (*Anatinae*). The three species of small waterfowl in the genus *Nettapus* are named "*pygmy geese*". They seem to represent another ancient lineage, with possible affinities to the Cape Barren goose or the spur-winged goose (http://www.newworldencyclopedia.org/entry/Goose).

Lamprecht (1987) reported that geese are monogamous, living in permanent pairs throughout the year; however, unlike most other permanently monogamous animals, they are territorial only during the short nesting season. Paired geese are more dominant and feed more, two factors that result in more young. Grzimek *et al*. (2004) reported that true geese are mostly herbivorous and feed by grazing. They also take invertebrates if the opportunity presents itself; domestic geese will try out most novel food items for edibility. All geese eat a largely vegetarian diet, and can become pests when flocks feed on arable crops or inhabit ponds or grassy areas in urban environments. Although geese largely require wetlands or water-bodies for survival, they feed mostly on land (Grzimek *et al*. 2004).



Appendix XXXII continued: *Alopochen aegyptica* (Geese)

Appendix XXXIII: Some characteristics of the Crowned crane

Crowned cranes are birds of the Balearica genus: Black crowned crane (*Balearica pavonina*) and Grey crowned crane (*Balearica regulorum*). So, the term "crowned crane" is used by some authorities to refer generally to the genus Balearica ("The Cranes", 2014).

The grey crowned crane, Balearica regulorum (Bennett, 1834)

The grey crowned crane occurs in dry savannah in Africa south of the Sahara, although it nests in somewhat wetter habitats. They can also be found in marshes, cultivated lands and grassy flatlands near rivers and lakes in Uganda and Kenya and as far South as South Africa. It does not migrate.

The grey crowned crane occurs in dry savannah in Africa south of the Sahara, although it nests in somewhat wetter habitats. They can also be found in marshes, cultivated lands and grassy flatlands near rivers and lakes in Uganda and Kenya and as far south as South Africa. It does not migrate. There are two subspecies. The East African *B. r. gibbericeps* (crested crane) occurs in the east of the Democratic Republic of the Congo and in Uganda, of which it is the national bird represented in its national flag, and Kenya to eastern South Africa. The other subspecies, *B. r. regulorum* (South African crowned crane), breeds from Angola south to South Africa. Grey crowned cranes are omnivores, eating plants, seeds, grain, insects, frogs, worms, snakes, small fish and the eggs of aquatic animals. The birds also associate with grazing herbivores, benefiting from the ability to grab prey items disturbed by antelopes and gazelles (BirdLife International, 2013).

The black crowned crane, Balearica pavonina (Linnaeus, 1758)

The black crowned crane, just like the grey species occurs in dry savannah in Africa South of the Sahara, although it nests in somewhat wetter habitats. There are also two subspecies. *B. pavonina* black crowned crane eats insects, reptiles, and small mammals (BirdLife International, 2012).

The grey crowned crane and the closely related black crowned cranes are the only cranes that can roost in trees, because of a long hind toe that can grasp branches.



Appendix XXXIII continued: *Balearica pavonina* (Black crowned cranes)

Appendix XXXIV: Some characteristics of the Domestic pigeon, *Columba livia domestica* (Gmelin, 1789)

The domestic pigeon is a pigeon that was derived from the rock pigeon. The rock pigeon is the world's oldest domesticated bird (Blechman, 2007). Domestic pigeons can often be distinguished from feral pigeons because they usually have a metal or plastic band around one (sometimes both) legs which shows, by a number on it, that they are registered to an owner (Porter, 2008).

Feral pigeons, together with Rock (i.e. "wild") and domestic pigeons are all the same species (*Columba livia domestica*) and will readily interbreed. Feral pigeons are derived from domestic pigeons that have returned to the wild. They are also called city doves, city pigeons, or street pigeons (Science and Nature, 2008). Feral pigeons find the ledges of buildings to be a substitute for sea cliffs, have become adapted to urban life, and are abundant in towns and cities throughout much of the world *(*Cornell Lab of Ornithology, 2016*)*.

Seeds form the major component of the diet, but it varies greatly according to species. Some ground feeding species (granivorous species) eat fruit and take insects and worms. The feral pigeon found in urban areas exists exclusively on a diet of seed (normally from human sources) and human refuse, such as fast food waste. Wood pigeons have a varied diet which includes vegetables and berries (Pigeon Control Resource Centre, 2009).



Appendix XXXIV continued: *Columba livia domestica* (Domestic pigeons)

Appendix XXXV: Some characteristics of the Peacock

The term peacock is properly reserved for the male; the female is known as a peahen, and the immature offspring are sometimes called peachicks (Britannica Online Encyclopedia). The general term is peafowl. Peafowl is a species of birds that include three species of birds in the

genera *Pavo* and *Afropavo* of the Phasianidae family, the pheasants and their allies. There are two Asiatic species (the blue or Indian peafowl originally of India and Sri Lanka and the green peafowl of Myanmar, Indochina, and Java) and one African species (the Congo peafowl native only to the Congo Basin). Male peafowl are known for their piercing call and their extravagant plumage. The latter is especially prominent in the Asiatic species, who have an eye-spotted «tail» or «train» of covert feathers which they display as part of a courtship ritual (https://anahitaquatuzyan.wordpress.com/2017/04/12 pecoke-and-bigest-butterfly-in-the-world/). Peafowls are omnivores and eat most plant parts, flower petals, seed heads, insects and other arthropods, reptiles and amphibians. Wild peafowl look for their food scratching around in leaf litter either early in the morning or at dusk. They retreat to the shade and security of the woods for the hottest portion of the day. These birds are not picky and will eat almost anything they can fit in their beak and digest. They actively hunt insects like ants, crickets and termites; millipedes; and other arthropods and small mammals (http://animals.nationalgeographic.com/animals/birds/peacock, 2015) Domesticated peafowl may also eat bread and cracked grain such as oats and corn, cheese, cooked rice and sometimes cat food. It is noticed by keepers that peafowl love protein rich food including larvae that infest granaries, different kinds of meat, as well as vegetables including dark leafy greens, broccoli, carrots, beans, beets, and peas. They also relish many fruits (Amistadt, 2014).

Peafowl are forest birds that nest on the ground, but roost in trees. They are terrestrial feeders. All species of peafowl are believed to be polygamous. In common with other members of the Galliformes, the males possess metatarsal spurs or "thorns" on their legs used during intraspecific territorial fights *(*Britannica Online Encyclopedia). In courtship, vocalization stands to be a primary way for peacocks to attract peahens *(*Yorzinski and Annop, 2012).



Appendix XXXV continued: *Pavo cristatus* (Peafowl)

Appendix XXXVI: Some characteristics of the Chicken, *Gallus gallus domesticus*

Chicken is a domesticated fowl, a subspecies of the red jungle fowl. It is suggested that domestic chickens originated from junglefowls in Southeast Asia (Hiromi *et al*., 2010).

Chicken originally referred to young domestic fowl (Dohner, 2001). The species as a whole was then called domestic fowl, or just fowl. The domestic chicken is descended primarily from the red jungle fowl (*Gallus gallus*) and is scientifically classified as the same species *(*Wong *et al.*, 2004). Chickens are gregarious birds and live together in flocks. They are omnivores (Info on chiken care, 2003). In the wild, they often scratch at the soil to search for seeds, insects and even larger animals such as lizards, small snakes or young mice (Gerard, 2008).

The development of several modern commercial breeds of chickens has been reported e.g. broilers breeds, egg-layers breeds *(*Hardiman, 2007), Shika brown, Shika white. Although chickens are omnivores, modern breeds are given access to a special diet of high protein feed.



Appendix XXXVI continued: *Gallus gallus domesticus* (Chickens)

Appendix XXXVII: Some characteristics of the Guinea fowl

Guineafowls are birds of the family Numididae in the order Galliformes. While modern guineafowl species are endemic to Africa, the helmeted guineafowl has been introduced widely elsewhere (Lever, 2005).

The guinea fowl is an omnivorous bird and therefore has a diet that consists of both plants and other animals. Guinea fowl primarily feed on worms and insects on the ground, along with seeds, berries and small mammals and reptiles (A-Z Animals- Animal Facts… 2008-2016). All guineafowls are social, and typically live in small groups or large flocks. Though they are monogamous, some species such as the helmeted guinea fowl (*Numida meleagris*) and the Kenya crested guinea fowl (*Guttera (pucherani) pucherani*) have been reported to be facultatively polygamous in captivity (Madge and McGowan, 2002).



Appendix XXXVII continued: *Numida meleagris* (Guinea fowl)

Appendix XXXVIII: Some characteristics of the White stork, *Ciconia ciconia*

It is a large bird in the stork family Ciconiidae. There are two subspecies, which differ slightly in size, C. c. ciconia and C.c. asiatica. C. c. ciconia, described by Linnaeus in 1758, breeds from Europe to northwest Africa and westernmost Asia, and in southern Africa, and winters mainly in Africa south of the Sahara Desert (Elliott, 1992), though some birds winter in India (Ali and Ripley, 2001). C. c. asiatica, described by Nikolai Severtzoy in 1873, breeds in Turkestan and winters from Iran to India. It is slightly larger than the other subspecies (Elliot, 1992; Rasmussen and Anderton, 2005). Like all storks, the white stork has long legs, a long neck and a long straight pointed beak. The sexes are identical in appearance, except that males are larger than females on average (Elliot, 1992). The plumage is mainly white with black flight feathers and wing coverts (Grande et al., 2004). White storks consume a wide variety of animal prey. They prefer to forage in meadows that are within roughly 5 km (3 mi) of their nest and sites where the vegetation is shorter so that their prey is more accessible (Johst et al., 2001). Their diet varies according to season, locality and prey availability. Common food items include insects (primarily beetles, grasshoppers, locusts and crickets), earthworms, reptiles, amphibians, particularly frog species such as the edible frog (*Pelophylax* kl. *esculentus*) and common frog (*Rana temporaria*) and small mammals such as voles, moles and shrews. Less commonly, they also eat bird eggs and young birds, fish, molluscs, crustaceans and scorpions. They hunt mainly during the day, swallowing small prey whole, but killing and breaking apart larger prey before swallowing (Cramp, 1977). The white stork is a gregarious bird; flocks of thousands of individuals have been recorded on migration routes and at wintering areas in Africa. Non-breeding birds gather in groups of 40 or 50 during the breeding season (Cramp, 1977).



Appendix XXXVIII continued: *Ciconia ciconia* (White stork)

Appendix XXXIX: Some characteristics of the Cane rat/grass-cutter, *Thryonomys swinderianus* (Temminck, 1827 in Hoffmann, 2008)

The cane rats belong to a genus of rodent found throughout Africa south of the Sahara, the only members of the family Thryonomyidae (Woods and Kilpatrick, 2005). The author also reported that the greater cane rat (*Thryonomys swinderianus*) is one of two the species of cane rats, a small family of African hystricognath rodents. Cane rats live in small groups led by a single male. They are nocturnal and make nests from grasses or burrow underground. If frightened, they grunt and run towards water. They live by reed-beds and riverbanks in Sub-Saharan Africa (http://eol.org/pages/326380/details). Woods and Kilpatrick (2005) reported that They are heavily built rodents, with bristly brown fur speckled with yellow or grey. They live in marshy areas and along river and lake banks, and are herbivores, feeding on aquatic grasses in the wild (Bishop, 1984). It was reported that as humans expanded into the cane rat's native habitats, the cane rats likewise expanded from their native reeds into the plantations, particularly the sugar cane plantations from which they derive their name, and that their tendency to adopt plantations as habitat, where they feed on agricultural crops such as maize, wheat, sugar-cane and cassava, often earns them the label of agricultural pest (Bishop, 1984; http://eol.org/pages/326380/details), even though the peoples of the region also utilize the cane rat as a food source (as bushmeat), considering the meat a delicacy. Consequently, they are often called grasscutters in Ghana and other regions of West Africa (Matthews, 2008).



Appendix XXXIX continued: *Thryonomys swinderianus* (Cane rat/Grass-cutter)

Appendix XXXX: Some characteristics of the Rabbit

Rabbits are small mammals in the family Leoparidae of the order Lagomorpha, found in several parts of the world. There are eight different genera in the family classified as rabbits, including the European rabbit (*Oryctolagus cuniculus*), cottontail rabbits (genus *Sylvilagus*; 13 species), and the Amami rabbit (*Pentalagus furnessi*, an endangered species on Amami Oshima, Japan). There are many other species of rabbit, and these, along with pikas and hares, make up the order Lagomorpha (www.onlineveterinaryanatomy.net/ sites/.../asset_9120_Rabbit%20svms%202091.pptx).

Rabbits are herbivores that feed by grazing on grass, forbs, and leafy weeds. In consequence, their diet contains large amounts of cellulose, which is hard to digest. Rabbits solve this problem via a form of hindgut fermentation. Rabbits reingest their own droppings (rather than chewing the cud as do cows and many other herbivores) to digest their food further and extract sufficient nutrients (Oaktreevet.co.uk. Retrieved 2010).

Rabbit habitats include meadows, woods, forests, grasslands, deserts and wetlands. Rabbits live in groups, and the best known species, the European rabbit which is the most widely domesticated, lives in underground burrows, or rabbit holes ("Rabbit Habitats". Retrieved 2009). Domesticated rabbits have mostly been bred to be much larger than wild rabbits, though selective breeding has produced a range sizes from "dwarf" to "giant", which are kept as pets and food animals across the world (http://eol.org/pages/327977/details).



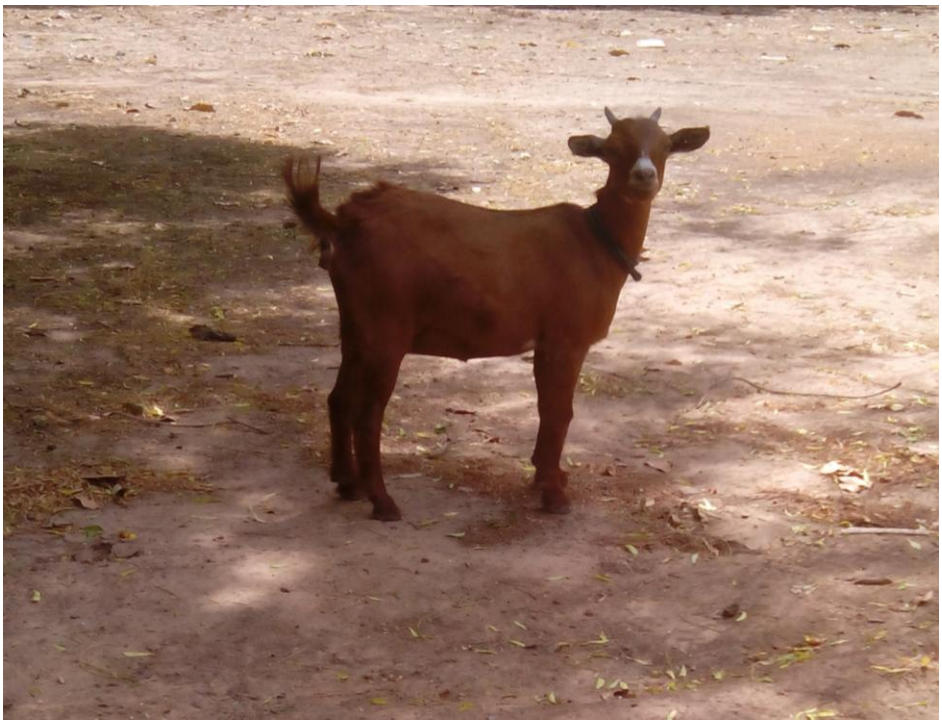
Appendix XXXX continued: *Oryctolagus cuniculus* (Rabbit)

Appendix XXXXI: Some characteristics of the Goat, *Capra aegagrus hircus* (Linnaeus, 1758)

The goat is a member of the family Bovidae. The domestic goat (*Capra aegagrus hircus*) is a subspecies of goat domesticated from the wild goat of Southwest Asia and Eastern Europe (Hirst, 2008).

Goats are ruminant and reputed to be willing to eat almost anything, including tin cans and cardboard boxes. While goats will not actually eat inedible material, they are browsing animals, not grazers like cattle and sheep, and (coupled with their highly curious nature) will chew on and taste just about anything remotely resembling plant matter to decide whether it is good to eat, including cardboard, clothing and paper (such as labels from tin cans) ("Learn About Goats").

There are several recognized breeds of domestic goat among which, the Sahelian goat, the West African Dwarf goat, the Red Sokoto goat. The Sahelian goat, also known as the Sahel or desert goat derives its name from the Sahel region in Africa and as its alternative name would suggest is a breed most suited to desert or semi desert environments and is intolerant of areas with high humidity (Deneice, 2016). The true West African Dwarf goat is considered to be confined to 15 countries in West and Central Africa, essentially confined to humid forest zones (South African Mohair Growers' Association). The Red Sokoto goat is one of the Savanna goat group but with relatively small size. It prefers semi-arid areas with a single rainfall season of 4-6 months duration, areas where sorghum and groundnuts are cultivated. It is found in southern Niger and northern Nigeria between latitudes 12°N and 14°N and longitudes 4°N and 10°E (South African Mohair Growers' Association).



Appendix XXXXI continued: *Capra aegagrus hircus* (Goat)

Appendix XXXXII: Some characteristics of the Sheep, *Ovis aries* (Linnaeus, 1758)

The sheep (*Ovis aries*) is a quadrupedal ruminant mammal, member of the order Artiodactyla, the even-toed ungulates, just like all ruminants. Sheep can be found in all Earth's climatic zones.

They are particularly well adapted to arid zones of all latitudes. Although the equatorial jungles are not conducive to sheep they can be found in the arid regions of the equatorial, tropical and sub-tropical zones (www.volcanocafe.org/biology-sheep-and-human-survival).

Domestic sheep are relatively small ruminants, usually with a crimped hair called wool and often with horns forming a lateral spiral. They differ from their wild relatives and ancestors in several respects, having become uniquely neotenic as a result of selective breeding by humans (Budiansky, 1999). Sheep are flock animals and strongly gregarious. They are exclusively herbivorous mammals. Most breeds prefer to graze on grass and other short roughage, avoiding the taller woody parts of plants that goats readily consume (Pugh, 2001).



Appendix XXXXII continued: *Ovis aries* (Sheep)

Appendix XXXXIII: Some characteristics of the Cattle, *Bos Taurus*, (Linaeus, 1758)

Also known as cows, cattle are a prominent modern member of the subfamily Bovinae, are the most widespread species of the genus *Bos,* and are most commonly classified collectively as *Bos*

taurus. They are the most common type of large domesticated ungulates (Huffman, 2006). "Cow" is in general use as a singular for the collective "cattle".

Cattle are ruminants, meaning their digestive system is highly specialized to allow the use of poorly digestible plants as food. They avoid grazing areas contaminated by the faeces of other cattle more strongly than they avoid areas contaminated by sheep (Forbes and Hodgson, 1985), but they do not avoid pasture contaminated by rabbit faeces *(*Daniels *et al*., 2001).



Appendix XXXXIII continued: *Bos Taurus* (Cow)

Appendix XXXXIV: Some characteristics of the Donkey, *Equus africanus asinus*, Linnaeus, 1758

The donkey also known as ass (Grubb, 2005; International Commission on Zoological Nomenclature 2003) is a domesticated member of the horse family, Equidae. It has been ruled

that if the domestic species and the wild species are considered subspecies of one another, the scientific name of the wild species has priority, even when that subspecies was described after the domestic subspecies (International Commission on Zoological Nomenclature, 2003). This implies that the proper scientific name for the donkey is *Equus africanus asinus* when it is considered a subspecies, and *Equus asinus* when it is considered a species.

Donkeys are herbivorous animals adapted to marginal desert lands although a number of them are kept for breeding or as pets in developed countries. Also, there are working donkeys which are often associated with those living at or below subsistence levels (http://www.worldanimal.foundation/advocate/donkeys). There are three categories of donkeys. Miniatures are 36 inches and under. Standards are between 36 and 54 inches. Mammoths are over 54 inches. All measured to the shoulder (Wanda Embar, 2008-2015). Unlike wild and feral horses, wild donkeys in dry areas are solitary and do not form harems. Each adult donkey establishes a home range; breeding over a large area may be dominated by one jack (French, 1997).



Appendix XXXXIV continued: *Equus africanus asinus* (Donkeys)

Appendix XXXXV: Some characteristics of the Camel

Camel is an even-toed ungulate within the genus *Camelus*, bearing distinctive fatty deposits known as "humps" on its back (https://www.pinterest.com/pin/ 432908582912666023). "Camel"

is also used more broadly to describe any of the six camel-like mammals in the family Camelidae: the two true camels and the four New World camelids: the llama, alpaca, guanaco, and vicuna of, South America (Century Dictionary and Cyclopedia. wordnik. 2012*;* Bornstein, 2010).

The two surviving species of camel are the dromedary, or one-humped camel (*C. dromedarius*), which inhabits the Middle East and the Horn of Africa; and the Bactrian, or two-humped camel (*C. bactrianus*), which inhabits Central Asia (https://www.pinterest. com/pin/432908582912666023).

Camels have a series of physiological adaptations that allow them to survive in deserts, for instance withstanding long periods of time without any external source of water (Roberts, 1986). Their thick coats insulate them from the intense heat radiated from desert sand; a shorn camel must sweat 50% more to avoid overheating (Schmidt-Nielsen, 1964 Cited in ("Coat of fur on the camel", 2003). Camels eat plants. Most camels surviving today are domesticated (Fedewa, 2000; Walker, 2009). The only wild camels left are the Bactrian camels of the Gobi Desert (National Geographic, 2012).



Appendix XXXXV continued: *Camelus dromedarius* (Camels)

Appendix XXXXVI: Some characteristics of the Horse, *Equus ferus caballus* (Linnaeus, 1758)

The horse is an old-toed ungulate mammal belonging to the taxonomic family equidae. It is one of two extant subspecies of Equus ferus (Grubb, 2005).

Horses are herbivores with a digestive system adapted to a forage diet of grasses and other plant material, consumed steadily throughout the day (Giffin *et al*., 1998; Kreling, 2005). They are adapted to survive in areas of wide-open terrain with sparse vegetation, surviving in an ecosystem where other large grazing animals, especially ruminants, could not (Budiansky, 1997). Horses are classified into three distinct breeds: *"*Hot blood", "Cold bloods" and "Warmblood" breeds. *"*Hot bloods" tend to be spirited, bold, and learn quickly. They are bred for agility and speed (Price *et al*., 1998). They tend to be physically refined—thin-skinned, slim, and long-legged (De Filippis, 2006). Cold bloods are muscular, heavy draft horses, as they are bred not only for strength, but also to have the calm, patient temperament needed to pull a plow or a heavy carriage full of people (Belknap, 2004). "Warmblood" breed, a riding horse with more refinement than a draft horse, but greater size and milder temperament than a lighter breed (Edwards, 1994). Strictly speaking, the term "warm blood" refers to any cross between cold-blooded and hot-blooded breeds (Belknap, 2004).



Appendix XXXXVI continued: *Equus ferus caballus* (Horse)

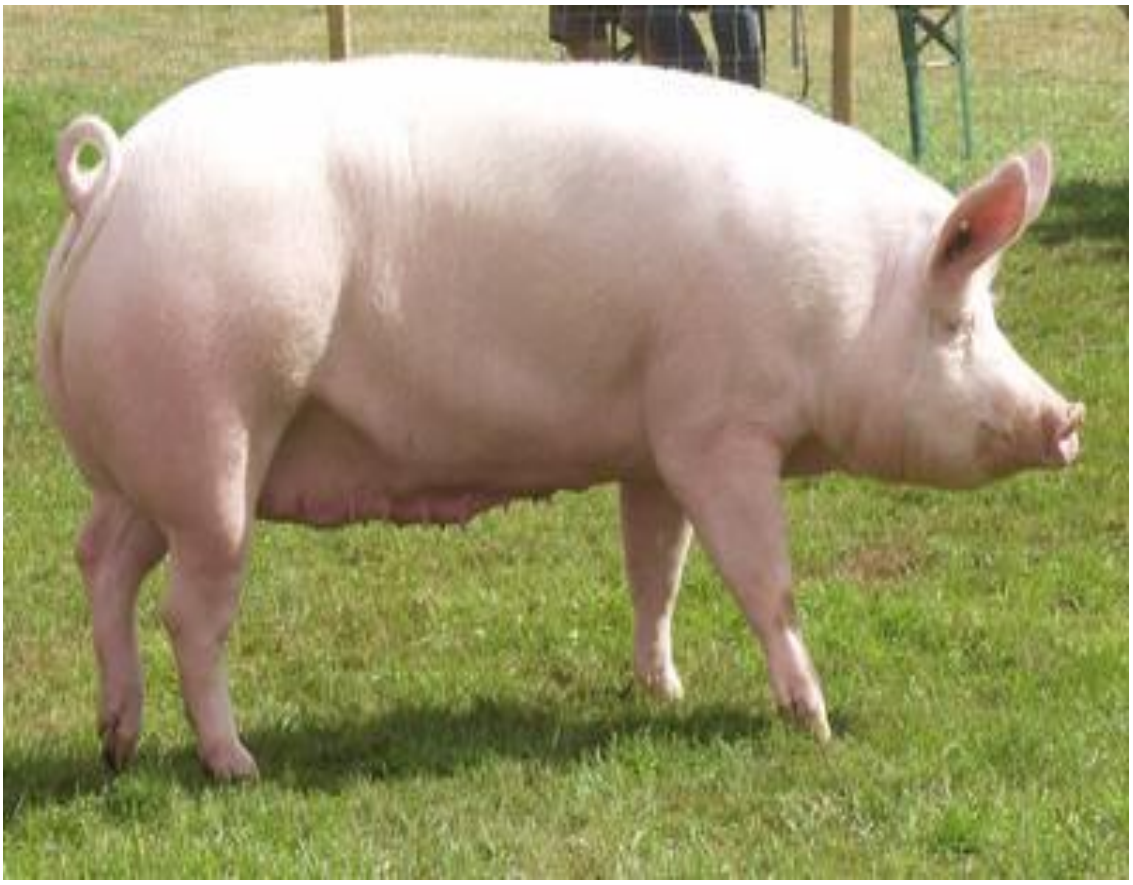
Appendix XXXXVII: Some characteristics of the Pig, *Sus scrofa domesticus*, (Linnaeus, 1758 in Gentry *et al.*, 2004)

The term pig refers to any of the animals in the genus *Sus*, within the Suidae family of even-toed ungulates. Pigs include the domestic pig and its ancestor, the common Eurasian wild boar (Sus scrofa), along with other species. Like all suids, pigs are native to the Eurasian and African continents (Merriam-webster.com. 2012. Retrieved 2013). The domestic pig is most often considered to be a subspecies of the wild boar, which was given the name *Sus scrofa*; following from this, the formal name of the domestic pig is *Sus scrofa domesticus* (Gentry *et al.*, 2004).

Even-toed ungulates are generally herbivorous, but the domestic pig is an omnivore, like its wild relative, and are highly versatile in their feeding behaviour. They can consume a wide range of food, similar to humans. They consume both plants and animals. In the wild, they are foraging animals, primarily eating leaves, grasses, roots, fruits, and flowers. Older pigs will consume three to five gallons of water per day (ncsu.edu*).*

Domestic pigs seek out the company of each other and often huddle to maintain physical contact, although they do not naturally form large herds. A behavioural characteristic of domestic pigs which they share with carnivores is nest building and bed making which is an important part in the process of pre and post-partum maternal behaviour, and occur during the last 24 hours before the onset of farrowing and becomes most intense during 12 to 6 hours before farrowing (Clutton-Brock, 1987; Algers and Uvnäs-Moberg, 2007).

Because of the similarities between pigs and humans, pigs are used for human medical research (http://www.foxnews.com/health/2014/05/09/). In November 2012 scientists managed to sequence the genome of the domestic pig. The similarities between the pig and human genomes mean that the new data may have wide applications in the study and treatment of human genetic diseases (Groenen *et al.*, 2012).



Appendix XXXXVII continued: *Sus scrofa domesticus* (Domestic pig)

Appendix XXXXVIII: Some characteristics of the Jackal

The jackal is a small omnivorous mammal of the genus *Canis*, which also includes the wolf and dog. Jackals are originally found in Africa, Asia and southeast Europe. There are four main species of jackal: the golden jackal *(Canis aureus)*, the side-striped jackal *(Canis adustus)*, the black-backed jackal (*Canis mesomelas)* and the Ethiopian wolf jackal (*Canis simensis*). Although the majority of the diet of the jackal is made up of meat, many jackal individuals (particularly those jackals living in the tropics of Southeast Asia) will often eat plant matter if no meat can be found (https://a-z-animals.com/animals/jackal/). Jackal usually hunts alone or with only one other jackal, and rarely in large group as their most common socialunit is a monogamous pair, which defends its territory from other pairs by vigorously chasing intruding rivals and marking landmarks around the territory with their urine and feces (Animal diversity web). Jackals inhabit crevices in rocks and dens made by other animals. Although many jackal individuals live a nocturnal existence, particularly those jackals that inhabit areas close to humans, some jackals in more remote areas are slightly more diurnal and will be out hunting during the cooler times of the day (https://a-z-animals.com/animals/jackal/).

Modern research has clarified the relationships among the "jackal" species. Despite their similarities, jackals do not all stem from the same branch on the canid family tree. The side-striped jackal and black-backed jackal belong to a branch of canids that includes the dhole and African wild dog, while the golden jackal, on the other hand, belongs to a branch that includes the Ethiopian wolf (*Canis simensis*), the coyote, and *Canis lupus*, the grey wolf/domestic dog (Lindblad-Toh *et al*., 2005). Because of this deep divergence between the black-backed/side-striped jackal and the golden jackal (as well as the rest of the "wolf-like" canids), it has been proposed to change the two species' generic name from *Canis* to *Lupulella* (Dinets, 2015).



Appendix XXXXVIII continued: *Canis aureus* (Jackal)

Appendix XXXXIX: Some characteristics of the Monkey

Monkeys are haplorhine ("dry-nosed") primates, one of the two distinct lineages of primates, the other one being strepsirrhines (Groves, 2005). There are many species of monkeys among which the Patas monkey and the Tantalus monkey.

- **The Patas monkeys, *Erythrocebus patas* (Schreber, 1775 (Kingdon *et al*., 2008))**

Also known as the Military Monkey, the Hussar Monkey and the Red Guenon, the Patas Monkey is a medium to large sized species of Old World Monkey that is found inhabiting the open grasslands of Central Africa. They are natively found in a broad band throughout Central Africa that is bordered by the Sahara Desert to the north and the moist tropical conditions of the equatorial forests to the south. They can be found as far west as Senegal to Ethiopia in the east and as far south as Tanzania in the east and Cameroon in the west. Patas Monkeys inhabit savanna plains, open woodlands and grass steppe that is well vegetated. They are known to be quite adaptable animals that are also found in arid areas including the southern fringes of the Sahara Desert, flooded deltas and even in moist forest where land has been cleared by people. Due to the fact that Patas monkeys rely more on the open country than they do dense jungle, they have even been able to move into areas that have been affected by deforestation and can also be found in agricultural plantations (https://a-z-animals.com/animals/patas-monkey/).

Patas monkeys consumes a wide range of both plant matter and small animals in order to survive. The omnivorous animals primarily feed on Acacia fruits, galls and leaves along with other seasonal fruits, flowers and tree gum. They are also known to eat insects, lizards and bird's eggs along with raiding crops where they are often known to cause damage to the crops

themselves. Due to their predominantly terrestrial nature, more than 85% of their food is thought to be collected on ground-level (https://a-z-animals.com/animals/patas-monkey/).

- **The Tantalus monkeys, *Chlorocebus tantalus* (Ogilby, 1841 (Kingdon and Gippoliti, 2008))**

The Tantalus monkey is an Old World monkey from Africa that ranges from Ghana to Sudan. *Chlocebus tantalus* has three recognized subspecies: *Chlorocebus tantalus tantalus*, *Chlorocebus tantalus budgetti* and *Chlorocebus tantalus marrensis* (Groves, 2005).



Appendix XXXXIX continued: *Erythrocebus patas* (Red Patas monkey)



Appendix XXXXIX continued: *Chlorocebus tantalus* (Tantalus monkey)