TRANSMISSION PATTERNS OF TRYPANOSOMES IN FLY VECTOR POPULATIONS AND BOVINE HOST IN SOUTHWESTERN NIGERIA

BY

PAUL OLALEKAN, ODENIRAN
DVM (Ibadan), MVSc. Veterinary Parasitology (Ibadan)
Matric No: 134484

A Thesis in the Department of Veterinary Parasitology and Entomology,
Submitted to the Faculty of Veterinary Medicine
in partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

JANUARY, 2020

CERTIFICATION

I certify that this work was carried out by Dr P.O. Odeniran in the Department of Veterinary Parasitology and Entomology, University of Ibadan.

……

Supervisor
I.O. Ademola,
DVM, M.Sc., Ph.D. (Ibadan), FCVSN
Professor, Department of Veterinary Parasitology and Entomology,
University of Ibadan, Nigeria

DEDICATION

I dedicate this dissertation to **God Almighty**, the **author** and **finisher** of my **faith**.

ACKNOWLEDGEMENTS

Now, thanks be unto God, which always cause me to triumph in Christ, and make manifest the savour of *his* knowledge by me in every place.

First, my appreciation goes to my supervisor, Prof. Ademola I.O., for his fatherly counsel, support, discipline, teachings and guidance. Thank you, Sir, for impacting so much knowledge, professional and spiritual. I have been moulded and helped greatly by your mentoring. You started the "work of building" from my undergraduate days till now. My prayer request is the Lord bless and increase you greatly. I appreciate the love from your family, Prof. Ademola, Oreoluwa, Inioluwa, Ifeoluwa and Ireoluwa, exceeding grace in Jesus name.

My appreciation goes to the Commonwealth Scholarship Commission (CSC) for offering me a Ph.D spilt-site scholarship and the University of Edinburgh for accepting me as a foreign Ph.D student and co-funding my research work. The CSC engaged me academically and socially. I have acquired knowledge from this exposure. Thanks for the travel and laboratory grants.

Special appreciation to my cosupervisor, Prof. Susan Welburn for accepting me into your laboratory and for the mentoring. Many thanks to Dr. Ewan MacLeod, for your tutelage in molecular techniques and research writing. You made my stay in Edinburgh worthwhile. Thanks for the "Haggis" and the associated Scotish tales. I also appreciate Pauline Mcmanus, for always providing solutions to every administrative problem throughout my stay in Edinburgh, Dr. Kim Picozzi and Jenner, as special instructors in seminars and office on trypanosomosis related matters and social discussions. I also enjoyed your cake during my sendforth and your special gifts. Emily and Kathrine, veterinarians from Glasgow and Berlin, respectively. I enjoyed our conversations. Ross Watson, a gentle and down-to-heart colleague in the laboratory. I was blessed meeting you. Joshua David Jones (JDJ), the Cambridge graduate from Wales. Thanks for teaching me meta-analysis and arranging for the paint-ball games. Malimba Lisulo, my Zambian friend, thanks for your advice always. Freya Webber, a colleague from Sterling, many thanks for the laboratory tutelage. Tarsha Smith, veterinarian and scholar, thanks for the moment shared and members of the DIPM, University of Edinburgh. Special thanks to Bro. Akindele Onifade for helping with modelling.

Special gratitude to Prof. Adejinmi and Prof. Abosede O. Omonona for your support in cash and kind during the course of this programme. I cannot forget Dr. Adediran for your assistance on my admission in the postgraduate school. Many thanks to Prof. Oyeyemi and Dr. Ayinmode for providing reference letters when it was needed; Dr. Fagbohun, Dr. Amosun and Prof. Oluwayelu for their advice; Mr. Obebe for lending out some FTA cards for sample collection; Dr. Adebiyi for contacts of cattle sellers in the market; Dr. Ismael for contacts of Fulani cattle herdsmen; Dr. Philip Ojo for assisting in sample collection; Dr. Famakinde for providing contacts for Abeokuta farms, Dr. Victor Oyebamiji as an observer, Mr. Ayodeji Ayeni, and Bro. Elijah Akintunde for your assistance in cash and kind during sample collection, Mr. Alade for assisting in sample collection in Lagos State and Mr. Paul of DHL for sample transportation.

I appreciate colleagues and fellowship pals for their constant prayers, encouragement and support. Engr. Jesutunde Adetokun, a confidant and brother; Mr. Femi Babatunde, Mr. Solomon, Dr. Odeyemi Olalekan, Dr. Foluke Esho, Dr. Ogunbadewa Anthony, Mr. Tope Bankole, members of Ibadan Varsity Christian Union Postgraduate Fellowhip (IVCU), members of Dalkeith Baptist Church, Edinburgh, my special Nigerian hosts- Dr. Musa and Dr. Ama Shallangwa, My Edinburgh hosts- Rev. Jeremy and Mrs. Karren Shaw- thank you for your hospitality, My Nigerian-Scottish family friend- Mr Abraham and Mrs. Kisten, my flatmate- “Bigbros”, Mr. Osagie, and my unit group in Dalkeith Baptist Church, Edinburgh.

Gratitude to all the field workers and friends, my Fulani friend-Isa, thanks so much for your assistance, to late Mr. Amidu, may your soul rest in peace, thanks for the assistance in meeting other Fulani nomads and frequent interpretation of Fulani to Yoruba language. All doctors and staffs in Lafenwa abattoir, Ogun State; Agege abattoir, Lagos State; Onyearugbulem abattoir, Ondo State; Sango cattle market, Ondo State; Sabo abattoir, Osogbo, Osun State; Ikere abattoir, Ekiti State; Akinyele cattle market, Oyo State; Gaa Seriki cattle settlements, Igboora cattle market, Eruwa and Iddo cattle settlements, FUNNAB farm, Abeokuta, Ogun State; UI research farm, Ibadan, Oyo State.

Many thanks to my parents- Pastor and Evangelist Odeniran, and Deacon and Mrs. Omolabi, may the LORD continue to enrich your lives and make you eat the fruits of your labour. I have been blessed by your counsels, prayers, gifts and supports. My acknowledgement goes to my siblings, Debby Mama and husband- Dr. Ojediran, you are blessed beyond limits, Isyboi- the Lord establish you on every side, Dantos- the Lord satisfy you with *his* mercy. My

regards to Feyi and Adedamola Rasak, for your prayers and kindness always, Olaoluwa Omolabi and Taiwo Omolabi, the Lord bless you both and perfect His will for your lives. God's loving kindness and tender mercies will be multiplied in you all. My appreciation goes to the following elders for their contributions to the success of this programme, Tolafeg (Prince and Mrs. Oyegbemi), God bless you richly for the constant blessings, and Dr. Nworgu for your advice.

Most importantly, my gratitude goes to my wife, Mrs. Folu Paul-Odeniran, for your love, support and care. You were there through the “thick and thin” of this programme. Your constant prayers, admonition and belief in what God only can do in my life have been my testimonies all through the years. How do I make account for the selfless service, the unfeigned kindness, the words of encouragement and your positive thoughts on my career? May the Lord beautify your life with successes and establish your career path in Jesus name. Amen

Now unto Him, who is able to build me up and preserve me unto perfection by his own spirit in the name of our Lord, Jesus Christ, be glory, honour and praise forever and ever. Amen.

ABSTRACT

Bovine trypanosomosis is still a major health problem causing severe morbidity and mortality, and the control of the disease is centered on *Glossina*, the main vector. Information on other vectors (*Tabanus* and stomoxyine) transmitting trypanosomes in southwestern Nigeria are lacking. This study was designed to investigate the transmission patterns of trypanosome in vectors and cattle to enable an effective control strategy.

Fifteen cattle settlements and institutional farms in the six southwestern states were randomly selected and Nzi traps set to capture transmitting vectors between April 2016 and March 2017. The vectors were identified using morphology, 28S rRNA PCR and Sanger sequencing. Fly abundance with environmental variables, distribution, endosymbionts, bloodmeal sources and feeding behaviour were investigated using standard methods. Insecticidal effects of cypermethrin against fly vectors were assessed using standard methods. Blood (125 µL) was randomly collected from 745 cattle during the same period in some of the locations. The vectors and cattle blood were screened for trypanosomes using 18S rRNA PCR, while *Trypanozoon* DNA positives were screened for RoTat 1.2 VSG and TgsGP genes. Livestock owners (n = 209) were interviewed using structured questionnaire to determine control methods. Relevant publications in databases from 1960-2017 were screened using PRIMSA checklist to analyse the southwest Nigeria estimates of trypanosome prevalence in livestock and *Glossina*. Data were analysed using descriptive statistics and one-way ANOVA at $\alpha_{0.05}$.

The identified transmitting vectors belong to the genera *Glossina*, *Tabanus* and *Stomoxys*. The total apparent density of trapped flies [*Glossina* (0.6 flies/trap), *Tabanus* (0.2 flies/trap), Stomoxyine (36.2 flies/trap)] was highest at temperature of 26-28°C, humidity >80.0% and rainfall of 150-220 mm/month. Distribution of flies increased with vegetation density and decreased in areas with high human population density (>100/km^2). *Sodalis glossinidus* (31.3%) was the only endosymbiont in *Glossina*. Sequenced cytochrome-B mitochondrial DNA segments revealed eight different host species as bloodmeal sources. On the average 78.0%, 19.6% and 10.2% *Glossina*, *Tabanus* and Stomoxyine, respectively were fully engorged. Cypermethrin was effective on Stomoxyine using restricted insecticidal application protocol, but ineffective using conventional pour-on protocol. Overall, 71.1%, 33.3% and 22.2% *Glossina*, *Tabanus* and Stomoxyine, respectively were positive for trypanosomes. Trypanosomes were identified in *Glossina* (*T. vivax* 36.4%, *T. brucei* 53.5%, *T. congolense*

5.4% and *T. simiae* 4.7%), *Tabanus* (*T. vivax* 43.5%, *T. evansi* 39.1%, *T. simiae* 8.7% and *T. godfreyi* 8.7%), and Stomoxyine (*T. vivax* 69.2%, *T. evansi* 20.5%, *T. congolense* 5.1% and *T. simiae* 5.1%). Trypanosome DNA was detected in 23.8% of cattle examined with significant increase in Osun State compared to other states. The species identified were *T. vivax* (43.4%), *T. congolense* (31.8%), *T. brucei* (18.2%) and *T. evansi* (6.6%). Livestock owners (93.9%) used trypanocides, while 60.5% used insecticides. Published articles revealed overall prevalence of bovine trypanosomosis at 21.3% and trypanosome prevalence of 42.6% from 4,808 *Glossina* species.

The mechanical vectors (*Tabanus* and Stomoxyine) are as important as biological vector (*Glossina*) in the transmission of bovine trypanosomosis in southwest Nigeria. The distribution and vectorial capacity of captured vector species warrants a new effective control strategy against trypanosomes.

Keywords: Bovine trypanosomosis, *Glossina*, *Tabanus*, Stomoxyine

Word count: 498

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAT-	African animal trypanosomosis
AFLP-	Amplified fragment length polymorphisms
ANOVA-	Analysis of variance
BICOT-	Biological Control of Tsetse
cAMP-	Cyclic andenosine monophosphate
CATT-	Card agglutination test for trypanosomiasis
CI-	Confidence interval
CDC-	Centre for disease control and prevention
CNS-	Central nervous system.
CRIN-	Cocoa Research Institute of Nigeria
DNA-	Deoxyribonucleic acid.
dNTPs-	Deoxynucleoside triphosphates
ELISA-	Enzyme linked immunosorbent assay
F/df-	Degree of freedom for ANOVA analysis
FAO	Food and Agriculture Organisation of the United Nations
FTA card-	Flinders technology associate card
FUNAAB-	Federal University of Agriculture Abeokuta
g-	Grams
GDP-	Gross domestic product
GIS-	Geographical information system
HAT-	Human African trypanosomiasis
HP-	Head and proboscis
I^2-	Inconsistency level
IAEA-	International Atomic Energy Agency
IFAT-	Indirect fluorescent antibody test.
ILCA-	International Livestock Centre for Africa
ILRAD-	International Laboratory for Research on Animal Diseases.
ILRI-	International Livestock Research Institute
ISCTRC-	International Scientific Council for Trypanosomosis Research and Control
ITC-	Insecticide-treated cattle
ITS-	Internal transcribed spacer
ITT-	Insecticide-treated targets
KCl-	Potassium chloride

KH_2PO_4-	Potassium dihydrogen phosphate
LC_{50}-	Lethal concentration
$MgCL_2$-	Magnesium chloride
ml-	millilitre
MUSCLE-	Multiple sequence analyses
NaCl-	Sodium chloride
NAFDAC-	National Agency for Food and Drug Administration and Control
Na_2HPO_4-	Sodium hydrogen phosphate
NAPRI-	National Animal Production Research Institute
NCBI-	National Centre for Biotechnology Information
NITOR-	Nigeria Institute of Trypanosomiasis and Onchocerciasis Research
NITR-	Nigerian Institute for Trypanosomiasis Research
NTEP-	Nigerian Tsetse Eradication Campaign
OIE-	Office Internationale des Epizooties.
PATTEC-	Pan African Tsetse and Trypanosomiasis Eradication Campaign
PCV-	Packed cell volume
PCR-	Polymerase chain reaction
PDEBI-	Phosphodiesterase brucei inhibitor
PRIMSA-	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QS-	Quorum sensing
RAP-	Restricted Instecticidal Application Protocol
RAPD-	Randomly amplified polymorphic DNAs
RFLP-	Restriction fragment length polymorphisms
RIT-seq-	RNA interference target sequencing
rRNA-	Ribosomal ribonucleic acid
RNA-	Ribonucleic acid
RNAi-	Ribonucleic acid interference
RoTat-	Rode Trypanozoon antigen type
SE-	Standard error
SIT-	Sterile Insect Technique
TA-	Thorax and abdomen
TgsGP-	Trypanosoma brucei gambiense glycoprotein
UI-	University of Ibadan
UNEP-	United Nations Environment Programme

USD-	United State Dollars
UV-	Ultra-violet
VSG-	Variant Surface Glycoprotein
w/w-	Weight per weight
WHO-	World Health Organization
α	significant value

CHAPTER ONE

INTRODUCTION

African animal trypanosomosis (AAT) is caused by extracellular protozoans of the genus *Trypanosoma* and economically important species are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*. AAT causes significant losses ranging from poor weight to mortality have been reported in Nigeria (Swallow, 2000). Clinical signs are not pathognomonic; however, common signs are fever, myalgia, arthralgia, lymphadenitis, salivation, lacrimation, anorexia, pica, recumbency, anaemia, abortion, morbidity and death (Seyoum *et al.,* 2013). The sequence of anaemia observed in *Trypanosoma* infection has been typed as normocytic normochromic anaemia in early stages, followed by macrocytic hypochromic anaemia during persistent infection and microcytic hypochromic anaemia in chronic stages (Stephen, 1986). The disease occurrence in Nigeria has been attributed to the presence of the biological vector (tsetse flies) and mechanical vectors (biting flies) in their significant numbers.

The complex transmission cycle of the disease has included wildlife reservoirs among other warm-blooded vertebrates (Reichard, 2002). The developmental reproductive stages of the mature infective form of trypanosomes (metacyclic trypomastigote) occur in the fly biological vector (Peacock *et al.,* 2012). Animals become infected when the infective form is transferred during bloodmeal. Trypanosomes posses a prominent surface antigen (VSG) which enables them to avoid the host immune mechanisms (Vincendeau and Bouteille, 2006). There are several distinct species of trypanosomes differing in structural, biological and physiological forms (Maudlin *et al.,* 2004). Many factors have been identified as risk factors of AAT in Nigeria, such as vegetational type, climatic factors, tsetse species and behaviour, presence of vertebrate host and human activities. An approximately 80% of the landmass is affected with tsetse flies (Figure 1.1; Anene *et al.,* 1991), hence the country remains endemic to the disease and nearly 19.4 million cattle population in the country are at risk (FAO, 2014), despite control efforts being initiated in the 1960s and 1980s, respectively (Jordan, 1986). Due to poor surveillance activities and lack of continuous development in the livestock health and disease management, AAT persists with increasing economic losses (Majekodunmi *et al.,* 2013a).

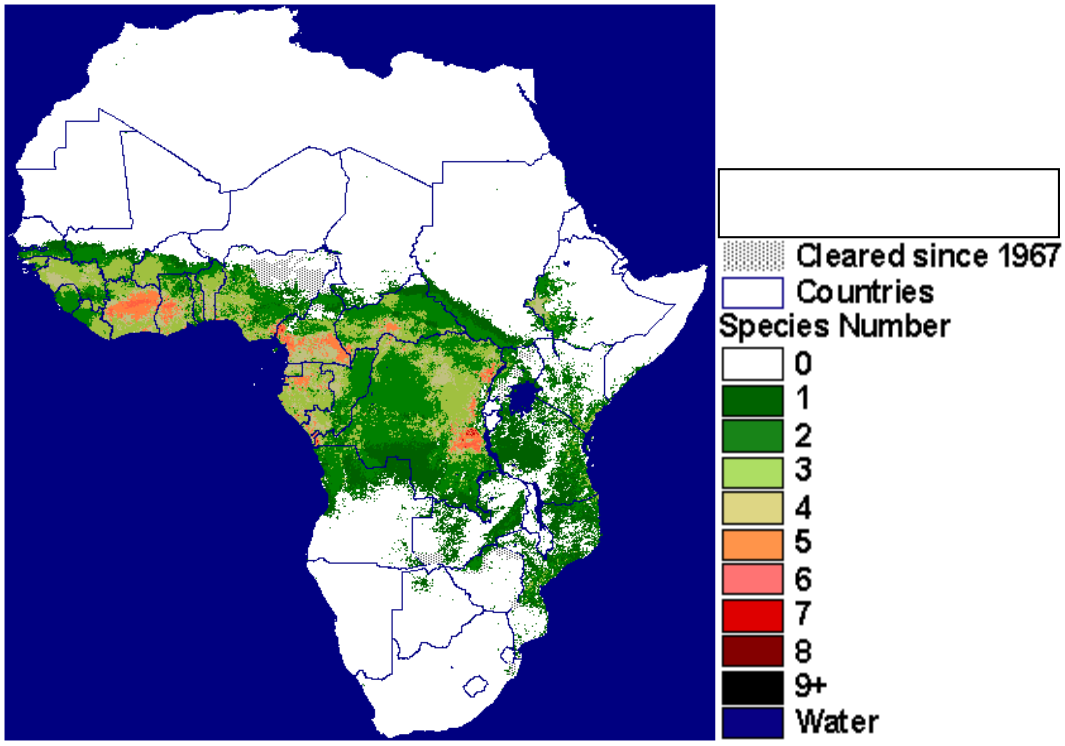


Figure 1.1: Predicted areas of all tsetse species distribution in Africa

(Source: **https://ergodd.zoo.ox.ac.uk/tseweb/allspecies.htm, accessed 16th August 2017).**

Microscopy remains the basic diagnostic approach for AAT using different techniques such as wet mount, thick and thin blood film, haematocrit centrifugation technique, buffy coat technique and standard trypanosome detection method (OIE, 2008). Notably, these methods are not very sensitive to pick up the organism at low trypanosomes/mL (OIE, 2013; Takeet *et al.,* 2013). Few studies analysed with serology and molecular techniques, respectively have been reported (Ijagbone *et al.,* 2004; Majekodunmi *et al.,* 2013a; Takeet *et al.,* 2013). Proper understanding on the epidemiology of AAT could be important in developing strategic integrated control measures to combat the disease and boost the animal production sector of the country. There have been arguments on some issues such as; regions with high endemicity of AAT in Nigeria, trypanosome prevalence in small ruminants, presence of wildlife reservoirs and sensitivities of techniques to specific *Trypanosoma* species (Leak, 1998). The initiative of PATTEC-Nigeria has not been holistic in eliminating the vectors and disease across the country, due to lack of information on AAT trend.

There is massive transhumance from cattle-producing areas in the Sudan vegetation zones in northern Nigeria to guinea savannah zones in West Africa (Majekodunmi *et al.,* 2014), precise information on transhumance around rainforest zones and coastal areas are limited. As dry season approach, there is less grass to feed upon, hence due to the densely populated southern parts and green grass vegetation, Pastoralist move their cattle down south (Azuwike and Enwerem, 2010). This southern movement starts in November in Nigeria, altering the tsetse belt and approaching regions of densely populated biting flies. The cattle movement and migration plans affect the ecological stability, thereby changing the disease epidemiology in southern regions. Decades ago, cattle graze on farmlands where harvest is complete and crop residues are available, while cattle dung serves as manure in return for this supplementary feeding. However, it has been reported that some Fulani Pastoralist stay in the fly-belt during the rainy season, causing emergence of new species of tsetse fly in this vegetation, increased dispersion of biting flies, conflicts between the nomads and local communities including farmers among other social complexities (Azuwike and Enwerem, 2010; Majekodunmi *et al.,* 2013b; Abubakar *et al.,* 2016).

Tsetse flies, *Glossina* species (Diptera: Glossinidae), are significantly important biological vectors of trypanosomes causing animal and human African trypanosomosis (AAT and HAT). The tsetse population in a geographical location could determine types of infection in the vertebrate host (Leak, 1998). Dignostic techniques such as dissection are common in

studies on *Glossina* species previously reported in southwest Nigeria (Yesufu and Mshelbwala, 1973). Environmental variables and intrinsic factors of fly vectors could affect the circulating trypanosomes (Majekodunmi *et al.,* 2013b).

Tabanidae and Muscidae (Veer *et al.,* 2002) are dipteran families reported as potential mechanical vectors of AAT (Sumba *et al.,* 1998). Ahmed *et al.* (2004) suggests that varied weather conditions may influence the abundance of *Tabanus*, as the availability of its adults are largely affected by environmental variables. Tabanidae has approximately 4000 species associated with 144 genera (Roskov *et al.,* 2013). Four subfamilies have been identified in the family (Tabaninae, Pangoniinae, Scepsidinae and Chrysopsinae) (Lessard *et al.,* 2013). Most economically important are the Chrysopinae and Tabaninae (Mullens, 2002). Despite the high economic importance as transmitting vectors of several diseases (including viruses, bacteria and protozoa (Banerjee *et al.,* 2015)), taxonomic studies of *Tabanus* in Nigeria are scanty.

Stomoxys (Diptera: Muscidae: Stomoxyini), commonly referred to as stable flies have 18 recognised species (Zumpt, 1973), among these, twelve are exclusive to Africa and one (*Stomoxys calcitrans*) is ubiquitous (Dsouli-Aymes *et al.,* 2011). Stomoxyine flies have matching size and colour pattern with house flies but their mouthparts have pointed proboscis for piercing and sucking blood (Masmeatathip *et al.,* 2006). Both sexes are blood-sucking and they feed on livestock, wildlife and humans. The major limiting factor in the elimination efforts of AAT can be related to the distribution of *Tabanus* species and *Stomoxys* species in Nigeria whose vectorial capacity and species potential have not been investigated (Anene *et al.,* 1991). However, proper identification of *Glossina*, *Tabanus* and stomoxyine is a prerequisite for generating biological data and vector-host specific interaction, to strategically control trypanosomosis in humans and livestock (Banerjee *et al.,* 2015).

In-depth understanding of the transmission dynamics of AAT has been effectively elucidated using bloodmeal studies (Muturi *et al.,* 2011). Mitochondrial cytochrome-b gene amplification has been used for identifying the vertebrate bloodmeal in tsetse fly (Steuber *et al.,* 2005). Since the development of its primers, several attempts have been made to improve the technique (Mwandiringana *et al.,* 2012). Parasites establishment in the midgut and subsequent maturity either in the mouth parts or salivary glands is important for transmission process depending on the *Trypanosoma* species involved (Van den Abbeele *et al.,* 1999).

Commonly used methods in controlling AAT in many parts of Nigeria is the combination of insecticides on vectors and chemotherapy (Kingsley, 2015). Even at that, reports of AAT in cattle herds persists across the country (Majekodunmi *et al.,* 2013a), hence more studies need to be done on the efficacy of the veterinary preparations administered. Vector control need not to be limited to insecticide treated cattle (ITC), there is a need for a more comprehensive approach like insecticide treated targets (ITT) (Vale *et al.,* 1999).

1.1 Study Design

Southwestern Nigeria falls in tsetse flies and biting vectors dense zone. A number of pastoralists in Nigeria walk their livestock down to southern part of the country in dry season to search for quality feed for their animals. The burden of tsetse and biting flies are high during the rainy season in these geographical areas. Fly survey and environmental data will be assessed from all the study sites in southwestern states using Nzi traps and weather tools to generate data on fly abundance and distribution.

The seasonality of flies will be examined along wet and dry seasons. Morphometric and molecular identification of the flies will be determined using microscopy and conventional polymerase reactions, to provide better understanding into the epidemiology of bovine trypanosomosis. Randomised sampling will be used for cattle selection in all the study sites. Trypanosomes will be identified from extracted cattle blood and fly vectors DNA using conventional polymerase reactions to determine the prevailing *Trypanosoma* species and their interactions. The prevalence, genetic diversity and the phylogenetic positions of identified trypanosomes will be determined for similarities with previously reported species.

Alighting and feeding behaviour of flies will be evaluated to understand the transmission dynamics of the disease and how these behaviours affect the disease epidemiology. Control strategy involving restricted insecticidal application protocol will be instituted to improve the existing method of fly vector control with Fulani application approach using *in vivo* cypermethrin bioassays. Questionnaires on how farmers' practices, knowledge and attitude affect the prevalence of bovine trypanosomosis will be conducted to assess their understanding on the epidemiology of the disease and correlate it with field results to provide useful information to the resourced farmers. A meta-analysis of studies involving trypanosomosis and trypanosome-transmitting vectors in Nigeria between 1960 – 2017 will be conducted using MedCalc® to determine if there in an increasing or decreasing trend of bovine trypanosomosis in southwest Nigeria.

1.2 Justification

In southwestern Nigeria, the prevalence of transmitting vectors of bovine trypanosomosis is unknown in recent years. The available data on these vectors are outdated, and the changing climate and ecological influence on vector fauna could suggest a change in the vector distribution map. The most recent distribution map was designed four decades ago, hence for proper assessment of bovine trypanosomosis, there is a need to examine the transmitting vectors.

The vectoral capacity and potential of these transmitting vectors is underestimated. *Glossina* species, which is the biological vector has been the main focus, even though, not much work has been done to molecularly assess the trypanosome distribution in them. Also, the mechanical vectors, could have a higher capacity and potential to transmit bovine trypanosomosis, hence, there is a need to examine all the vectors for *Trypanosoma* species.

Precise information on insecticidal resistance of these vector flies are limited. The changing marketing structure and polity could have effect on the type of insecticides used by the farmers. The frequency of use and application timing could either control the vectors or increase resistance among the vector population, which is critical in the epidemiology of bpovine trypanosomosis.

The current prevalence of bovine trypanosomosis across southwestern Nigeria using molecular tools is limited. The knowledge of disease prevalence will be important in formulating control plans in the country.

1.3. Aim

This research project aimed to assess the transmission patterns of bovine trypanosomes and its prevalent vectors in southwestern Nigeria.

1.4 General Objective

To use molecular techniques to detect the *Trypanosoma* species found in bovine blood and its transmitting vectors in southwestern Nigeria**.**

1.4.1 Specific Objectives

(1) To determine the catch composition of trypanosome-transmitting vectors of trypanosomosis in southwestern Nigeria.

(2) To evaluate the vectors for trypanosomes and endosymbionts by molecular studies.

(3) To assess the sources of vector bloodmeal using molecular techiques

(4) To evaluate transmission patterns of *Trypanosoma* species, by molecular studies of the bovine blood in relation to their sources.

(5) To evaluate the feeding behaviour of the fly vectors on bovine host and its control

(6) To evaluate the livestock owner's perception on bovine trypanosomosis.

(7) To analyse studies of trypanosomes found in bovine host and *Glossina* carried out in southwestern Nigeria.

CHAPTER TWO

LITERATURE REVIEW

2.1. Trypanosomosis in Nigeria

The cattle production sector is threatened with diseases in Nigeria. One neglected and significant parasitic diseases that has caused a sizable percentage of losses in cattle is trypanosomosis (Swallow, 2000). *Glossina* spp, *Tabanus* spp, *Stomoxys* spp and many other biting flies have been incriminated in transmitting infection (Abebe and Jobre, 1996). Only one-fifth of Sahel savannah, Mambilla plateaus, Obudu and Jos are free from tsetse abundance (ILARD, 1990), however; there have been reports of tsetse and trypanosomosis in some of these areas due to influx of cattle from neighbouring Cameroon and areas not covered by the control programmes (Jordan, 1986), resulting in trypanosomosis being of serious economic importance in the livestock industry (Majekodunmi *et al.,* 2013b).

Studying *Trypanosoma* species in transmitting vectors is significant to understanding the dynamics of AAT transmission, assessment of human and animal infections, spatio-temporal monitoring and control intervention impacts. The major keepers of cattle in Nigeria are Fulani herdsmen (Ducrotoy *et al.,* 2016). They engage in different agricultural practices such as mixed farming, transhumance and pastoralism. These systems are major determinants in the spread of trypanosomosis in Nigeria because of their ecological influence (Anene *et al.,* 1991).

The problem of conflicts on land-use resulted in the Nigerian government enacting a grazing bill act in 1965. Most pastoralists engage in discrete settlement patterns and or transhumance which affects the epidemiology of the disease (Ducrotoy *et al.,* 2016) and hence control relies on trypanocides. Management systems of livestock production need to be improved and educating herders on proper use of insecticides will reduce trypanosome drug resistance and socioeconomic losses in the livestock industry. Animal husbandary practices, knowledge and attitude have direct effects on the persistence of Afican animal trypanosomosis (Majekodunmi *et al.,* 2013a).

2.2 Trypanosomes

Trypanosomes are extra-cellular protozoan parasites of mammals caused by *Trypanosoma* species and transmitted by insect vectors either biologically (tsetse fly) or mechanically (*Reduviid, Tabanids* and *Stomoxys*) and via coitus depending on the organism involved. The species of trypanosomes are grouped into; Stercoraria (subgenera: *Herpetosoma Megatrypanum* and *Schizotrypanum*): contaminative transmission from the hindgut occurs. Salivarian (subgenera: *Nannomonas, Duttonella* and *Trypanozoon*): inoculative transmission from anterior gut occurs (Hoare, 1972). Trypanosomes are classified as follows: Kingdom- Animalia, Sub-kingdom- Protozoa (Levine *et al.*, 1980), Phylum- Sarcomastigophora, Subphylum- Mastigophora, Class- Zoomastigophora, Order- Kinetoplastida, members of kinetoplastids are flagellated extracellular protozoans identified by the presence of kinetoplast (Stuart *et al.*, 2008). Family- Trypanosomatidae and Genus- *Trypanosoma*.

Trypanosomes are monophyletic and parasitize almost all animal groups ranging from fish to humans as well as plants and insects. They are widely dispersed throughout the sub-saharan Africa primarily in tropical areas covering an area of 10 million km^2 (OIE, 2013). The Salivarian species which are more abundant in Africa possess variant surface glycoprotein (VSG) gene demonstrating antigenic variation.

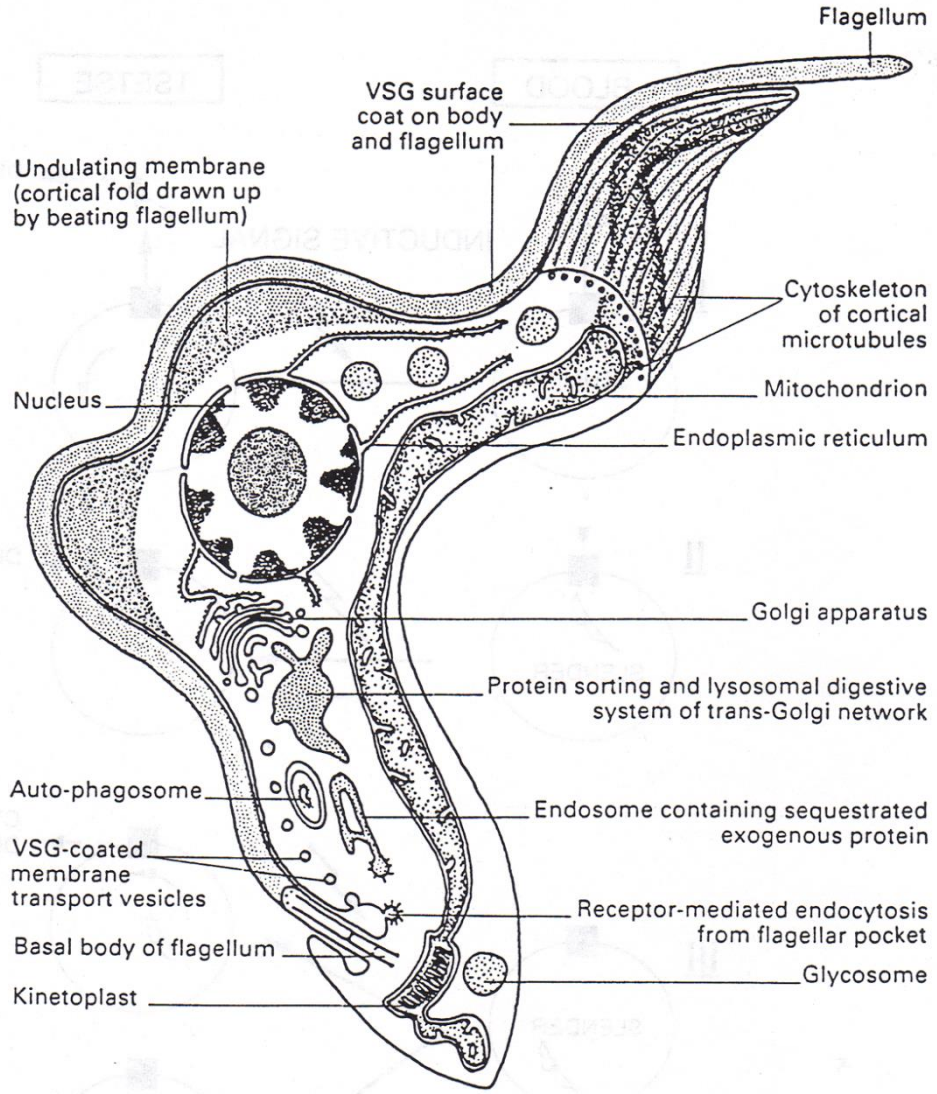


Figure 2.1: Diagram of *Trypanosoma brucei* (Courtsey: Vickerman *et al.*, 1993)

However, some trypanosomes will undergo switching different VSG leading to replacement of the surface coat with a protein not recognised by the antibodies that are present in the serum at that particular moment in time (Figure 2.1). Therefore, resulting in a new wave of parasitemia and these new parasites will increase in numbers since the expressed VSG genes are not familiar to the immune repertoire of the host. AAT or Nagana as called locally, is caused by *T. brucei, T. congolense* and *T. vivax.* However, there are reports that *T. vivax* thrives outside *Glossina* infestation zones, and reported to be mechanically transmitted (Abebe and Jobre, 1996). *Trypanosoma evansi* is also acyclically transmitted by *Tabanus* and *Stomoxys*, while *Trypanosoma equiperdum* transmission has been attributed to coitus, from one animal to the other.

2.3. Transmission of Trypanosomes

The transmission involves vertebrate host (man, domestic and wild animal) and an invertebrate host (tsetse fly) which has several species; *Glossina palpalis, G. tachnoides and G. morsitans* depending on the vegetation. Nigeria has wide vegetation, but the southwestern part of the country is basically rainforest zone. The infective metacyclic stage of trypomastigotes introduced by the bite of the infected tsetse fly develop into long forms and multiply by binary longitudinal fission. These become 'stumpy' through intermediate forms. Subsequently the parasites invade the blood streams, resulting in parasitaemia. The trypomastigote forms, particularly the short forms are picked by tsetse along with its blood-meal and undergo a series of complex biological development inside the insect host before becoming infective to mammals.

The development that takes place in the tsetsefly (the short stumpy forms of trypomastigote ingested by the insect) causes a morphological change in the mid-gut. Long slender forms (the kinetoplast lying midway) pass to the posterior end of the extra-peritrophic space where they continue to multiply for some days. By the 15th day, they escape from the anterior end of this space and enter the lumen of the proventiculus (Chatterjee, 2009). Then they migrate forwards to the buccal cavity; pass on to the hypopharynx and eventually reach the salivary glands through the opening of the salivary ducts. Under optimum conditions, many midgut infected tsetse flies will have their salivary glands infected (Akoda *et al.,* 2009). Here in the salivary glands, they multiply and change their morphology, first into epimastigote and then metacyclic stage-short stumpy forms of trypomastigote which are infective to man (Roditi *et al.,* 2008). The period to complete evolution of the infective forms inside tsetse is about 20

days. These flies remain infective for the rest of their lives, a period extending up to 185 days (Chatterjee, 2009). There is no evidence of a hereditary transmission of trypanosomes in the fly to its offspring. The tsetse flies produce a single larva and about 6 to 12 larvae during their life span, hence reproduction is very limited (Chatterjee, 2009).

In mechanical transmission, *Tabanids* and stomoxyines infect the susceptible host by contaimination after initially picking the blood forms from an infected host. The period between the two feeds is important for a successful transmission. Importantly, the mode of transmission is dependent on location, trypanosomes species and the availability of susceptible host. *Tabanids* are big, and could effectively feed on large amount of blood and hence act as efficient mechanical vectors.

2.4. The parasites lifecycle

In *Trypanosoma brucei* lifecycle, not less than several distinct forms have been reported between the vertebrate and invertebrate hosts namely slender/long, stumpy/short, procyclics, mesocyclics, epimastigotes and metacyclics forms (Chappuis *et al.,* 2005). Usually, trypomastigotes in the saliva of tsetse are inoculated into the vertebrate host where they transform to long form. Movement from inoculation point to the bloodstream through the lymphatic vessel and differentiation by binary fission to form short-stumpy forms (non-dividing) which is tsetse infective stage when picked (Chappuis *et al.,* 2005). Tsetse ingest short stumpy forms from infected blood meal in the vertebrate host (Peacock *et al.,* 2012).

For *T. brucei,* parasite transforms to procyclic trypomastigote after 24 hours in the midgut and multiply (Van den Abbeele *et al.,* 1999) Parasites then migrate as mesocyclics through proventiculus between 6 - 28 days' post exposure (Peacock *et al.,* 2012). Migration of immature forms (epimastigote) to salivary glands and further development to non-dividing metacyclic trypomastigote- host infective form occurs (Aksoy, 2003), and it is thought to possess surface coat like that of bloodstream forms (Leak, 1998).

Trypanosoma congolense appears like *T. brucei* in all the bloodstream forms, however, epimastigotes attach to mouthparts of tsetse instead of salivary glands where it transforms to infective metacyclics. *T. congolense* lifecycle is shorter than *T. brucei* with a total of 15.5 days with standard deviation of 4.6 days (Dale *et al.,* 1995). One of the barriers of tsetse infected with *Trypanosoma congolense* is the forward migration from the posterior segment

of the midgut (Leak, 1998), however, this has also been found to be true of *Trypanosoma brucei* (Dipeolu and Adam, 1974).

Trypanosoma vivax is slightly different in which all the lifecycle is completed in the proboscis (Chatterjee, 2009), although infections can sometimes be detected in the anterior gut and cibarium (Moloo and Gray, 1989). Feeding causes the transmission of the long forms, and differentiation to other forms begins in a cycle (Osoriol *et al.*, 2008). Moloo and Gray (1989) found epimastigotes and trypomastigotes in the region of cibarium and oesophagus of dissected tsetse 48 hours after an infected feed (Figure 2.2).

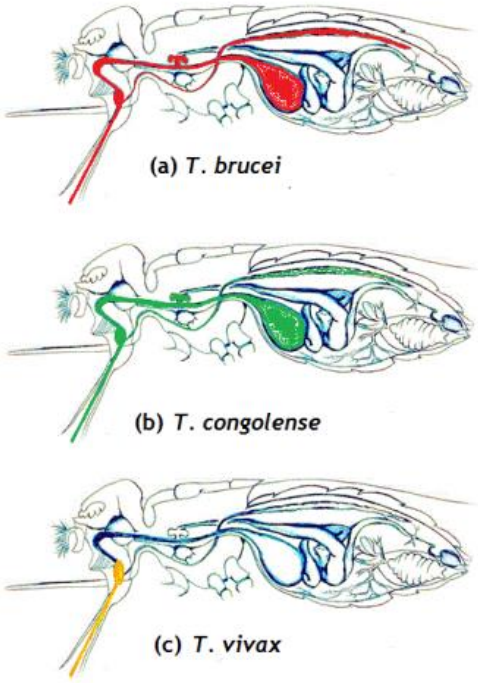


Figure 2.2: *Trypanosoma* species developmental locations in the biological vector (tsetse fly). (*courtesy- Maun)

2.5. Early research centres

There have been several centres established since the 1920s to research animal diseases in Nigeria. These include the Veterinary Reseach Centre in Vom, Jos that was set up in 1924 and the Shika Research Station in 1928 (later renamed in 1976 as- National Animal Production Research Institute). The first centre to specifically investigate African trypanosomes was created in 1947 and was called the West African Institute for Trypanosomiasis Research. It later transformed in 1964 to the Nigerian Institute for Trypanosomosis Research (NITR). Specific aims of this agency were to conduct research for the elimination and control of trypanosomosis, promoting animal and human health. Nigerian universities in 1960s conducted researches on animal disease through their veterinary faculties towards improving agricultural development with AAT being a major target.

2.6. Vectors of trypanosomosis in Nigeria

2.6.1. Tsetse vectors

Tsetse are found in Nigeria and the distribution map was constructed (Davis, 1977). There are three groups of *Glossina* species, found in Africa, namely the savannah, riverine and forest groups and they are commonly found in grazing and drinking habitats of host animals (Baldry, 1967). The savannah species (Morsitans), includes (*G. morsitans, G. pallidipes, G. longipalpalis, G. austeni* and *G. swynnertoni*), is found in southeastern and central Africa. Riverine group (Palpalis) includes *G. palpalis, G. fuscipes, G. tachinoides* and two less species, and occur primarily along watercourses in western and central Africa and forest group (Fusca) which include *G. tabaniformis, G. fusca, G. medicorum, G. longipennis, G. brevipalpis,* with eight other species that are often dispersed around the central and western Africa (Jordan, 1993). Out of the notable identified tsetse species, eleven have been reported in Nigeria (Baldry, 1967; NITR, 1983; Table 2.1; Figure 2.3), but only five are commonly found in cattle rearing areas (Davis, 1977). Optmal survival and developmental temperature are 16˚C – 40˚C and 22˚C – 24˚C, respectively; while tsetse flies are not found at elevations above approximately 1500m (Lehane, 2005).

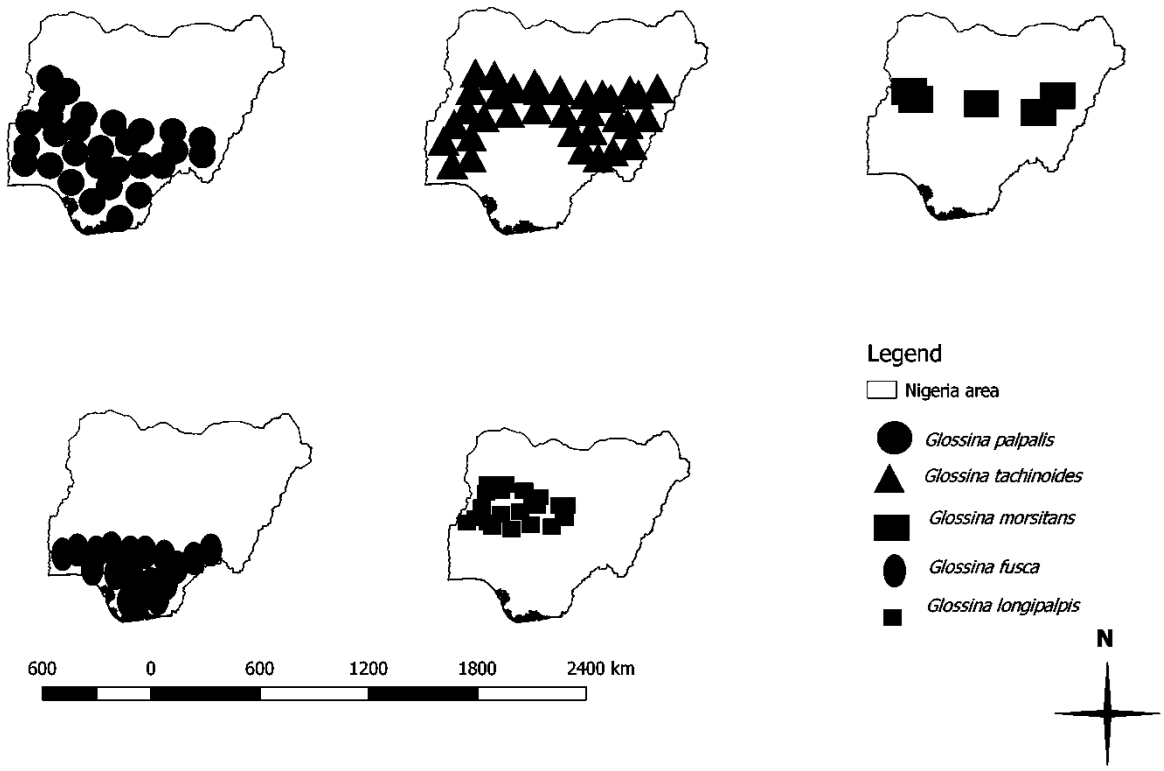


Figure 2.3: Tsetse distribution map of commonly found species in Nigeria. Source: Davis, 1977; Onyiah, 1983.

In Nigeria, *G. morsitans* are found in discrete areas of middlebelts while *G. palpalis* dispersion transcends from the northcentral to southern rainforest zones (Figure 2.3). Early surveys suggest *G. tachinoides* (Westwood) spreads around the north and southern states (Baldry, 1967). Palpalis group have been reported in a survey done between 1994 – 1999 in Kotangora town, Niger State where 7, 24 and 39 tsetse were caught in 1994, 1995 and 1999 respectively in a peridomestic habitat (Ahmed *et al.,* 2004).

A total of 0.09 flies/trap/day apparent density was reported in Plateau State (Kalu, 1996), which is pronounced free of tsetse flies after reclamation by several control programme in northeast of the country (Jordan, 1986). Shortly before the introduction of sterile insect during the project in Lafia, the apparent densities of trapped *G. palpalis* ranged from 0.10 – 19.30/flies/trap/day (Takken *et al.,* 1986). *G. tachinoides*, *G. palpalis palpalis* and *G. m. submorsitans* have been reported from Yankari Game Reserve, Bauchi State and Wuya, Niger State with apparent tsetse densities of 128.03, 27.31 and 101.21/flies/trap/day for the three species respectively (Isaac *et al.,* 2016).

Table 2.1: *Glossina* species diversities found in Nigeria

Palpalis	Morsitans	Fusca
[&]*G. palpalis*	[&]*G. morsitans*	[&]*G. fusca*
[&]*G. tachinoides*	[&]*G. longipalpalis*	*G. nigrofusca*
G. caliginea		*G. medicorum*
G. pallicera		*G. haningtoni*
		G. tabaniformis

[&]species of importance (Davis, 1977)

Traps often used to sample tsetse in Nigeria include biconical trap (Challier and Larviessier, 1973) and nitse trap (Omoogun, 1994). High infection rates and increased transmission for all trypanosome species have been reported for the palpalis group (Leak, 1998) while Fusca group of tsetse have high transmission and infections of *T. vivax* and *T. congolense* than *T. brucei*. On the contrary, Moloo *et al.* (1992) reported higher infection rates for the Morsitans group.

Hence, the available tsetse species found in a geographical location could determine type of infection in the vertebrate host. Variation in vector capacity could be determined by physiological and genetic factors such as age, sex, physiology, immune status, biochemical status, nutrition, and endosymbionts. All these vary geographically and could affect the species of trypanosomes at a given time. The riverine tsetse are efficient vectors of human sleeping sickness possibly because of close human and fly contact which may be reflected in their peridomestic larviposition sites (Leak, 1998).

2.6.2. Non-tsetse transmitted trypanosomosis

The ecological preferences of tabanid flies have been reported to be very diverse with a characteristic broad geographical distribution which only excludes extreme areas. Tabanids are specific with vegetation and different areas in a country may harbour distinct species of fly (Barros, 2001). Outside Nigeria, African tabanids and stable flies in Burkina Faso and Central African Republic, respectively have been reported to be competent of *Trypanosoma vivax* transmission (D'Amico *et al.,* 1996; Desquesnes and Dia, 2003). *Trypanozoon* and *Nannomonas* have been observed to be transmitted by *S. taeniatus* and *S. niger* (Sumba *et al.,* 1998), *Stomoxys calcitrans* was also reported to transmit *T. brucei* (Taylor, 1930) and *A. agrestis* transmitted *T. congolense* (Desquesnes and Dia, 2003). In Nigeria, there are wide seasonal patterns which may affect tabanid abundance and its distribution (Barros, 2001). There are different tabanids in Nigeria, and it is important to evaluate the habitat preference which may be due to differences in larva requirements (Lane, 1976), and this may largely affect the distribution of the existing tabanids.

Tabanids inflict painful bites and are aggressive feeders; they cause host defensive reactions, such as tail twitching, hoof stamping and ear flicking. Reported *Tabanus species* include, *T. biguttatus*, *T. taeniola*, *T. latipes*, *T. pluto*, *T. subangustus*, *T. fasciatus*, *T. fuscipes*, *T. gratus*, *T. pertinens*, *T. par*, *T. albipalpus*, *T. secedens*, *T. thoracinus* and *T. neocopinus* (Dipeolu,

1977; Ahmed *et al.,* 2005). Other members such as *Haematopota* and *Chrysops* could be potential mechanical vectors of trypanosomosis in Nigeria. In Nigeria, very few studies have been done, and the role of biting flies in transmission dynamics is not well-understood. Ahmed *et al.* (2005) reported the most abundant species in his report as *T. biguttatus*, compared to *Tabanus taeniola* by Dipeolu (1977). The differences in the reports suggest, there could have been a change in the vector distribution map in the country due to activities by livestock owners.

The adaptive mechanism of *Tabanids* which makes them potential mechanical vectors of trypanosomosis include, autogeny (blood meal for egg maturation), Telmophagy (feeding from pool of blood which increases the chance of imbibing the pathogen), Long engorgement time (increases fly exposure to infected blood) and Feeding interuption (changing host before complete feeding and through contamination mechanical transmission occurs) (Foil, 1989). *Stomoxys* have not been characterized in recent experimental work in Nigeria. The distribution of *Stomoxys* compared to other biting flies is between 60 – 75% in reported studies (Dipeolu, 1977; Ahmed *et al.,* 2005). Most common species reported are *Stomoxys calcitrans* and *Stomoxys nigra*. Vectoral potential and infection rate is not known, but they could be economically important for trypanosomosis prevalence.

2.7. African animal trypanosomosis prevalence in Nigeria

There have been many studies investigating the prevalence of AAT in Nigeria over the past sixty years. Microscopical techniques dominated and it was not until the 21st century that serological and molecular tests started to be utilised. One of the first studies to be undertaken reported 30% prevalence, and based on morphology the causative agents were suggested as *T. brucei* (0.09%), *T. congolense* (14.1%) and *T. vivax* (14.7%) (MacLennan, 1956).

Studies revealed general decrease in the AAT incidence after control programmes were initiated with reports of trypanosomosis prevalence in northern Nigeria (Onyiah, 1997). Around the same time in the southern states' prevalence was found to be between 2.7 – 14% (Opasina and Ekwuruke, 1987; Ikede *et al.,* 1987). It became difficult to establish regional prevalence as some reports were from abattoir studies, in which case the animals were transported to those areas and stayed there for short period, therefore it would be unclear where infections would have been picked up. Many studies were undertaken on the Jos Plateau and these are still on-going because it was earlier reported to be free of tsetse flies (Ducrotoy *et al.,* 2016). Longitudinal survey on bovine trypanosomosis across the Jos Plateau

in Central Nigeria reported 46.8% with polymerase chain reaction (Majekodunmi *et al.,* 2013b).

Seasonal differences reported variations between the *Trypanosoma* species. Takeet *et al.* (2013) survey used both microscopy and PCR in Ogun and Kaduna States, respectively. The prevalence of 15.1% and 63.7%, respectively were reported for both techniques with highest overall prevalence of *T. congolense* (48.7%) compared to other species. This study compared bovine infection in southwest and north-central states in Nigeria (Takeet *et al.,* 2013).

2.8. Bovine trypanosomosis in Africa

African animal trypanosomosis (AAT) is a major setback in the livestock industry reducing off-take of animal protein and other products, while human African trypanosomosis (HAT) causes sleeping sickeness which compromises the ability of communities to produce food efficiently by cultivating the land and raising livestock (Welburn *et al.,* 2006). An estimated loss of US$1.3 billion has been associated with AAT, both directly and indirectly in Africa (Welburn *et al.,* 2006). AAT affects livestock yields and causes devaluation of farmers assets (Kristjanson *et al.,* 1999). It has been estimated that between 46 - 62 million are at risk of AAT in sub-Saharan Africa, with potentially huge socioeconomic losses (Swallow *et al.,* 2000; Holt *et al.,* 2016). Also, several risk factors have been listed to affect the prevalence of AAT in livestock herd such as fly vector density, livestock management system, ecological factors, trypanotolerance, availability of trypanocides and insecticides (Leak *et al.,* 1998). In sub-Saharan Africa, AAT has been perceived to be a major constraint in livestock production (Swallow, 2000), hence routine treatment of livestock with trypanocides, not necessarily after proper diagnosis have been the normal practice (Machila *et al.,* 2003).

Most prevalent trypanosome in Eastern Africa that affect cattle are *T. congolense* and *T. vivax*, although there have been several reports of *T. brucei brucei* and *T. brucei rhodesciense* with lower prevalence. In Ethiopia for instance, prevalence ranges between 1.0-37.0% depending on the abundance of vector flies and other favourable conditions (Fetehanegest *et al.,* 2012). The most affected areas with AAT in this region are the wettest and agriculturally productive areas (Fetehanegest *et al.,* 2012), hence affecting the maximum yield. Metadata analysed from trypanosome prevalence in Ethiopia indicated the presence of heterogenity among studies influenced by study year and locations (Leta *et al.,* 2016). Ebhodaghe *et al.* (2018) conducted meta-analysis on bovine trypanosomosis in Africa, obtaining an overall prevalence of 15.1% (31.2-17.0) in 180 studies. The least trypanosome prevalent country was

Senegal (2.3%) and highest Gabon (46.4%). Prevalence in southern Africa was equally high, with Mozambique and South Africa recording 22.9 and 30.9%, respectively. The country with most studied articles on trypanosomosis was Ethiopia, followed by Nigeria and Uganda, respectively. The reason for high trypanosome prevalence in sub-Saharan Africa has been attributed to the small ruminant reservoirs of trypanosomes in cattle-rearing areas (Sinshaw *et al.,* 2006), high vector population, resistant strains of blood and vector trypanosomes, cattle host preference and presence of wildlife reservoirs (Leak, 1998; Sinshaw *et al.,* 2016).

Veterinary services in sub-Saharan Africa have been silent on AAT over the past 20 years, following privatization trend, especially in Eastern and Southern Africa. For instance, the Plan for Modernization of Agriculture, in Uganda and Agricultural Sector Investment Programme in Zambia (Welburn *et al.,* 2006). In this setting, individual farmers take ownership in control of trypanosomosis supported by animal health personnel, local organisations and extension workers. All these workers are often poorly equipped to diagnose and conduct proper treatment and control services (Chilonda and Huylenbroeck, 2001; Engel and Okidegbe, 1997).

2.9. Control campaigns

The national programme known as Nigerian Tsetse Eradication Campaign (NTEP) commenced with an improved selective application of insecticides at northeastern regions in 1955 (Putt and Shaw, 1982). The success achieved encouraged expansion of the working areas to the western and eastern sections in the tropical rainforest area. Prior to this period, different activities engaging targeting trypanosomosis included settlement strategies, bush clearance, chemoprophylaxis and chemotherapy. In 1978, US$ 72.6 million was spent on ground spraying to oust tsetse flies from an area of 196,500km^2 (Putt and Shaw, 1982). Afterwards, between 1979 – 1987, landmass of 1,500 km^2 was targeted in Nassarawa State for the Biological Control of Tsetse (BICOT) project (this includes 450 km^2 of linear riverine forest) which involved mass rearing of 1.5 million laboratory-bred sterile male *G. p. palpalis* with ratio of 10:1 (Leak, 1998). The wild population was reduced to less than 10% using continuous trapping and insecticide treated-targets (ITT) for 6 – 12 weeks (Leak, 1998), then sterile insect technique (SIT) was instituted in the elimination strategy. Also, an approximately 180,000 sterile virgin-female *Glossina* were released and recaptured for the first time in Nigeria to confirm eradication (Leak, 1998). The poor financial base to extend and maintain barriers and target areas halted the programme and reinvasion occurred (Leak, 1998).

Parasitological assessment of tsetse and cattle reared in BICOT study area of Nasarawa state where SIT was carried out to combat tsetse vectors in the 1980s was undertaken. Of the 200 slaughtered and 200 settled cattle evaluated, prevalence of 9% and 10.5% respectively was reported. A total of 466 tsetse was captured with majority reported to be *Glossina palpalis palpalis* and 1.9% were positive for trypanosomes when dissected (Oluwafemi *et al.*, 2007). Sterile insect technique is thought to be cost-competitive compared to other conventional control programmes for AAT (Leak, 1998). Several repeated failures recorded on past tsetse control efforts in Nigeria have been due to trivialization and inconsequentially excluding local communities in tsetse control initiatives. The limited number of trained extension officers in Nigeria poses a problem to cover numerous communities, even though the country has demonstrated potentials to alleviate the disease in the past.

However, there are overwhelming challenges such as inadequate finances. Researchers have been focusing on sub-humid zones to promote the production of livestock (Majekodunmi *et al.,* 2013b; Ducrotoy *et al.,* 2016), cattle welfare and associated diseases; modification of livestock production is currently occurring where rainforest regions are being flooded with cattle production and improved marketing is observed due to increased migration of Fulani pastoralist (Azuwike and Enwerem, 2010). However, trypanosomosis is not funded in this zone, which has left livestock owners to find alternative measures. There need for livestock products are increasing while transmitting vectors remain constraints, hence emphasising the need for improvement of livestock research in Nigeria. Major national trypanosomosis projects have been halted since the late 1980s because of financial inadequacies and lack of interest from the government.

There have only been a few studies undertaken using routine examination and rarely molecular tools (Takeet *et al.,* 2013). Different control measures adopted in the past like chemotherapy and SIT are not sustainable in Nigeria because of drug resistance and problem of reinvasion respectively (Jones-Davis, 1967; Ilemobade, 1979; Leak, 1998; Geerts *et al.,* 2001; Oluwafemi *et al.,* 2007). PATTEC was officially inaugurated in the 26th ISCTRC conference in 2001. The PATTEC programme assisted some African countries to access loans (US $70 million) to implement first phase of their projects through the African Development Bank in 2004. Some other African countries including Nigeria settled for self-funded approach or foreign investors to start the implementation of the PATTEC initiative. The emphasis of PATTEC control strategy was to sustain the elimination of tsetse and adopt

an area-wide approach in an area (Vreysen *et al.,* 2000). The effect of PATTEC Nigeria on implementing trypanosomosis and tsetse elimination is not yet felt among local farmers considering the financial limitations.

2.10. Diagnosis of trypanosomosis and Nigeria research status

In the field in Nigeria, quick diagnosis of AAT is often used for diagnosis, this involves physical examination like lymphadenitis, emaciation, recumbency, anorexia and general body weakness. Blood samples to examine trypanosomes using blood smears and buffy coat examination from susceptible host (OIE, 2013 Staining techniques such as Giemsa stainig can also used for identification. These methods are mostly adopted in poor resourced areas that are endemic. Most of the screened animals for AAT in Nigeria were done with microscopy technique; however, they lack specificity and sensitivity (OIE, 2013). Differential diagosis include anaplasmosis, babesiosis, haemonchosis, ehrlichiosis, rabies and theileriosis. It is arrived by specific observations, clinical history, epidemiology, evolution and laboratory diagnosis. Techniques such as rodent sub-inoculation and *in vitro* methods have been reported (Ezeh *et al.,* 2014). However, xenodiagnosis is not a frequent practice in Nigeria. Serological assays are more efficient but mixed, past and current infections may not be distinguished. They are basically antigen-antibody reactions and those commonly used include ELISA (enzyme-linked immunosorbent assay), CATT (card agglutination test) and IFAT (indirect fluorescent antibody test) (Karshima *et al.,* 2012). Several molecular methodologies of diagnosis have been developed over the years, and this is currently recommended for both human and animal trypanosomosis (Wastling and Welburn, 2011). Some of which are PCR, RFLP, AFLP and RAPD (Masiga *et al.,* 2000) etc. Disadvantages of molecular tools in Nigeria include cost implications, general perception of its complexities and ill-equipped laboratories.

2.11. Trypanocidal drug resistance and substandard drugs

Trypanosome resistance report in Africa is still on-going, the concept of its significance among farmers is extremely low (Sinyangwe *et al.,* 2004). Resistance often develops because of underdosage of trypanocidal drugs to trypanosomes and poor diagnosis (Geerts and Holmes, 1998). Not less than a third of African countries have reported trypanocidal resistance (Delespaux *et al.,* 2008). Drug resistant trypanosomes have long been detected in Nigeria as far back as 1960s and ‘70s (Ilemobade, 1979). Little effort has been made to prevent drug resistance, despite persistent trypanosome infection on the field even when

common trypanocide treatments are done (Ilemobade, 1979). Notably, there are no current information on trypanocide resistance in Nigeria despite reports of livestock deaths due to AAT.

Various methodologies show a wide range differences in resistance problems from one region to another (Sinyangwe *et al.,* 2004). Increased patronage of substandard veterinary preparations has been attributed as risk factor to drug resistance (Teko-Agbo *et al.,* 2008). Chemotherapy seems to be the most valuable measure than any method in sub-Saharan Africa. Kingsley *et al.* (2015) reported a concise survey on substandard veterinary drugs which is the major cause of drug resistance in Nigeria. In one of his interviews with a CEO in veterinary drug business, it was reported that trypanocides form a large chunk of their net gain, but in the past 3 - 4 years, several trypanocides have flooded the market from China, Middle East, India and Egypt, these drugs are said to be very cheap and their effectiveness cannot be guaranteed. Studies have reported increasingly substandard drugs circulating in developing countries (Erhun *et al.,* 2001). Previously, public awareness was initiated, however, the authorities are yet to make decisive efforts in controlling drug inflow to the country (Kingsley, 2015). Although NAFDAC was established to tackle this problem, there was a neglect on veterinary drugs.

There exists wide gap in tackling trypanocide resistance in Nigeria, a study stressed correctly gauging dosages administered to animal based on its weight (Machila *et al.,* 2008), however, this is not often the case when being administered by livestock owners. It has been discovered that pastoralists find it hard to distinguish between curative and prophylactic trypanocides (Leak, 1998). The use of multiple trypanocides from different product classes was observed because livestock owners reported several responses to single treatment (Kingsley, 2015). In Cameroon, 69% of veterinary drugs did not comply with requirements this included all diminazene and isomethamidium based drugs (Teko-Agbo *et al.,* 2008). Three compounds (isometamidium chloride, diminazene aceturate and homidium salts) have been in the market for more than half-century (Holmes *et al.,* 2004). Trypanocides like ethidium have been reported to act as carcinogens, which might be used in HAT (Holmes *et al.,* 2004). Continuous chemotherapy and drug doses used in treatment are important factors in establishing drug resistance (Geerts and Holmes, 1998). The use of trypanocides to control trypanosomosis is well established and the concept is widely publicised. Studies have reported the possibility of trypanocide resistance in regions with high tsetse abundance using

normal trypanocide doses (Geerts and Holmes, 1998). Multiple drug resistance in Nigeria has been reported (Ilemobade, 1979), however, no recent studies are investigating the presence of trypanocide resistance isolates. In other African countries drug resistance does represent a problem, for example in Togo (Tchamdia *et al.,* 2017) and Ethiopia (Dagnachew *et al.,* 2015). Novel studies are required to investigate AAT drug resistance in Nigeria. The problem of drug resistance to AAT has been attributed to the weak legislation that afford farmers and unskilled workers to administer trypanocides without any consequences rather than veterinarians (Matovu *et al.,* 1997).

2.12. Advances on trypanosomosis research

2.12.1. High throughput phenotyping

Genetically distinct cells can be surveyed in a complex population with the development of Ilumina sequencing. Theis is important in trypanosome genome research, providing a genome-wide concept for RNA interference-based phenotypic research (RNAi target sequencing, RIT-seq) (Alsford *et al.,* 2011). Hence, combing Illumina and RNAi sequencing, identification of trypanosome genes responsible for growth in both procyclic and bloodstream form during development can be identified. Hence, the variant surface glycoprotein (VSG) can be screened, identified and monitored. These have provided molecular insights into the trafficking pathways and resistance mechanisms for anti-trypanosomal drugs (Cayla *et al.,* 2019).

2.12.2. Cell-cell and environmental communication

African trypaosomes have been observed to exhibit social behaviours throughout their life-cycle stages. Social or swarming motility on culture plates shown by *T. brucei* procyclic forms (Oberholzer *et al.,* 2010). cAMP plays specific role in this behaviour, for instance with the knockdown of the cAMP-specific phosphodiesterase PDEBI found to inhibit social motility (Oberholzer *et al.,* 2015), and knockdown of certain adenylate cyclases will produce hypersocial phenotype (Lopez *et al.,* 2015). This is important in the differentiation of *T. brucei* form slender to stumpy bloodstream forms, this signaling components have been observed to be conserved in *T. congolense* (Cayla *et al.,* 2019). Moreover, T. congolense produces QS signal that is responsible for differentiation of *T. brucei* to stumpy forms in cases of mixed infections (Silvester *et al.,* 2017)

2.12.3. Transgenesis

This targets the endosymbiont, *Sodalis glossinidius* in tsetse flies. It involves an evolutionary concept of introducing a gene that controls the development of *Sodalis* in tsetse body parts to prevent the establishment of *Trypanosoma* species in tsetse flies. Research is on-going on this conceptual framework.

CHAPTER THREE

MATERIALS AND METHODS

3.1. STUDY ONE

Fly survey: abundance, distribution, morphological and molecular identification of transmitting vectors of bovine trypanosomosis in southwest Nigeria.

3.1.1. Fly survey: study areas

The study area is a tropical rainforest zone which has varied vegetation ranging from dry rainforest to lowland rainforest in approximately 78,000km^2. The study site coordinates are within the latitudes 6°64'04.66"N – 7°67'77.14"N and longitudes 2°75'11.18"E – 5°20'46.13"E. Between April 2016 and March 2017, fifteen livestock centres in nine areas across southwest Nigeria were visited for fly surveying (Figure 3.1). The centres consist of livestock settlements and institutional farms. These were selected randomly across the southwest based on herd capacity within the study site clusters. All the study sites were visited in the wet (April - October) and dry (November - March) seasons.

3.1.2. Name and location (Geography, demography, topology and climate)

The study sites were Akingbite, Eruwa, University of Ibadan (UI), Abeokuta (FUNAAB), Adebayo, Ponpoola, Idiroko, Sango and Igangan.

Akingbite site: There is intense cultivation; the area is dominated by farmland. Riverine vegetation occurs only in narrow strips often cleared at many points. It consists of wide and relatively deep stream/river. No wild animals were seen during study. The climatic conditions in the area were; average temperature – 27°C, mean relative humity – 86% and wind speed – 2 secs. The area is located in Ido Local Government Area (LGA) of Oyo State. Nzi traps were set in two cattle settlements in this study site.

Eruwa site: Fulani settlements were observed in patches with presence of mild cultivation. The study site was surrounded by primary forest with wild rodents' present. The study area

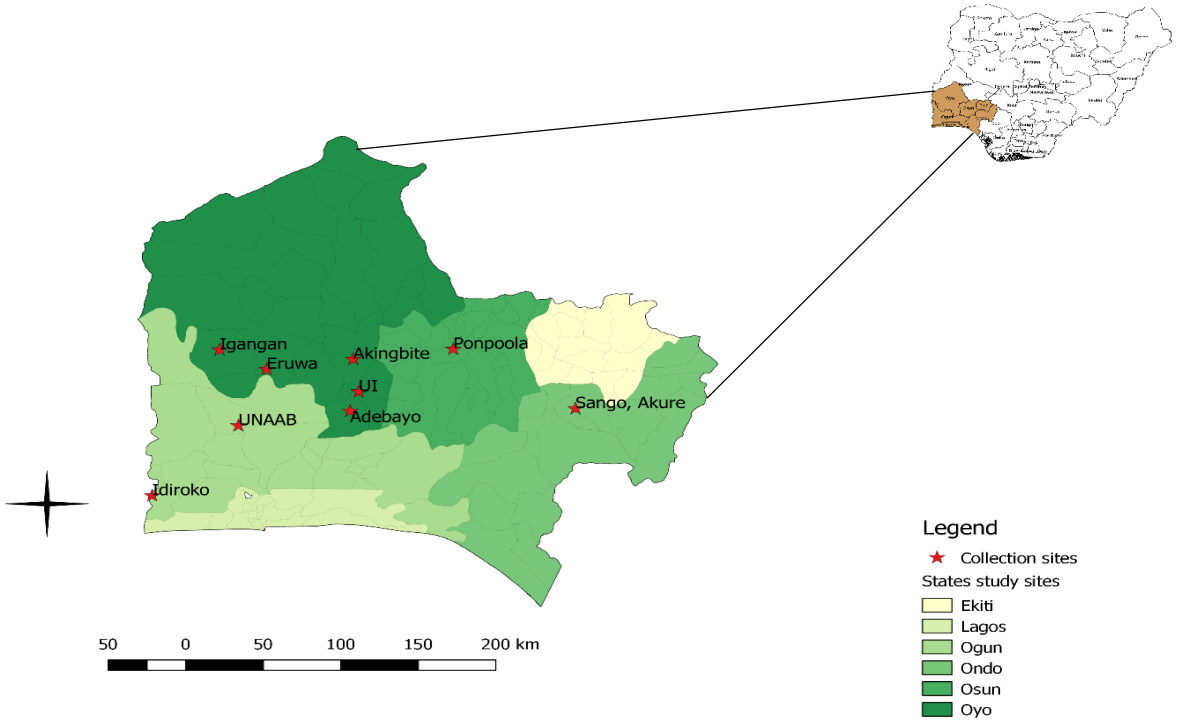


Figure 3.1: Fly trap locations of study sites in southwest Nigeria

had a wide stream adjacent to the site. The study site is characterised by thick vegetation with a thousand cattle population. The climatic conditions showed an average temperature – 28°C, mean relative humity – 87% and wind speed – 2 secs. Nzi traps were set to trap vector flies in three cattle settlements in this study area. The area is located in Ibarapa East LGA of Oyo State.

University of Ibadan (UI) site: This is an institutional farm with cattle kept for research purposes. The cattle were managed in semi-intensive system and routinely assessed. There are scanty secondary forest areas and cultivated grassland around the study area. This area is a tropical rainforest zone with no wildlife seen. The climatic conditions showed an average temperature – 26°C, mean relative humity – 89% and wind speed – 2 secs. The area is situated in Ibadan North LGA of Oyo State.

Abeokuta (FUNAAB) site: This is an institutional farm with cattle kept for research purposes. Cattle kept in semi-intensive system and routinely monitored. It consists of large expanse of paddock for grazing surrounded by secondary forest and cultivated areas. This is a typical rainforest zone with no wildlife observed. The climatic conditions showed an average temperature – 27°C, mean relative humity – 88% and wind speed – 2 secs. The study site is located in Odeda LGA of Ogun State.

Adebayo: This study site is characterised by mixture of secondary and primary forests. The vegetation is dense with conglomerate of rivers along its eastern borders. It is adjacent to the Cocoa Research Institute of Nigeria (CRIN). There are discrete farm settlements owned by community elites but monitored by Fulani herdsmen. The climatic conditions showed an average temperature – 26°C, mean relative humity – 86% and wind speed – 2 secs. Cattle population in this site was less than 100. The area is situated in Oluyole LGA of Oyo State.

Ponpoola: The study site has thick vegetation, connecting rivers, scanty forest and farming areas. The farm is fenced; however, cattle often trek out to graze. The climatic conditions showed an average temperature – 26°C, mean relative humity – 86% and wind speed – 2 secs. Total cattle population was 113 after few losses to trypanosomosis as reported by the veterinarian in charge of the farm. Blood samples from cattle and vector flies were collected in this study site. The area is situated in Abere, Ede North LGA of Osun State.

Idiroko: The location was characterised by surrounding primary forests and presence of thick vegetation. There exists a river at the east section of the farm that transverse surrounding

hamlets. The climatic conditions showed an average temperature – 27°C, mean relative humity – 87% and wind speed – 2 secs. Human population was less than 5000 while cattle population was one thousand. The area is situated in Ipokia LGA of Ogun State.

Akure (Sango): This site is a cattle market located in Akure along Ikere Ekiti road. The market consists of a mixture Yorubas and Hausa-Fulani tribe. The climatic conditions showed an average temperature – 28°C, mean relative humity – 85% and wind speed – 2 secs. There is demarcation between their market spaces while consumers and retailers visit the market to buy cattle. The area is situated in Akure North LGA of Ondo State.

Igangan: The study site (also known *"Gaa Seriki"*) is in Ibarapa LGA with a large expanse of land that encamps discrete Fulani nomad for settlements. The settlement is divided into residential section which have discrete huts and grazing lands for cattle feeding. The vegetation is tropical rainforest in nature. Few wildlife populations were reported in this area and the land is plain and rocky. The climatic conditions showed an average temperature – 28°C, mean relative humity – 84% and wind speed – 2 secs. Bovine and human population was in excess of 10,000 and 1,000, respectively. Vector flies were collected from four cattle settlements in this study site. The area is situated in Ibarapa North LGA of Oyo State.

3.1.3. Nzi traps

Nzi trap (Mihok, 2002) was designed locally using Sunbrella® Pacific Blue, Sunbrella® Black, white polyester mosquito mesh and attached to the roof is an improvised plastic collecting jar. Nzi traps used were non-baited.

3.1.4. Fly collection and processing

The trapped transmitting vectors in southwestern Nigeria were classified as fed or unfed. A total of 60 Nzi traps were used to capture adult dipteran flies (4 traps per cattle settlement and institutional farm set at 500 m equidistant to each other) and fly vector collection was done every 12 hours to avoid dryness. The traps were site bound for five days in each season per site. All trapped vectors were preserved in 95% ethanol and air dried.

3.1.5. Fly morphometric and morphological studies

Before the commencement of molecular studies, specimens were morphologically identified and species names were evaluated with Systema Dipterorum (Pape and Evenhuis, 2013). Not

all species were found in the Systema directory. Nzi-trapped flies were observed using graduated reticule embedded stereomicroscope (Euromex®, UK) and photographs were captured with Nikon A900 digital camera. The process was dependent on two important aspects; (i) alignment of ocular micrometre with morphological landmarks, (ii) proper transcription and conversion of ocular units to metric system equivalents. Various parts of the flies were measured and compared with identification keys in the literature. *Glossina* spp. were morphologically characterised using identification keys (FAO, 2008), while stomoxyine were identified using wing patterns, thoracic and abdominal markings, legs, frons, mouth-parts and genitalia (Zumpt, 1973). *Tabanus* species were identified using several keys from different authors (King, 1910). Most reports were not of Nigerian species; hence it was necessary to use specific keys from authors who identified species from Nigeria in the early 20th century (Austen, 1909). Body description which involves shape, colour, sizes of distinct parts of the body were major indices used in identification. All the vectors were morphologically identified.

3.1.6. DNA extraction of flies

DNeasy® Blood and Tissue kit Qiagen (Hilden, Germany) was used to extract fly DNA using the manufacturer's description. Prior to extraction, tsetse flies stored in ethanol were sterilised in a buffer solution (g/l NaCl 8.0, Na_2HPO4 1.15, KCl 0.2, KH_2PO_4 0.2, pH 7.3) (Sigma-Aldrich, Germany). All vector flies were examined in two parts namely, mouth (HP) and gut (TA) parts. Both parts were properly labelled and extracted separately to identify mature and imature trypanosomes found in the vector flies. Briefly, 180 µl tissue lysing buffer (ATL buffer) and 20µl of proteinase K was added to fly sample and crushed properly with autoclaved pestle (one/sample to prevent contamination). The mixture was vortexed (MS2 Minishaker, IKA®, Wilmington, USA) and incubated for 1 hr at 56°C in a preheated incubator (Grant, Barrington, Cambridge, UK). This process was repeated, then 200 µl genomic lysing buffer (AL buffer) was added to the mixture and vortexed for 10 – 15 seconds. Thereafter, absolute ethanol (200 µl) was added and vortexed. About 500 µl of mixed solution was added to microcentrifuge spin column and centrifuged (Biofuge pico, Heraeus instrument, Germany) at 8,000 rpm for one minute, after which flow through tube was disposed while the spin column was transferred to another collection tube. Genomic DNA wash buffer 1 (AW1) (500 µl) was introduced and centrifuged at 8,000 rpm for one min, flow through disposed and genomic DNA wash 2 (AW2) added and centrifuged at

maximum 13,000 rpm for three mins to completely dry the membrane. Flow through was disposed and lidless microcentrifuge introduced. Then 200 µl eluting buffer (AE) was added and centrifuged at 8,000 rpm for one min and then DNA yield was transferred to storage tube using pipette (Nichipet EX, Japan) and stored at -20°C.

3.1.7. Molecular analysis and vector DNA sequencing for fly identification

Whole flies were extracted with blood and tissue kit (Qiagen, Germany). Confirmation of tsetse flies were with ITS-2 primers; ITSA: TGT-GAA-CTG-CAG-GAC-ACA-T, ITSB: TAT-GCT-TAA-ATT-CAG-GGG-GT, used as inner and outer primers. Each PCR master mix contained 5 µl of 5 × PCR buffer, 1.2 mM $MgCl_2$, 16.6 µl of double distilled water, 0.2 mM of dNTPs, 0.5 units of Taq polymerase, 0.75 µl of 10 pmol/µl each of primers and 1µl of DNA template. Controls were included (positive and negative). PCR thermal conditions involved initial denaturation of 94°C for 5 minutes; annealing at 94°C for 1 minute, 52°C for 1 minute and 72°C for 2 minutes at 30 cycles; final extension at 72°C for 5 minutes followed by indefinite hold at 4°C. Amplified PCR products were separated by 1.2% agarose gel electrophoresis containing GelRed nucleic acid stain while 100 bp DNA ladder was used as marker to visualize sizes of DNA fragments. PCR gel products were purified using the QIAquick PCR gel extraction kit (Qiagen, Germany) and products were sequenced. BLASTn searches were used to identify 28S rDNA gene entries in the GenBank database with the highest nucleotide sequence data identities. Phylogenetic tree construction involved G-blocks curation.

3.1.8. Statistical analysis

All analyses were done using version 5 GraphPad Prism for Windows (GraphPad, San Diego, CA, USA) and WINIPEPI, United Kingdom. Measurements of fly body parts was entered in Excel spreadsheet. Standard deviation of total body length of trapped flies was calculated. Percentage distribution of trapped vectors were reported. The qGIS software (version 2.18) was used to examine the spatio-temporal distribution across the study sites. Neighbour Joining Length and Maximum Likelihood was assessed using MEGA7 software, however phylogeny tree construction was done with an online phylogeny software. Apparent density was used to evaluate relative abundance as flies trapped per day (FAO, 2008). Each species of captured flies during the five consecutive days were added to form total samples collected for the month. A transformation of the type $Y = \log_{10} (X + 1)$ was done on mean abundance

to standardise the data and normalise the variance (Mavoungou *et al.*, 2012), to explain species diversity of the vector flies. Two-way ANOVA was used to test associations of site and month on log-transformed catches of *Stomoxys* spp., *Tabanus* spp. and *Glossina* spp.

3.2. STUDY TWO

Molecular identification of *Trypanosoma* species, endosymbionts and bloodmeal analysis in transmitting vectors

3.2.1. PCR evaluation of trypanosomes DNA in vector flies

Identification of *Trypanosoma* spp. in fly vectors were done with ITS1 PCR assay (Njiru *et al.,* 2005). The primer detects trypanosomes DNA between 250 – 700 bp. Species-specific *Trypanosoma evansi* primer, RoTat 1.2 gene (Claes *et al.,* 2004), and TgsGP specific primers for *Trypanosoma b gambiense* (Radwanska *et al.,* 2002) was used on all *Trypanozoon*-positive samples. Amplified products were separated in 1.5% agarose stained with GelRed™ (Biotium, USA). Transilluminator (BIO-RAD) was used to view the DNA products, while exACTGene DNA ladder (Fisher Scientific) was used to compare the band sizes.

3.2.2. Evaluation of endosymbionts

3.2.2.1. PCR assay for Wigglesworthia glossinidia

W. glossinidia was screened for using primers to amplify 129 bp fragment of the flagellin (FliC) gene (Soumana *et al.,* 2013). Reactions were done in 25 µl volumes containing 5 × Mango Taq buffer, 0.2mM of each dNTP (Rovalab, Germany), 50 mM $MgCl_2$, primers concentration at 0.1 µM and 1Unit of BioTaq DNA polymerase (Bioline, UK). Thermal conditions were initial denaturation step at 94˚C for 5 minutes, followed by 35 cycles of 94˚C for 40 seconds, 58˚C for 40 seconds, and 72˚C for 90 seconds with final extension at 72˚C for 5 minutes. Thermal cycling for this and all other PCRs were carried out in a Bio-Rad (MJ Research Inc., USA). *Wigglesworthia* DNA from *G. palpalis* was the positive control while distilled water serves a negative control. The PCR targets, primer sequences and expected product sizes for all PCR assays are shown in Table 3.1.

3.2.2.2. PCR assay for detection of Sodalis glossinidius

S. glossinidius were screened for in tsetse flies with primers that amplify 95 bp fragment of the GroEl gene (Matthew *et al.,* 2007). Total volume for reaction was 25 µl and contained 1 µl of eluted DNA, 5 × Mango Taq buffer, 50 mM $MgCl_2$, 0.1 µM of both primers, 0.2mM of each dNTP (Rovalab, Germany), primers concentration at 0.4 µM and 1Unit of BioTaq DNA polymerase (Bioline, UK). Thermal conditions were at 94˚C (5 mins) for denaturation, 35

cycles of 94˚C (40 secs), 58˚C (40 secs) and 72˚C (90 secs) for annealing, and 72˚C (5 mins) for elongation. Zambia tsetse DNA samples initially positive for *S. glossinidius* were used for positive control and distilled water for negative control. Information on the primer sets have been reported in Table 3.1.

3.2.2.3. PCR assay for detection of Wolbachia

Primer sets which amplify a 500bp fragment of the *Wolbachia* surface protein (wsp) gene (Cheng *et al.,* 2000) was used in the PCR assays to screen fly DNA samples for the presence of *Wolbachia*. Reaction was carried out in 25 µl volumes containing 5 × Mango Taq buffer, 50 mM $MgCl_2$, 0.1 µM of both primers, 0.2mM of each dNTP (Rovalab, Germany) and 1Unit of BioTaq DNA polymerase (Bioline, UK) (Table 4.1). Thermal conditions were at 94˚C (5 mins) for denaturation, 35 cycles of 94˚C (40 secs), 58˚C (40 secs) and 72˚C (90 secs) for annealing, and 72˚C (5 mins) for extension. Kenyan tsetse DNA samples positive for *Wolbachia* were used for positive control and distilled water for negative control.

3.2.3. Analysis of bloodmeal from fly vectors

Vector abdominal contents were extracted and PCR analysis using standard procedures were used for mitochondrial cytochrome b fragments amplification (Mwandiringana *et al.,* 2012). PCR amplification was performed with 2 × Accuzyme-mix, 20 pmol/µl of both primers (Table 3.1), double distilled water and 5 µl of genomic DNA in 25 µl solution. Gel electrophoresis was done and positive band size were purified and sequenced. The PCR was done with denaturation step (95°C for 10 minutes), followed by 45 cycles of 95°C for 30 seconds, 52°C for 30 seconds and 72°C for 45 seconds for annealing step, while the elongation step is 72°C for 5 minutes.

3.2.4. Purification of DNA

DNA gel products positive for trypanosomes were separated into sterilized eppendorf tubes. Three volumes of buffer QG and 1 volume of gel were mixed and incubated at 50°C for 10 minutes. One volume of isopropanol was introduced and centrifuged at 13,000 rpm for 1 minute. Buffer QG (500 µl) was added to mini-spin column and centrifuged, then washing with 750 µl of buffer PE and centrifuge at 13,000 rpm for 2 minutes before eluting the DNA with 30 µl EB. Nanodrop spectrophotometer, ND-1000 (Labtech, UK) was used to measure the absorbance of extracted DNA at 260 nm (A_{260}) and 280 nm (A_{280}) while Thermo-

Scientific® software was used to assess the measurement. The assessment ratio gave percentage quality of the extracted DNA.

3.2.5. Sequencing of trypanosome and bloodmeal DNA

All samples positive for cytochrome b oxidase and six samples each positive for *Trypanosoma* species were examined using Sanger sequencing (GATC-biotech, Germany). Data from the National Centre for Biotechnology Information (NCBI) were used to identify and compare similar sequences on the GenBank (http://blast.ncbi.nlm.nih.gov). Phylogenetic analyses of individual alignment were constructed using MUSCLE sequence alignment (www.phlogenetic.fr).

3.2.6. Statistical analysis

All analyses were done using GraphPad Prism for Windows (GraphPad, version 5.02; 2010, San Diego, CA, USA) and WINIPEPI, United Kingdom. Values for significant differences were observed at $\alpha_{0.05}$.

Table 3.1: The primer sets for polymerase chain reactions.

Primer set	Sequences (5' - 3')	Lengths (bp)	Species identification	References
Trypanosome ITS-1	CF: CCG-GAA-GTT-CAC-CGA-TAT-TG BR: TTG-CTG-CGT-TCT-TCA-ACG-AA	Varied[1]	*T. vivax, T. brucei, T. congolense, T. simiae and T. godfreyi*	Njiru *et al.*, 2005
TgsGP gene	F: GAT-GTG-CTG-CTT-TGC-GTA-TG R: GGG-CCT-TCA-TCA-CAT-AAG-AAT-T	308	*Trypanosoma brucei gambiense*	Radwanska *et al.*, 2002
RoTat 1.2 VSG	F: GCG-GGG-TGT-TTA-AAG-CAA-TA R: ATT-AGT-GCT-GCG-TGT-GTT-CG	205	*Trypanosoma evansi*	Claes *et al.*, 2004
Cytochrome oxidase	CB1: CCA-TCC-AAC-ATC-TCA-GCA-TGA-TGA-AA CB2: GCC-CCT-CAG-AAT-GAT-ATT-TGT-CCT-CA	359	sources of vector bloodmeal	Steuber *et al.*, 2005
Flic	F: TAT-ACG-AGG-ATC-TTT-AGG-AGC R: GAT-ACT-TCT-GTG-GCA-AAA-TCT-GC	129	*Wigglesworthia glossinidia*	Soumana *et al.*, 2013
SodGRoEl	F: CCA-AAG-CTA-TCG-CTC-AGG-TAG-G R: TTC-TTT-GCC-CAC-TTT-CGC-CAT-A	95	*Sodalis glossinidius*	Matthew *et al.*, 2007
Wsp	81F: TGG-TCC-AAT-AAG-TGA-TGA-AGA-AAC 691R: AAA-AAT-TAA-ACG-CTA-CTC-CA	610	*Wolbachia*	Chen *et al.*, 2000

3.3. STUDY THREE

Molecular identification of *Trypanosoma* species in bovine host

3.3.1. Study areas

The study sites were cattle locations in southwest Nigeria. The coordinates are located between latitude 6° - 8° 00' 00"N and longitude 3° - 5° 00' 00"E. However, the livestock centres where the study was done were detailed areas of vector collections (cattle settlements and institutional farms), together with cattle markets and abattoir in southwest Nigeria. The inhabitants of the settlements are mainly Fulbe (Fulani migrants) in cattle farms. Mixed ethnic groups in the abattoir and cattle markets. Apart from study areas mentioned in study one where flies and blood samples were collected on the same site, other additional locations were blood from cattle were sampled include (Figure 3.2);

Abeokuta (Lafenwa): A major abattoir located in the central part of the Abeokuta city. Bloodspot on FTA cards were made from slaughtered cattle. This study area is located in Abeokuta North LGA of Ogun State.

Onyearugbulem: A major abattoir located in the central part of the Akure city. Blood were collected on FTA cards from slaughtered cattle. This study area is located in Akure North LGA of Ondo State.

Ikere: An abattoir located in Ikere-Ekiti city. No trap was set here, however, blood samples were collected from few slaughtered cattle. This study area is located in Ikere LGA of Ekiti State.

Agege: A major abattoir located in the Lagos state. An average of 720,000 cattle slaughtered per year. No trap was set here, however, blood samples were collected from slaughtered cattle. This study area is located in Agege LGA of Lagos State.

Akinyele: A major cattle market in Ibadan, located in Akinyele LGA of Oyo State. The market is well patronized by retailers and consumers. It has several sections such as small ruminant and cattle sections. There is a section for moribund cattle which are quickly sold for lesser price. The market consists of Yorubas, Hausa-Fulani tribe, and other tribes such as Igbo, Tiv and Nupe. No trap was set here, however, blood samples were collected from slaughtered cattle.

3.3.2. Sampling technique and study sites

Population of cattle stands at approximately 2.4 million (FAO 2014) with about 97% being trade cattle in markets and those ready for slaughter at the abattoir, while 3% are found in farm settlements (Swallow and Jabbar, 1994). Thirty-six cattle points (from four cattle sources) across the six southwest states which were considered as clusters from 16 locations which individual cattle were randomly selected (Table 3.2). Random sampling of cattle found in major cattle markets (trade cattle), Fulani cattle farms, institutional cattle farms and abattoir during wet and dry season was carried out between April 2016 – March 2017. I also sampled at least 70 – 100% from herds ≤ 50 considering major indices so that our statistical analysis would be valid. Following migration activities, improved marketing and transhumance, different classes of animals were sampled to compare prevalence and seasonal variation. Abattoir cattle were first examined and then slaughtered. Several meat retailers and wholesalers come to buy cattle in this study site. The cattle markets are cited far away from residential buildings and livestock owners have a cut-out area in the market. Fulani cattle are those raised by pastoralists which graze around the rural and urban areas and sometimes around the market for easy disposition. The Fulbe pastoralists had settled in discrete patches for 5 – 20 years or longer and apart from cattle, they owned small ruminants and practised mixed farming. Institutional cattle farms are university-based, where cattle are often raised for teaching and research.

Randomised sampling technique was applied on cattle groups based on management practices. Average prevalence of 14.3% used for this study was based on published articles between 1987 and 2016 in southwest Nigeria which gives sampling size of 741 (Appendix II).

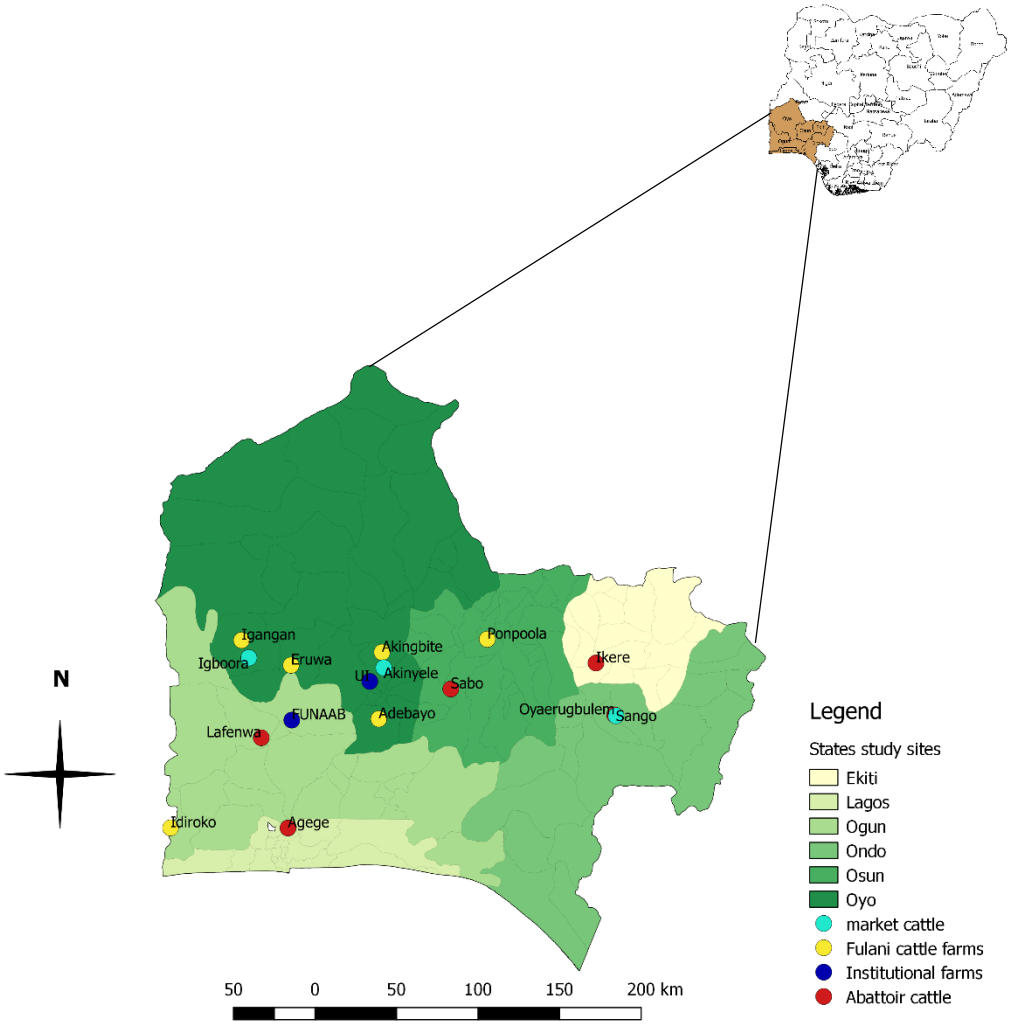


Figure 3.2: Sample collection sites of bovine blood based on management.

Table 3.2: Sampling locations for vector flies and cattle blood.

State	Location	Flies trapped	Trapping settlements	Blood collected	Cattle points	Sources
Oyo	Akinyele	-	-	144	1	Market
	Eruwa	✓	3	09	4	Farm
	Akingbite	✓	2	05	4	Farm
	Igangan	✓	4	13	6	Farm
	UI	✓	1	15	1	Inst. Farm
	Igboora	-	-	08	3	Market
	Adebayo	✓	1	16	3	Farm
Osun	Ponpoola	✓	1	20	3	Farm
	Sabo	-	-	09	1	Abattoir
Ogun	FUNAAB	✓	1	148	1	Inst. Farm
	Idiroko	✓	1	11	3	Farm
	Lafenwa	-	-	23	1	Abattoir
Lagos	Agege	-	-	251	1	Abattoir
Ekiti	Ikere	-	-	13	2	Abattoir
Ondo	Onyearugbulem	-	-	10	1	Abattoir
	Sango	✓	1	50	1	Market & *Farm
TOTAL			15	745	36	

*indicates that only flies were collected in the farm located close to the market, while blood samples were from the market cattle.

3.3.3. Collection of cattle blood sample

Venipuncturing of middle ear vein, bloodspots (2 – 3 drops) were made on Whatman FTA™ card (Sigma-Aldrich, USA) for DNA extraction. Blood spot on the cards were labelled with proper identification of the animal including age, sex, breed, body condition and location. The cards were thereafter air-dried and stored in sealed pouches (Sigma-Aldrich, USA) (Ahmed *et al.,* 2011). A total of 745 cattle blood samples were randomly collected (362 male and 383 female) consisting Sokoto Gudali, White Fulani, Kuri, N'dama, Red Bororo, Ambala and Muturu. Age was estimated as follows; ≤ 1 year, >1 and ≤ 3 years and > 3 years. Seasonal collection of blood samples was wet (480) and dry (265). Body score was based on physical fitness and presence/absence of suggestive clinical signs (Takeet *et al.,* 2013). Cattle with good conformation, gait, skin, without discharges were classified as "good", those with mild cases e.g. few clinical signs but nutrition unaffected with good conformation were classified "intermediate", while those with obvious signs like recumbency, severe lacrimation, profuse salivation, anaemia and anorexia were classified "poor".

3.3.4. Extraction of DNA from FTA cards

Four-stage extraction process was identified; punching, washing, fishing and eluting. All the instruments needed such as punches, pipette tips, eppendorf tubes, PCR tubes were all sterilised (Airclean® 600 PCR station, Star lab, China) with ultraviolet rays for 20 minutes. UV-treated hole-punch (Fiskas, China) was disinfected (Distel high-level disinfectant wipes, Cambridgeshire, UK) and used to punch five discs (3 mm/disc) into 1.5 ml eppendorf tube. After punching the needed discs, another 5 discs was punched from a blank Whatmann® filter paper to clean up before punching from another sample (Picozzi *et al.,* 2002). Lastly, 5 discs from plane Whatmann® filter paper was punched for negative control. For washing, 1ml of FTA purification reagent (Scientific Laboratory, UK) was added into each eppendorf tube in a rack using 5ml fine-tip pastette NS (Alpha laboratories, Hamsphire, UK). The rack containing the tubes is placed on rocking platform shaker (Heidolph duomax 2030, Germany) for 15 minutes, while hand-shaking is done at seven-minute interval. FTA reagent was removed and the process was repeated with a new FTA purification reagent to purify nucleic acids on cards, ensure quality DNA by removing haem, PCR inhibitors and other potential contaminants.

One millilitre of 1× TE solution (Sigma-Aldrich Ltd. Gillingham, UK) used to remove residual FTA purification reagent was introduced into the tube and the entire process of shaking and reagent removal was repeated. The discs are transferred to PCR tubes by dragging the discs from eppendorf tube using sterilised pipette tips in fishing. Samples were left overnight at room temperature. For elution, Chelex solution (5% w/v) was prepared from the properly weighed (Adventurer^{TM} OHAUS, China) UV-treated Chelex crystals. Chelex solution (100µl) was added into PCR tubes containing the discs and heated at 90 °C (30 mins) in a Peltier thermal cycler (MJ Research Inc., USA) (Becker *et al.,* 2004). The samples were then frozen at -20 °C.

3.3.5. Amplification of DNA using PCR

3.3.5.1. PCR analysis using internal transcribed spacer (ITS-1 rDNA)

Master-mix contains 1 µl of 50 mM $MgCl_2$, 5 µl of 5 × Mango Taq buffer, 1 µl of both forward 5'- CCG-GAA-GTT-CAC-CGA-TAT-TG -3' and reverse 5'- TTG-CTG-CGT-TCT-TCA-ACG-AA -3' primers (Njiru *et al.,* 2005), 0.2 µl of 5U/µl Taq DNA, 0.2 µl of 25 mM dNTPs, and 12.6 distilled water. PCR cycling was done with Bio-Rad Dyad (MJ Research Inc., USA). Total reaction volume was 25 µl containing 5 µl genomic DNA. Reactions were denaturation at 72 °C (5 mins) followed by 35 cycles of 94 °C (40 seconds), 58 °C (40 seconds), 72 °C (90 seconds) and a final extension 72 °C (5 minutes). The expected base pairs which depend on the species/sub species are between 250 bp – 700 bp.

3.3.5.2. PCR analysis with Trypanosoma brucei gambiense glycoprotein (TgsGP)

Trypanozoon positives were analysed with TgsGP PCR of 308 base pair. PCR master mix in a 25 µl final volume contains 10 × CoralLoad PCR buffer (Qiagen), 5U/µl Hotstar® DNA polymerase, 50 mM $MgCl_2$ (Bioline, UK), 25 mM deoxynucleoside triphosphates (dNTPs) (Rovalab, Germany), 10 pmol/µl of forward 5'- GCT-GCT-GTG-TTC-GGA-GAG-C -3' and reverse 5'- GCC-ATC-GTG-CTT-GCC-GCT-C -3' primers (Radwanska *et al.,* 2002), 1 µl genomic DNA and double distilled water (Sigma-Aldrich, UK). Reaction steps were denaturation (95 °C - 15 mins), annealing (45 cycles of 94 °C - 60 seconds, 63 °C of 60 seconds, 72 °C - 60 seconds) and final extension (72 °C - 10 minutes).

3.3.5.3. PCR analysis of RoTat 1.2 gene

Again, *Trypanozoon* positives were analysed with RoTat 1.2 PCR of 205 base pair. Master mix in a 25 µl final volume contained 0.2 µl of 25 mM dNTPs, 5 µl of 5 × Mango Taq buffer, 11.6 µl distilled water, 0.2 µl of 5U/µl Taq DNA, 2 µl of 0.8 µM both forward 5'- GCG-GGG-TGT-TTA-AAG-CAA-TA -3' and reverse 5'- ATT-AGT-GCT-GCG-TGT-GTT-CG -3' primers (Claes *et al.*, 2004). Reaction steps were; denaturation at 94 °C (4 mins), annealing (40 cycles at 94 °C – 60 secs, 94 °C – 60 secs, 72 °C – 60 secs) and elongation (72 °C – 5 mins)

3.3.6. Gel electrophoresis of amplified DNA from cattle blood

Agarose gel was prepared from 1.5 g of agarose in 100 ml of 1 × Tris-borate–EDTA (TBE). The mixture was placed in a micro chef SM 11 (Proline) and gently rocked every 15 seconds until a clear solvent is obtained. The gel-bottle is placed on plate stirrer for 3 minutes to allow it cool. GelRed™ nucleic acid stain (10 µl) (Biotium Inc., USA) was added and properly mixed after which the gel is poured on the assembled plate with gel-combs. A solid gel is formed and combs removed gently, 10 µl PCR amplicon was introduced into each well with 8 µl of 100-bp (exACTGene™) molecular ladder (Fisher Scientific, UK) on either side to determine PCR fragment sizes. The plate was then placed in the gel-container with 1 × TBE and connected to 100 V for 60 minutes and assessed under a UV transilluminator (Gel-Doc™ 2000) with the Quantity One software (Bio-Rad Laboratories, Inc.).

3.3.7. DNA purification with QIAquick® gel extraction kit and quantification

Exactly 3 volumes of buffer QG (pH ≤ 7.5) and 1 volume of gel were mixed and incubated at 50 °C for 10 minutes vortexing every 2 – 3 minutes. One volume of absolute isopropanol was introduced and centrifuged at 13,000 rpm for 1 minute. Buffer QG (500 µl) was added to mini-spin column and centrifuged, then washing with 750 µl of buffer PE and centrifuging at 13,000 rpm for 1 minute before eluting the DNA with 30 µl EB for direct sequencing. Purified DNA was quantified with nanodrop spectrophotometer, ND-1000 (Labtech, United Kingdom).

3.3.8. Sequencing of purified DNA from cattle blood

Samples positive for *Trypanozoon*, *Duttonella* and *Nannomonas* group were used for Sanger sequencing and sequenced using small subunit ribosomal RNA- 18S rRNA (Njiru *et al.*, 2005) and variable surface glycoprotein targets respectively. The analysed results were done with CLC sequence viewer version 7.8.1 (Qiagen, Germany) and then blast searches on National Centre for Biotechnology Information. The result with the highest nucleotide sequence identities were reported.

3.3.9. Statistical analysis

Tukey's multiple comparison ANOVA test was used for pairwise analysis, while regression analyses was used for some indices like age, breed etc. Sex and seasonal variation were assessed with chi-square and their odd-ratios. Variations in species distribution in respect to seasonal variations and cattle sources were analysed with Pearson chi-square.

3.4. STUDY FOUR

Feeding and alighting behaviour of trypanosome transmitting vectors to cattle host

3.4.1. Experimental design

The research was carried out between April and October 2016 at cattle farm settlements in southwestern Nigeria where fly vectors were abundant. White Fulani cattle (mean weight approximately 250 kg) were used for the adults while the calves used in this study were less than 70 kg and below 12 months. Cattle were camped in paddock (approx. 20 m diameter) and allowed to graze during experiment. Visual components were incorporated for assessment (video cameras). After experiments, they were freed into grazing lands daily.

3.4.2. Inclusion criteria of cattle and study sites

All the cattle used in this study were White Fulani breed. A total of three cattle in respect to age (cow, bulls and calves) were isolated and examined in each study site for the feeding behaviour. The study sites selection was based on preliminary study results using stationary traps, available workers and owners' consent. Tag number of each cattle for the experiment was recorded. In all the preferred study sites, the examined animals were restricted in smaller paddock and allowed to graze around while others go out to graze for adequate fodders and water (a semi-intensive system in all sites).

The direct observation of fly vectors on cattle host lasted 40 days which spanned through the seven wet months and study sites were free of insecticide use during behavioural experiment. The study was carried out for five consecutive days in each site. Observational period was between 9 am – 12 pm the five consecutive days in each cattle farm. Total number of vector flies alighting and or feeding on cattle host was recorded at the end of 60 seconds and examined for 5-10 minutes by three observers. Videos of the behavioural activities were recorded. Comparison of results from observers was done.

3.4.3. Study duration and study sites

All experiments were done in rainy season (April – early October), while study sites are in the rainforest vegetation. The range of mean annual precipitation, temperature and humidity stands 1500 mm – 2000 mm, 23°C – 32°C and 73 – 90%, respectively. Behavioural studies related to *Glossina* spp. were done in Idiroko and Adebayo study sites. Both sites are

characterised by surrounding primary forests. There exist rivers at the east and west parts of the cattle farms that cut across to other nearby towns. An approximately 5,000 human and 2,000 cattle population, respectively are resident in these sites.

The feeding behaviour studies on stomoxyine in all the study sites which are Igangan, Akingbite, Ponpoola, Abeokuta, UI, Adebayo, Eruwa and Idiroko was evaluated. However, Akingbite study site has a cattle herd settlement of approx. 200 cattle herd surrounded with active cultivating area. It has a sparse human population with less dense vegetation. Igangan study site (Gaa Seriki) is characterised with a wide-area land that encamps Fulani pastoralists in discrete form. The vegetation is a dry tropical rainforest, contained mixed rocky and plain terrain, sparse vegetation and few wildlife populations. Cattle and human population were in excess of 10,000 and 1,000, respectively. FUNAAB and UI were the institutional cattle farms for the studies. Both farms were characterised by the presence of secondary forest with not fewer than 200 cattle in each respectively. Presence of primary forest, dense vegetation, barricade fence and surrounding farming areas are typical of Osogbo study area. Eruwa study site is characterised by approx. 1,000 cattle while vegetation is dense. Fly behaviour regarding *Tabanus* spp. was done in all study sites except Abeokuta, because it was not found in the study area (Figure 3.3).

3.4.4. Identification of landing flies

Landing *Glossina* and *Tabanus* spp. could easily be identified from three meters away, since the observers are well acquainted on species types. Besides the videos made identification easy. Previous months, Nzi traps were used to capture flies for abundance, while the general body description and colouration were properly studied using identification keys- *Tabanus* spp. (Austen 1909) and *Glossina* (FAO 2008). Stomoxyines were not readily identified to species level at few meters away, hence, record was made at genera level.

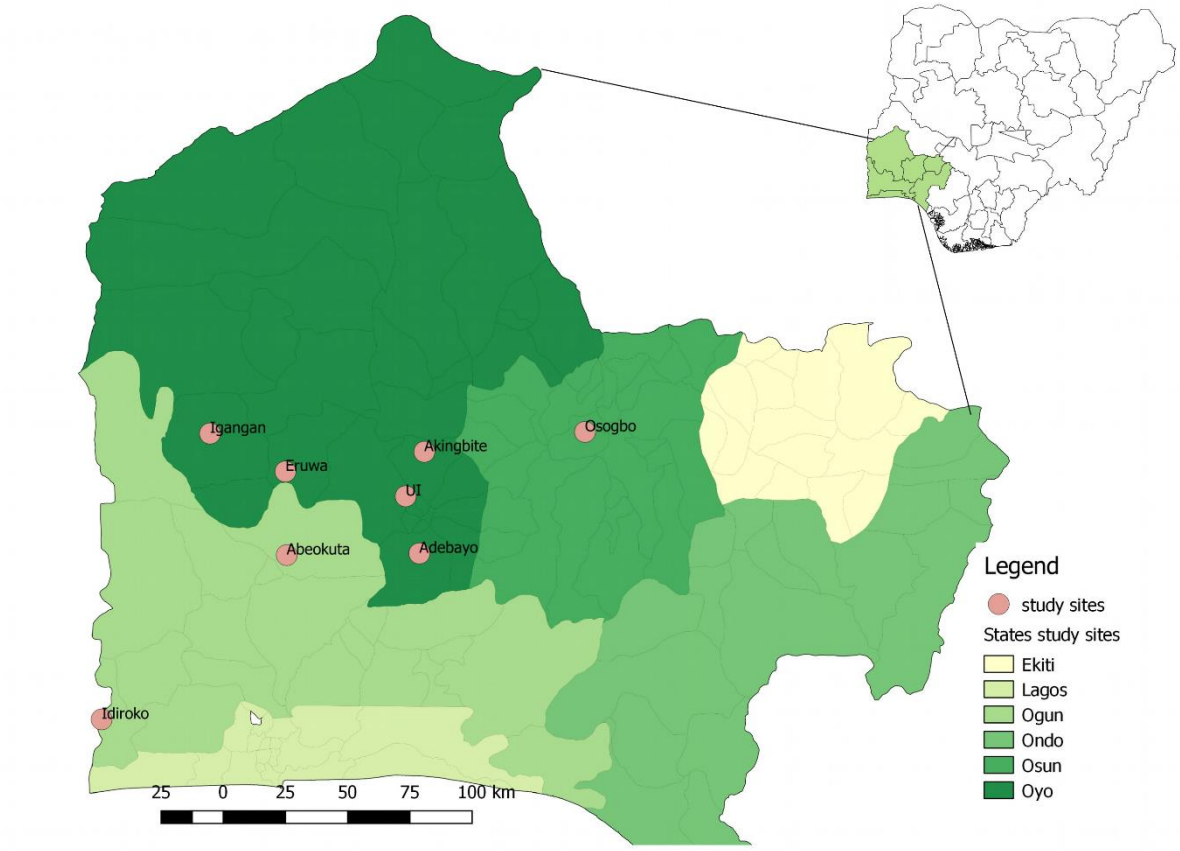


Figure 3.3: Study locations for feeding behavioural responses of fly vectors.

3.4.5. Landing behaviour observations

The body regions for the landing flies were based on previous illustrations (Torr *et al.,* 2007; Figure 3.4). Three observers monitored cattle (cow, calves and bulls– each at a time) from 3 m distance (Muzari *et al.,* 2010a) which helped to avoid bias from visual stimuli and human odours (Vale, 1974). Observations were made at 60 seconds interval for 10 minutes to record fly numbers using a counter. Landing and alighting fly vector species were recorded using colour markings, size and shape of their body parts. While the landing position of the flies on cattle hosts were reported. All vector flies alighting and landing were recorded and classified based on specific body sites (Figure 3.4).

3.4.6. Feeding behaviour and engorgement success of flies

Studies on feeding responses to cattle hosts observed individually in the paddock (20 m diameter) was assessed. Prevailing flies have been caught from this site prior to this experiment. Total counts were added together to obtain daily estimate of each of the fly density (Schofield and Torr, 2002). Immediate taking-off after landing without penetration of the proboscis is regarded as unfed. Those that fed without completing feeding do not have distended abdomen, hence they are known to be partially fed. Flies were observed to be fed once the abdomen became fully distended with blood before alighting. Feeding duration in seconds was estimated for every observed fly using a stop-watch on body sites (Figure 3.4). Repeated fly visit of flies could not be differentiated, while and a fly commence feeding when proboscis is seen entering the skin. This was same for *Tabanus* species in Igangan, Akingbite, Eruwa, Osogbo, Idiroko, UI and Adebayo. Stomoxyine was also assessed in all the sites were *Tabanus* spp. was examined with Abeokuta inclusive.

3.4.7. Cattle host defensive behaviour

The defensive behaviour were represented as: Tail flicking– when the tail flicks above the back height; Hoof stamping– deliberate and vigorous stamping of the foot; Ear twitching– when both ears flick simultaneously. Observations were repeated three times on cattle, in which the numbers of fly vectors were assessed per minute using a counter and stop-watch, while observers take their rest for 20 minutes (Torr and Hargrove, 1998).

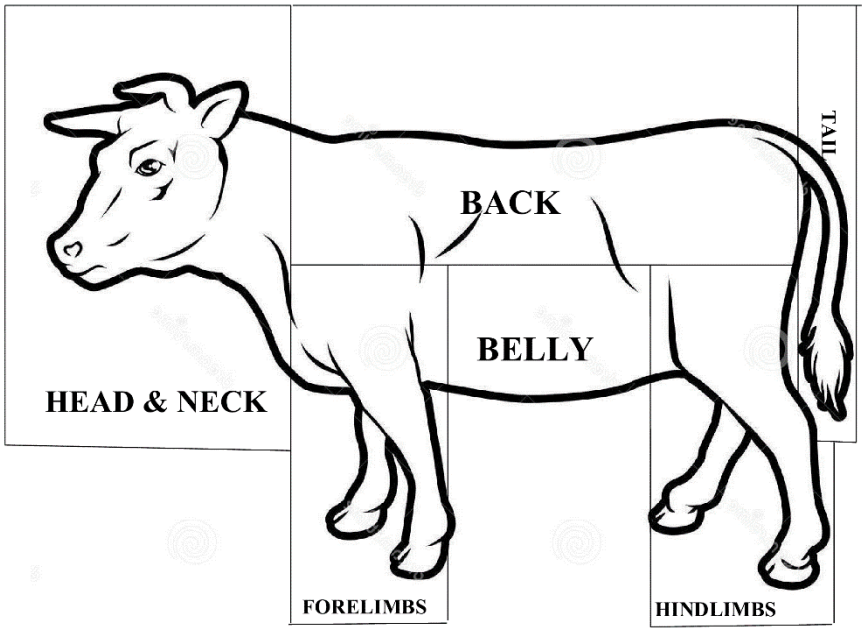


Figure 3.4: Fly alighting sites on cattle host

3.4.8. Statistical analysis

GraphPad Prism (version 5) was used for analysis. General behavioural assessments were quantitatively analysed while median was used for data with outliers. To analyse proportional differences of vector species landing on specific body region, number of fly vectors species landing on the body and the total number of the genera observed on daily collection serve as the response variable and the binomial denominator, respectively (Torr *et al.*, 2007). Alighting sites of *Tabanids* were analysed using pair-wise comparisons of Bonferroni's multiple tests.

$$\text{Percentage engorgement success} = \frac{\text{total engorged flies}}{\text{total biting/feeding flies}} \times 100.$$

3.5. STUDY FIVE

Insecticidal activity of cypermethrin and analogues against stomoxyine vectors: *in vivo* and *in vitro* bioassay

3.5.1. Stomoxyine flies

Morphologically speciated *Stomoxys niger* trapped in the study sites were selected for the *in vitro* study. Only the fed stomoxyines were used in the study, hence, feeding subjects were not necessary during the 48 hrs. A total of 550 stomoxyines were used in the experiment.

3.5.2. Insecticides- Cypermethrin and analogues

The *in vitro* insecticidal bioassay of stomoxyines were validated using cypermethrin formulations. Group A- contains graded concentrations of insecticides; Provethrine 30 PANTEX® (Holland) containing Cypermethrin (30 g l^{-1}). Group B- contains the graded concentration of cypermethrin (Ectopouron® – components include leaves and seed oil of *Azadirachta indica* (25%), *Cymbopogon nardus* – 5% and synthetic cypermethrin – 10%). Group C- contains Fulani insecticide concoction which is a mixture of cypermethrin (Cyperfly® 6%) and oil of a leave (*Senna occidentalis*) locally called *"Bangaru angasa"*. Group D is the study control.

3.5.3. Restricted Insecticidal Application Protocol (RAP)

RAP involves insecticidal application protocol in which body regions of the host where vectors are thought to concentrate while feeding is targeted. The application is restricted to economically reduce the amount of insecticide used for effective control strategy. This helps to limit the environmental pollution from the insecticide compounds, encourages enzootic stability and invertebrate fauna (Vale *et al.*, 2004; Torr *et al.*, 2007).

3.5.4. Fulani Application Approach (FAA)

This approach involves the crude application of insecticides by the Fulani herdsmen without interference. Application of dispensed insecticides were often used on all the body regions. These insecticides were purchased from street vendors in local markets. The Fulani livestock keepers were often engaged in second application of the insecticides at approximately 72 h

after the initial application to further improve the effectiveness of the preparation. Also, adjuvants like plant or seed oil, mineral oil were observed to be used as mixtures with the insecticides at 1:10 ratio to avoid or minimise the potential toxicity associated with the drug.

3.5.5. Grouping of cattle for the in vivo bioassay

For the *in vivo* insecticidal study, the RAP method, Fulani method, and control groups were evaluated. Three cattle were used per group in this study. RAP method (group A)- cypermethrin (Panthex®) was used for restricted insecticide application (RAP) as previously described by Torr *et al.* (2007) but has not been experimented in Nigeria. Fulani method (group B)- cypermethrin (either Ectopouron® or Cyperfly® with leave (*Senna occidentalis*) oil) was used based on the type of insecticides available in the nearby markets to the farm settlements. For most insecticidal treatement by Fulani herdsmen, application is often repeated after three days. However, for the RAP method, application was once all through the six days. Examination was done for the six days post-application and assessed every three hours for the first 12 hours and subsequently every 24 hours. Group C was the control without any drug applied.

3.5.6. Experiment one

3.5.6.1. Insecticidal bioassay (in vivo) on fly behaviour

Glossina spp. and *Tabanus* spp. trapped were not enough for bioassay, hence experiment focused on the stomoxyine flies. In a case where the insecticide prescribed dose was 1 ml/10 kg, RAP method affords the opportunity to use on 20% of the recommended dose. This is concentrated in body regions where flies landed (e.g. the belly and legs represent 20% of the total body size) (Torr *et al.*, 2007). For the FAA group, the Fulani herdsmen were allowed to administer the insecticides exactly as in routine practice. Stomoxyines were observed for alighting flies/min, landing duration and feeding duration; while cattle were evaluated for hoof-stamping/min, tail-twitching/min, and ear-twitching/min in the insecticidal resistance study.

Timing was three hours/day between 9 am to 12 pm for five consecutive days in cattle farm settlements, although weather conditions were important factors considered during the experiment. Live stomoxyine flies observed on the host were assessed with a counter and stopper for the insecticidal activity. Experimented animals were isolated for the six-day

period and prevented from body contact with other animals. Nine cattle were used in Akingbite, Eruwa and Igangan study sites with three cattle in each group (RAP method, Fulani method, and control)

3.5.7. Experiment two

3.5.7.1. In vitro insecticidal assay

Residual contact assay recommended forresidual monitoring were used to evaluate the insecticide concentrations (Torr *et al.,* 2007). A clean glass jar of 100 ml was treated with insecticide and acetone at 1:1. After allowing the evaporation of the acetone, fly vectors were introduced appropriately to into the treated jar for 30 secs to make contact and then moved into to a clean jar with a perforated lid. After 48 hrs period, survivability assessment was done (Kernaghan and Johnston, 1962, Torr *et al.*, 2007).

Ten stomoxyine flies in each jar contained graded concentrations of insecticides (30 µg/ml, 20 µg/ml, 10 µg/ml, 5 µg/ml, 1 µg/ml and 0.5 µg/ml). These were in three groups (Group A- Pantex®, Group B- Ectopouron®, Group C- Fulani concoction). The *in vitro* experiment made use of 550 captured stomoxyine flies for the insecticidal resistance evaluation at room temperature done in triplicates. Ten stomoxyine were used for group D (control experiment) without insecticide.

$$\% \text{ insecticidal activity} = \frac{\text{Total number of flies exposed} - \text{Number of unaffected flies at 48 hrs}}{\text{Total number of flies exposed}} \times 100$$

3.5.8. Statistical analysis

One-way ANOVA analyses was used to compare the insecticidal bioassay of the *in vivo* experiment. Percentage change of experimental groups was calculated using the formula below $\frac{\text{No of flies at the end of Exp} - \text{No of flies at the beginning of Exp}}{\text{No of flies at the beginning of Exp}} \times 100$

Where, No is number; Exp is experiment.

Log concentration in µg/ml was plotted against percentage insecticidal activity to determine the LC_{50} of the non-linear regression curve model of cypermethrin against stomoxyine flies.

3.6. STUDY SIX

Perception and practises of livestock owners in southwest Nigeria in relation to bovine trypanosomosis

3.6.1. Questionnaire design

Self-explanatory questionnaire was administered to livestock keepers in southwestern Nigeria (Appendix VIII). Participatory rural appraisal involved a one-on-one evaluation with focus group (2-20 in the group) on general information about AAT and how it affects their livelihood by considering several factors. Livestock owners (farmers, herders, traders and butchers) were interviewed based on structured questions illustrated above. Language interpreter was used in some places to avoid language barrier.

3.6.2. Questionnaire sampling technique

A multistage random sampling was used to identify areas concentrated with livestock keepers. Ten areas (Iddo, Akinyele, Igangan, Eruwa, Ekiti, Igboora, Akure, Lagos, Osogbo and Abeokuta) with cattle sources such as: farm settlements, abattoir and cattle markets were selected for the study on bovine trypanosomosis (BT). A semi-structured questionnaire and verbal assessment was applied to 209 randomly selected cattle owners (focus groups of 2 – 20) in southwestern Nigeria.

At each household, a focus group discussion was held with all the workers employed by the cattle owner. The questionnaire was design to achieve the socioeconomic and perception on bovine trypanosomosis by livestock owners (Appendix VIII). The importance of interviewing in groups was to generate a viable data from collective and accurate responses to questions. This was done to understand the perception of cattle owners on bovine trypanosomosis and its vectors, constraints from their practises, socioeconomic assessment and government interventions. The consent of the livestock owners was obtained and identities were kept confidential.

3.6.3. Statistical analysis

Descriptive analysis was used for livestock owner's occupation and practises. Livestock mortality was analysed with poisson regression analysis. Socioeconomic parameters were measured for disease listed and were analysed with the t test. All values were analysed with GraphPad prism (version 5) and significance were calculated at $\alpha_{0.05}$.

3.7. STUDY SEVEN

Meta-analysis of African animal trypanosomosis in Nigeria

3.7.1. Parameters specification for sample survey

The study followed PRISMA guideline (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). All information according to PRISMA checklist were considered in the study.

3.7.2. Search strategy for meta-analysis

Screened publications Google Scholar, Web of Science, PubMed Central, Global Health and Ovid MEDLINE were made till September 07, 2017. Important words searched include bovine, trypanosomes, porcine, horses, small ruminant, camels, tsetse, *Glossina* and trypanosomosis Studies not found in the database were directly requested from the University Library which were ordered through University ILLiad Odyssey enabled mechanism.

3.7.3. Inclusion criteria

All articles recovered were screened and only those with natural infections and outbreak reports were included in the manuscript. Experimental cases were all excluded. Eligible studies were sorted (Figure 3.5). Inclusion criteria were location of study, study type, prevalence, *Glossina* species, *Trypanosoma* species, prevalence of trypanosomes in tsetse and livestock, diagnostic method and sampling year. The PRISMA Checklist 2009 (Appendix VII) was used to ensure inclusion of all relevant information in the analysis (Moher *et al.*, 2010). Bias assessment was evaluated with some questions such as: was the research objective stated clearly? Was the sampling method properly described? Were the study areas clearly pointed out? Were humans categorised into different subgroups? All case reports and studies which did not meet the inclusion criteria were thoroughly reported in the text for completeness.

3.7.4. Statistical analysis

MedCalc® software and WINPEPI statistic package (United Kingdom) were used for analyses. Random and fixed effects were calculated using heterogeneity I^2 statistic. Chi-square analysis was used for diagnostic subgroup analyses. One-way ANOVA compared prevalence and study years of AAT analyses. Illustation map of AAT distibution was

generated with qGIS (version 2.8.10). Raw data was entered into Microsoft Excel spread sheet and 95% confidence interval for descriptive analysis. Values for significant differences were reported at $\alpha_{0.05}$.

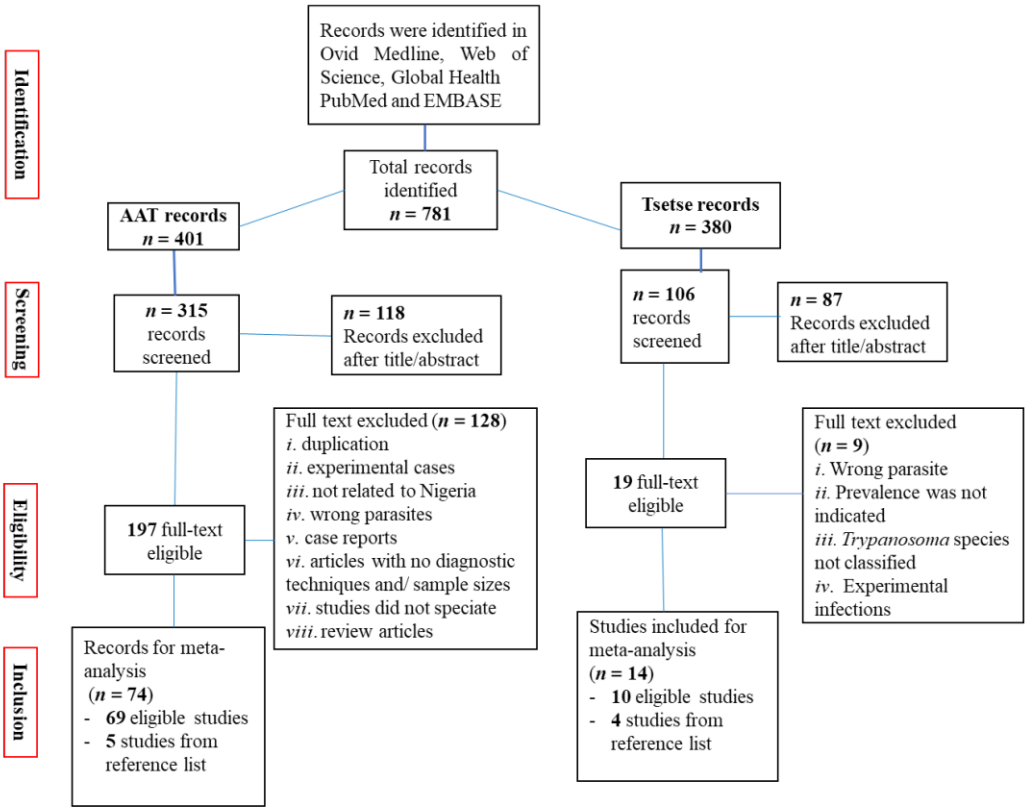


Figure 3.5: Flow chart on the systematic searches on literature

CHAPTER FOUR

RESULTS

4.1. STUDY ONE

4.1.1. Spatio-temporal distribution of trypanosome-transmitting vectors

Glossina palpalis and *Glossina tachinoides* were identified from the trapped flies in southwest Nigeria. It was only in Adebayo, Oyo State and Idiroko, Ogun State that *Glossina* species were trapped throughout the trapping season (Figure 4.1). Locations of trapped *Tabanus* species and apparent densities are shown (Figure 4.2). *Tabanus taeniola* Palisot de Beauvois (1807) was most abundant and was observed in Akingbite, Eruwa, UI and Igangan. *T. subangustus* Ricardo (1908) was most distributed and trapped in Akingbite, Eruwa, UI, Idiroko and Sango in Akure.

Throughout the sampling, none of the traps caught *Tabanus* in FUNAAB, Abeokuta despite an apparent density of >50 per trap of *Stomoxys* species trapped in the location (Figure 4.3). *Tabanus par* Walker (1854) and *T. thoracinus* Palisot de Beauvois (1806) were both trapped in Adebayo and UI, both in Oyo State and catches were made in wet and dry seasons. *Tabanus rubidus* Wiedemann (1821) was only trapped in UI. *Tabanus pertinens* Austen (1912) was trapped in Igangan, Oyo State and Poonpola, Osun State. *Tabanus biguttatus* Wiedemann (1830) was trapped in Akingbite, Oyo state and Ponpoola, Osun State while *Tabanus brucei* Ricardo (1908) was trapped at Adebayo and UI. *Stomoxys* species was trapped in all the study sites. The two species of stomoxyine fly observed in all sampled areas were *Stomoxys niger* and *Stomoxys calcitrans*.

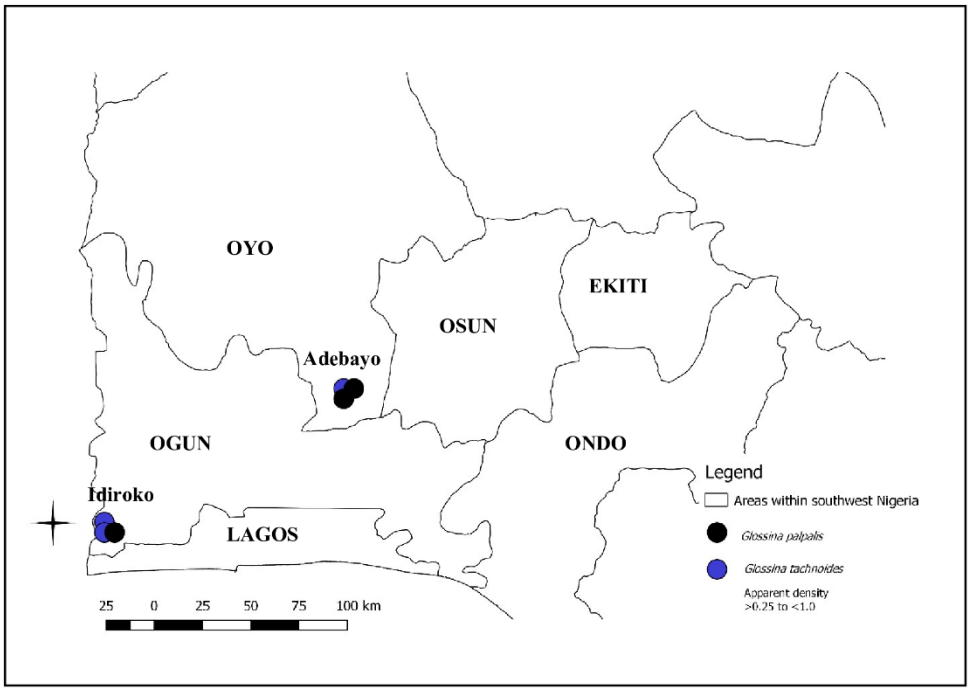


Figure 4.1: Spatio-temporal distribution of trapped *Glossina* species.

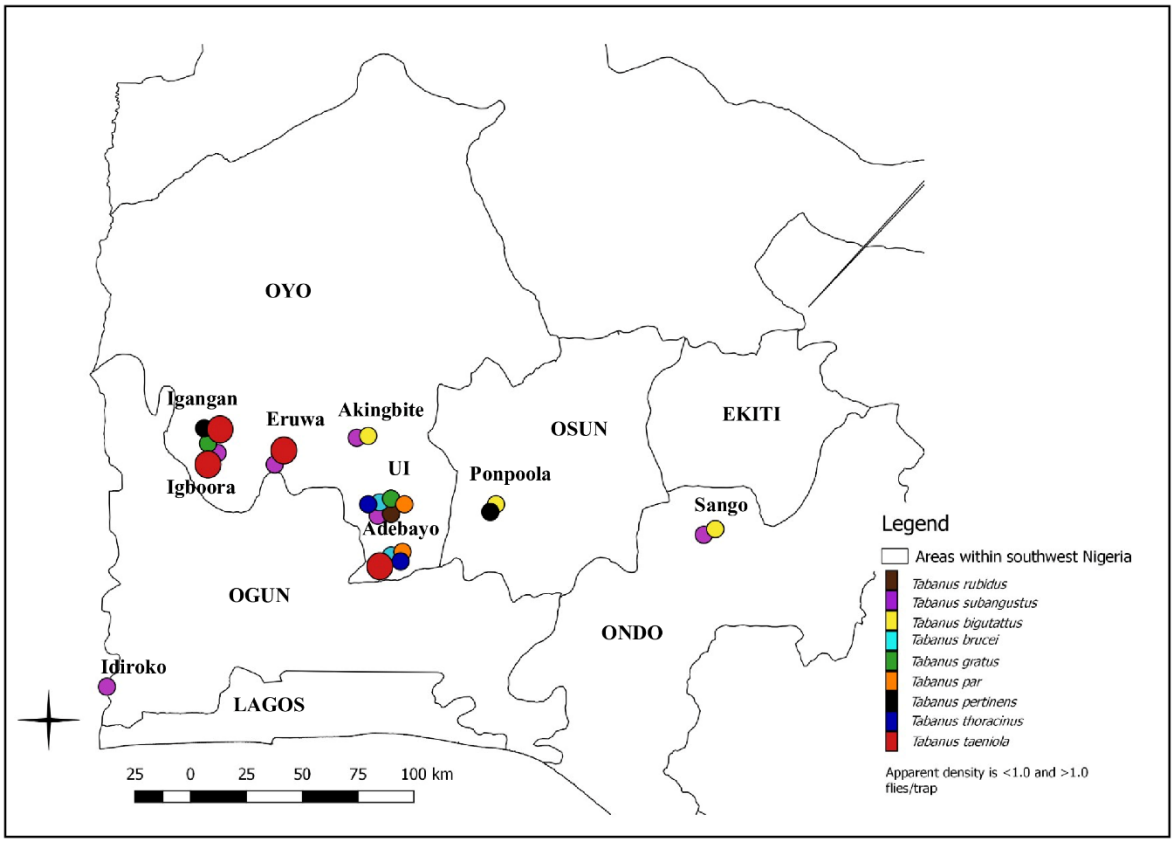


Figure 4.2: Spatio-temporal distribution of trapped *Tabanus* species.

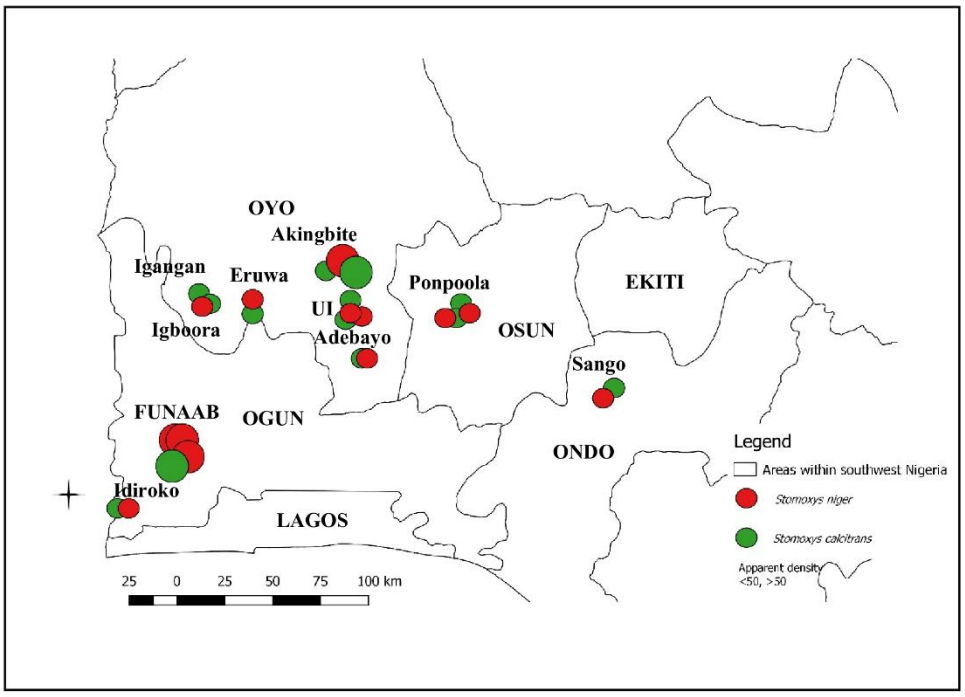


Figure 4.3: Spatio-temporal distribution of trapped *Stomoxys* species.

4.1.2. Abundance of transmitting vectors of importance

Out of 135 *Glossina* spp. trapped, 52.6% were identified as *Glossina tachinoides* and 47.4% were *Glossina palpalis*. The apparent fly density for *G. palpalis* was 0.5 flies/trap/day for rain season and 0.08 flies/trap/day for dry season, while *G. tachinoides* reported 0.6 flies/trap/day in rain and 0.02 flies/trap/day in dry season (Figure 4.4). The highest apparent density of 1.8 flies/trap/day was observed in the month of May for *Glossina* species.

A total of 52 *Tabanus* spp. of nine taxa were caught in this study namely; *Tabanus gratus* Loew (3.85%), *Tabanus taeniola* Palisot de Beauvois (28.85%), *Tabanus biguttatus* Wiedemann (13.46%), *Tabanus subangustus* Walker (21.15%), *Tabanus thoracinus* Palisot de Beauvois (5.77%), *Tabanus par* Walker (9.62%), *Tabanus rubidus* Wiedemann (1.92%), *Tabanus pertinens* Austen (7.69%) and *Tabanus brucei* Macquart (7.69%) (Figure 4.5; Table 4.1). The abundance of *T. taeniola* was observed between the end and beginning of dry season, its short absence observed was when temperature was > 30 °C with humidity below 65%. The other groups in the family Tabanidae trapped were *Chrysops* and *Haematopota* which makes 21.7% and 2.9% of total *Tabanid*, respectively. Identified *Chrysops* were *C. longicornis* (Macquart), *C. distinctipennis* (Austen) and *C. silacea* (Austen).

The only identified *Haematopota* was *H. pertinens* (Austen). A total of 8,697 stomoxyine flies were trapped, *Stomoxys niger* and *Stomoxys calcitrans* make up 72.38% and 27.62%, respectively. Relative abundance of *Stomoxys calcitrans* and *Stomoxys niger* were highest at mean temperature of 27 – 28 °C, humidity above 80% and rainfall of 100 – 250 mm. As the rainfall continue to increase with over 20 days/month in August – September (> 300 mm) the relative abundance reduced by 47.4%, and more reduction up to 83.37% was observed in dry months (October – March with < 80 mm) when compared with rainy months. Monthly relative abundance is shown in Figure 4.4, 4.5 & 4.6. The vector species relative apparent density from all collection sites is shown Table 4.2.

4.1.2.1. Other trapped flies

Total dipteran flies trapped in this study were 13,895; comprising male (5,551) and female (8,344) flies. Of these, 64.17% (CI: 63.37 – 64.96%) were blood-sucking flies. The sex ratio of trapped flies indicates 60.05% (CI: 59.23 – 60.86%) female and 39.95% (CI: 39.14 – 40.77%) male for total catches and 60.3% (CI: 59.32 – 61.35%) female for haematopagous flies. One *Haematopota species* and three species of *Chrysops* are part of other biting flies

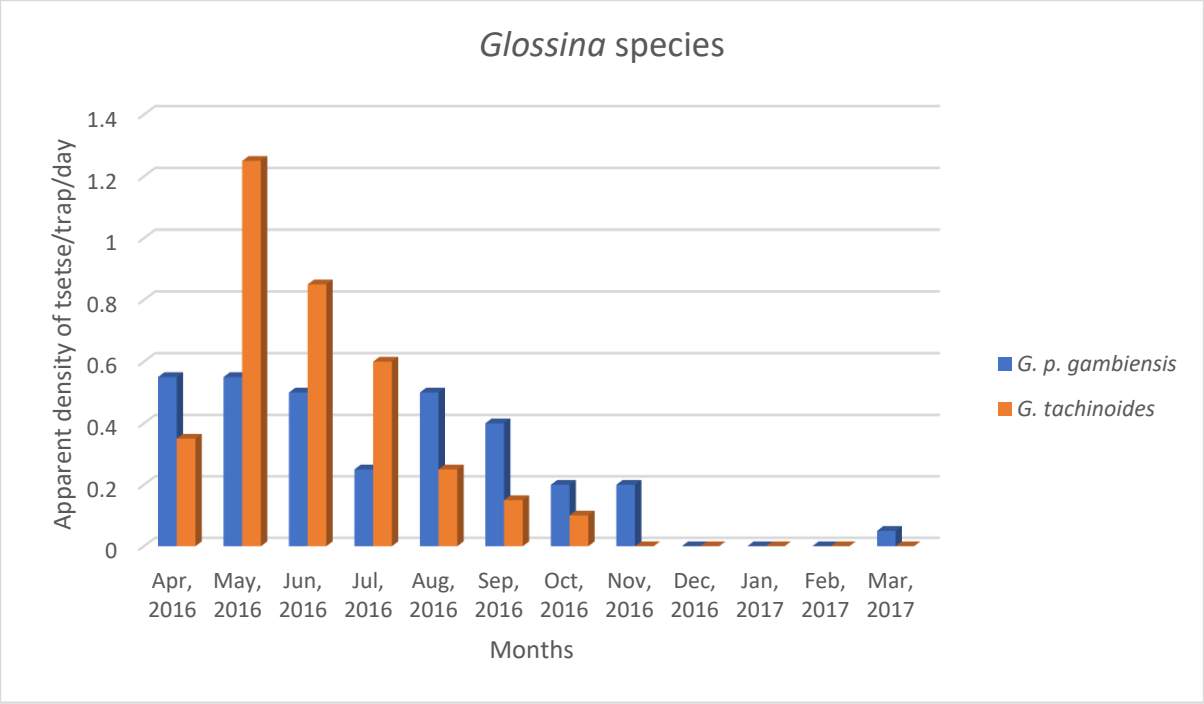


Figure 4.4: Relative apparent density of *Glossina* spp. abundance

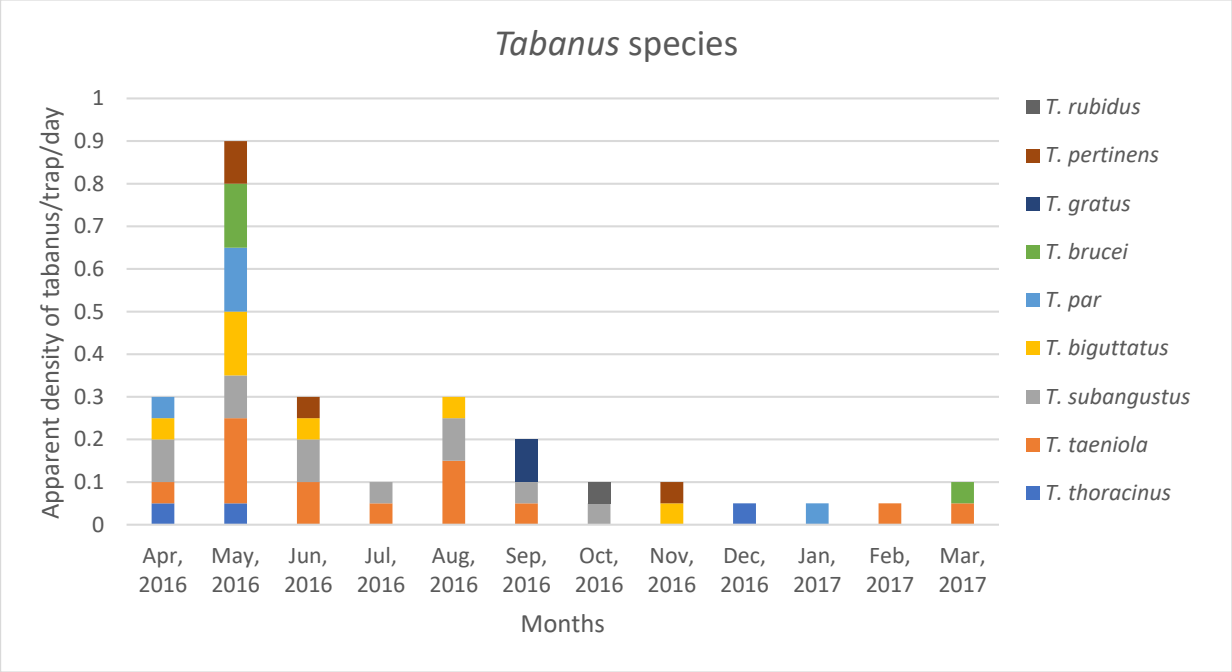


Figure 4.5: Relative apparent density of *Tabanus* spp. abundance

Table 4.1: Abundance and feeding characteristics of captured transmitting-vector flies.

Vector	Variable	Level	Total	% spp. Dist.	95% CI
Glossina	Species	*G. palpalis*	64	47.4	39.2-55.8
		G. tachinoides	71	52.6	44.2-60.8
	Sex	F	72	53.3	44.9-61.5
		M	63	46.7	38.5-55.1
	Blood meal	Fed	43	31.9	24.6-40.1
		Unfed	92	68.1	59.9-75.4
		Total	135		
Tabanus	Species	*T. biguttatus*	7	13.5	6.7-25.3
		T. brucei	4	7.7	3.0-18.2
		T. gratus	2	3.8	1.1-13.0
		T. par	5	9.6	4.2-20.6
		T. pertinens	4	7.7	3.0-18.2
		T. rubidus	1	1.9	0.3-10.1
		T. subangustus	11	21.2	12.2-34.0
		T. taeniola	15	28.8	18.3-42.3
		T. thoracinus	3	5.8	2.0-15.6
	Sex	F	42	80.8	68.1-89.2
		M	10	19.2	10.8-31.9
	Blood meal	Fed	10	19.2	10.8-31.9

		Unfed	42	80.8	68.1-89.2
		Total	52		
Stomoxyine	Species	*S. calcitrans*	2402	27.6	26.8-28.4
		S. niger niger	6295	72.4	71.4-73.3
	Sex	F	5253	60.4	59.4-61.4
		M	3444	39.6	38.6-40.6
	Blood meal	Fed	4642	53.4	52.3-54.4
		Unfed	4055	46.6	45.6-47.7
		Total	8697		

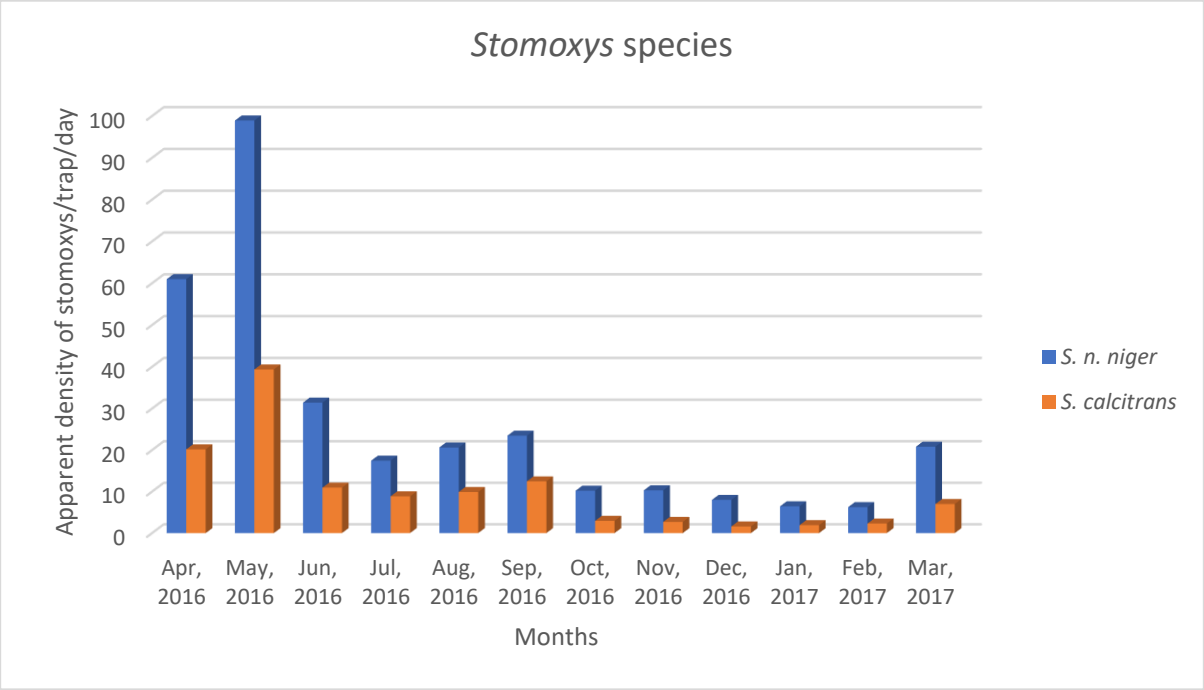


Figure 4.6: Relative apparent density of *Stomoxys* spp. abundance

captured. *Chrysops* reported in this study are *Chrysops silacea*, *Chrysops longicornis* and *Chrysops distinctipennis* with genera prevalence of 40.0% (CI: 19.82 – 64.25), 53.33% (30.12 – 75.19%) and 6.67% (CI: 1.19 – 29.82) respectively. Only *Haematopota pertinens* was caught by Nzi trap with prevalence of 2.90% (CI: 0.80 – 9.97%) of total *Tabanidae*. Non-biting flies caught during the survey have been reported. *Musca domestica* was most abundant (76.78%, CI: 75.59 – 77.93%) non-biting flies amongst *Chrysomyia spp*, *Lucilia spp.* and others (Table 4.2).

4.1.2.2. Seasonal variables relationship with catches

There was rainfall throughout the study months with peak in August, 2016 and lowest in January, 2017. The months of November to March were observed to be dry season and April to October were wet season. However, the usual August break did not surface in 2016 due to climate change. The average of 94 mm in April indicates that early rain was delayed, but there were few days of heavy rainfall between April and May. Temperature peaks were observed between the months of November to March (Figure 4.7). Also, decrease in temperature between June and July could be responsible for the steep drop observed in transmitting vectors graph. There seems to be homogenous percentage humidity across all the study site throughout the year with minimum and maximum values at 72 and 91%, respectively. The weather variables observed in southwest Nigeria were similar in the study sites and no marked difference between derived savannah, wet rainforest and lowland rainforest. Hence, clustered environmental surrogates have been reported (Figure 4.7).

Relative abundance of *Stomoxys calcitrans* and *Stomoxys nigra* were highest at mean temperature of 27 – 28 °C, humidity above 80% and rainfall of 100 – 250 mm. As the rainfall continue to increase with over 20 days/month in August – September (> 300 mm) the relative abundance reduced at about 22.06%. More reduction of up to 83.44% were observed in dry months when compared with rainy months. All the *Tabanids* were in relative low numbers, such as *Tabanus gratus* representing 0.02% of all haematopagous flies trapped. They were not favoured by elevated temperature (> 30 °C) despite the high percentage humidity. As early rain begins, the presence of *Tabanus taeniola* was noticed in mid-March and soon after other species were also captured. The *Glossina* species were scanty in the dry months with only 3.70% of total tsetse catches.

Table 4.2: Total catches of dipteran flies using Nzi traps in southwest Nigeria.

Fly species	Season		Sex		Haematophagous		TOTAL
	Wet	dry	Male	Female	fed	Unfed	
Glossina p. palpalis (Robineau-Desvoidy)	59	5	29	35	27	37	64
Glossina tachnoides (Westwood)	71	-	34	37	16	55	71
Tabanus taeniola (Pal.Beauvois)	13	2	3	12	4	11	15
Tabanus subangustus (Ricardo)	11	-	2	9	1	10	11
Tabanus biguttatus (Wiedemann)	6	1	1	6	2	5	7
Tabanus par (Walker)	4	1	3	2	1	4	5
Tabanus brucei (Ricardo)	3	1	1	3	-	4	4
Tabanus gratus (Loew)	2	-	-	2	1	1	2
Tabanus rubidus (Wiedemann)	-	1	-	1	-	1	1
Tabanus pertinens (Austen)	4	-	1	3	-	4	4
Tabanus thoracinus (Pal.Beauvois)	2	1	1	2	1	2	3
Chrysops silacea (Austen)	5	1	2	4	1	5	6
Chrysops longicornis (Macquart)	7	1	1	7	3	5	8
Chrysops distinctipennis (Austen)	1	-	1	-	-	1	1
Haematopota pertinens (Austen)	2	-	2	-	-	2	2
Stomoxys nigra (Macquart)	5259	1036	2388	3907	4325	1970	6295
Stomoxys calcitrans (Linnaeus)	2092	310	1056	1346	317	2085	2402

Simulium damnosum (Latreille)	15	-	4	11	4	11	15
Chrysomya putoria (Wiedemann)	15	4	2	17	-	-	19
Chrysomya bezziana (Villeneuve)	1	-	-	1	-	-	1
Lucilia sericata (Meigen)	29	2	14	17	-	-	31
Fannia canicularis (Linnaeus)	908	114	296	726	-	-	1022
Fannia scalaris (Fabricius)	12	-	-	12	-	-	12
Hippobosca variegata (Megerle)	63	8	30	41	-	-	71
Musca domestica (Linnaeus)	3114	709	1680	2143	-	-	3823
TOTAL	11698	2197	5551	8344	4703	4213	13895

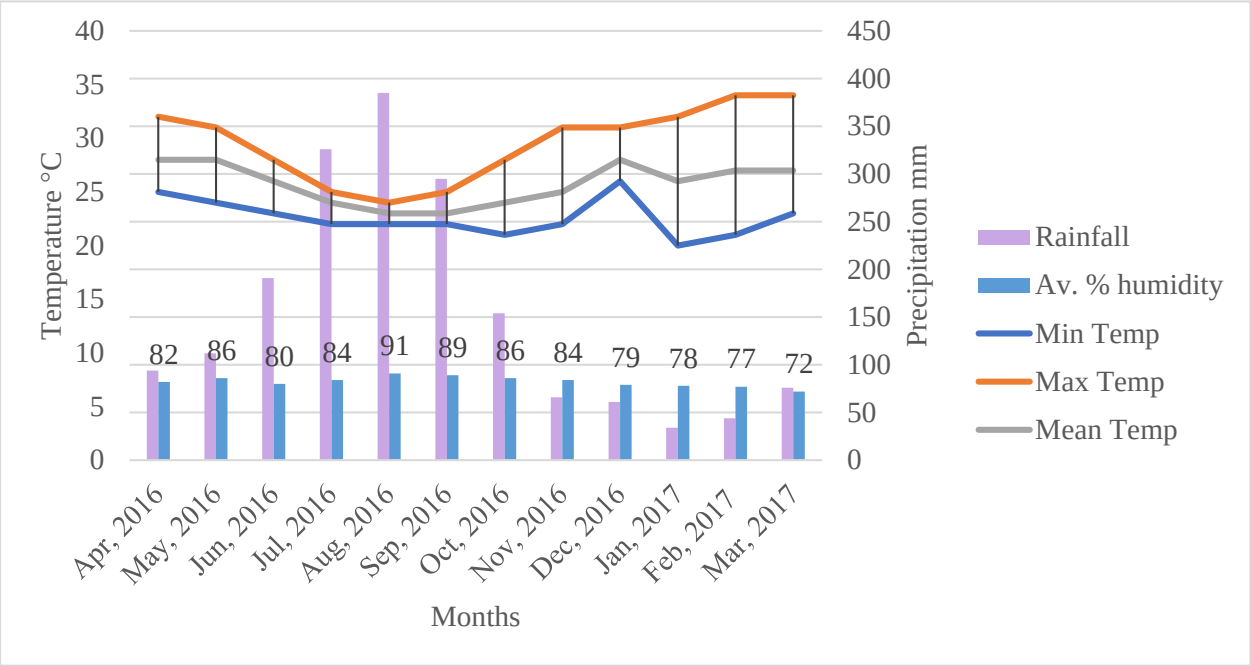


Figure 4.7: Clustered environmental surrogates of the study sites in southwest Nigeria.

4.1.3. Morphological studies of Tabanus species

The morphology of trapped vectors was studied for the first time in Nigeria since the efforts of British medical officers. No heterogenous variation was observed within vector species, however, several species were identified including species not previously described in Nigeria (e.g. *Tabanus brucei* and *Tabanus rubidus*).

4.1.3.1. Tabanus gratus (Loew, 1858)

Sample specimen ♀: Figure 4.8A. Width of head 5.15 mm, head length 1.98 mm, thorax length 4.85mm, length of abdomen 7.72 mm, length of wing 13.1mm, body length 13.6mm, length of sclerite 0.56 mm and distance between claspers at origin 0.71 mm. Only female samples trapped and length range was 11.9 mm – 12.3 mm (±0.15). Description- Wing was clear, r_5 was a bit narrowed, and the length of basicosta was 20µm (Figure 4.8Ai). The scutellum of the thorax was brownish-black, a discontinuous median marking with four other visible lines (Figure 4.8Ai). The abdomen had predominant or ground colour with contrasting patterns of median lines in the tergites.

The first two abdominal tergites have black median line which was discontinuous while the 3rd – 7th tergites have concolour lines which were also discontinuous. At the posterior abdomen tergites were dorsomedial parallel-sided irregular lines seen from the 4th tergite (Figure 4.8Aiii). The anterior part of antennae is white and remaining part golden orange. White short hairs are evidently present arounf the parafacials and face with a brownish probocis (Figure 4.8Aiv). The last stermite with the reduced ones were black (Figure 4.8Av). The coxae, femur and tronchanter were black, tibia yellowish with posterior 1/4th, tarsal and metatarsal segments also black (Figure 4.8Avi). Pulvinus and empodium were well-developed and claws brownish-black (Figure 4.8Aii).

4.1.3.2. Tabanus brucei (Ricardo, 1908)

Sample specimen ♀: Figure 4.8B. Width of head 5.69 mm, head length 2.15 mm, length of thorax 6.60 mm, body length 16.91 mm, length of wing 14.4 mm. Samples examined revealed ♀ 15.9 – 16.2 mm (±0.17), ♂ 14.8 mm. General yellow body colouration. The prescutellum and mesonotum appear fused and artistically separated with thin markings while the scutellum was perfectly carved out (Figure 4.8Bi). A shade of greyish yellow on the

thorax though may not be seen with naked eyes. The tergites were devoid of lines or stripes as also observed in the stermites (Figure 4.8Bii). Wings hyaline was strongly infuscated with clove-brown (Figure 4.8Biv). The 1st and 2nd basal cells have clear spaces except for the thin markings around their margins. The observed transverse band extending across the wing does not reach the hind margin and ends irregularly. The tip of the wing has a grey border and composed of hairs generally not infuscated. The r_5 is opened. The length of angle just in front of basicosta was 17.7 µm. The coxae, femur and tronchanter are yellow while the fore-limb tibia has a robust characteristic shape more obvious than the hind limb (Figure 4.8iii). The tibia, tarsal and metatarsals are black. This is the first report in Nigeria.

4.1.3.3. Tabanus taeniola (Palisot de Beauvois, 1807)

Sample specimen ♀: Figure 4.8C. Width of head 5.84 mm, head length 2.97 mm, thorax length 5.15 mm, length of abdomen 10.79 mm, body length 17.92 mm. Samples collected revealed ♀ 17.3 – 18.7 mm (±0.38), ♂ 16.0 – 16.2 mm (±0.09). Body description- The head was robust with thick mouth parts (Figure 4.8Ci). Frons covered with short white hairs in female are parallel sided and was deep brown with a thin tomentum (Figure 4.8Cvi). The thorax had four visible lateral lines which did not extend to the brown scutellum (Figure 4.8Ci).

The abdomen fusiform, buff coloured with 3 obvious longitudinal brown stripes on the tergites. The median line was very pronounced and continuous while the sublateral lines were visible but discontinuous in the last two tergites. The last three abdominal stermites were black and plumose (Figure 4.8Ciii). The wings were clear and the r_5 was a bit narrowed and the length of basicosta was in 12 µm on the average (Figure 4.8Civ). The metatarsal has characteristic black lateral patches and last one is developed into paw-like shape having two black claws projected anteriorly with reduced pulvinus (Figure 4.8Cv).

4.1.3.4. Tabanus subangustus (Ricardo, 1908)

Sample specimen ♂: Figure 4.8D. Width of head 5.5 mm, head length 2.3 mm, thorax length 5.2 mm, length of abdomen 10.6 mm, body length 16.7 mm. Samples examined revealed ♀ 16.2 – 17.2 mm (±0.27), ♂ 16.7 – 16.8 mm (±0.05). Body description- The costa, subcosta and anterior r_1 were slightly infuscated. The veins accompanying them were brownish and striated. The r_5 was completely opened. The margin around the basicosta had a length of 20 µm (Figure 4.8Dvii). Prescutal lobe concolours with the rest of the mesonotum but slightly

different from the scutellum. There were faint markings on the thorax. Mid-dorsal abdominal plate stripe obviously seen and ends on the 7th tergite. The coxae and anterior 1/4th of the femora were dark-brown while the posterior femora, tronchanter, tibia and tarsal were light brown. The metatarsals appeared studded with round black patches while the black claws were curved outwards (Figure 4.8Div).

4.1.3.5. Tabanus par (Walker, 1854)

Sample specimen ♀: Figure 4.8E. Width of head 4.45 mm, head length 2.08 mm, thorax length 4.85 mm, length of abdomen 7.43 mm, length of wing 11.3 mm, body length 13.37 mm. Collected samples revealed ♀ 13.3 – 13.9 mm (±0.26), ♂ 11.7 – 12.0 mm (±0.15). Body description- Frons yellow with black hairs. The eyes lack bands and are juniper green (Figure 4.8Ei). Palpi yellow with pale-yellow and black short-fine hairs (Figure 4.8Eii). First two anterior antennae segments have whitish-brown hairs while remaining part yellow, while proboscis is also yellowish in colour.

A yellow unicolour on the tergites with no lateral markings. Thorax including the scutellum were concolour (Figure 4.8Eii). There are no thoracic lines. In male, the thorax was pollinose, which is not in female (Figure 4.8Eiii). No visible stripe on the tergites and stermite (Figure 4.8Eiv). Brownish transverse bands observed at the base of each tergite. The coxae, femur, tronchanter, tibia, tarsal and metatarsals were bronze brown to yellow in colour with whitish yellow hairs. Wing is transparent with yellow veins (Figure 4.8Ev)

4.1.3.6. Tabanus biguttatus (Wiedemann, 1830)

Sample specimen ♂: Figure 4.8F. Width of head 6.8 mm, thorax length 6.3 mm, abdomen length 10.4 mm, length of head 2.8 mm, body length 18.7 mm. The fly samples revealed ♀ 19.6 – 21.3 mm (±0.62), ♂ 18.7 mm. Fly was globose and completely black (Figure 4.8Fi). The thorax including the scutellum and part of the head was pollinose which is absent in female. The thorax and abdomen were completely black, no visible stripes or lines. The wing was saturated and bit narrowed at r_5 and there is a curved margin around the basicota (Figure 4.8Fvi).

However, the basicosta was setated in female. All the limb segments were black in colour including the claws. Major parts of the cell were infuscated with roundish transparent section posterior to the discal cell (Figure 4.8Fvi). The segments have continuous black colouration.

The head is robust with flagellum of 3.0 mm, proboscis 4.6 mm, labium 4.8 mm and labrum 3.2 mm (Figure 4.8Fiv). The coxae, femur, tronchanter, tibia, tarsal and metatarsals are all black (Figure 4.8Fv). The claws were V-shaped in appearance with curved ends. The empodium was visible and brownish.

4.1.3.7. Tabanus pertinens (Austen, 1912)

Sample specimen ♀: Figure 4.8G. Width of head 5.45 mm, head length 2.97 mm, thorax length 5.74 mm, length of abdomen 8.71 mm, body length 15.84 mm, length of wing 10.80 mm. Collected samples revealed ♀ 15.7 – 16.1 mm (±0.17), ♂ 15.1 mm. Body description: Elongate species, eyes present in male and composed of short pale hair while front in female was broad but frontal callus absent. The body colouration was not concolour and it had four noticeable stripes of elongate black and or clove-brown present which were not continuous (Figure 4.8Gi). The scutellum had a clove-brown colouration, a bit different from the prescutellum and mesonotum, Thorax longitudinally stripped with grey in female possessing an erect fine yellowish hair mixed with fine black. The pleurae and pectus have light grey pollinose.

The median and sublateral margins ends on the 6th tergite. Generally, the abdomen had mixed colouration. The shape was narrow, elongate and tapering. Presence of dark stripes on the dorsum with fine black hair with long fine whitish hair on the lateral sides. Hind borders of ventral stermites 2nd – 6th segment had cream-buff (Figure 4.8Gii). Wing hyaline, veins are mummy-brown and can be inconspicuous, characteristic pin-point on basicosta and r_5 is opened. Length of angle around basicosta was 16.1 μm and halters were cream-coloured (Figure 4.8Giii). Coxae grey with whitish hairs, femur grey but less grey or turns deep brown distally. Tibia long (5.07 μm) with mixture of white and black hairs giving a general brownish colouration (Figure 4.8Giv). The last two stermite with the reduced ones have roundish-black appearance (Figure 4.8Gv). Tarsal and metatarsal were deep brown (Figure 4.8Gvi).

4.1.3.8. Tabanus thoracinus (Palisot de Beauvois, 1806)

Sample specimen ♀: Figure 4.8H. Width of head 5.25 mm, head length 2.14 mm, thorax length 5.07 mm, length of abdomen 8.22mm, length of wing 11.9 mm, body length 14.98 mm. Collected samples revealed ♀ 14.2 – 15.1 mm (±0.27), ♂ 11.9 – 13.5 mm (±0.25). Body description- A yellow unicolour on the tergites with no lateral markings. Thorax including the

scutellum were concolour. There were no thoracic lines in all segments (Figure 4.8Hi). In male, the thorax was pollinose (Figure 4.8Hi, Hii)), but not so in female (Figure 4.8Hiii, Hiv). No visible stripe on the tergites. Brownish transverse bands observed at the base of each tergite. The coxae, femur, tronchanter, tibia, tarsal and metatarsals were buff-brown to yellow in colour (Figure 4.8Hvi). It appears like *T. par*, except the wing was slightly infuscated (Figure 4.8Hv) and thorax was wider and differences in limbs were noticed.

4.1.3.9. Tabanus rubidus (Wiedemann, 1821)

Sample specimen ♀: Figure 4.8I. Width of head 3.21 mm, head length 2.80 mm, thorax length 3.43 mm, length of abdomen 6.82mm, length of wing 9.3 mm, body length 12.98 mm. Body description: slender species with visible wide black median stripe which was concolour (Figure 4.8Ii). There was sublateral markings appearing step-like and ending at the 5th tergite (Figure 4.8Iiii). Frons are parallel-sided with cranial divergence, while basal callosity triangular with a median extension (Figure 4.8Iii). Limbs appear yellowish with prominent black hairs. The coxae, femur, tronchanter, tibia, tarsal and metatarsals were buff-brown to yellow in colour (Figure 4.8Iiii). The wing was transparent and r_5 was opened (Figure 4.8Iiv). Only one specimen was captured during the trapping seasons.

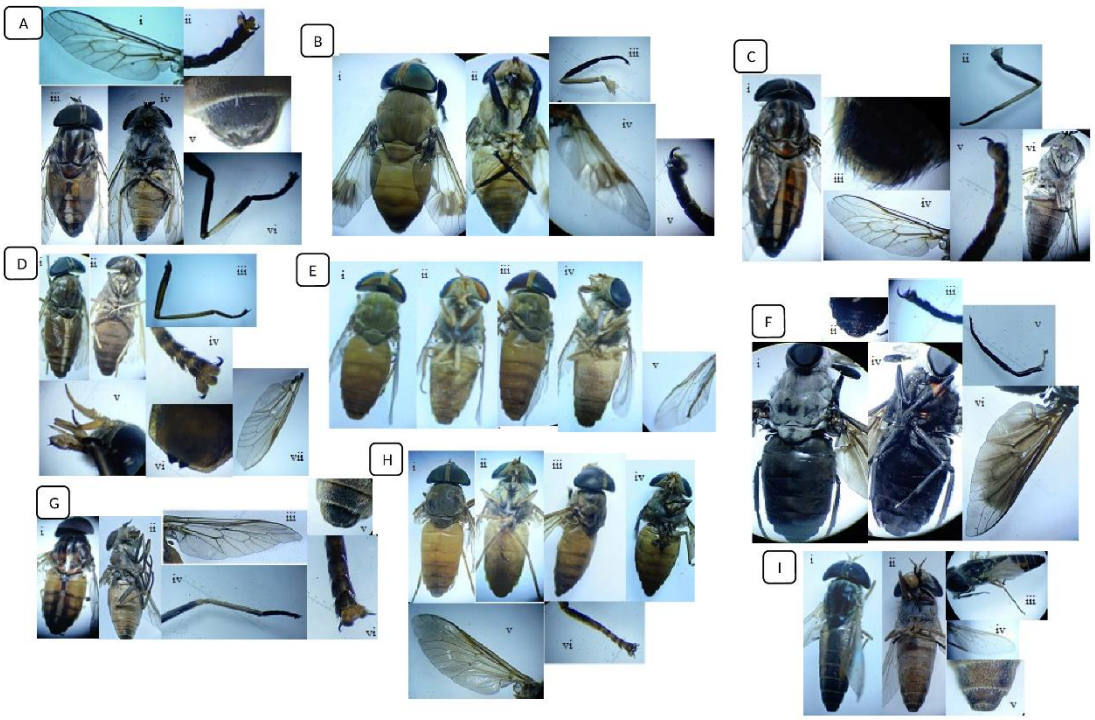


Figure 4.8: Morphological analysis of *Tabanus* species in Nigeria.

Footnote: A- *Tabanus gratus* (i- right wing, ii- metatarsus and claw, iii- dorsal part, iv- ventral part, v- last segment showing the claspers, vi- leg parts). B- *Tabanus brucei* (i- dorsal part, ii- ventral part, iii- leg parts: costa, trochanter, femur, tibia, tarsus, metatarsus, claws, iv- left wing, v- metatarsus with curved claws). C- *Tabanus taeniola* (i- dorasl part, ii- leg parts, iii- posterior ventral tergites with radiating black hairs, iv-right wing, v- magnified posterior sections of the hind leg with lateral black stripes, vi- ventral part. D- *Tabanus subangustus* (i- dorsal part, ii- ventral part, iii- hindleg, iv- magnified metatarsals with claws, v- head with attached mouthpart, vi- last segment with claspers, vii- left wing. E- *Tabanus par* (i- ♂ dorsal part, ii- ♂ ventral part, iii- ♀ dorsal part, iv- ♀ ventral part, v- transparent left wing). F- *Tabanus biguttatus* (male) (i-dorsal part, ii- last segment showing the genitalia, iii- metatarsal and claws, iv- ventral part, v- hindleg, vi- right wing). G- *Tabanus pertinens* (i- dorsal part, ii- ventral part, iii- right wing, iv- hindleg, v- posterior segments with claspers, vi- metatarsal with claws). H- *Tabanus thoracinus* (i. ♂ dorsal part, ii- ♂ ventral part, iii- ♀ dorsal part, iv- ♀ ventral part, v- infuscated left wing vi- tibia, metatarsal.

4.1.4. Morphological studies of trapped Stomoxys species

4.1.4.1. Stomoxys calcitans (Linnaeus, 1758)

Sample specimen ♂: Width of head 2.18 mm, head length 1.19 mm, thorax length 2.18 mm, length of abdomen 2.57 mm, length of body 5.94 mm. Collected samples revealed ♀ 5.2 – 6.9 mm (±0.05), ♂ 4.1 – 6.2 mm (±0.03). Body description: Frons was twice long as wide at vertex in male, one-half times in female, the proboscis project upwards from beneath. Presence of median black spot and two sub-lateral dark round spots on the 2nd and 3rd abdominal segments were characteristic. Palpi was yellow and proboscis brownish-red. Abdomen had grey to brown pollinosity, thorax dark and mesonotum had two pairs of sublateral stripes separated faintly by copper-brown colour. The scutellum had a well-formed dark cranial spot. Hindlimb was brown with posterior 1/3rd of femur and basal part of tibiae yellow. Wing was hyaline, r_5 broad and halter yellowish.

4.1.4.2. Stomoxys niger (Macquart, 1851)

Sample specimen ♀: Width of head was 2.12 mm, head length 1.16 mm, thorax length 2.2 mm, length of abdomen 2.46 mm, length of body 5.34 mm. *Stomoxys* examined revealed ♀ 4.2 – 6.0 mm (±0.02), ♂ 4.0 – 5.6 mm (±0.02). Variable species were observed with some very dark colouration. Frontal stripe continuous, black, parafrontalia and facialia have dense, silvery-white pollinosity. Palpi yellowish and proboscis was black. The prominent median stipe in the 2nd and 3rd segment which is not concolour was observed. The difference with that of *S. calcitrans* was that it was not as wide. The basal marking of first and second segment was continuous while the third segment appeared like double cone. Wing hyaline, setulosity of r_{4+5} was variable in many samples observed. It is also known as *Stomoxys nigra* in several texts.

4.1.5. Morphological studies of trapped Glossina species

4.1.5.1. Glossina palpalis gambiensis (Vanderplank, 1911)

It was identified as *G. palpalis* at this stage. Sample specimen ♀: Width of head was 2.4 mm, head length 1.2 mm, thorax length 3.8 mm, length of wing 7.3 mm, length of proboscis from origin 3.3 mm, length of abdomen 3.7 mm, body length 8.7 mm (±0.05). The feathered arista on the third antennal segment were studded and short. The abdominal tergites appeared dark.

The superior claspers of the male were clawed with sharp edges and joined by a membrane like structure. The leg parts were buff-brown except the tarsal which is black. The inferior claspers of the male have a long thing neck and a small head. The tarsal segments were black in colouration.

4.1.5.2. Glossina tachinoides (Westwood, 1850)

Sample specimen ♂: Width of head was 2.3 mm, head length 1.2 mm, thorax length 3.9 mm, length of wing 8.0 mm, length of proboscis from origin 2.8 mm, length of abdomen 4.1 mm, body length 9.0 mm (±0.05). The abdominal segments were separated with buff-brown areas especially on the first tergite. The general abdominal colouration ranges from chocolate-brown to black. The thoracic contours were very visible. The superior claspers of the male also clawed and joined with a membrane-like structure. Inferior claspers of male have a short wide neck and a wide lobed head. The coxae, femur, tibia, tarsals were brown.

4.1.6. Phylogenetic analysis of transmitting vectors

Phylogenetic relationships of ITS2 sequences conducted on *Glossina*, *Tabanus* and stomoxyine were inferred by neighbour joining analyses produced with G-blocks curation to eliminate poorly aligned clades using multiple sequence analyses (MUSCLE) using Phylogeny software (Figure 4.9). Variation in length was observed in the nuclear 28S gene and besides distinct clusters were formed due to unique nucleotide sequence of individual species. In *Tabanus*, a total of twenty-seven *Tabanus* species were sequenced from 8 trapped species. Homogenous pattern from lineage shows they are from the same clad, and besides sequences of each identical species were the same. Total length of neighbour joining was 1.42 with nine alignments using MEGA7, subsequently dataset with little similarities were deleted from the tree. Closely related species were noticed from common nodal points (Figure 4.9). All sequenced species results were submitted in the GenBank with submission ID: #2030624. Stomoxyine and *Glossina* species constructed trees were unrooted, because they already illustrate the relatedness of the leaf nodes without any assumption about the ancestry and all sequences have known identities. Hence, no outgroup data input was introduced.

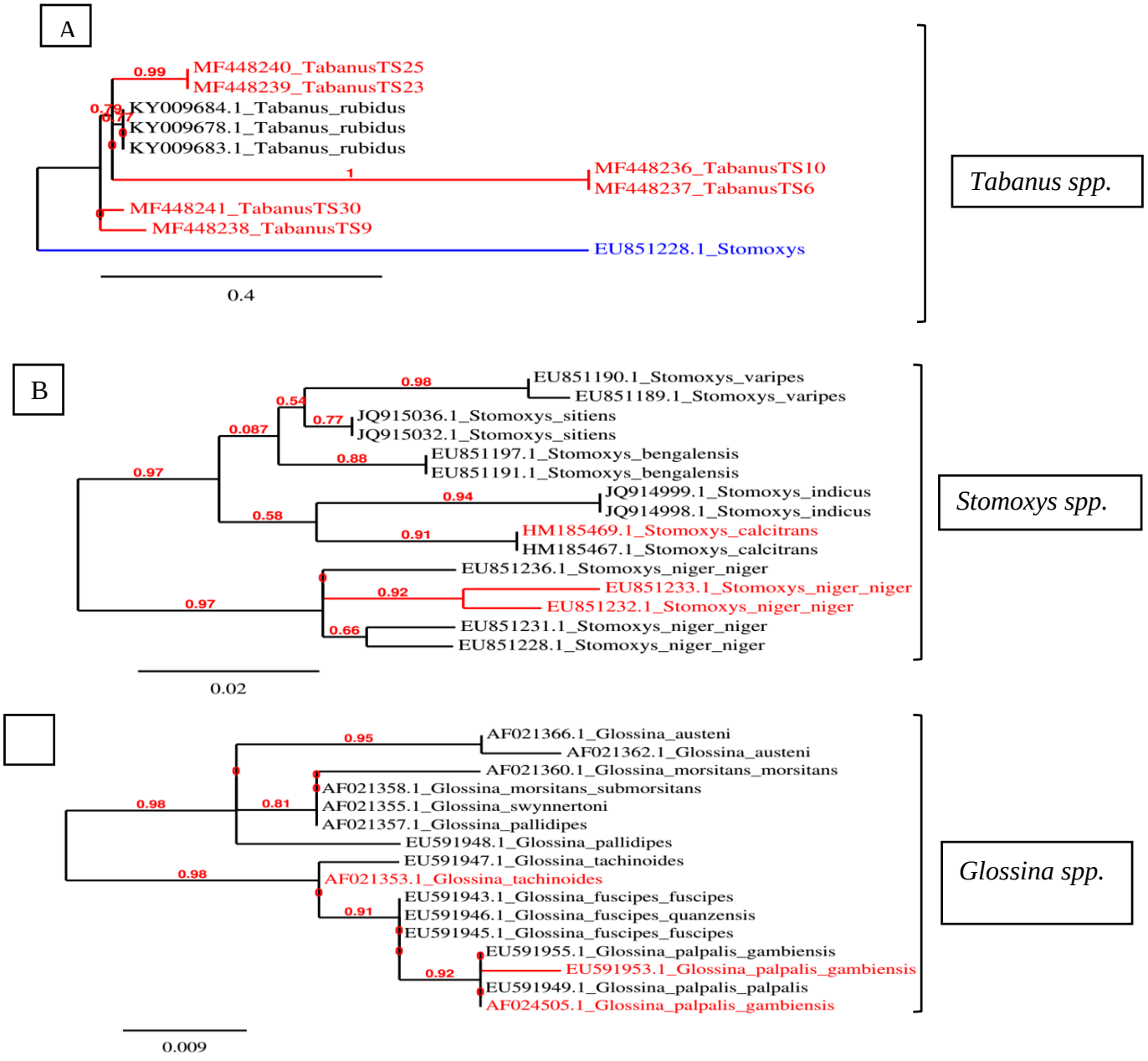


Figure 4.9: Phylogenetic analysis of fly vectors based on ITS2 nuclear sequences.

There was limited sequence voucher for *Tabanus* species in the database, hence the generated sequences were rooted with *Stomoxyini* as an outgroup. *Tabanus* species TS6 (273bp), TS8 (364bp), TS9 (287bp), TS10 (273bp), TS23 (542bp), TS25 (685bp), TS28 (<200bp), TS30 (610bp) and TS32 (580bp) were characterised, however, only six could be identified on BLAST result with ITS tool. TS6 (*Tabanus gratus*- MF448237), TS9 (*Tabanus rubidus*- MF448238), TS10 (*Tabanus pertinens*- MF448236), TS23 (*Tabanus taeniola*- MF448239), TS25 (*Tabanus subangustus*- MF448240) and TS30 (*Tabanus thoracinus*) were molecularly confirmed and assigned accession number from the GenBank. Short nucleotide fragment generated from *Tabanus par* (TS28) sequences could not be aligned with other species. Also, TS8 (*Tabanus biguttatus*) and TS32 (*Tabanus brucei*) only had little similarities and were removed.

The tree with the highest log likelihood was shown in Figure 4.8. High similarity matches (98% and 99%) were also obtained for two other *G. p. gambiensis* (EU591953 and EU591955) isolates, a *Glossina fuscipes quanzensis* isolate (EU591946), a *G. p. palpalis* isolate (EU591949) and a *G. tachinoides* isolate (AF021353). *Glossina tachinoides* with accession number AF021353 was also identified at 99% homology. Morphologically identified *S. niger* sequences were recognised as *Stomoxys niger niger*, and this is the first report from Nigeria with accession numbers EU851233 (98 – 99% homology) and EU851232 (98% homology). *Stomoxys calcitrans* sequences were all on the accession number HM185469 (96 – 99%).

4.2. STUDY TWO

4.2.1. Trypanosome prevalence in Glossina species

A total of 96 out of the 135 tsetse flies analysed gave a positive signal for trypanosome DNA (71.1%, 95%CI: 63.0-78.1). *T. b. brucei*, *T. vivax*, *T. congolense* and *T. simiae* DNA was amplified in 51.1% (95%CI: 42.8-59.4), 34.8% (95%CI: 27.3-43.2), 5.2% (95%CI: 2.5-10.3) and 4.4% (95%CI: 2.1-9.4) of tsetse respectively. In terms of single detections events, the prevalence in the tsetse flies were 31.1%, 14.8%, 2.9% and 0.7% for *T. b. brucei*, *T. vivax*, *T. congolense* and *T. simiae* respectively. Tsetse were also observed with more than one trypanosome species (Table 4.3). The flies where DNA from two *Trypanosoma* species was detected included *T. vivax* and *T. b. brucei* 15.6% (95%CI: 11.0-23.4), *T. vivax* and *T. simiae* 1.5% (95%CI: 0.8-6.3), *T. b. brucei* and *T. simiae* 1.5% (95%CI: 0.8-6.3), *T. b. brucei* and *T. congolense* 0.7% (95%CI: 0.1-4.1) and *T. vivax* and *T. congolense* 0.7% (95%CI: 0.1-4.1).

DNA from three trypanosome species included tsetse amplifying *T. vivax*, *T. b. brucei* and *T. simiae* 1.5% (95%CI: 0.4-5.2) and *T. vivax*, *T. b. brucei* and *T. congolense* 0.7% (95%CI: 0.1-4.1). Trypanosome DNA was positive in 52 (81.3%) out of 64 *G. p. palpalis*. Species distribution include *T. brucei* (76.6%), *T. vivax* (31.3%), and *T. simiae* (6.3%). Single infection shows *T. brucei* (46.9%) and *T. vivax* (4.7%). There were 15 *G. p. palpalis* (23.4%) where double infections of *T. brucei* and *T. vivax* were detected. Another two flies (3.1%) detected double infections of *T. simiae* and *T. brucei*. Multiple infections of *T. brucei*, *T. simiae* and *T. vivax* were observed in two other flies (3.1%). For *G. tachinoides*, 44 of the 71 (62.0%) flies were observed with trypanosome DNA. *T. vivax* was the highest at 38.0% followed by *T. b. brucei* at 28.2%, *T. congolense* at 9.9% and *T. simiae* at 2.8%. In terms of flies where a single trypanosome DNA detection was observed *T. vivax* was found in 23.9%, *T. b. brucei* in 16.9% and *T. congolense* in 5.6% of *G. tachinoides*. There were six (8.5%) *G. tachinoides* where *T. vivax* and *T. b. brucei* DNA was detected, two (2.8%) where *T. vivax* and T. simiae and one where *T. brucei* and *T. congolense* and one with *T. vivax* and *T. congolense*. Multiple infection *T. b. brucei*, *T. congolense* and *T. vivax* DNA was observed in a fly (1.4%).

Table 4.3: Trypanosomes identified in *Glossina* spp.

	No	Total	*T. vivax*	*T. b. brucei*	*T. congolense*	*T. simiae*	Mixed	Chi sq., $\alpha_{0.05}$
HP								
G. palpalis	64	(38) 59.4[a]	(18) 28.1[a]	(33) 51.6[a]	(0) 0.0	(2) 3.1	(14) 21.9[a]	$X^2 = 9.893$, $\alpha = 0.002$
G. tachinoides	71	(23) 32.4	(11) 15.5	(9) 12.7	(3) 4.2	(0) 0.0	(0) 0.0	
Prevalence		(61) 45.2	(29) 21.5	(42) 31.1	(3) 2.2	(2) 1.5	14 (10.4)	
TA								
G. palpalis	64	(31) 48.4	(8) 12.5	(25) 39.1[a]	(0) 0.0	(2) 3.1	(3) 4.9	$X^2 = 0.309$,
G. tachinoides	71	(31) 43.7	(20) 28.2[a]	(11) 15.5	(4) 5.6	(2) 4.2	(5) 7.0	$\alpha = 0.578$
Prevalence		(62) 45.9	(28) 20.7	(36) 26.7	(4) 3.0	(4) 3.0	(8) 5.9	
(HP) + (TA)								
G. palpalis	64	(17) 26.6	(6) 9.4	(9) 14.1 [a]	(0) 0.0	(0) 0.0	(1) 1.6	$X^2 = 3.275$,
G. tachinoides	71	(10) 14.1	(4) 5.6	(0) 0.0	(0) 0.0	(0) 0.0	(0) 0.0	$\alpha = 0.070$
Prevalence		(27) 20.0	(10) 7.4	(9) 6.7	(0) 0.0	(0) 0.0	(1) 0.7	
Whole fly								
G. palpalis	64	(52) 81.3[a]	(20) 31.3	(49) 76.6[a]	(0) 0.0	(4) 6.3	(21) 32.8[a]	$X^2 = 4.357$, $\alpha = 0.037$
G. tachinoides	71	(44) 62.0	(27) 38.0	(20) 28.2	(7) 9.9[a]	(2) 2.8	(12) 16.9	
Total prevalence	135	(96) 71.1	(47) 34.8	(69) 51.1	(7) 5.2	(6) 4.4	(33) 24.4	

Note: HP- head+proboscis, TA- thorax+abdomen, a- significantly different ($\alpha<0.05$)

Tissues where the trypanosomes settled at the time of capture were also analysed and the prevalence of trypanosomes in the head plus proboscis (HP) was 45.2% (95%CI: 37.0-53.6), while that of the thorax plus abdomen (TA) was 45.9% (95%CI: 37.8-54.3). The data by trypanosome species is summarised in Table 4.2. For *T. b. brucei*, DNA was found in in the thorax and abdomen in 39.0% of *G. p. palpalis* and 15.4% of *G. tachinoides*. *T. b. brucei* DNA was found in 37.5% of only the head and proboscis of *G. p. palpalis* and 12.7% of *G. tachinoides*. While for *T. vivax*, DNA was found in the head and proboscis of 28.2% and 15.5% *G. p. palpalis* and *G. tachinoides* respectively. *T. vivax* DNA was found in only the thorax and abdomen of 3.1% and 22.5% of *G. palpalis* and *G. tachinoides* respectively.

4.2.1.1. Endosymbionts in Glossina species

Of total 64 tsetse flies examined for endosymbionts, *Wigglesworthia glossinidia* was present in all (100%) and none (0%) was positive of *Wolbachia*. However, 31.3% (95%CI: 21.2-43.4) were positive of *Sodalis glossinidius*. The positive tsetse for *Sodalis glossinidius* were 35.7% (95%CI: 20.7-54.2) and 27.8% (95%CI: 15.9-44.0) of *Glossina palpalis* and *Glossina tachinoides*, respectively. Of the total tsetse, 51.6% (95%CI: 39.6-63.4%) were positive of trypanosomes. Flies positive of both *Sodalis glossinidius* and trypanosomes were 17.2% (95%CI: 9.9-28.2). Tsetse flies negative of both *Sodalis glossinidius* and trypanosomes were 34.4% (95%CI: 23.9-46.6). *Trypanosoma* species interaction with *S. glossinidius* has been reported in Figure 4.10.

4.2.2. Prevalence of trypanosomes in Stomoxys species

A total of 32 out of the 144 stomoxyine flies analysed were positive for trypanosome DNA (22.2%, 95%CI: 16.2-29.9). *T. vivax*, *T. evansi*, *T. congolense* and *T. simiae* DNA was observed in 18.8% (95%CI: 13.2-25.9), 6.3% (95%CI: 3.3-11.5), 1.4% (95%CI: 0.4-4.9) and 1.4% (95%CI: 0.4-4.9) of stomoxyine respectively. In terms of single detections events, the prevalence in the tsetse flies were 14.6% (95%CI: 9.7-21.3), 1.4% (95%CI: 0.4-4.9), 0.7% (95%CI: 0.1-3.8) and 0.7% (95%CI: 0.1-3.8) for *T. vivax*, *T. evansi*, *T. congolense* and *T. simiae* respectively. Double infections for trypanosome DNA was observed in 3.5% (95%CI: 1.5-7.9) of *T. vivax* and *T. evansi*, 0.7% (95%CI: 0.1-3.8) in *T. evansi* and *T. simiae* and 0.7% (95%CI: 0.1-3.8) in *T. vivax* and *T. congolense*.

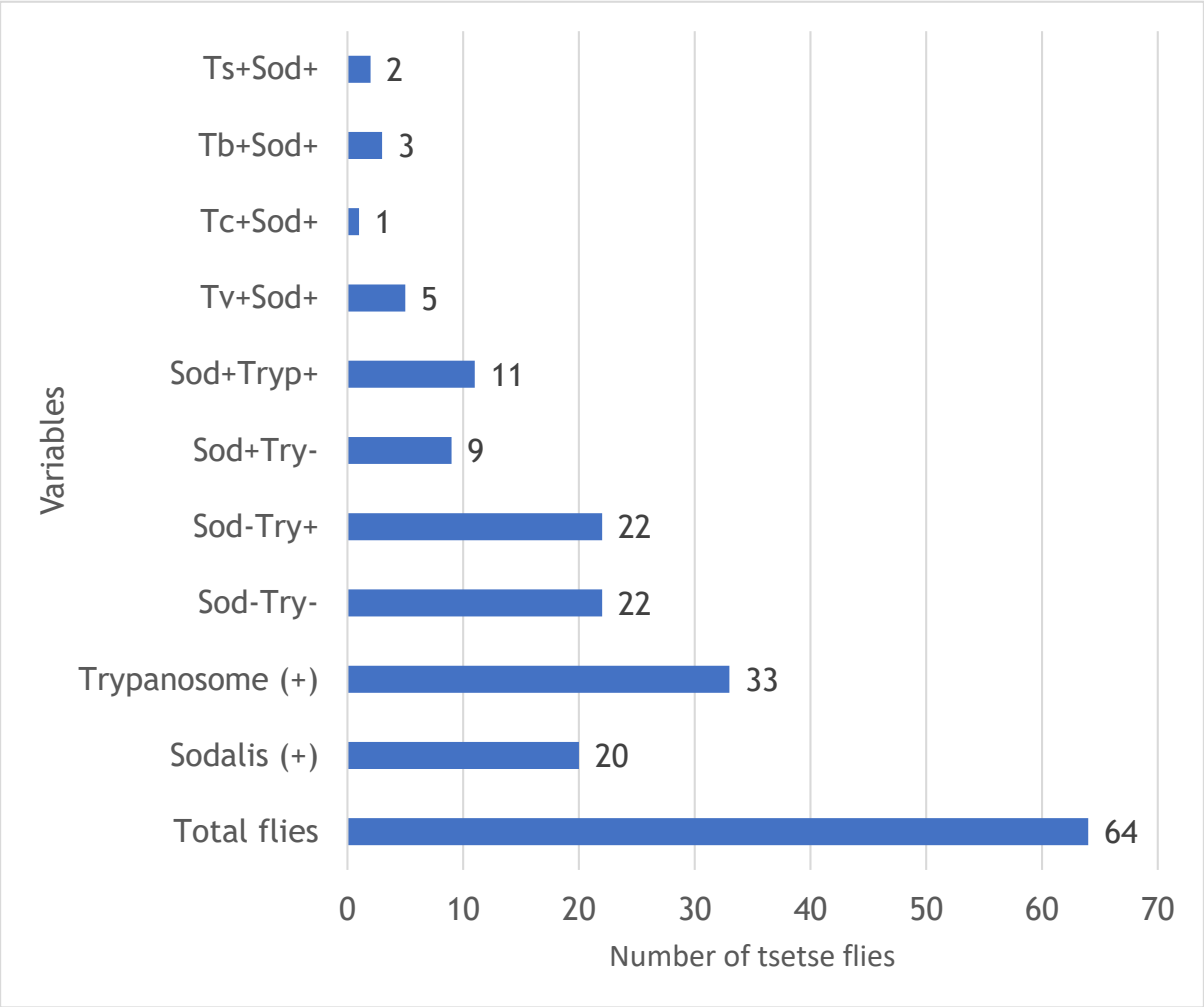


Figure 4.10: Trypanosome-*Sodalis glossinidius* association in tsetse flies

For *S. calcitrans*, 17 of total 72 (23.6%) tested fly species were positive for trypanosome DNA. *T. vivax* was highest at 19.4% followed by *T. evansi* at 6.9%, *T. congolense* at 1.4% and *T. simiae* at 2.8%. Single infections for trypanosome DNA observed were 13.9%, 1.4%, 1.4% and 1.4% in *T. vivax*, *T. evansi*, *T. congolense* and *T. simiae* respectively. Double infections were observed in 4.2% of *T. vivax* and *T. evansi*, 1.4% in *T. evansi* and *T. simiae*, 1.4% in *T. vivax* and *T. congolense*. For *S. niger*, 15 of total 72 (20.8%) tested stomoxyine were positive for trypanosome DNA. *T. vivax*, *T. evansi* and *T. congolense* prevalence was 18.1%, 4.2% and 1.4% respectively. In terms of *S. niger* flies where a single trypanosome DNA was observed, *T. vivax* was detected in 15.3%, *T. evansi* and *T. congolense* was found in 1.4% and 1.4% respectively. There were two (2.8%) *S. niger* where *T. vivax* and *T. evansi* DNA was detected.

In terms of tissue location of trypanosome DNA, the HP had 21 (14.6%) positive stomoxyine flies out of total 144 sampled. Infections observed were 17 (11.8%) *T. vivax*, 5 (3.5%) *T. evansi* and 2 (1.4%) *T. simiae*. Double infections were observed with 2 (1.4%) *T. vivax* and *T. evansi*, 1 (0.7%) *T. evansi* and *T. simae*. For TA, 21 (14.6%) flies were positive. *T. vivax* was found in 16 (11.1%), *T. evansi* in 3 (2.1%) and *T. congolense* in 2 (1.4%). Double infection was only found in one (0.7%) *T. vivax* and *T. evansi*. Stomoxyine species distribution according to tissue and combined infection of HP and TA has been described in Table 4.4.

Table 4.4: Trypanosomes identified in *Stomoxys* spp.

	No	Total	*T. vivax*	*T. evansi*	*T. congolense*	*T. simiae*	Mixed	Chi sq., P value
HP								
S. calcitrans	72	(15) 20.8[a]	(11) 15.3	(4) 5.6	(0) 0.0	(2) 2.8	(2) 2.8	X^2 = 4.516, α = 0.034
S. niger	72	(6) 8.3	(6) 8.3	(1) 1.4	(0) 0.0	(0) 0.0	(1) 0.0	
Prevalence		(21) 14.6	(17) 11.8	(5) 3.5	(0) 0.0	(2) 1.4	(3) 2.1	
TA								
S. calcitrans	72	(8) 11.1	(7) 9.7	(1) 1.4	(1) 1.4	(0) 0.0	(1) 1.4	X^2 = 1.394,
S. niger	72	(13) 18.1	(9) 12.5	(3) 4.2	(1) 1.4	(0) 0.0	(0) 0.0	α = 0.238
Prevalence		(21) 14.6	(16) 11.1	(4) 2.8	(2) 1.4	(0) 0.0	(1) 0.7	
(HP) + (TA)								
S. calcitrans	72	(5) 6.9	(4) 5.6	(0) 0.0	(0) 0.0	(0) 0.0	(1) 1.4	X^2 = 0.119,
S. niger	72	(4) 5.6	(1) 1.4	(0) 0.0	(0) 0.0	(0) 0.0	(3) 4.2	α = 0.731
Prevalence		(9) 6.3	(5) 3.5	(0) 0.0	(0) 0.0	(0) 0.0	(4) 2.8	
Whole fly								
S. calcitrans	72	(17) 23.6	(14) 19.4	(5) 6.9	(1) 1.4	(2) 2.8	(5) 6.9	X^2 = 0.161,
S. niger	72	(15) 20.8	(13) 18.1	(3) 4.2	(1) 1.4	(0) 0.0	(2) 2.8	α = 0.688
Total prevalence	144	(32) 22.2	(27) 18.8	(8) 5.6	(2) 1.4	(2) 1.4	(7) 4.9	

Note: HP- head+proboscis, TA- thorax+abdomen, a- significantly different (α<0.05)

4.2.3. Prevalence of trypanosomes in Tabanus species

One *Tabanus* species (*T. biguttatus*) was lost during processing to make a total of fifty-one. All the species of trypanosomes observed were only found in the TA tissue of *Tabanus* spp. No HP infection was detected. A total of 17 (33.3%, 95%CI: 21.9-47.0) out of 51 *Tabanus* species examined were positive. *T. vivax* 19.6% (95%CI: 11.0-32.5) infection was predominant, followed by *T. evansi* 17.6% (95%CI: 9.6-30.2), while *T. simiae* and *T. godfreyi* had a prevalence of 3.9% (95%CI: 1.1-13.2) each. Double infection with *T. vivax* and *T. evansi* showed a prevalence of 9.8% (CI: 4.3-20.9) and multiple infection with *T. vivax*, *T. evansi* and *T. simiae* was found in 1.9% (95%CI: 0.4-10.3).

Of 15 *Tabanus taeniola* examined, 8 tested positive for trypanosome DNA, with 7 *T. vivax*, 5 *T. evansi* and 4 were observed to harbour double infection of *T. vivax* and *T. evansi*. Out of 4 *Tabanus pertinens* examined, 3 tested positive for trypanosome DNA, comprising 1 *T. vivax* and 2 *T. godfreyi* infections respectively (Table 4.5). A total of 2 out of 5 *Tabanus par* tested for trypanosome DNA were positive. Distribution of infection was 1 *T. vivax*, 2 *T. evansi*, 1 *T. simiae*. Double infection was observed in one sample with *T. vivax* and *T. evansi* while multiple infection was observed in one sample with *T. vivax*, *T. evansi* and *T. simiae*. Of 9 *Tabanus subangustus* examined, 2 were positive with *T. evansi* infection only. Out of 6 *Tabanus biguttatus*, one was positive of *T. simiae* only. Of 2 *Tabanus gratus* examined, one was positive of *T. vivax*. *Tabanus brucei*, *Tabanus socialis* and *Tabanus thoracinus* were all negative of trypanosome DNA (Table 4.5). However, regression analysis shows that infection rate between infected *Tabanus* species was not significantly different ($r^2 = 0.6080$, $df = 5$, $\alpha = 0.0674$). Distribution of mixed trypanosomes between the HP and TA has been described in table 4.6.

4.2.4. Seasonal effect of trypanosomes on transmitting vectors

Seasonal assessment of flies indicates that *T. vivax* was mostly observed during the dry months. In the TA tissues, one of five *Glossina* spp was positive for *T. vivax* trapped in the dry months. Similarly, TA tissues of *Tabanus* examined revealed 57.1% (4/7) to be *T. vivax* and were identified to be *Tabanus taeniola*. However, out of the 72 stomoxyine flies examined during dry months, 6.9% (95%CI: 3.0-15.3) were positive of trypanosome DNA. Out of the 144 Stomoxys examined 5.6% of the TA tissues were positive for *T. vivax* DNA, while 2.8%

Table 4.5: *Tabanus* spp. with *Trypanosoma* DNA.

Tabanus spp.	No sampled	Positive with trypanosomes	*T. vivax*	*T. evansi*	*T. simiae*	*T. godfreyi*	Tv/Tev	Tv/Tev /Ts
T. taeniola	15.0	8.0	7.0	5.0	0.0	0.0	4.0	0.0
T. subangustus	11.0	2.0	0.0	2.0	0.0	0.0	0.0	0.0
T. biguttatus	6.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0
T. par	5.0	2.0	1.0	2.0	1.0	0.0	1.0	1.0
T. pertinens	4.0	3.0	1.0	0.0	0.0	2.0	0.0	0.0
T. brucei	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T. thoracinus	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T. gratus	2.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0
T. socialis	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL	51.0	17.0 (33.3)	10.0 (19.6)	9.0 (17.6)	2.0 (3.9)	2.0 (3.9)	5.0 (9.8)	1.0 (2.0)

Table 4.6: Tissue distribution of *Trypanosoma* species in *Glossina* and stomoxyines

	Head + Proboscis (HP)	Thorax + Abdomen (TA)	
		+ve	-ve
a. *Glossina- T. vivax*	+ve	10 (7%)	19 (14%)
	-ve	18 (13%)	88 (65%)
b. *Glossina-T. b. brucei*			
		+ve	-ve
	+ve	9 (6%)	33 (24%)
	-ve	27 (20%)	57 (42%)
c. Stomoxyine-*T. vivax*			
		+ve	-ve
	+ve	6 (4%)	8 (6%)
	-ve	13 (9%)	117 (81%)
d. *T. evansi* in stomoxyine			
		+ve	-ve
	+ve	0 (0%)	5 (4%)
	-ve	4 (2%)	135 (94%)

(HP & TA samples evaluated with ITS1 rDNA nested PCR methodology from prepped DNA.

(95%CI: 0.8-9.6) of HP tissues were positive for *T. vivax* and *T. congolense* DNA at a prevalence of 1.4% (95%CI: 0.3-7.5 each. In stomoxyine, the prevalence of trypanosomes during the rainy season (18.8%, 95%CI: 13.2-25.9) was significantly higher ($X^2 = 19.446$, $\alpha < 0.0001$) when compared to the dry season (3.5%, 95%CI: 1.5-7.9).

4.2.5. Blood meal analysis

The overall percentage of tsetse with bloodmeal was 31.9% (95%CI: 24.6-40.1). A total of 24 female tsetse out of 72 were positive with bloodmeal (33.3%, 95%CI: 23.5 – 44.8). This consists of 13 out of sampled 35 female *G. palpalis* (31.4%, 95%CI: 23.2 – 53.7) and 11 out of sampled 37 female *G. tachinoides* (29.7%, 95%CI: 17.5 – 45.8)). Male tsetse are also haematophagous and a total of 19 out of 63 were positive with bloodmeal (30.2%, 95%CI: 20.2 – 42.4) consisting of 13 out of total 29 male *G. palpalis* (44.8%, 95%CI: 28.4 – 62.4) showing a significant difference ($X^2 = 5.489$, $\alpha = 0.019$) when compared with *G. tachinoides* that has six positives with bloodmeal out of total 34 sampled (17.7%, 95%CI: 8.4 – 33.5).

Five vertebrate hosts' blood were observed. The percentage of human (*Homo sapiens*) at 96-99% homology, cattle (*Bos taurus* & *Bos indicus*) at 92-97% homology, giraffe (*Giraffa camelopardis*) at 92-94% homology, spotted hyena (*Crocuta crocuta*) at 91% homology and an unidentified host were, 88.4%, 7.0%, 4.7%, 2.3% and 2.3%, respectively. Figure 4.11 shows the percentage of *Glossina* with bloodmeal according to species. A total of 79.1% (95%CI: 64.8-88.6) tsetse flies out of those detected by cytochrome oxidase to have bloodmeal were positive of trypanosomes (Table 4.7). Preferences for stomoxyine flies (Figure 4.12) and *Tabanus* (Figure 4.13) have equally been reported.

The percentage of *Tabanus* species with bloodmeal was 19.6% (95%CI: 11.0-32.5), with 10 out of 51 total *Tabanus* spp. being positive. A total of four out of 15 *Tabanus taeniola* (26.7%, 95%CI: 10.9-52.0), two out six *Tabanus biguttatus* (33.3%, 95%CI: 9.7-70.0), one out of two *Tabanus gratus* (50%, 95%CI: 9.5-90.6), one out of five *Tabanus par* (20%, 95%CI: 3.6-62.5), one out of 11 *Tabanus subangustus* (9.1%, 95%CI: 1.6-37.7) and one out of three *Tabanus thoracinus* (33.3%, 95%CI: 6.2-79.2) were positive with bloodmeal. Four vertebrate hosts were observed to be the sources of bloodmeal in *Tabanus* species.

The percentage of human (*Homo sapiens*) at 98-99% similarity, cattle (*Bos taurus*) at 98-99% similarity, hippopotamus (*Hippopotamus amphibious*) at 99% homology and giant rat (*Leopoldamys sabanus*) at 82% homology, was 50% (95%CI: 23.7-76.3), 30% (95%CI:10.8-

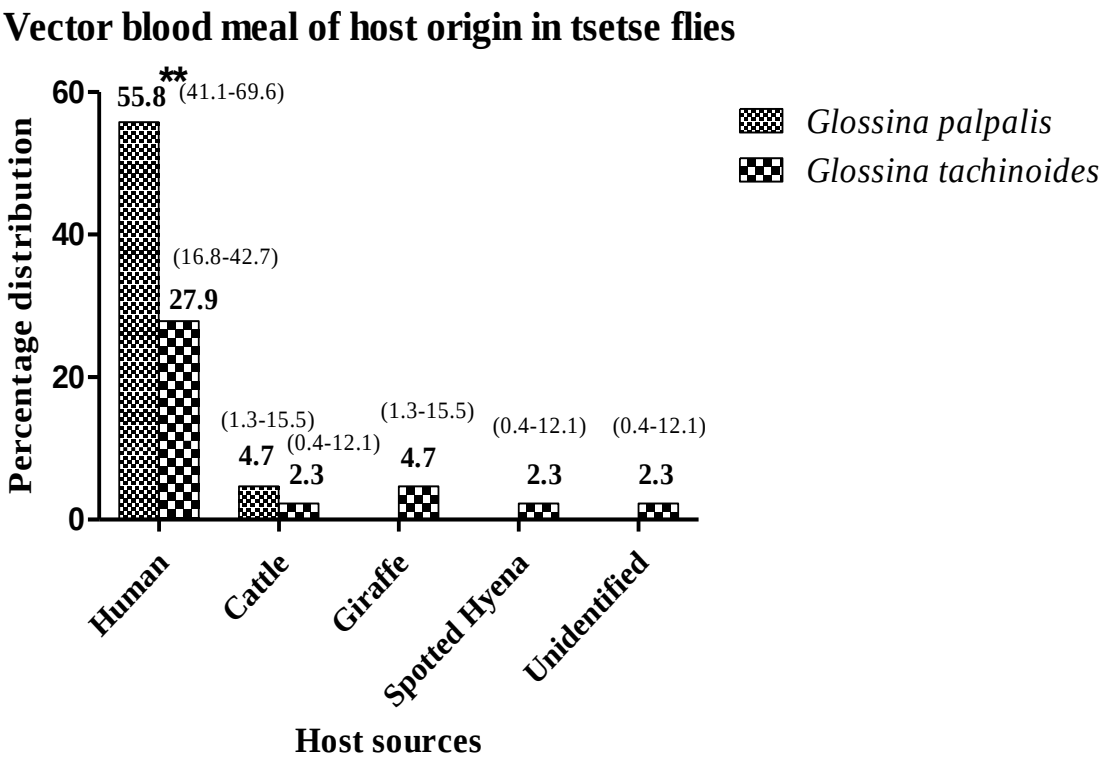


Figure 4.11: Host blood meal of *Glossina* spp.

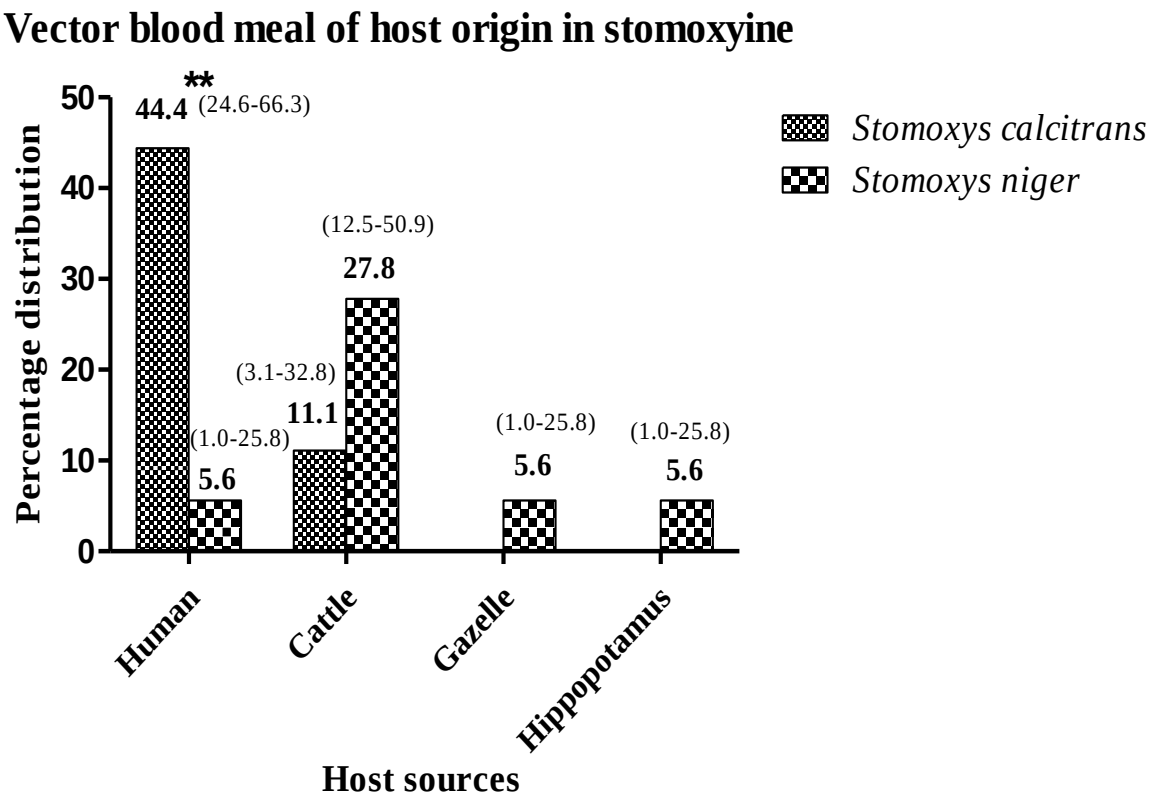


Figure 4.12: Host blood meal of stomoxyine flies.

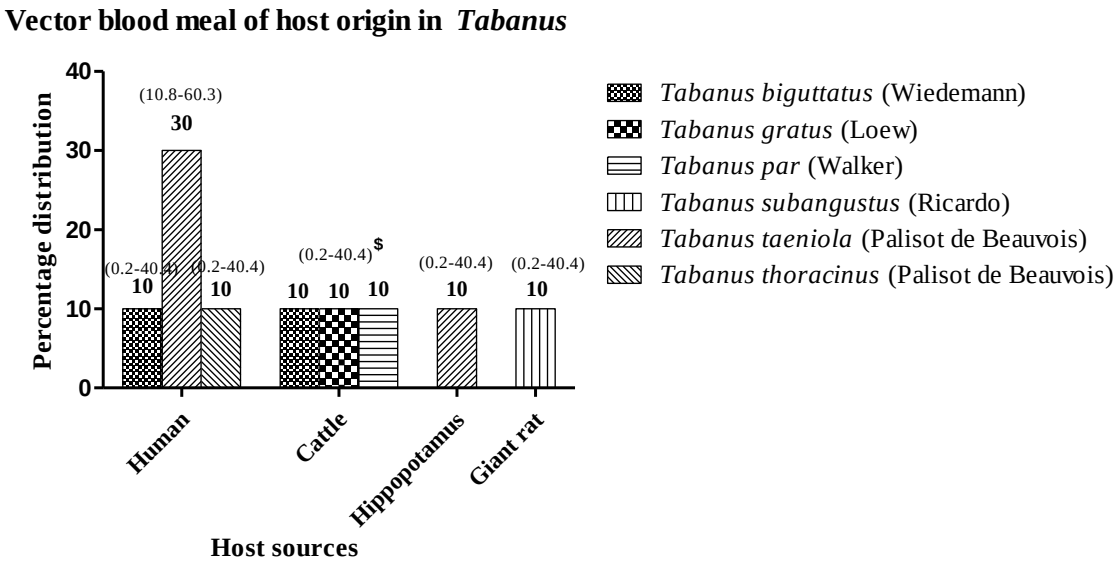


Figure 4.13: Host blood meal of *Tabanus* spp.

Table 4.7: Fly bloodmeal and trypanosomes interactions

	Bloodmeal	Trypanosomes	
		+ve	-ve
a. *Glossina*	+ve	34 (25.2%)	9 (6.7%)
	-ve	62 (45.9%)	30 (22.2%)
		+ve	-ve
b. stomoxyine	+ve	4 (2.8%)	14 (9.7%)
	-ve	28 (19.4%)	94 (65.3%)
		+ve	-ve
c. *Tabanus*	+ve	5 (9.8%)	5 (9.8%)
	-ve	12 (23.5%)	29 (56.9%)

60.8), 10% (95%CI: 1.8-40.4) and 10% (95%CI: 1.8-40.4), respectively. Bloodmeal according to *Tabanus* species preferences are shown in Figure 4.13. The relationship between bloodmeal in the abdomen and presence of trypanosomes is shown table 4.6.

For stomoxyine flies, a total of 18 out of 144 (12.5%, 95%CI: 8.1-18.9) were observed with bloodmeal. Out of 72 *Stomoxys calcitrans* tested, 10 were positive while 8 out of 72 *Stomoxys niger* were positive. Overall 126 stomoxyine tested negative indicates that percentage of teneral and/ hungry stomoxyine flies was 87.5% (95%CI: 81.1-91.9). Comparing sex, since both male and female are haematophagous, 11 out of 18 positive stomoxyine with bloodmeal were male 7.6% (95%CI: 4.3-13.2) which is non-significantly higher ($X^2 = 1.016$, $\alpha = 0.313$) than female stomoxyine with bloodmeal of 4.9% (95%CI: 2.4-9.7) in relation to total flies examined. Total of four hosts were observed as bloodmeal sources in stomoxyines. The percentage of vertebrate bloodmeal observed in human (*Homo sapiens*), cattle (*Bos taurus* and *Bos indicus*), gazelle (*Gazella subgutturosa*) and hippopotamus (*Hippopotamus camelopardis*) was 50%, 38.9%, 5.6% and 5.6%, respectively. Figure 4.12 shows *Stomoxys* species preferences of vertebrate bloodmeal.

4.2.6. Nucleotide analysis of trypanosomes from vector flies

Sequences from fly vectors of AAT showed various isolates of trypanosome species (Figure 4.14, 4.15 and 4.16). Tsetse flies from this study were identified in resembled isolates as follows: KM391828 (from cattle in Nigeria) at 98-99% similarity for *T. vivax*, FN712715.1 (from buffalo in China), at 99% similarity for *T. evansi evansi*, AF30677 (from Cote d'Ivoire), at 99% similarity for *T. brucei*, U22319 (from blood of *Rattus norvegicus*) at 97% for *T. congolense*, JN673387 at 93% similarity was identified previously in wildlife species from Tanzania. In *Tabanus* species, isolates KM391828 and KM391829 at 99% similarity for *T. vivax* were identified. *T. evansi* genotype AZ resembled isolate was detected with accession number KX898420 at 99% similarity. *T. godfreyi* resembled isolate ZWA6309 with accession number JN673383 was detected with 91% similarity, and it is a known Zambia strain. *T. simiae* resembled isolate with accession number JN673387 was detected at 93% similarity (Figure 4.15). *Stomoxys* species sequences, *T. vivax* resembled isolates KM391828 and KM391829, *T. evansi* resembled isolate KX898420, *T. simiae* resembled isolate JN673387 and *T. congolense* resembled isolate U22319 were all detected (Figure 4.16). Appendix VI shows the vertebrate sequences from vector bloodmeals.

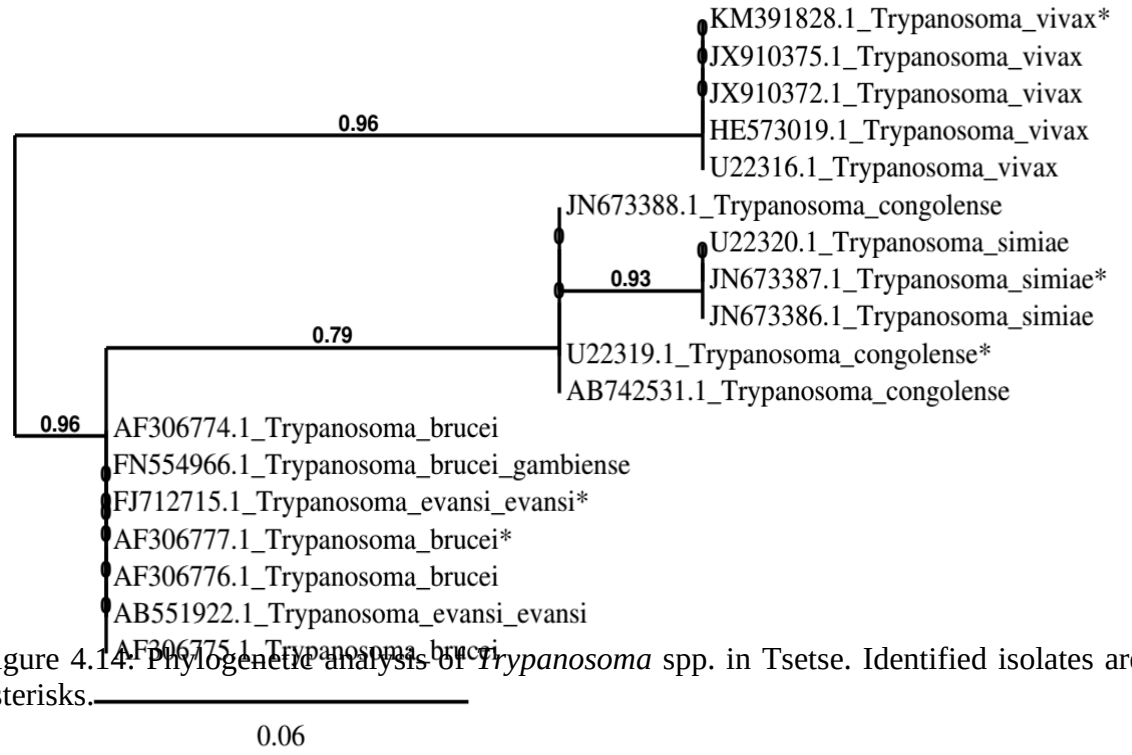


Figure 4.14: Phylogenetic analysis of *Trypanosoma* spp. in Tsetse. Identified isolates are in asterisks.

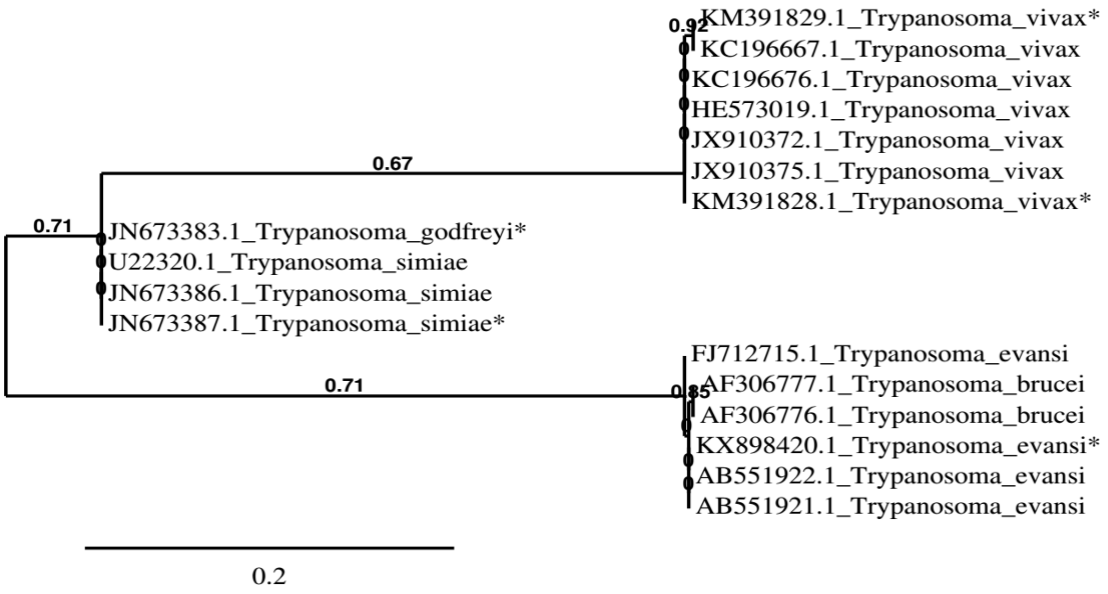


Figure 4.15: Phylogenetic analysis of *Trypanosoma* spp. in *Tabanus* spp. Identified isolates are in asterisks.

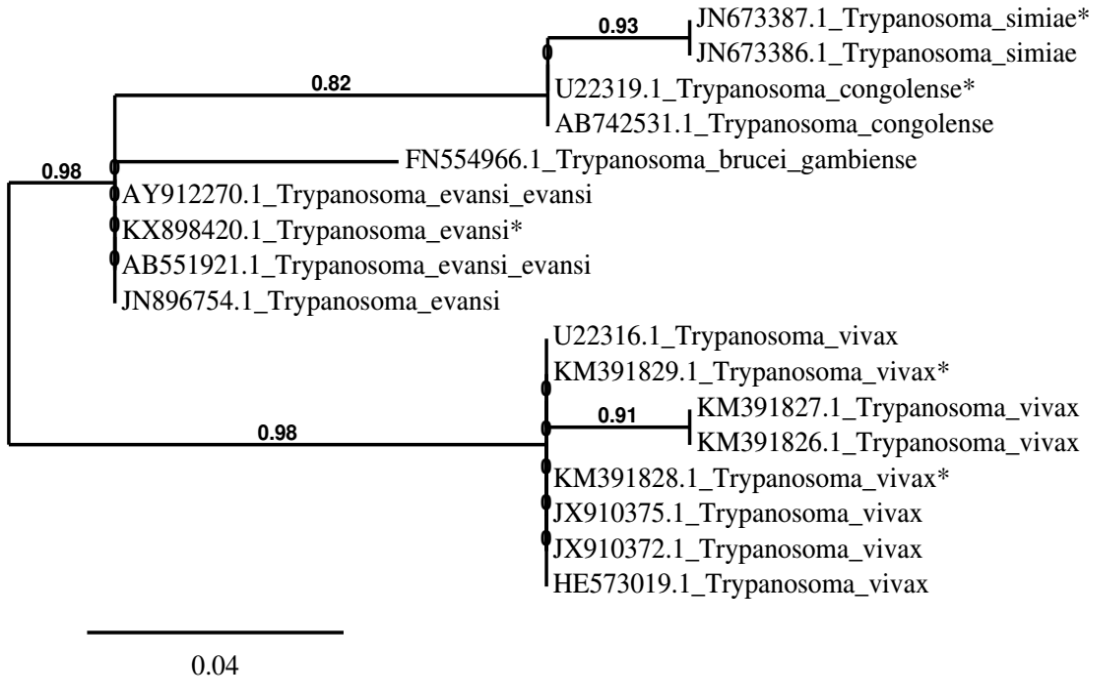


Figure 4.16: Phylogenetic analysis of *Trypanosoma* spp. in stomoxyine. Identified isolates are in asterisks.

4.3. STUDY THREE

4.3.1. Trypanosome prevalence in cattle blood

The 745 analysed bovine blood samples showed 177 cattle were positive for trypanosome DNA, with an overall prevalence rate of 23.8% (95%CI: 20.8-26.9). Total species distribution is illustrated in Figure 4.17. Seasonal variation showed significant increase in prevalence (X^2 = 6.301, α = 0.0120) in the wet season (26.7%) compared to the dry season (18.5%). *T. vivax* was reported mostly in dry season with a prevalence of 14.7% (95%CI: 10.96-19.49) and significantly different (X^2 = 4.056, α = 0.044) from 9.8% (95%CI: 7.4-12.8) observed in the wet season. While *T. congolense* had higher prevalence in the wet season with 11.0% (95%CI: 8.5-14.2) of cattle infected when compared (X^2 = 11.651, α = 0.001) to the dry season (3.8%; 95%CI: 2.1-6.8). Notably, *T. b. brucei* had a prevalence of 6.9% (95%CI: 4.9-9.5) in wet season which is significantly higher (X^2 = 12.245, α < 0.0001) compared to 1.1% (95%CI: 0.4-3.3) in dry season. *Trypanosoma evansi* (1.74%, 95%CI: 1.0-3.0) observed in cattle were only found in the wet season (Figure 4.18). *T. b. gambiense* DNA was observed to be absent in the cattle blood examined.

Mixed infections observed within the sampled cattle include double infections of *T. congolense* and *T. vivax* (0.67%, 95%CI: 0.3-1.6), *T. vivax* and *T. b. brucei* (0.54%, 95%CI: 0.2-1.4), *T. congolense* and *T. evansi* (0.27%, 95%CI: 0.1-1.0), *T. congolense* and *T. b. brucei* (0.54%, 95%CI: 0.2-1.4), *T. evansi* and *T. b. brucei* (0.40%, 95%CI: 0.1-1.2). There were also some triple infections observed consisting of *T. vivax*, *T. congolense* and *T. evansi* (0.13%, 95%CI: 0.0-0.8), and *T. congolense*, *T. b. brucei* and *T. evansi* (0.13%, 95%CI: 0.0-0.8). Cattle sex showed no significant difference (Appendix III) with prevalence results of females (25.6%, 95%CI: 21.5-30.2) and males (21.8%, 95%CI: 17.9-26.4).

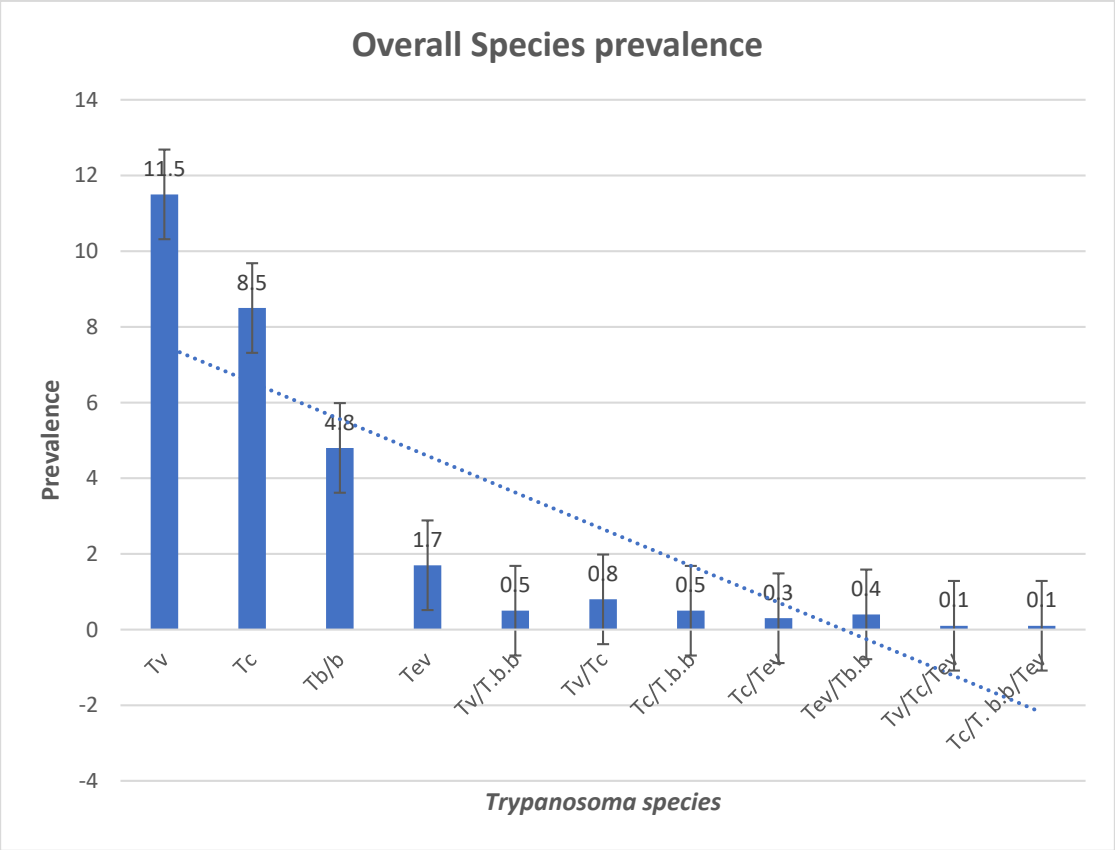


Figure 4.17: Overall prevalence of *Trypanosoma* species from cattle in southwest Nigeria. *Tc, T. congolense; Tv, T. vivax, Tb/b, T. brucei brucei and Tev, Trypanosoma evansi.

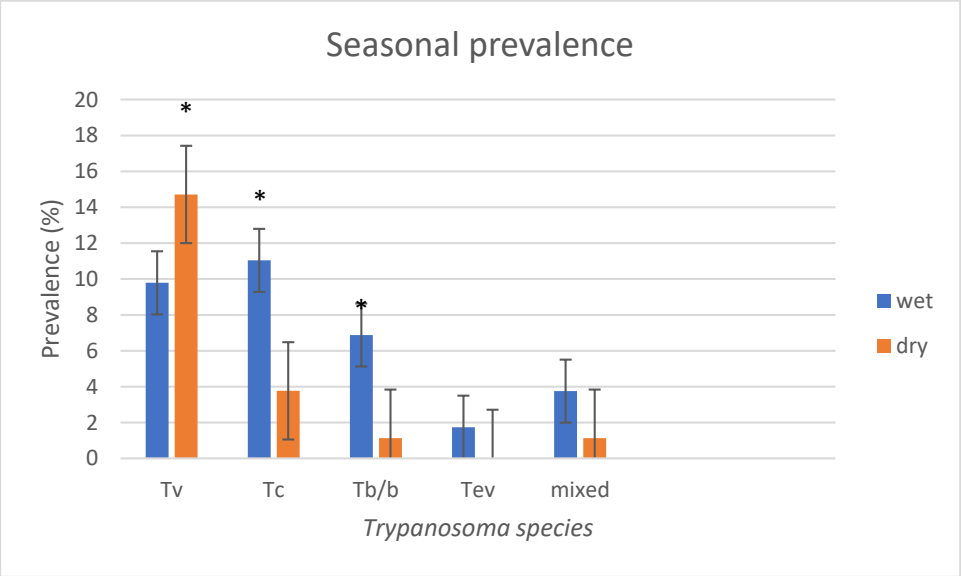


Figure 4.18: Prevalence of *Trypanosoma* species based on seasons.

*indicates significant difference ($\alpha < 0.05$). Tc, *T. congolense*; Tv, *T. vivax*, Tb/b, *T. brucei brucei* and Tev, *Trypanosoma evansi.*

4.3.2. Trypanosome prevalence based on cattle management sources

Cattle were sampled from four sources, abattoirs, cattle markets, institutional farms and Fulani cattle farms. Information on the original source of individual cattle from markets and abattoirs was not determined, however reports from cattle owners indicated over 90% were from the north. Some cattle can be at the same market for month/years. Trypanosome infection rates varied significantly between cattle sources. Most cattle from Fulani farms are from settlements in southwest Nigeria with owners engaging in pastoralism to satisfy these livestock while a few do undergo transhumance and may have been infected out of the study area. Cattle from institutional farms are engaged in pastoral farms owned by the institution in closed setting with proper management. Importantly, the log-transformed regression analysis revealed no association, however, Tukey multiple pairwise comparison observed significant differences among some groups (Apendix IV).

Abattoir cattle trypanosome prevalence was significantly higher compared to institutional cattle farms ($\alpha = 0.002$) and markets cattle ($\alpha = 0.001$) but significantly lower when compared with Fulani cattle farms ($\alpha < 0.0001$). Also, Fulani cattle prevalence (54.1%, CI: 42.9 – 64.9%) was significantly higher ($\alpha < 0.0001$) compared to other cattle sources, namely markets cattle 14.9% (CI: 10.61 – 20.41%) and institutional cattle farms 14.7% (CI: 10.10 – 20.97%). No statistical association ($\alpha = 0.973$) in the prevalence between institutional farms and cattle markets. Cattle sources in relation to distribution of *Trypanosoma* spp. showed (Figure 4.19), highest prevalence of *T. vivax* in Fulani cattle farms with 47.3% (95% CI: 36.3-58.5) of cattle infected, followed by abattoir (9.8%, 95% CI: 7.0-13.7), market cattle (7.4%, 95% CI: 4.6-11.9), and institutional farms (3.7%, 95% CI: 1.7-7.8).

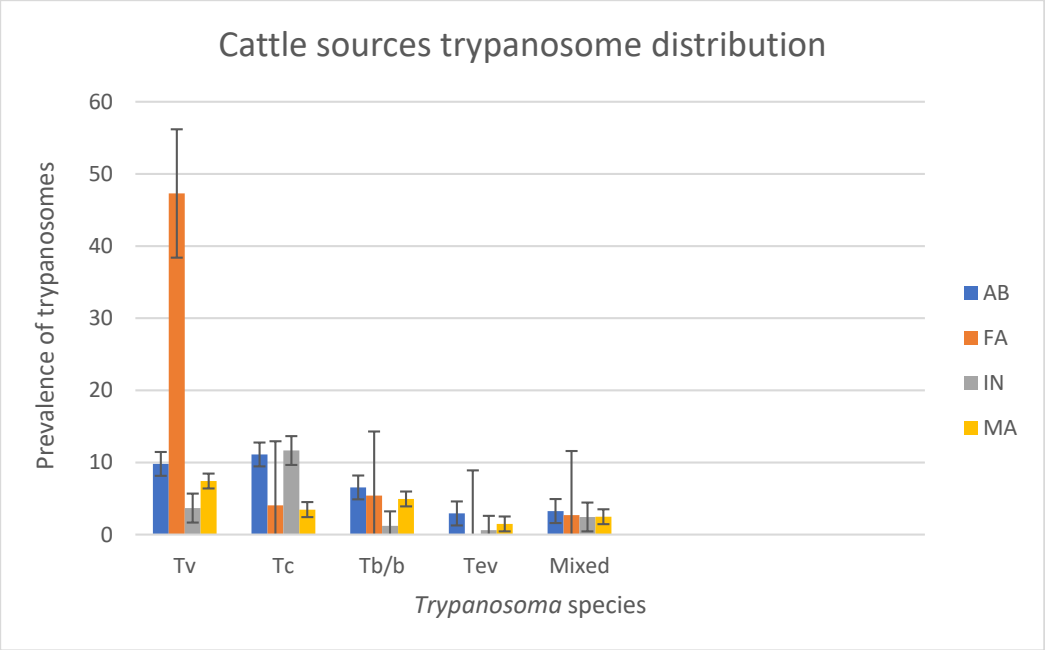


Figure 4.19: *Trypanosoma* species distribution in respect to cattle sources. Error bars are based on standard error means.

*indicates significant difference ($\alpha < 0.05$). Tc, *T. congolense*; Tv, *T. vivax*, Tb/b, *T. brucei brucei* and Tev, *Trypanosoma evansi*. AB – abattoirs, FA – Fulani cattle farms, IN – institutional cattle farms and MA – cattle markets.

T. congolense was mostly observed in institutional cattle farms and abattoir cattle with reported prevalence of 11.7% and 11.1%, respectively. Prevalence of *T. congolense* was lower in Fulani cattle (4.1%, 95% CI: 1.4-11.3) and market cattle (3.5%, 95% CI: 1.7-7.0). Prevalence of *T. b. brucei* infections were similar in Fulani cattle (5.4%, 95% CI: 2.1-13.1), abattoir cattle (6.5%, 95% CI: 4.3-9.9) and market cattle (5.0%, 95%CI: 2.7-8.9) but lower in institutional cattle (1.2%, 95% CI: 0.3-4.4). *T. evansi* was found in abattoir (2.9%, 95% CI: 1.6-5.5), market (1.5%, 95% CI: 0.51-4.3) and institutions (0.6%, 95% CI: 0.1-3.4), respectively. No *T. evansi* infection was observed in Fulani cattle.

4.3.3. Bovine trypanosome distribution in southwest Nigeria

The trypanosome prevalence according to states revealed highest in Osun State followed by Oyo, Ogun, Lagos, Ekiti and least in Ondo State. However, no association was observed in the prevalence (Appendix III). The prevalence of important trypanosomes in cattle population from this study has been reported (Figure 4.20). *T. vivax* was highest in Osun (20.7%) and Oyo (20.5%) states. *T. congolense* was more in Ogun state at 13.7%, then Lagos at 10.7%. *T. b. brucei* prevalence was highest in Ekiti (15.4%) followed by Osun state (6.9%).

Trypanosome distribution among cattle breeds reveals there is no association among the zebu breeds (White Fulani, Red Bororo, Sokoto Gudali) (Appendix III). However, Ambala, another zebu type (imported from Mali) was not infected at all. The taurine groups which are trypanotolerant (Muturu, Kuri and N'dama) could not be statistically evaluated because of the negligible numbers in this study. The overall zebu group prevalence was 24.0% which was higher but not significantly ($X^2 = 0.860$, $\alpha = 0.354$) to taurine groups 16.0%. Asymptomatic chronic cases were observed as our result showed 9.7% (95% CI: 5.62 – 16.16%) cattle with good health to be positive of trypanosome DNA. However, there is association in the prevalence when compared with those with intermediate ($X^2 = 16.366$, $\alpha < 0.0001$) and poor ($X^2 = 12.699$, $\alpha < 0.0001$) body score (Appendix III). Cattle $\leq$ 1 year showed lower prevalence (Appendix III) compared to other age groups. Those of >1 and $\leq$ 3, and > 3 years do not vary significantly ($X^2 = 0.041$, $\alpha = 0.840$) as observed in this study.

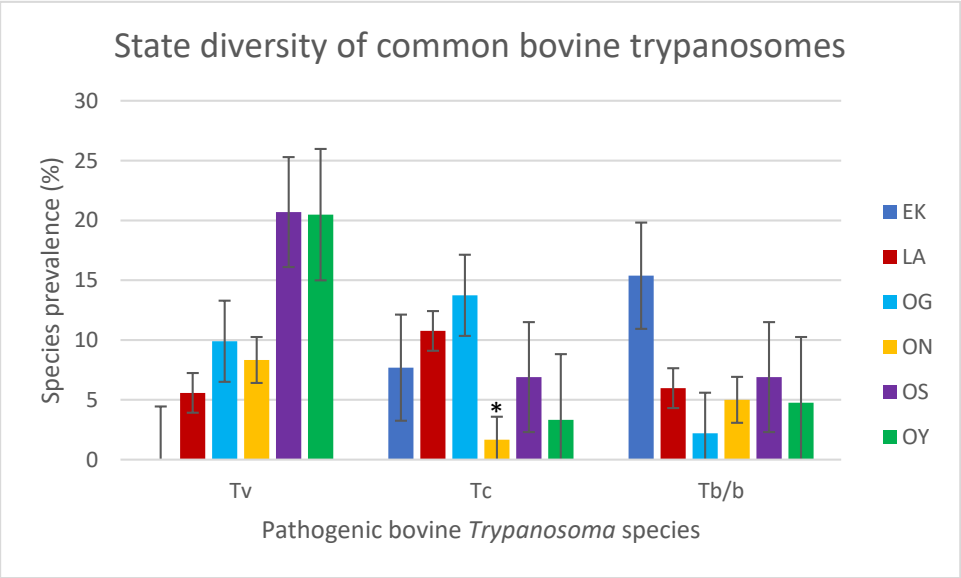


Figure 4.20: Important *Trypanosoma* species distribution according to states.

Abbreviations: Tc, *T. congolense*; Tv, *T. vivax and* Tb/b, *T. brucei brucei*. EK- Ekiti, LA- Lagos, OG- Ogun, ON- Ondo, OS- Osun and OY- Oyo.

4.3.4. Nucleotide sequence analysis of cattle blood

Of the purified *T. vivax* samples sequenced, *T. vivax* resembled isolate TvY486 (ILRAD 700) with KM391828 accession number was observed in Oyo, Ogun, Osun and Ondo states at 99 – 100% similarity to Nigerian strain (has also been reported in Ethiopia), and common in all the cattle sources while *T. vivax* with 98% similarity (Figure 4.21), *T. vivax* resembled isolate (KM391827) was reported from Agege abattoir in Lagos state with 98% similarity with another isolate in Ethiopia.

However, sequenced sample from Lafenwa abattoir, Ogun State had 100% similarity with the same description (maximum score, similar query, query cover, total score and e-value) for five different isolates (JX910375, KM391828, HE573019, JX910372 and U22316), found in Burkina Faso and Ethiopia. The ITS1 used did not classify *T. congolense* into either savannah or forest type, our sequences showed *T. congolense* riverine/forest- type isolates (U22319) which had 96 – 99% similarity with strain from Kenya, this was found in FUNAAB institutional farm, Ogun State and Akinyele cattle market, Oyo State.

Trypanosoma congolense genes isolates (AB742531) in FUNAAB institutional farm had 86% similarity with strain from Ghana. Non-RoTat 1.2 gene *T. evansi* was identified from a positive *Trypanozoon* base pair which was negative to RoTat 1.2 gene and TgsGP and assumed to be *Trypanosoma brucei brucei*. *T. evansi* resembled genotype AZ isolate (KX898420) from Agege abattoir, Igangan farm and Akinyele cattle market, Oyo State has 98% similarity with strain isolated from camel blood in Iran.

Trypanosoma congolense resembled isolate 32-SA (KX870079) with 81% similarity to the strain isolated from *Tabanid* blood in Zambia and South Africa, was identified in cattle population from Fulani cattle farms at Igangan and Eruwa. Submission ID: SUB4467120 was given for sequences submitted to the GenBank. Two *T. vivax* sequences (MH796907 and MH796908), four *T. congolense* sequences (MH79909-MH796912) and one *T. evansi* (MH796913) were accession numbers generated from this study.

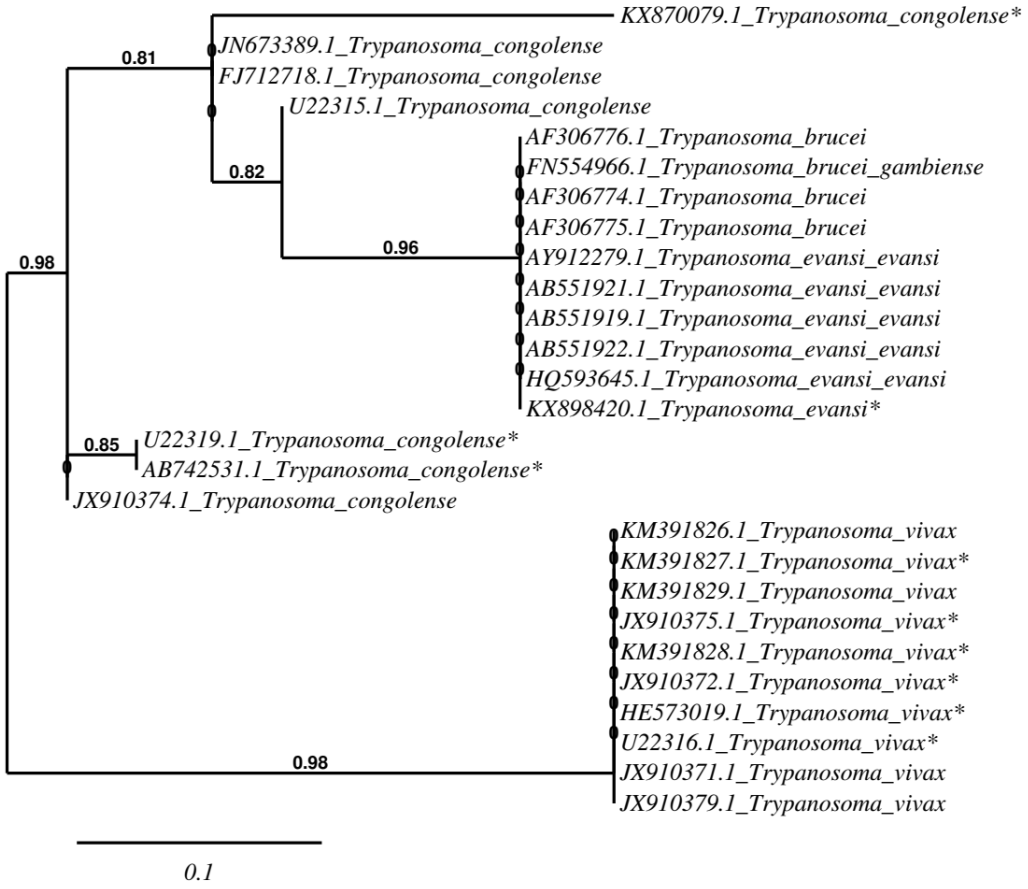


Figure 4.21: Phylogenetic relationship of the 18S rDNA of *Trypanosoma* strains detected in cattle.

Footnote: The phylogenetic tree was further edited by tree rendering alignment (http://phylogeny.fr) using multiple sequence alignment with high accuracy and throughput. Sequences generated in this study have their accession numbers and species names in asterisks.

4.4. STUDY FOUR

4.4.1. Landing positions of vector flies on cattle host

Fly vectors showed variations for landing preference on cattle hosts. Predominantly, the tsetse flies trapped were those of palpalis group. Total landing *Glossina* species shows no significant difference ($\alpha > 0.05$).

Bonferroni's multiple comparison test revealed higher significance ($\alpha < 0.05$) in overall *Glossina* spp. landing on forelimbs, hindlimbs and belly compared to other body parts. *G. tachinoides* were observed to prefer the forelimbs and belly more than other parts, while *G. palpalis* showed preference for forelimbs and hindlimbs in adult cattle. Landing preference in calves for both *G. palpalis* and *G. tachinoides* was the belly. Although, *G. tachinoides* were found on forelimbs and head at negligible numbers but *G. palpalis* landed on the forelimbs and hindlimbs. No *Glossina* species landed on the tail on the cattle examined (Figure 4.22A).

Tabanus taeniola landing preference was defined as forelimbs > belly > head and neck while *T. thoracinus* preferred the forelimbs, belly and hind limbs. *Tabanus par* failed to land on the head and neck and hind limbs while their preference was mostly on the back. *T. gratus* landing site was forelimbs and belly, but *T. biguttatus* preferred to land on the forelimbs. *T. subangustus* mostly landed on belly and forelimbs. *T. pertinens* landed more on hind limbs and forelimbs. *T. rubidus* landed mostly on forelimbs while *T. brucei* landed more on belly. Overall, *Tabanids* preferred lower body parts (limbs and belly) for both sexes. Bonferroni's pairwise comparison shows a significant difference ($F_{5, 48}$; $\alpha < 0.0001$) in the landing positions of *Tabanus* spp. In calves, *T. taeniola*, *T. pertinens* and *T. subangustus* mostly landed on the belly and limbs. The percentage of *Tabanus* spp. landing on body sites is shown is Figure 4.22B.

More stomoxyines preferred head and neck of cow compared to bulls and calves with no association (Figure 4.22C). Stomoxyines showed significant increase ($F_{5, 12}$; $\alpha < 0.0001$) in those landing on the head and neck compared to other landing positions. Meanwhile, no observable significance of stomoxyines between all the animal ages examined. The Bonferroni's *"post-hoc"* comparison test revealed association between stomoxyine body parts landing. Hence, major sites were head and neck, belly, forelimbs and hindlimb

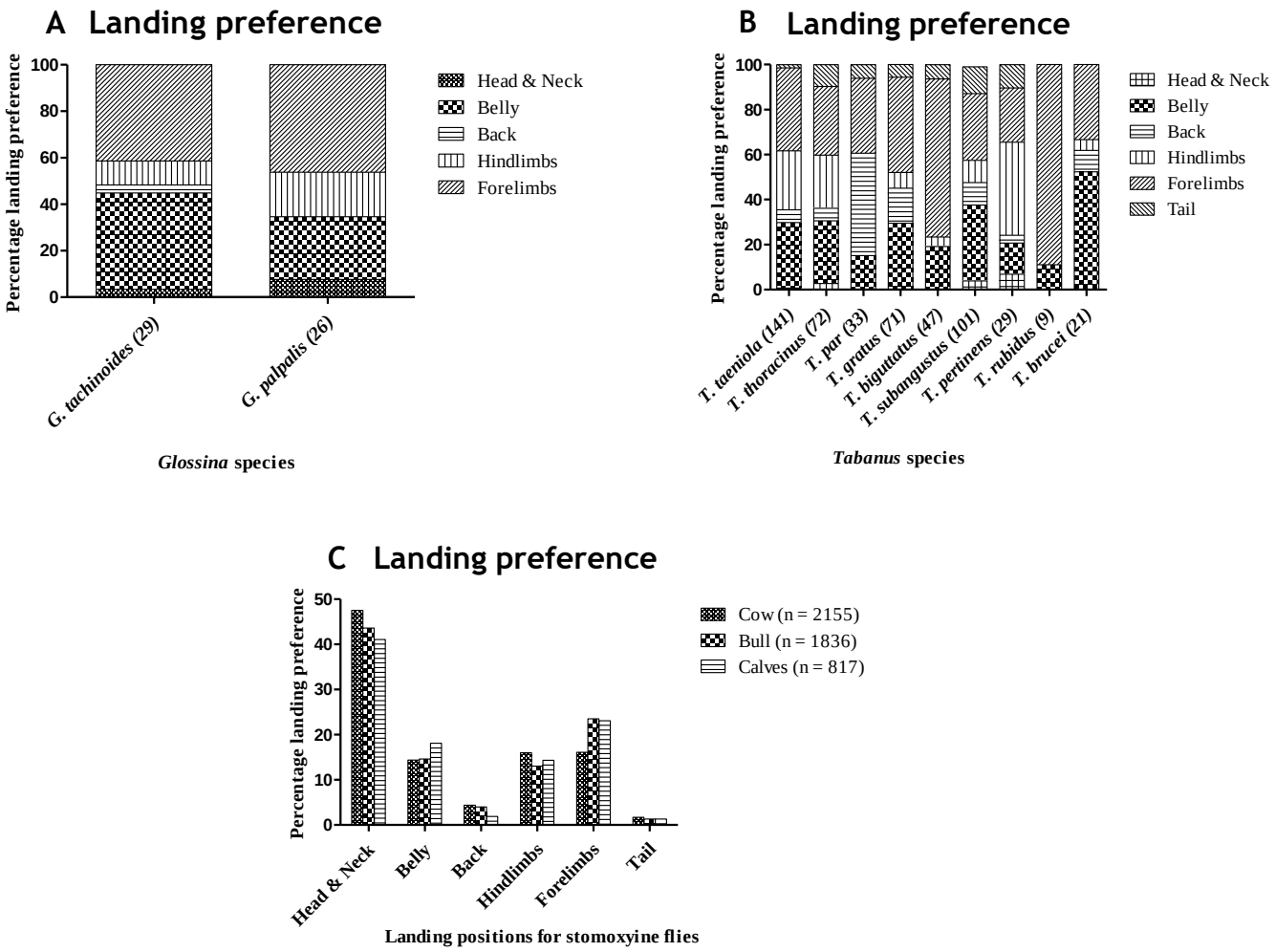


Figure 4.22: Landing preferences of transmitting vectors

4.4.2. Fly vector engorgement success

The engorgement success based on fly species has been reported in Appendix V. The Bonferroni's pairwise comparison showed significant difference in engorgement success rate ($\alpha = 0.0002$) between the transmitting vectors. There is no association ($\alpha = 0.9809$) in engorgement success of fly vectors according to cattle age. *Glossina* species revealed that 77.8% (95%CI: 61.9-88.3) of fed flies got engorged with 66.7% (95%CI: 41.7-84.8), 84.2% (95%CI: 62.4-94.5) and 100% (95%CI: 34.2-100.0) in cow, bulls and calves, respectively. Complete engorgement successes for *G. palpalis* was observed in calves and bulls, while 63.6% (95%CI: 35.4-84.8) was reported for cows.

Engorgement success in *Glossina tachinoides* was highest at 75% (95%CI: 30.1-95.4) in cow, even though there was no statistical association ($\alpha > 0.05$) in the engorgement success observed between *Tabanids* and *stomoxyines* There was variation in the engorgement success observed according study sites. In Adebayo, higher engorgement success was achieved in *G. palpalis* compared to Idiroko. For *Tabanids* 19.6% (95%CI: 15.6-24.3) of the fed flies were engorged, with cow being most engorged at 21.9% (95%CI: 16.3-28.7), followed by bull at 17.4% (95%CI: 12.0-24.4), while calves were least engorged at 11.1% (95%CI: 2.0-43.5).

In cow however, *T. thoracinus* was observed with highest engorgement success at 40.9% (95%CI: 23.4-61.3), while *Tabanus brucei* and *Tabanus rubidus* were not engorged after attempted feeding. Incidentally in bulls and calves, *T. taeniola* achieved the highest engorgement success. *Stomoxys* spp. that were engorged from the total feeding was 10.2% (95%CI: 8.9-11.6). Engorgement values were 11.2% (95%CI: 9.3-13.4), 9.6% (95%CI: 7.9-11.6) and 6.6% (95%CI: 3.2-13.0), respectively for bulls, cow and calves.

4.4.3. Vector flies feeding duration

Feeding duration recorded for *Glossina palpalis* was higher than *Glossina tachinoides* though with no significant difference. Tsetse fed more on the bulls compared to cow in the study sites, while the feeding duration to complete a meal was least in calves (median = 123 s). Calves were very reactive to *Glossina* spp. bites and hence a lower feeding duration was reported (Appendix V). The mean feeding duration is higher for *Glossina* spp. as compared to the biting flies while cattle defensive behaviour were lower during *Glossina* spp. Study site variations on feeding duration was observed on similar fly vectors though not significantly different.

Longest feeding durations were observed for *T. gratus*, *T. brucei*, *T. subangustus* and *T. taeniola* on cattle host. Higher feeding durations did not correspond to greater engorgement, while sizes among *Tabanus* spp. did not affect the time of feeding. *T. rubidus*, *T. thoracinus* and *T. par* fed less (Appendix V). *T. taeniola* was the most persistent *Tabanid* on the defensive host, staying more than 2 – 5 times than other species. *T. subangustus* switched on cattle more than other observed species on cattle host. Female were more attracted to tabanids than male although not significantly different. The feeding durations of vector species biting cattle (cow, bull and calves) are shown in Appendix V. Substantial number of *T. par* were scarred off in less than 30 seconds of feeding on the cattle and find it difficult to complete a bloodmeal session. *Stomoxys* spp. were observed engage longer feeding in cow and bulls in comparison to calves with no association ($\alpha > 0.05$) in the number of feeding duration. Engorgement success of stomoxyine is reduced when compared with those of tsetse *and Tabanids*. The feeding duration in stomoxyine flies for cow, bulls and calves was highest at 81, 68 and 33 s, respectively. The overall feeding duration has been reported (Appendix V).

4.4.4. Cattle defensive behaviour to vector flies

Cattle defensive behaviour studied involved tail flicking, hoof stamping and ear flicking. Calves showed a quicker response to flies more readily than cow and bulls. Their teeth also serve as scratching tool when there is bite from heavy flies such as *Glossina* and *Tabanids*. Adult cattle (bulls and cow) showed similar defensive response except for tail flicking where significant higher response was commonly observed in cows (Table 4.8). The rate of attack negatively correlates to the defensive behaviours (Figure 4.23). Hence, as the apparent density of fly vectors increased, cattle defensive behaviour increased. Continuous hoof stamping (calves- 14 / min, cow- 8 / min, bull- 8 / min) and ear flicks were reported on attacks from stomoxyine due to its overwhelming abundance. Therefore, statistical association between cattle hosts defensive behaviours have been reported (Table 4.8). The persistence of stomoxyine flies around the head and neck coupled with significant numbers around the limbs makes dislodgement difficult for cattle hosts. Of the total 524 *Tabanus* spp. landing on calves, cow and bulls, 75.7% (95%CI: 59.9-86.6), 28.1% (95%CI: 23.1-33.7) and 32.4% (95%CI: 26.5-38.9), respectively were observed to be dislodged by the host within five seconds, preventing them from relaxed feeding state. However, *Glossina* spp. could feed continuously without significant host response from the adult cattle compared to the calves.

Irritation caused by stomoxyine was highest in all the study sites due to its overwhelming abundance.

Table 4.8: Tukey *"post-hoc"* pairwise comparison test of cattle defensive behaviours

Tukey multiple comparison test	Mean difference	Q-ratio	95%CI	α - value summary
Tail flicking				
Calves vs cow	8.576[a]	11.79	6.040 - 11.11	α < 0.0001
Calves vs bulls	12.33[a]	16.96	9.798 - 14.87	
Cow vs bulls	3.758[a]	5.166	1.222 - 6.293	
Hoof stamping				
Calves vs cow	6.49[a]	29.77	5.73 - 7.24	α < 0.0001
Calves vs bulls	5.91[a]	27.13	5.15 - 6.67	
Cow vs bulls	-0.58[b]	2.643	-1.335 - 0.1836	
Ear flicking				
Calves vs cow	11.79[a]	13.82	8.815 - 14.76	α < 0.0001
Calves vs bulls	12.55[a]	14.71	9.573 - 15.52	
Cow vs bulls	0.7576[b]	0.8883	-2.215 - 3.730	

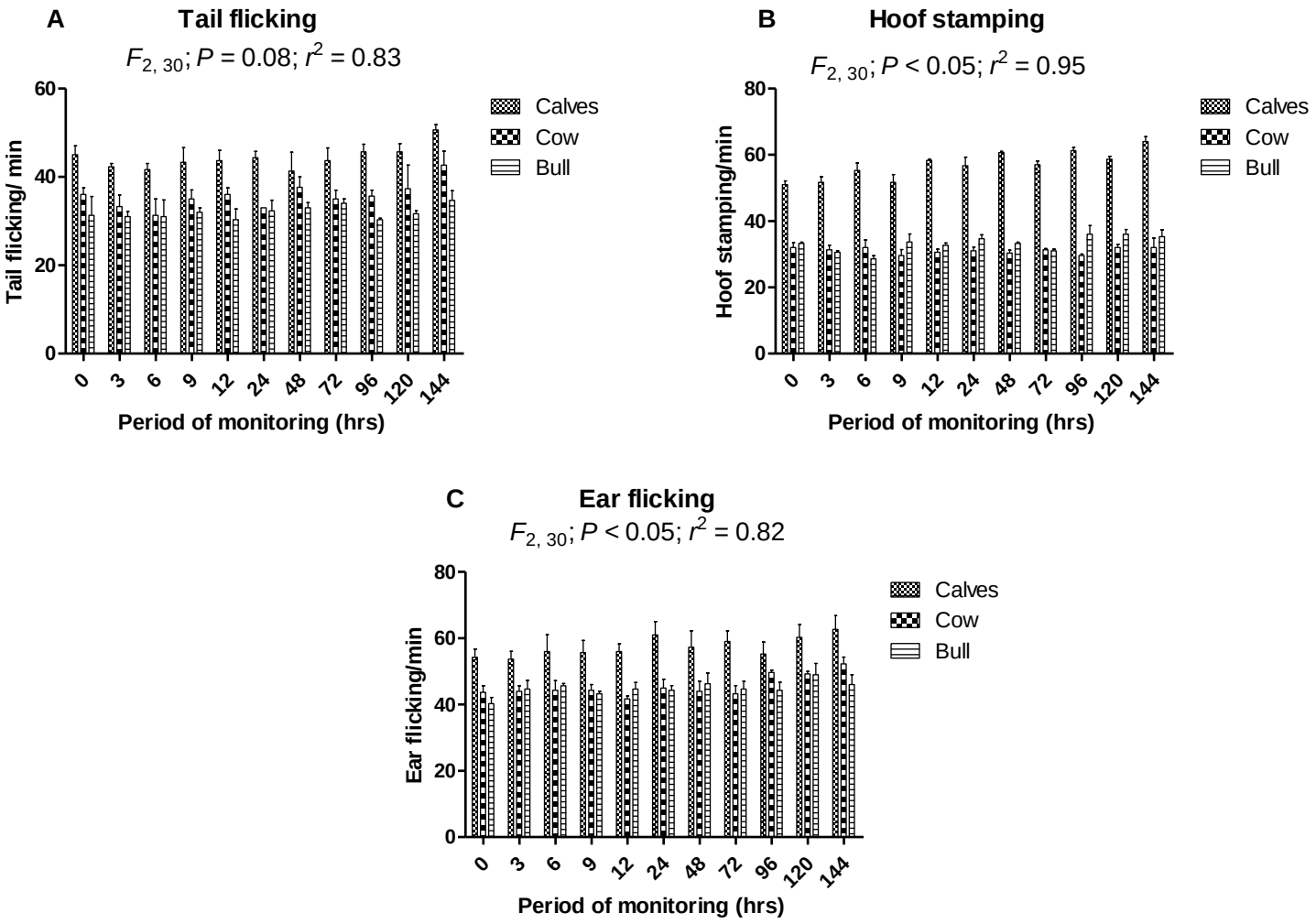


Figure 4.23: Cattle defensive behaviours monitored over a period.

4.5. STUDY FIVE

4.5.1. Insecticide-treated cattle defensive behaviour to stomoxyines

Tail twitching: average counts of stomoxyine flies that fed ranged from 0.10 to 0.25 per time point in the RAP group, while moderate counts of stomoxyine flies that fed ranged from 0.01 to 0.2 was observed in the FAA group. Control group showed percentage increase of 85.7% in stomoxyine flies. For hoof stamping: the control group showed 29.1% percentage increase in abundant stomoxyine during the experiment with no association between the start and end. The RAP method was 96.9% more effective compared to 42.3% FAA against the stomoxyine flies. Ear twitching; RAP and Fulani methods reported 87.5% and 11.8% repelling rate, while the control group revealed 20.0% in ear flicking defensive response (Figure 4.24). Assessments on defensive behaviour of cattle revealed lack of association ($\alpha > 0.05$) of control group compared to FAA group. Although, RAP group did show significant increase ($\alpha > 0.05$) in effectiveness in comparison to control and FAA groups (Table 4.9).

4.5.2. Feeding behaviour during insecticidal treatment

The feeding stomoxyine flies increased by 46.9% within 144 hrs in control group. However, the RAP and Fulani groups showed an effectiveness against stomoxyine flies by 94.6% and 46.3%, respectively. Even though the alighting flies in control group increased by 28.6% over six-day period, the RAP and Fulani treated showed 97.1% and 75.7% reduction in alighting stomoxyines. Feeding duration showed percentage decrease of 87.5% and 75.8% for RAP and Fulani methods, respectively. however, the control groups fed longer with average feeding time of 48 seconds. The flies feeding on treated groups (RAP and FAA) exhibited low engorgement success at 6 and 17% rate respectively. The comparison test reported significant difference ($\alpha > 0.05$) in feeding stomoxyine flies among the treated (RAP and FAA) and untreated (control) cattle groups (Figure 4.25)

4.5.3. In vitro bioassay

Insecticidal stomoxyine bioassay validated on the field exhibited lethal concentration (LC_{50}). The best LC_{50} of group A (Pantex®) was observed at 1.52 µg/ml (95%CI: 0.6-3.9, $r^2 = 0.53$) followed by group C, the Fulani concotion containing mixture of dispensed cypermethrin and

extracted oil of *Senna occidentalis* which revealed a LC_{50} of 20.62 µg/ml (95%CI: 10.5-40.3, r^2 = 0.32), while group B (Ectopouron®), exhibited LC_{50} of 22.68 µg/ml (95%CI: 13.2-38.9,

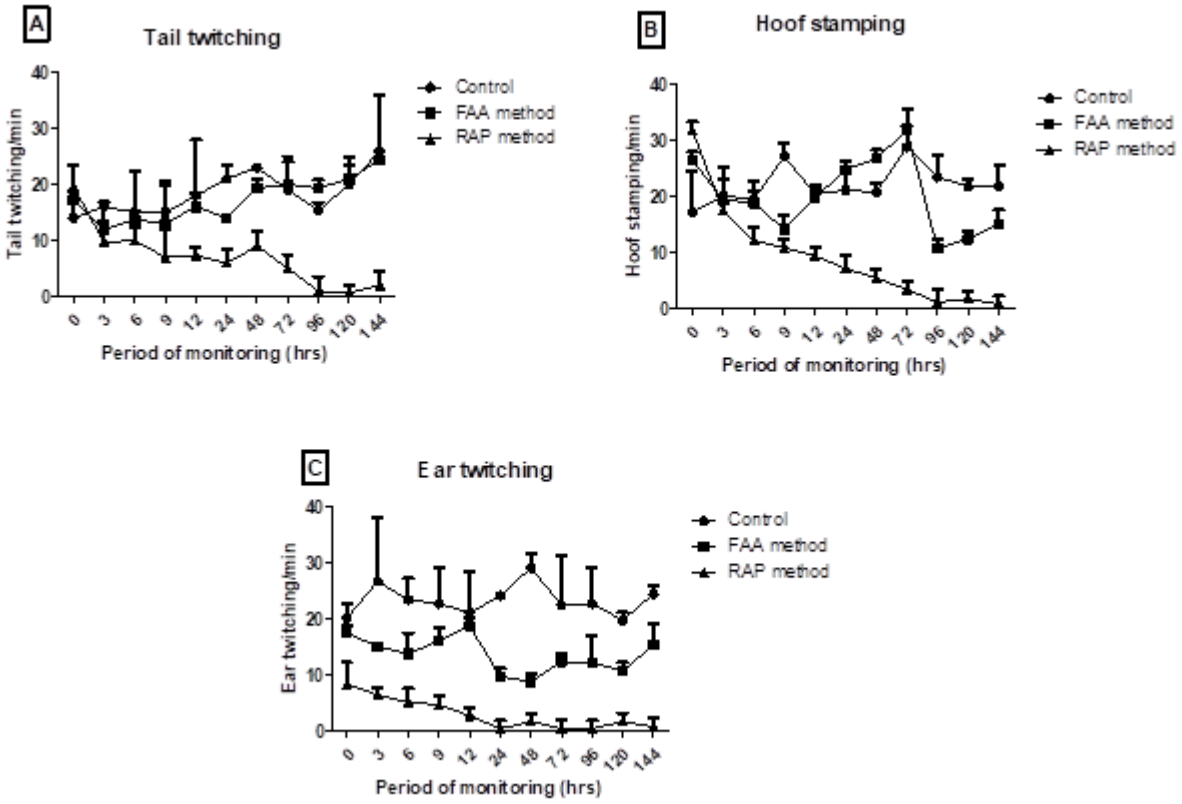


Figure 4.24: Insecticidal treated cattle defensive behaviours to stomoxyines per minute count over six-days exposure.

Table 4.9: One-way ANOVA analyses of host-vector interactions on insecticide application

Behavioural response	Tukey pairwise comparison test	Mean difference	Q	α-value	95%CI
Feeding behaviour	Control vs FAA	40.6	10.5	$\alpha < 0.05$	27.1-54.1
	Control vs RAP	61.5	15.9	$\alpha < 0.05$	48.0-75.1
	FAA vs RAP	20.9	5.4	$\alpha < 0.05$	7.4-34.4
Tail twitching	Control vs FAA	1.1	0.8	$\alpha > 0.05$	-3.5-5.8
	Control vs RAP	11.4	8.5	$\alpha < 0.05$	6.7-16.0
	FAA vs RAP	10.2	7.7	$\alpha < 0.05$	5.6-14.9
Hoof stamping	Control vs FAA	2.0	1.0	$\alpha > 0.05$	-5.2-9.1
	Control vs RAP	12.8	6.2	$\alpha < 0.05$	5.6-20.0
	FAA vs RAP	10.8	5.4	$\alpha < 0.05$	3.7-18.0
Ear twitching	Control vs FAA	9.7	11.0	$\alpha > 0.05$	6.6-12.7
	Control vs RAP	20.3	23.1	$\alpha < 0.05$	17.3-23.4
	FAA vs RAP	10.7	12.1	$\alpha < 0.05$	7.6-13.7

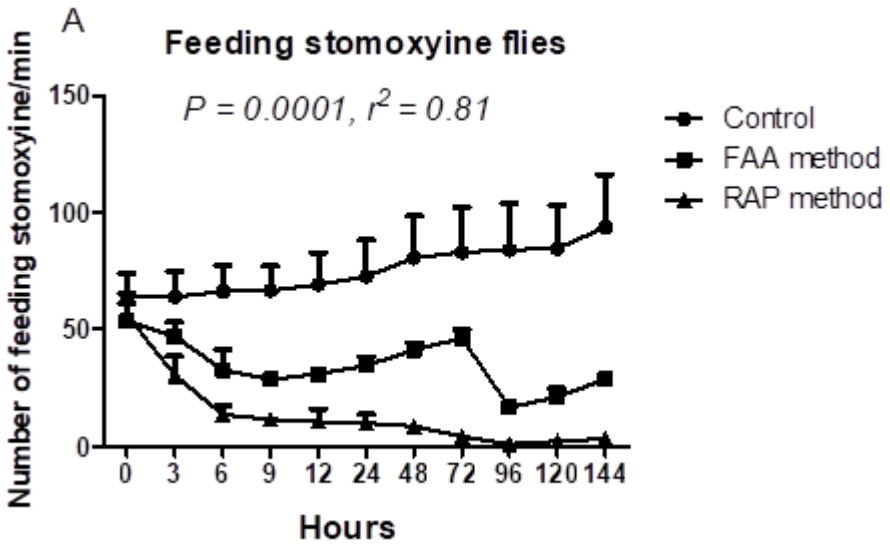


Figure 4.25: Assessment of stomoxyine flies feeding response per minute time over six-day period after insecticidal treatment.

r^2 = 0.60). Group A showed significant difference (α > 0.05) in efficacy when compared to other groups. However, group B and C showed no association (α > 0.05) in effectiveness. By the end of experiment (48 hrs), the survival rates of Pantex®, Ectopouron® and Fulani concoction at highest doses was observed to be 0.7. 50 and 46.7%, respectively. Figure 4.26 reported the LC_{50} values of insecticidal activity against stomoxyine flies.

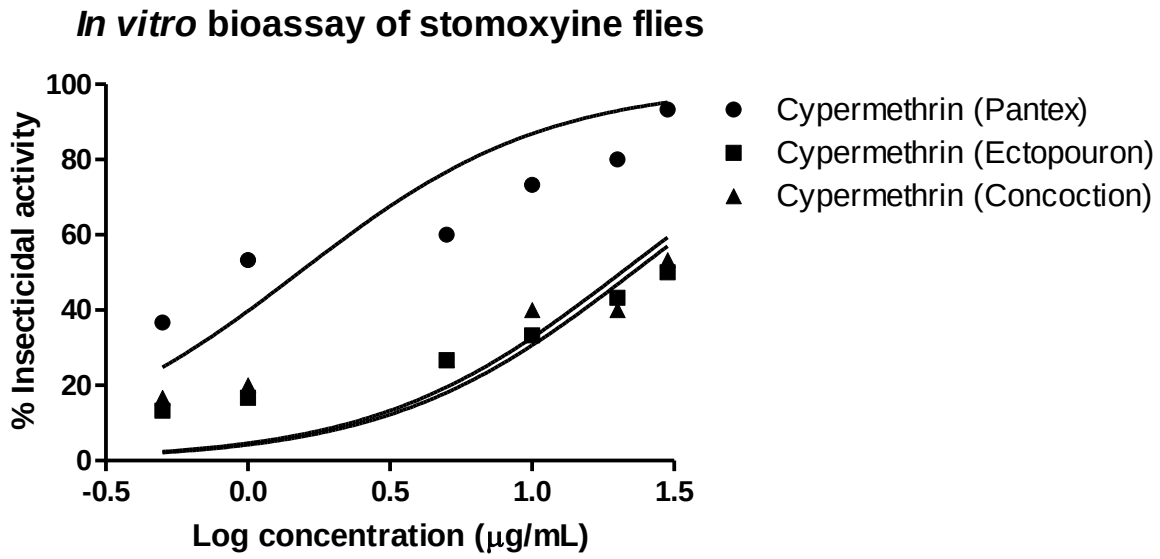


Figure 4.26: Insecticidal *in vitro* bioassay of cypermethrin formulations on stomoxyines.

4.6. STUDY SIX

4.6.1. Cattle keepers in Southwestern Nigeria

Cattle owners population distribution showed that Hausa- Fulani make up 75.6% (CI: 69.4 – 80.9%, SE: 0.03), Yoruba group were 20.6% (CI: 15.7 – 26.6%) and 3.8% (CI: 2.0 – 7.4%) were from other tribes. The study reports diverse practices among these keepers, however, (Figure 4.27; Table 4.10), market evaluation showed that 95.7% (CI: 95.2 – 96.2%) of examined cattle were bought from outside southwest and mostly by road transportation, while transhumance cattle were reported to be 1% (CI: 0.79 – 1.30%), settled cattle in the region were 3.3% (CI: 2.9 – 3.8%, SE: 0.00). Most of the cattle survey revealed that not less than 80% were purchased from northern part of the country (Figure 4.28). This is critical in understanding the transmission patterns of trypanosomosis in a tropical rainforest zone. Dynamics of practices and its significant effect on livestock production and AAT were of utmost interest in this study. Greater practice of zero grazing (66%, CI: 59.4 – 72.1%) was observed compared to other practices in this report.

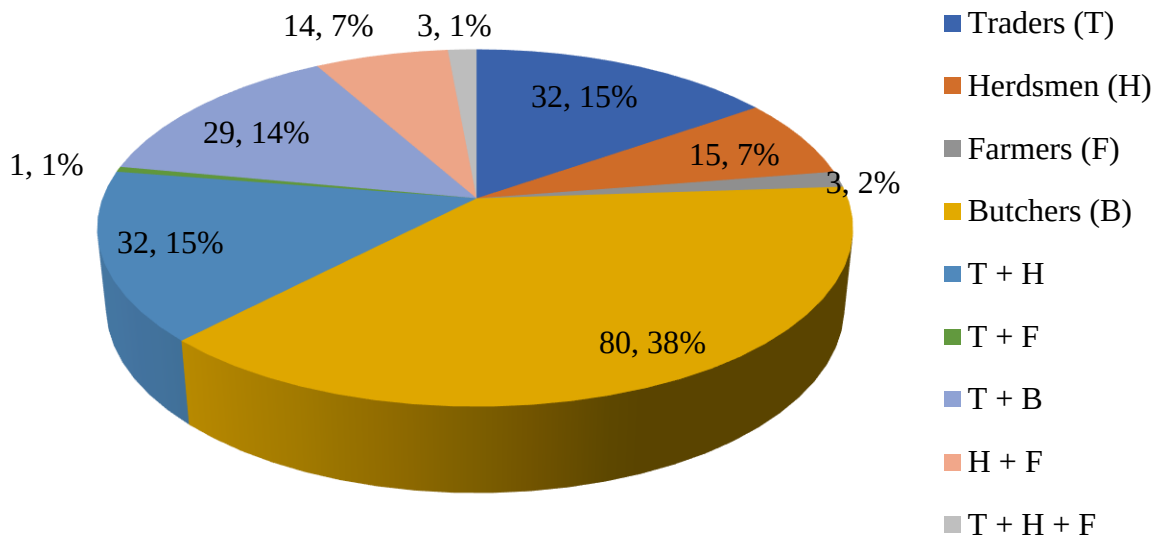


Figure 4.27: Livestock owners' nomenclature in southwest Nigeria

Table 4.10: Farmers' practices in southwest Nigeria

Practices	Akinyele	Igangan	Igboora	Iddo	Eruwa	Ekiti	Lagos	Akure	Ogun	Osogbo	Total
Zero grazing (ZG)	16	-	-	-	-	3	65	18	12	24	138 (66.02%)
Nomadism (N)	-	-	-	-	-	-	-	-	-	-	-
Mixed farming (MF)	2	1	-	-	-	-	-	3	3	5	14 (6.70%)
Pastoralism (P)	-	2	5	-	4	-	-	-	-	2	13 (6.22%)
ZG + N	-	-	-	2	-	-	1	-	-	1	4 (1.91%)
ZG + MF	-	-	-	-	-	-	-	-	-	-	-
ZG + P	4	-	-	-	-	-	-	-	-	1	5 (2.39%)
N + MF	-	-	-	10	-	-	-	-	3	-	13 (6.22%)
N + P	-	2	-	-	-	-	-	-	-	1	3 (1.44%)
MF + P	-	1	5	-	7	-	-	-	-	-	13 (6.22%)
ZG+ N + MF	-	-	-	-	-	-	-	-	-	-	-
ZG + N + P	4	-	-	-	-	-	-	-	-	-	4 (1.91%)
ZG + MF + P	2	-	-	-	-	-	-	-	-	-	2 (0.97%)
N + MF + P	-	-	-	-	-	-	-	-	-	-	-
Total Cattle owners	28	6	10	12	11	3	66	21	18	34	209

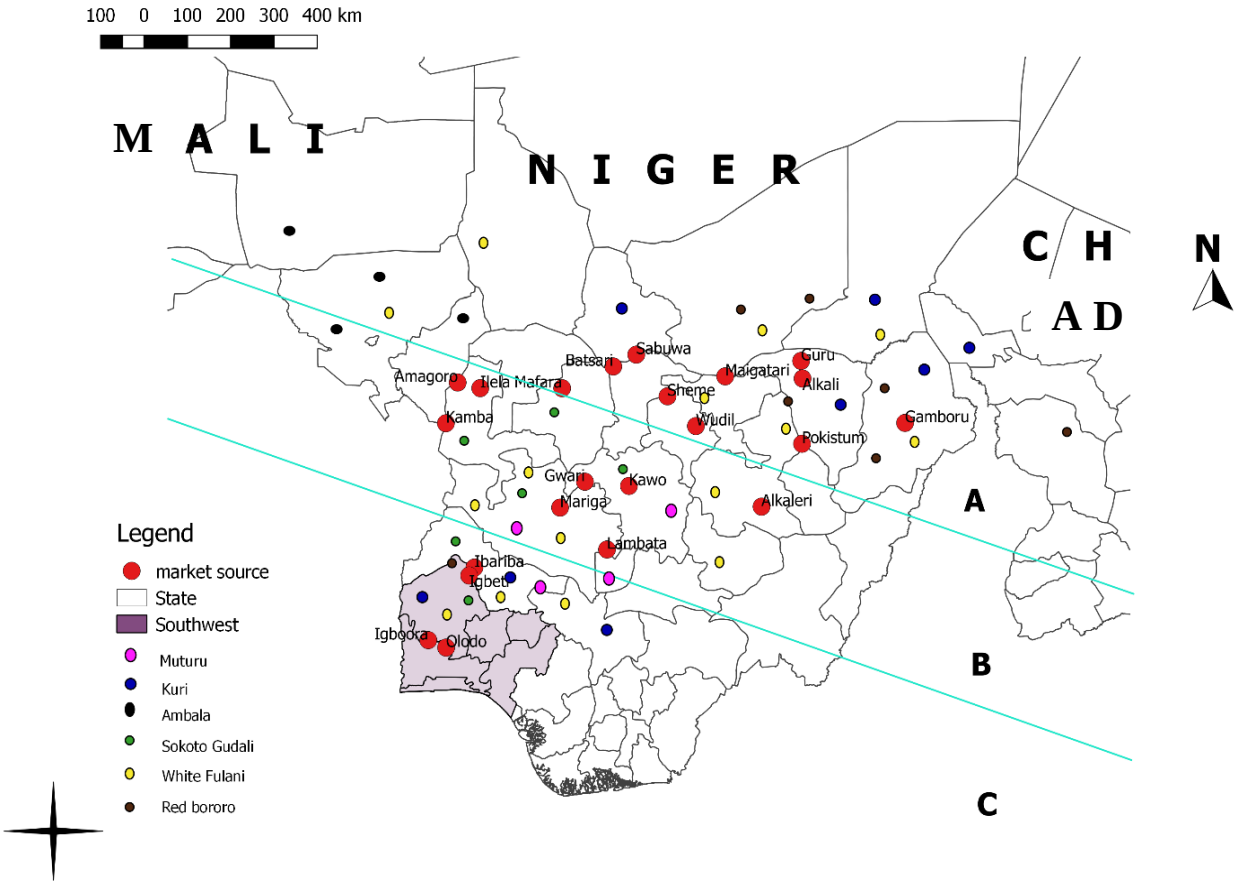


Figure 4.28: Market situation in relation to AAT

A general decrease in nomadism was noticed as no single livestock keeper embacked on the practice alone. Although, combined practice of nomadism and zero-grazing was observed in 1.9% (95%CI: 0.8 – 4.8%). Zero grazing practice was common for trade cattle in this study area. In order to maximise profit, combine practices was a common act among livestock owners, of which 21.1% (95%CI: 16.7 – 27.1%, SE: 0.03) were involved. A total of 89.5% (95%CI: 84.6 – 92.9%) livestock keepers have concise understanding on how climatic conditions adversely affect viable production of cattle. Not less than 76.1% (95%CI: 96.9 – 81.4%) reported that both seasons (wet and dry) have effects on livestock productivity, while 10.5% (CI: 7.52 – 14.54%) maintained that climate have no detrimental effects on their cattle. The education strata of cattle keepers revealed that 85.6% (95%CI: 80.3 – 89.8%) were not opportuned to attend primary education and believed that their knowledge of cattle keeping was inherited. Only a negligible proportion of the cattle keepers 2.4% (95%CI: 1.0 – 5.5%) could clearly explain the epidemiology of AAT, providing the local names of the transmitting vector flies.

4.6.2. Perceived problems associated with sustainable animal production

AAT stands after FMD with 70.8% (95%CI: 64.3 – 76.6%) of cattle keepers confirming it, while 16.2% (95%CI: 11.2 – 23.0%) of them reported death of their animal from the disease (Table 4.11).

4.6.3. Transportation methods

The main mode of transporting cattle to southwestern Nigeria is by road. Although most parts are connected by railway, none of the cattle dealers subscribe to its use. Long articulated lorries were major carriers of cattle in about 80 – 100 capacity from the far-north, while small trucks could be used in cases of 20-40 animals. Transportation could cost cattle owners up to US$ 17.7 – US$ 35.5 on an animal depending on size. Death of livestock were also reported from transport stress, however, only 6.2% (95%CI: 3.7 – 10.4%, SE: 0.02) admit that AAT could cause cattle mortality from transport stress.

Purchasing preference was traced to breeds, susceptibility to AAT and general body conditions. Commonly traded breeds in southwest Nigeria include Sokoto Gudali, White Fulani, Red bororo, while less traded were Ambala, Muturu and Kuri. Off-loading fee in kraals could cost between US$ 0.35 – US$ 0.70 per animal, while travel cost (police fees, toll fees etc.) varied.

Table 4.11: Cattle owner's account of disease prevalence

Diseases	Number of cattle keepers reporting diseases. n=209	95%CI	Numer/Percentage of mortality cases
Foot and mouth disease (FMD)	169 (81%)	75.0 – 85.6	9 (5.3%)
Trypanosomosis	148 (71%)	64.3 – 76.6	24 (16.2%)
Tuberculosis	115 (55%)	48.3 – 61.6	-
Helminthosis	58 (28%)	22.1 – 34.2	
Contagious bovine pleuropneumonia (CBPP)	47 (23%)	17.4 – 28.6	3 (6.4%)
Mange	22 (11%)	7.1 – 15.4	-
Acariosis	17 (8%)	5.1 – 12.6	
Fasciolosis	16 (8%)	4.8 – 12.1	-
Dermatophilosis	11 (5%)	3.0 – 9.2	-
Mastitis	6 (3%)	1.3 – 6.1	-
Actinobacillosis	3 (1%)	0.5 – 4.1	-
Malnutrition	2 (1%)	0.3 – 3.4	-
Myiasis	1 (1%)	0.1 – 2.7	-

Footnote: There is significant increase ($\alpha < 0.05$) in cattle mortality compared to other diseases mentioned.

Retailers and consumers are always present in most cattle markets to buy animals of their choice, this is based on the amount of money they have on them. They can also be concerned with animals that have obvious signs of disease. Weak and recumbent cattle on arrival were sold cheap and some were often administered with drugs such as combination of vitamins, antibiotics and trypanocides in attempt to rescue them from transportation stress.

4.6.4. Transhumance practices and prevalence of AAT

Transhumance was only observed in the wet season with 13.4% (95%CI: 9.4 – 18.7%) of cattle keepers engaging from this study. From this number, 82.1% (95%CI: 64.4 – 92.2%) were both traders and herdsmen while 17.9% (95%CI: 7.8 – 35.6%) were herdsmen only. A ratio of 1:100 associated to AAT mortality in cattle herd have been identified in transhumance according to 85.6% (95%CI: 80.3 – 89.8%), although some 14.4% (95%CI: 10.2 – 19.8%) reported there were no mortality cases. There is correlation between migration and seasons in response to AAT in the study areas (Figure 4.29). There was consensus in the response to the months of highest (April) and lowest (December) Not less than 75% of interviewered livestock keepers opined that prevalence is always high during early and peak wet seasons annually (Figure 4.29). Migration from southwest Nigeria to savannah tropical areas begins in February and spans to mid-March, while these same set of nomads arrive in October when grasses and water becomes scarse in the savannah. Livestock keepers mentioned resistance to trypanocide as a major set back. Of those interviewed, 63.2% (95%CI: 56.4 – 69.4) make quick sale on unresponsive cattle to trypanocide to avoid major economic losses.

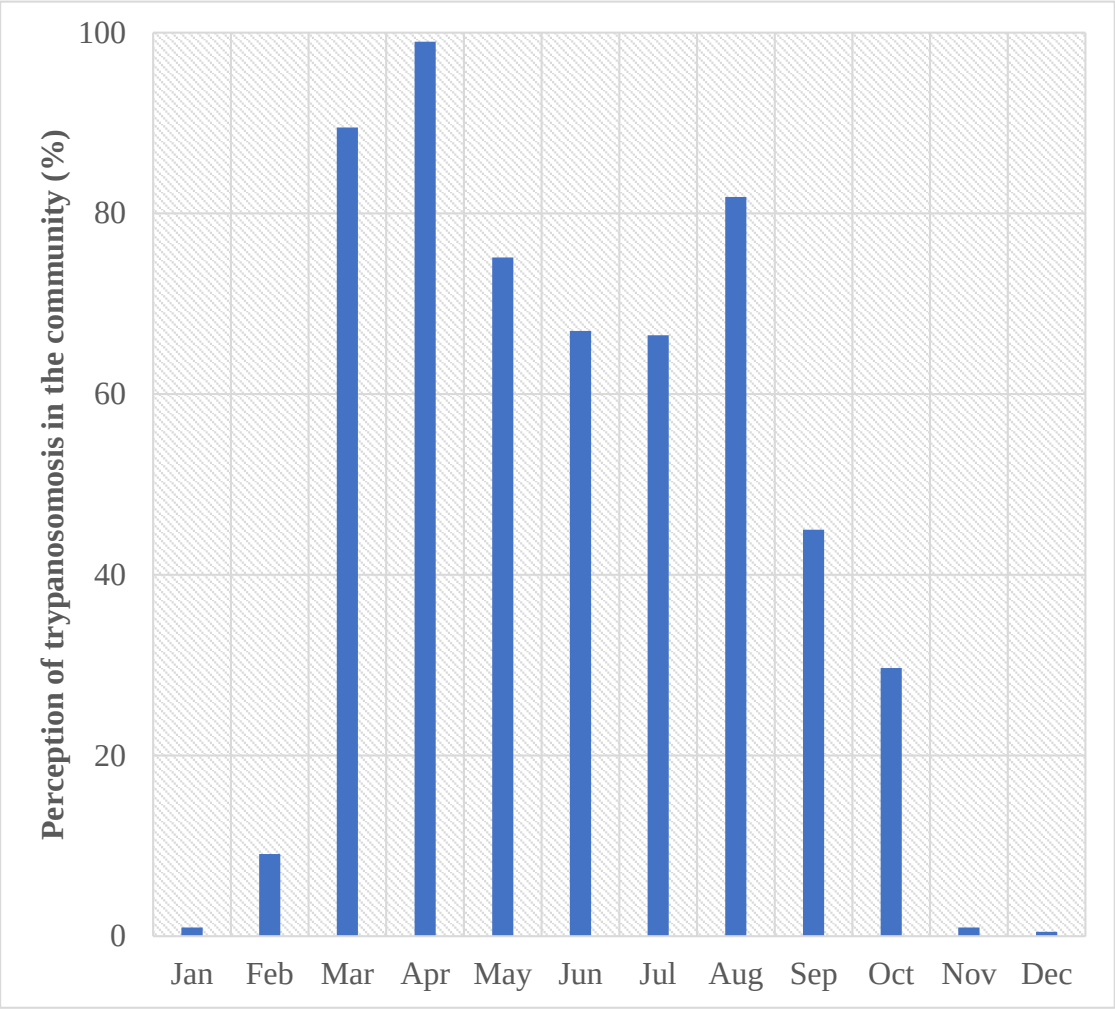


Figure 4.29: Monthly perception of bovine trypanosomosis in southwest Nigeria

4.6.5. Assessment of economic condition to AAT

4.6.5.1. Vector threat

Fly vectors (*Glossina*, Stomoxyines *& Tabanids*) of BT in southwestern Nigeria were recognized by cattle keepers as threats to their animals. Mechanical transmitting vectors (stomoxyines and *Tabanids*) were observed all year round and ubiquitous, 73.2% (95%CI: 66.8 – 78.8%) perceived them as threat due to the constant irritation and disturbance on both the keepers and their animals, but only 1.4% (95%CI: 0.5 – 4.1%) reported biting flies as vectors. Although, *Glossina* populations have decreased in recent reports and they comply with seasonal distribution. A total of 91% (95%CI: 86.2 – 94.1%) considered tsetse flies as a major problem but only 1.9% (95%CI: 0.8 – 4.8%) reported them as transmitting vector. In total 2.4% (95%CI: 1.0 – 5.4%) of respondents recognised both tsetse flies and biting flies as trypanosome transmitting vectors. A sizeable 78.5% (95%CI: 72.4 – 83.5%) of the livestock keepers reported sand-eating and early rain lush as the cause of AAT, while 19.1% (95%CI: 14.4 – 25.0%) are indifferent on the causal agent.

4.6.5.2. Trypanocides usage and cost implication

The study reported that 97.0% (95%CI: 92.5 -98.8%) of the cattle owners prefer to administer at least a synthetic drug during AAT crisis. Of this percentage, 3.1% (95%CI: 1.2 – 7.7%) administer antibiotics only (Table 4.12). Therefore, a total of 93.9% (95%CI: 88.6 – 96.9%) administer trypanocides only or with other drug combinations. According to the National Agricultural Sample Survey in 2011, Nigeria has 19.5 million head of cattle, with an estimated 2.34 million head of cattle in southwest Nigeria from 3 % (reared & transhumance) (Swallow and Jabbar, 1994) and 9% from market evaluation. The report from this study was used to simulate the overall cost of trypanocides to the livestock industry in southwest Nigeria. The estimated annual trypanocides used by cattle keepers in southwest Nigeria stands at approximately US$ 8.4 million, provided the use is same in the fly-belt area (Table 4.12). Not less than 3.0% (95%CI: 1.2 – 7.5%, SE: 0.015) livestock keepers preferred locally made traditional drugs and they all refused to divulge the drug content.

Table 4.12: Estimated cost of trypanocides treatment for 2,185 cattle examined.

Treatment cost (USD)	Percentage in sample population	Annual trypanocides losses (USD)	Estimated annual trypanocides losses in southwest Nigeria (thou$)
1.77	7.8%	302	303
3.55	70.0%	5,430	5,454
5.32	21.0%	2,441	2,452
7.09	1.2%	186	186
Total	100.0%	8,359	8,396

US $1= ₦282 (August 2016). *total loss excluded those without trypanocides, thou$- thousand dollars. Total of US$ 8.4 million for the whole of the country was estimated.

4.6.5.3. Insecticides use by cattle keepers

Approximately 60.5% (95%CI: 51.5 – 68.5%) cattle keepers prefer the use of synthetic insecticides. However, it is difficult to quantify the use of these compounds because the pattern of use is not specific. The use among cattle keepers varies significantly, while application mostly correlates with presence of fly vectors. The most commonly available preparation is cypermethrin (3%), which cost approximately $US 15 / bottle / litre. This should be sufficient for 50 animals weighing 200 kg (10 kg/ml), however, most of the herders use same quantity for over 500 animals. The analysed data on insecticides exclude butchers because they hardly use it on their animals because of the short period of keeping the animals before slaughtering. Those involved in combined practices administer it on their cattle. A total of 60.5% (95%CI: 51.5 – 68.5%) administer insecticides on their cattle, 19.4% (95%CI: 13.5 – 27.1%) preferred smoke generated *Senna occidentalis* known as *"Bangaru angasa"* in local language, 5.4% (95%CI: 2.7 – 10.8%) administer concoction that include herbicides and 14.7% (95%CI: 9.6 – 21.9%) reported that do not control flies. Of those that use insecticides, cypermethrin, permethrin, ivermectin and chrysanthemum usage among cattle keepers was 84.6% (95%CI: 75.1 – 91.0%), 10.2% (95%CI: 5.3 – 19.0%), 2.6% (95%CI: 0.7 – 8.9%) and 2.6% (95%CI: 0.7 – 8.9%), respectively.

4.6.5.4. Economic and production losses of cattle to AAT

AAT has significantly affected the production of cattle in Nigeria (Table 4.13). However, indirect losses are often unaccounted due to the problem of agicultural management practices in Nigeria. The crippled agro-allied industrial system has limited the profit from the industry. Direct losses from AAT were observed. For instance, losses of ≥ US$ 426 was generated from the mortality of a single animal from AAT. Livestock keepers were passionate to account on losses during the early and late stages of AAT on their animals in which the late stage losses could be up to ≥ 80% of actual cost.

4.6.5.5. Management and drug administration by cattle keepers against AAT

A total of 82.3% (95%CI: 76.6 – 86.9%) prefer not to use veterinarians, by administering drugs to their animals while a few that do request veterinarians (17.7%, 95%CI: 13.1 – 23.5%, SE: 0.0264) only do so in rare occasion, in spite of increasing prevalence and cattle deaths. Cattle keepers have varying treatment approach which could include combination of drugs (Appendix IV).

Table 4.13: Bovine economic scale assessment of AAT.

Cost of animal (USD)	Expected selling cost (USD)	Cost at early-stage infection (USD)	Cost at late-stage infection (USD)	Proportion in the study (%)
≤ 177	≥ 213	≤ 71 – 142	≤ 71	3.0
178 – 355	248 – 426	142 – 287	≤ 106 – 177	51.0
356 – 709	427 – 887	287 – 638	≤ 177 – 248	37.0
> 710	≥ 1064	≤ 709	≤ 284	9.0

*(1 USD = ₦282, August 2016)

4.6.6. Government intervention measures

Only 1.9% (95%CI: 0.75 – 4.82%) of the cattle keepers were aware of government intervention schemes like PATTEC, which they acclaimed free trypanocides were injected to their animals several years back in northern Nigeria. Livestock keeper are prepared to be part of any established control scheme and organized workshops; although, 0.96% (95%CI: 0.26 – 3.42%) are not ready to part of any governmental or non-governmental scheme.

4.7. STUDY SEVEN

4.7.1. AAT prevalence between 1960-2017

4.7.1.1. Meta-data analysis of AAT studies

Collated inclusive AAT studies (*n* = 74) was 16% prevalence (95%CI: 12.3-20.3) (Table 4.14). Funnel plot to show that there is no bias in the analysis is reported (Figure 4.30). Point estimates of each study have been described (Appendix IX). Results of sub-group analyses are presented in Table 4.14. Trypanosomes prevalence declined in the first two decades of assessment and increases afterwards with no significant difference in the five decades (Figure 4.31). Higher AAT infection was observed in southern Nigeria (*n* = 24, $\alpha > 0.05$), even though more studies have been conducted in the north (*n* = 54).

Southwest Nigeria AAT infections were reported in 12 eligible studies. Cattle was the most studied animal (*n* = 55) showing 17.0% (95%CI: 12.3-22.2) prevalence, while diagnostic-based techniques showed highest prevalence for PCR (25.5%), followed by ELISA (21.0%) and microscopy (13.0%). Microscopy technique was higher for *T. vivax*, while *T. congolense* prevalence was higher for serology and PCR, respectively (Table 4.14). Trend of AAT infection over decades is reported (Fig. 4.31).

4.7.2. Tsetse-trypanosome infections-metaanalysis.

Glossina infected with trypanosomes trapped in the field reported prevalence of 17.3% (95%CI: 4.5-36.0) for the combined studies (*n* = 14) (Table 4.15). Prevalence point for each study has been presented (Appendix IX). Study inconsistency shows significant difference ($\alpha < 0.0001$). Southern study reports show significant increase in prevalence compared to the north (Figure 4.32). Trypanosome prevalence was *G. morsitans* > *G. tachinoides* > *G. palpalis* (Table 4.15). Identification by dissecting flies under stereomicroscope was mostly adopted (Table 4.15). Order of prevalence in the *Glossina* species were *T. vivax, T. congolense and T. brucei*. The latter shows direct consistency in the studies examined for *Glossina morsitans* ($\alpha = 0.1586$). In southwest Nigeria, out of 4,808 *Glossina* species analysed, 42.6% were positive for trypanosomes while bovine blood prevalence of 21.3% was obtained. Geographic map shows the intensity of studies carried out in Nigeria (Figure 4.33).

Table 4.14: AAT and tsetse prevalence between 1960 and 2017

Attribute	Total studies included/total samples examined	Prevalence [95%CI]	Measure of heterogeneity [Cochran's Q]	% Variation; I^2 [95%CI]	P value
National	74 (53,924)	16.1% (12.3-20.3)	11830.19	99.4% (99.3-99.4)	$\alpha < 0.0001$
Northern region	54 (32,134)	15.9% (11.1-21.4)	8311.08	99.4% (99.3-99.4)	$\alpha < 0.0001$
Southern region	24 (22055)	19.9% (14.0-26.5)	2890.36	99.2% (99.1-99.3)	$\alpha < 0.0001$
Southwest zone	12 (9912)	21.3% (12.4-31.7)	1391.57	99.2% (99.1-99.4)	$\alpha < 0.0001$
Cattle	55 (40,863)	17.0% (12.3-22.2)	9383.29	99.4% (99.4-99.5)	$\alpha < 0.0001$
Microscopy	45 (31,135)	13.0% (10.0-16.2)	2749.86	98.4% (98.2-98.6)	$\alpha < 0.0001$
T. vivax	32 (16,942)	8.2% (5.7-11.1)	1183.98	97.4% (96.9-97.8)	$\alpha < 0.0001$
T. congolense	32 (16,942)	2.8% (1.7-4.3)	725.89	95.7% (94.8-96.5)	$\alpha < 0.0001$
T. brucei	32 (16,942)	1.7% (0.7-3.1)	1076.37	97.1% (96.6-97.6)	$\alpha < 0.0001$
Serology	2 (1175)	21.0 (17.9-24.4)	1.57	36.4% (0.0-0.0)	$\alpha < 0.0001$
T. vivax	2 (1175)	7.1% (5.7-8.7)	0.2	0.0% (0.0-0.0)	$\alpha = 0.67$
T. congolense	2 (1175)	9.0% (7.4-10.7)	0.34	0.0% (0.0-0.0)	$\alpha = 0.60$
T. brucei	2 (1175)	2.0% (0.7-4.1)	3.23	69% (0.0-93)	$\alpha = 0.07$
PCR	7 (8,672)	25.5% (10.5-44.4)	793.26	99.2% (99.0-99.4)	$\alpha < 0.0001$
T. vivax	5 (8022)	9.5% (2.1-21.2)	231.15	98.3% (97.4-98.9)	$\alpha < 0.0001$
T. congolense	3 (7754)	25.1% (16.7-34.4)	37.26	94.6% (87.9-97.7)	$\alpha < 0.0001$
T. brucei	6 (8522)	4.5% (2.3-7.4)	56.5	91.2% (83.5-95.3)	$\alpha < 0.0001$

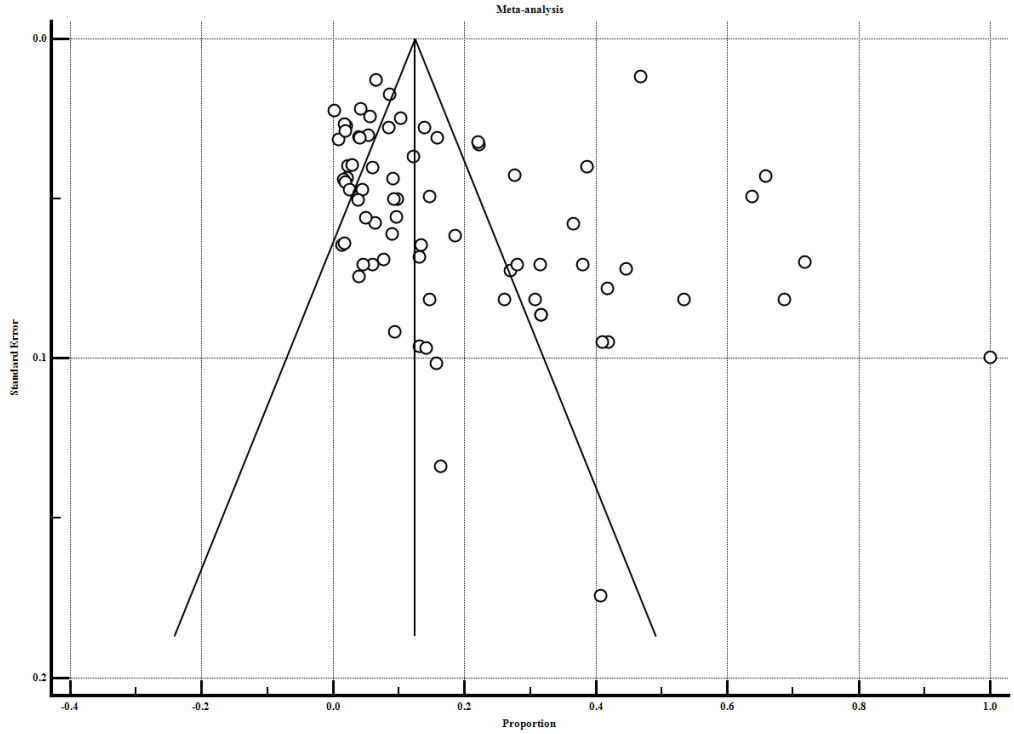


Figure 4.30: Funnel plots of the AAT studies.

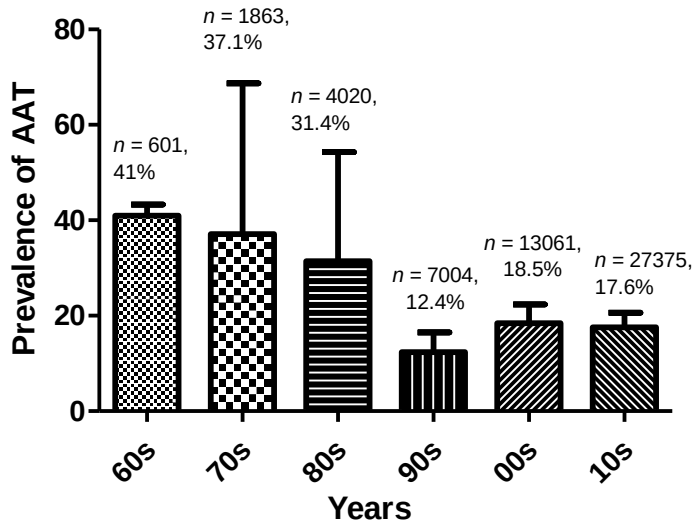


Figure 4.31: AAT trend over decades.

One- way ANOVA reveals that there is no association ($\alpha = 0.1676$, $F = 1.616$, $r^2 = 10.6\%$) between reported AAT prevalence of six decades.

Table 4.15: Prevalence of tsetse-trypanosomes between 1960 and 2017

Attributes	No of studies examined/total samples examined	Prevalence [95%CI]	Measure of heterogeneity [Cochran's Q]	% Variation/ I^2 [95%CI]	α value
National	14 (12,552)	17.3% (4.5-36.0)	6287.8	99.8% (99.8-99.9)	$\alpha < 0.00$
Northern region	9 (3107)	10.5% (5.1-17.5)	244.7	96.7% (95.3-97.7)	$\alpha < 0.00$
Southern region	5 (9445)	30.4% (0.6-78.6)	487.9	99.9% (99.9-100.0)	$\alpha < 0.00$
Southwest zone	4 (4808)	42.6% (12.6-76.0)	669.9	99.6% (99.4-99.7)	$\alpha < 0.00$
G. morsitans	5 (4883)	49.7% (30.7-68.8)	310.6	98.7% (98.1-99.1)	$\alpha < 0.00$
T. vivax	5 (4883)	36.8% (21.9-53.2)	221.4	98.2% (97.2-98.8)	$\alpha < 0.00$
T. congolense	5 (4883)	5.9% (2.6-10.3)	52.0	92.3% (85.0-96.1)	$\alpha < 0.00$
T. brucei	5 (4883)	0.2% (0.0-5.2)	6.6	39.4% (0.0-77.6)	$\alpha = 0.16$
G. tachinoides	11(5,793)	11.5% (6.1-18.5)	226.4	95.1% (93.1-96.6)	$\alpha < 0.00$
T. vivax	10 (5646)	4.9% (1.0-11.4)	263.3	96.6% (95.1-97.6)	$\alpha < 0.00$
T. congolense	10 (5646)	2.0% (0.5-4.5)	85.6	89.5% (82.8-93.6)	$\alpha < 0.00$
T. brucei	10 (5646)	1.2% (0.2-3.2)	77.3	88.4% (80.7-93.0)	$\alpha < 0.00$
G. palpalis	11 (1874)	4.5% (1.6-8.8)	118.4	91.6% (86.9-94.6)	$\alpha < 0.00$
T. vivax	6 (1610)	1.6% (0.3-3.9)	38.8	87.1% (74.3-93.6)	$\alpha < 0.00$
T. congolense	5 (1546)	1.5% (0.0-5.2)	69.9	94.3% (89.5-96.9)	$\alpha < 0.00$
T. brucei	5 (1546)	1.2% (0.0-5.2)	87.4	95.4% (91.9-97.4)	$\alpha < 0.00$

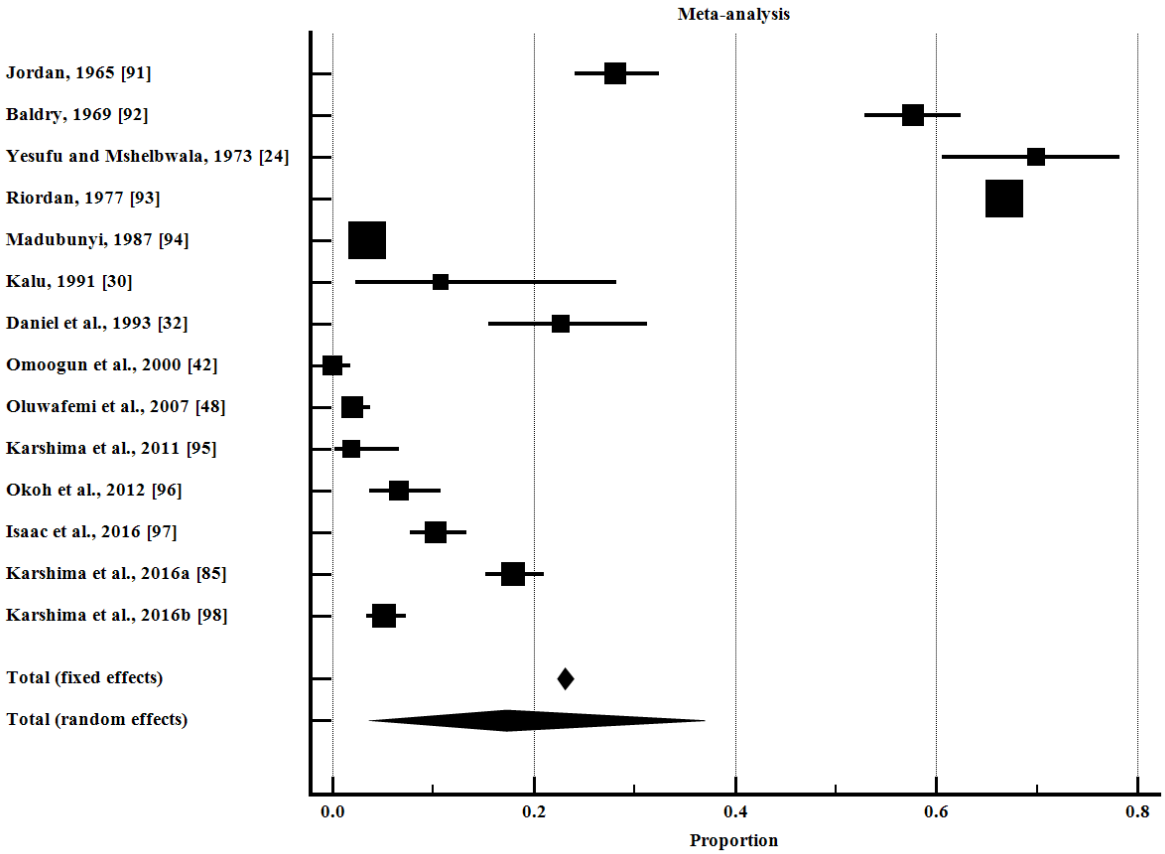


Figure 4.32: Studies on trypanosome-infected *Glossina* species

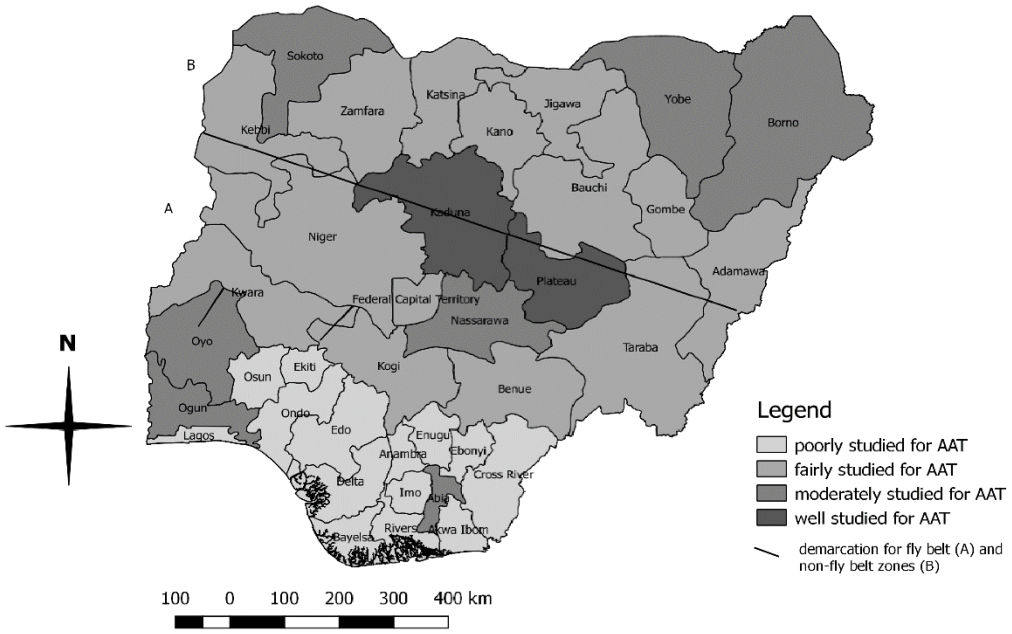


Figure 4.33: Nigeria's AAT distribution map on studies conducted between 1960 – 2017.

CHAPTER FIVE

DISCUSSION

5.1.1. Glossina species

In this study, *Glossina* species were observed to be *Glossina palpalis* and *Glossina tachinoides* morphologically. Similar species have been identified previously in southern-Oyo and northern Nigeria-Niger and Bauchi States (Yesufu and Mshelbwala, 1973; Isaac *et al.,* 2016). Despite the occurrence of *Glossina palpalis* in southwestern Nigeria, the subspecies has not been classified as either *palpalis* or *gambiensis* before now through sequencing. High similarity matches (98% and 99%) were also obtained for two other *G. p. gambiensis* (EU591953 and EU591955) isolates, a *G. p. palpalis* isolate (EU591949) and a *G. tachinoides* isolate (AF021353).

Glossina subspecies was difficult to confirm morphologically using taxonomic identification keys. Of recent field samples, *G. p. gambiensis* had been mostly reported in Burkina Faso (Hoppenheit *et al.,* 2014), Mali (Vreysen *et al.,* 2013) and Guinea (Solano *et al.,* 2009). *G. p. gambiensis* is economically important because of its potential in biological transmission of *Trypanosoma brucei gambiense* (Vreysen *et al.,* 2013), and the species is often found in sleeping sickness foci (Solano *et al.,* 2009). Studies have also shown that this tsetse fly often takes most of its bloodmeals from humans (Hpooenheit *et al.,* 2013).

Palpalis group presence in two foci covering approximately 50 km^2 area in this study could be due to the presence of rivers with dense vegetation. *G. p. gambiensis* spread has been observed in the dry season along water courses. Flies can also spread perpendicular to the river systems in wet season, although there are limited studies to confirm (Vreysen *et al.,* 2013). Before now, Fusca group of *Glossina* was thought to dominate these parts of Nigeria (Davis, 1977), however, cattle and human movement together with migration (Abubakar *et al.,* 2016), could have changed the vector distribution pattern over the years. Rainfall from 150 – 220 mm/month favours its relative abundance while dry season generally recorded 0.0 – 0.3 apparent densities. Hot months with > 30 °C do not favour tsetse abundance in this study.

5.1.2. Tabanus species

Tabanids belonging to three different genera representing 13 species were observed in this study namely; *Tabanus*, *Chrysops* and *Haematopota*. The emphasis was on *Tabanus* spp. because of its established competence to mechanically transmit trypanosome species (Taioe *et al.,* 2017). Besides, 75.4% of total tabanids trapped were observed to be *Tabanus* species. The last study of *Tabanus* species in southwest Nigeria was four decades ago (Dipeolu, 1977). Then, species like *T. par*, *T. rubidus*, *Tabanus brucei* and *T. gratus* were not reported. However, species like *T. socialis*, *T. neocopinus* and *T. pluto* reported then were not found in this study. *T. taeniola*, *T. biguttatus*, *T. thoracinus* and *T. subangustus* were reported in both studies (Dipeolu, 1977).

Low numbers of *Tabanids* trapped could be because the traps were not baited. *T. taeniola* Palisot de Beauvois is the most widely distributed and abundant *Tabanus* species in southwest Nigeria (Dipeolu 1977). This was true with its abundance in our study. In its first collection during the national collection by the British medical officers, it was reported to be the most abundant throughout Africa and its spread ranged from Egypt, Sudan, Senegal to Delegoa Bay and the Transvaal (Austen, 1909). This was reported during subsequent national survey of Dipteran flies in Nigeria (Crosskey and Crosskey, 1954; Dipeolu, 1977). Though, similar species to *T. taeniola* were said to exist; the distinct abdominal stripe modification differentiates them at the time of collection and most opposing species were not found in Nigeria such as *T. socious* Walker, *T. macrops* Walker, *T. variatus* Walker and *T. dorsivitta* Walker. *T. subangustus* Ricardo is believed to originate from Nigeria from Natural history in the British Museum. However, some specimens were caught in Senegal and Gambia (Austen, 1909). It was most widely distributed in this study. Changes in agricultural practices could have direct effect on the spread. Early samples were from Zungeru, Shonga, Amara, Zaria, Benue, Abutshi and Odut (Austen, 1909).

A later report suggests its abundance in both northcentral and southwest Nigeria (Crosskey and Crosskey, 1954). Even though it was only found in Ilorin four decades ago (Dipeolu, 1977), it was not an established species in southwest Nigeria as at that time. *Tabanus brucei* Ricardo is a distinct species only reported in Eastern Africa during its first encounter especially in regions of Uganda and Katanga district of the Congo Free State (Austen, 1909). Crosskey and Crosskey, (1954) did not report this species in their collection, neither was it mentioned in later reports (Dipeolu, 1977; Ahmed *et al.,* 2005). It was found in both seasons

in this study in Adebayo area (northwest border between Oyo and Ogun state) and University of Ibadan. This is the first report on this species in Nigeria and the obvious deep green eyes without band and wing markings were distinctive. It can easily be confused with *T. fasciatus* Fabricius, *T. africanus* Gray and *T. latipes* Macquart, all of which have been reported (Ahmed *et al.,* 2005; Dipeolu, 1977). *Tabanus par* Walker is regarded as one of the most widely distributed *Tabanus* in sub-Saharan Africa (Austen, 1909).

It was first found in Senegal, Egypt, Mozambique, Congo, Ivory Coast, Zimbabwe, Gambia and northern Nigeria (Austen, 1909). Its abundance was noticed at the beginning of rains during its collection in this study. It shares similar features with *T. thoracinus* Palisot de Beauvois, which has its origin from Benin Republic and well-distributed in other west African countries. This similarity might have affected its reporting in the past, however the transparent wing pattern, shorter body length, tarsi differences and body width differentiates it. Ahmed *et al.,* (2005) also reported *Tabanus par* Walker in his collections, however the species was missing in Dipeolu's collection. While Dipeolu (1977) reported *T. thoracinus*, it was absent in Ahmed's collection (Ahmed *et al.,* 2005). *T. par* and *T. thoracinus* from this study were found in Adebayo and UI, Oyo state and catches were made in wet and dry seasons. The short sequence obtained from *T. par* suggests that DNA barcoding of highly conserved region could be used in future studies. *T. rubidus* was found for the first time in Nigeria in this study, having 79% homology with species from Thailand, even though phylogenetic tree revealed them in separate clusters. Due to the differences in nucleotides, sequence was submitted to the GenBank. *Tabanus* spp found in Nigeria were monophyly to Afrotropical region and may be genetically distinct from those in other Neotropical or Nearctic regions (Morita *et al.,* 2016), that could be the more reason for the absence of its nucleotide in the observed species. Hence sequences were deposited in GenBank for processing after proper morphometric analysis.

5.1.3. Stomoxyine flies in southwestern Nigeria

Stomoxyine flies account for 97.5% of total haematophagous flies captured in this study. *Stomoxys calcitrans* is most widely distributed stomoxyine in Africa and it has been reported to spread from Algeria and Egypt to Cape Colony, and from Gambia to the East Africa Protectorate (Austen, 1909). It has been reported in Europe, Middle East, South America, North America and New Zealand and it is regarded as synanthropic fly (Zumpt, 1973).

Documented report in Nigeria suggests its abundance in early and later surveys (Crosskey and Crosskey, 1954; Dipeolu, 1974, 1977; Ahmed *et al.*, 2005).

In this study however, *Stomoxys niger niger* was most abundant among the trapped vectors and distributed across the study sites. *Stomoxys calcitrans* was also trapped throughout the study sites but with lower apparent density and low percentage catches in the dry season. The general reduction of stomoxyine flies during heavy rainy months could be due to washing away of the larvae. The seasonal pattern of relative abundance characterising all the studied vector species is a well-known phenomenon. Studies have shown different vector species encountered seasonal fluctuations with increased catches at the beginning of rains and decline along the dry season (Bitome-Essono *et al.*, 2015). However, this is modulated by regional climatic parameters. Albert *et al.*, (2015) explains that environmental surrogates such as rainfall, temperature, humidity, wind-speed and vegetation-type as major drivers of vector distribution and abundance.

5.2.1. Detection of trypanosomes in the trapped fly vectors

It was observed from this study that trypanosomes were positive in the transmitting vectors trapped in various institutional fams and cattle settlements. None of the trapped tsetse flies were positive for *T. b. gambiense* using the TgsGP primers. Therefore, the *Trypanozoon* group were attributed as *T. brucei brucei* having screened them with RoTat 1.2 primers for *T. evansi*, of which none was positive. Even though 88.4% of the engorged tsetse flies had human (*Homo sapiens*) DNA in their bloodmeals, yet; they were observed to be negative for *T. b. gambiense*. This probably mean that apart from feeding on humans, tsetse flies also feed on hosts susceptible to *T. b. brucei* (wildlife and livestock).

The ITS 18S PCR assay revealed different trypanosomes such as *T. vivax, T. congolense, T. brucei brucei* and *T. simae*. This indicate the active transmission patterns of trypanosomes by these vectors. The complexity of trypanosome transmission observed from this study showed the presence of only biting flies in farms situated around densely populated urban areas while rural areas with scanty human population have pockets of tsetse foci. The presence of *T. vivax* in TA showed that some tsetse flies recently fed before being trapped (Isaac *et al.*, 2016), although trypanosomes have been identified in the anterior gut and cibarium (Moloo and Gray, 1989). However, more *T. vivax* were observed in HP as expected. The presence of *T. congolense* observed in the study was low compared to a previous studiy (Isaac *et al.*,

2016). The low prevalence of *T. congolense* in tsetse flies observed in this study as compared to the often-high reports in cattle (Takeet *et al.,* 2013), could be due to the inability of the organism to become established in the tsetse (Peacock *et al.,* 2012). Mixed infections observed could be because trypanosome species seldom occupy the same tissue and there is less competition (Mwandiringana *et al.,* 2012), similarly, same situation was identified in head plus proboscis and thorax plus abdomen in this study.

The connection between the two-trypanosome species in separate tsetse tissues is not yet known. Comparatively, no significant difference was observed between *Trypanosoma* species in *Glossina palpalis* and *Glossina tachinoides* from this study, this could be because they both belong to the riverine group. This is the first study using molecular tools to identify trypanosomes in *Tabanus* and stomoxyine flies in Nigeria. The reported prevalence of trypanosomes in biting flies observed from this study explains that they are efficient mechanical vectors (Anene *et al.,* 1991), especially especially when tsetse is absent as observed in the dry season. Infection was highest in *T. pertinens* and *T. taeniola*, while diversity was seen mostly observed in *T. par*. The prevalence in the identified species indicates that any of the *Tabanus* could be infected, even though only the HP infections are most likely relevant epidemiologically in biting flies. Hence gut infection observed in this study could indicate recent feeding. In Burkina Faso, *Tabanids* have been incriminated to harbour *T. vivax* (Desquesnes and Dia, 2003). *T. vivax* in areas outside tsetse belt have been observed in Chad (Delafosse *et al.,* 2006). In this study, the prevalence of bloodmeals in *Tabanus* is quite high considering the number of trapped flies and only female are haematophagous. Hence, their intermittent feeding style could have deleterious effect on the herd. Vertebrate host identified from stomoxyine meals and feeding habits observed makes them an effective vector for trypanosomes.

The reported trypanosomes in both species examined, *Stomoxys niger* and *Stomoxys calcitrans* indicates the vector capacity for trypanosomes. In this study, stomoxyine was observed to harbour *T. vivax, T. evansi* and *T. simiae*, although *T. simiae* was confined only to the mouth tissue. The importance of stomoxyine in the epidemiology of AAT in southwest Nigeria could be of interest because of its abundance in the region. Previous studies have reported *Stomoxys taeniatus* and *Stomoxys niger* to transmit *T. brucei* and *T. congolense* in a laboratory mouse model (Sumba *et al.,* 1998). *T. vivax* DNA observed in *Tabanus* species and stomoxyine in dry months could associated to high *T. vivax* prevalence often reported

during the dry season in cattle from southwest Nigeria. AAT could be a continuous disease problem in cattle herd all through the year due to the presence of reservoir hosts and biting flies as rightly observed. Hence, elimination of tsetse flies may not necessarily lead to eradication of bovine trypanosomosis. While it is unfair to consider the mere presence of trypanosome DNA in haematophagous flies as a proof of transmission by these vectors, it reveals their occurrence in the vertebrate hosts' blood inhabiting the fly collection environment (Votypka *et al.,* 2015). An obvious setback in using PCR assay to detect trypanosome DNA is perhaps if the *Trypanosoma* species were in their infective stage (Mekata *et al.,* 2008), because DNA detection cannot differentiate the maturity stage of trypanosomes. In this study, *T. godfreyi* was present in *Tabanus*, a trypanosome species often observed in pigs (McNamara *et al.,* 1994). This suggests other *Trypanosoma* species could be harboured by biting flies. *T. godfreyi* has been identified in tsetse flies in Nigeria (Isaac *et al.,* 2016), but not from *Tabanus,* as the case in this study. The presence of *T. godfreyi* in *Tabanus* in this study indicates that the tabanids could have fed from pig or warthog at a time. Trypanosomes isolated from tsetse flies and biting flies from this study further corroborate them to be efficient vectors. *T. congolense* was isolated from stomoxyine flies, however; the successful mechanical transmission will need to be investigated experimentally.

5.2.2. Interaction of endosymbionts with tsetse flies

The presence of *W. glossinidius* in all the tsetse flies examined is expected due to its obligate characteristics (Wang *et al.,* 2013). Besides the fly larvae has been observed to be infected, howbeit; vertically with the symbiotic pathogen, hence all adults have been observed to be carriers (Wang *et al.,* 2013). The commensal endosymbiont, which is often related to the infectivity of tsetse fly with trypanosomes is *S. glossinidius*. In this study the endosymbint (*S. glossinidius*) present in both Nigerian tsetse fly species examined was lower compared to the study carried out in Cameroon (Farikou *et al.,* 2010). The environmental differences could be responsible (Rio *et al.,* 2006). The negative prevalence of *Wolbachia* in these examined *Glossina* species need to be further investigated, even though very low prevalence of *Wolbachia* has been reported for these tsetse species (Ouedraogo *et al.,* 2018). Endosymbionts are important pathogens that could help in understanding host-pathogen role in eradication programme (Wang *et al.,* 2013).

5.2.3. Cytochrome B oxidase analysis of vector flies.

Sanger sequencing of cytochrome b oxidase positive samples enhanced the determination of vertebrate hosts spectrum from vector bloodmeal. The results from this study suggest that negative flies to cytochrome oxidase could either be classified as total hungry (matured, could be trypanosome positive or negative) and or teneral flies (immature flies, trypanosome negative). Proper vector control strategies could be designed along concise understanding of host preferences (Bauer *et al.*, 1995). The high prevalence of human blood could be attributed to the settlement patterns which means livestock owners could be predisposed to human African trypanosomosis (HAT), especially if *T. b. gambiense* is introduced in the herd. Individual sequences of positive cytochrome b- gene samples were used for the detection of all the vectors. The spectrum of wildlife bloodmeals identified indicates the presence of reservoir hosts in the epidemiology of AAT in this study area. However, human blood was most detected than any other vertebrate in all the vectors, contrary to the feeding preference for cattle based on its size (Torr *et al.*, 2007). The detection of bloodmeals from all host types within the vector species explains host choice and availability relationship (Muzari *et al.*, 2010a). Livestock owners live with their animals in these settlements and that could be the reason for high bloodmeals from humans and cattle.

5.3.1. Prevalence in southwest Nigeria

Tropical rainforest is an integral part of humid zone in Nigeria and it has long been classified unfit for livestock production owing to abundance of trypanosome-transmitting vectors (Abubakar *et al.*, 2016). There have been various reported outbreaks of bovine trypanosomosis especially in sedentary herds (Anene and Ezekwe 1995). However, selective control efforts by Nigerian government in the past and poor diagnostic reporting of the disease has changed the perception of its epidemiology such that there have been inconsistent reports of its prevalence from different authors (Oniyah, 1997; Majekodunmi *et al.*, 2013b; Takeet *et al.*, 2013; Abubakar *et al.*, 2016). Nigeria Institute of Trypanosomosis and Onchocerciasis Research (NITOR) has been responsible for planning, implementing, supervising, monitoring, evaluating and controlling bovine trypanosomosis in Nigeria. As a matter of concern, socioecological factors such as migration activities, conflicts, transhumance, changes in settlement patterns and adoption of mixed farming by livestock owners have further complicated the epidemiology of the disease (Azuwike and Enwerem, 2010).

The molecularly characterised trypanosomes from cattle blood in the southwestern states were presented with associated factors on season and management sources. The reported prevalence of AAT in southwest Nigeria from this study is 23.8%, which is quite high. Although, lower prevalence were observed in market cattle and institutional cattle farm due to constant use of chemotherapy. Ikede *et al.,* (1987) reported cattle in southwest states using standard trypanosome detection technique which is the conventional microscopic method with 14.4% prevalence result. The higher prevalence in this study could be attributed to the improved diagnostic technique (PCR) used over parasitological methods. The impact of bovine trypanosomosis as animal health indicator in this study was found to be significant in the rainy season (26.7% versus 18.5% in the dry season) and this may be due to vector abundance. The prevalence in the dry season is complicated with low nutrition and abundance of biting flies. A section of southwest (Ogun state) reported higher prevalence compared to the 24.7% reported in this study (Takeet *et al.,* 2013). Although, Takeet *et al.* (2013) reported 76.6% in Ogun state with no reference to season using species specific primers for the PCR methodology, the animal group sampled were mostly abattoir cattle which could be due to high incidence of infection in slaughtered cattle at that time. Prevalence was low in Ondo and Osun States, in spite that there have been no organised tsetse control measures, this was observed in Ogun and Bendel States in Ikede's report (Ikede *et al.,* 1987). However, Ikede *et al.* (1987) reported high prevalence in Oyo (19.4%) and Ondo (28.2%) respectively. In this study, highest prevalence was observed in Osun (34.5%) which was Oyo State as at three decades ago, making it comparable. The least prevalence was Ondo State in this study. Hence, there may have been a change in the distribution of bovine trypanosomosis in southwest Nigeria.

5.3.2. Species diversity and prevalence

Trypanosoma congolense and *T. vivax* were more identified during wet and dry seasons, respectively. Different authors have argued the predominance of either species because the period of sampling was observed to affect the species distribution. A previous work reported *T. vivax* to predominate in late wet season and *T. congolense* in early wet season from northern Nigeria (Majekodunmi *et al.,* 2013a). The predominance of *T. vivax* in dry season from this study could be due to abundance of infective biting flies all through the year. Also, Anene *et al.,* (1991) argued that AAT persistence could be maintained in the flock by tabanids and stomoxyine flies even when *Glossina* speies are absent. The presence of non-

RoTat 1.2 VSG gene *T. evansi* as identified by sequencing is a drawback in reporting *Trypanozoon* group in *Trypanosoma* species distribution because some positives were regarded to be *T. b. brucei* because the RoTat 1.2 VSG gene does not confirm all the *T. evansi* groups (Salim *et al.,* 2011). Generally, *Trypanozoon* group identified from this work seems higher compared to previously reported studies (Takeet *et al.,* 2013; Majekodunmi *et al.,* 2013b), this could be that ITS1 rDNA detects this group of parasites readily.

Although, *T. b. gambiense* is known to be strictly a human disease with little involvement from other reservoirs, recent studies have reported its presence in domestic livestock (Njiokou *et al.,* 2010), however, there have been no infections found in cattle, even in countries with endemic *T. b. gambiense* in humans and detected infections found only in domestic pigs (Simo *et al.,* 2006). The current work also found no *T. b. gambiense* in cattle and this may be due to the inability of the organism to establish infection in cattle. Even though, southwest Nigeria is not known as endemic focus for *Trypanosoma b. gambiense*, no documented screening of humans and animals have been done. This is the first-time cattle will be screened by TgsGP PCR for *T. b. gambiense* in southwest Nigeria. Although sequencing of the ITS-PCR DNA product revealed a high match of one of the *Trypanozoon* positives with *T. b. gambiense* resembled DAL972 isolate (FN554966) but given the lack of TgsGP-PCR positivity of same product, it is likely that this isolate was not *T. b. gambiense*. Before now, the status of bovine trypanosomosis has been skewed to abattoir cattle in southwest of the country.

5.3.3. Management practices and trypanosomosis

The highest trypanonosome prevalence was reported in Fulani cattle farms characterised by movements and migration. Due to logistics and restrictions from Fulani herdsmen, only few works can be traced to the Fulbe (Fulani nomads) cattle source (Abubakar *et al.,* 2016). When cattle are crowded together in a spot such as watering and feeding points, there is increase exposure to transmitting vector flies (Mamoudou *et al.,* 2016). Increased marketing activities down south could have caused Fulani migration and transportation of cattle to fly areas. Institutional cattle with lowest prevalence was because there is proper routine activites on the animals such as consistent use of insecticides and trypanocides. However, a record of 14.7% in pastoral farming engaged by institutions could still mean that the disease is maintained in the flock either by trypanotolerant breeds and presence of trypanocide resistant-strains of trypanosomes as observed in this study. Prevalence in abattoir cattle was higher than in

market trade cattle and no association between trade cattle and institutional cattle (Appendix III).

Bovine trypanosomosis was reduced when cattle remain in markets for long periods due to low vector flies in places with dense human population (Reid *et al.,* 2000), coupled with consistent use of trypanocide. Another assertion made three decades ago was mainly because 90% of market cattle are now transported in lorries to southern markets hence, virtually eliminating possible risk of infection from *Glossina* threat (Ikede *et al.,* 1987). However, the 14.9% prevalence in market cattle is still worrisome, as cattle can still be lost to AAT. Abattoir cattle prevalence (27.1%), could be attributed to cattle host immune system, transport stress and infection rate from purchasing source. Cattle sources have positive correlation with AAT in specific locations with time. Increased movements down south and unstructured settlement patterns have direct impact on epidemiology of AAT.

5.3.4. Breed, age, sex and season variables influence on trypanosomosis

Low prevalence of AAT was observed in trypanotolerant breeds compared to zebu breeds with good body score. Age prevalence analysis showed that calves ≤ 1-year old infection rate was lower as compared to adults and aged cattle as earlier reported (Takeet *et al.,* 2013). This could be due to maternal immunity conferred and higher infection in adults could attributed to constant exposure to transmitting vectors on pastoralism, while the younger ones are left behind in the paddock. The sex prevalence of bovine trypanosomosis in this study showed slightly higher prevalence in females with no significance when compared with male. This corresponds to previously conducted studies in Nigeria (Onyiah, 1997; Sam-Wobo *et al.,* 2010). Further investigations are required in several aspects; to examine humans involved with cattle occupation for *T. brucei gambiense*, developing a species-specific diagnostic tool to identify all the groups of *T. evansi* and implementing a cost-effective control against AAT and its transmitting vectors. In conclusion, it was observed that bovine trypanosomosis is affected by cattle sources and other risk factors such as migration, season and age group.

5.4.1. Behavioural patterns and dynamics

The vector fly behaviours are important to study AAT dynamics and epidemiology. Landing sequence of *Glossina* species observed on the belly and limbs from this study has been previously reported (Torr and Hargrove, 1998; Vale *et al.,* 1999). Preference of tsetse to the belly of cattle could be attributed to the swift host response around other body parts (Torr and

Mangwiro, 2000). Inspite of consistent seasonal and interspecies variations, majority of tsetse flies have landing affinity for the belly and limbs (Torr *et al.,* 2007). Tsetse was not observed on the tail probably because it is a major defensive body part. Studies were focused for the wet season because it was difficult to study the landing site for dry season in all locations as the vector flies were scantily present. This was due to environmental surrogates such as temperature (> 32 °C) and humidity (< 60%) in cattle farm locations in the dry season. Both single and multiple feeding options in *Glossina* species proved its adaptable feeding behaviours (Muturi *et al.,* 2011), but the single host animal (cattle) in the herd was well-fed on.

Tabanids efficient capacity as mechanical vector is expressed in their ability to engage in increased interrupted feeding (Muzari *et al.,* 2010b). Most of *Tabanus* spp. preferred limbs and belly, only *T. par* landed more on the back in this study. This could be the reason for its easy dislodgement from the host during attempted feeding sessions. However, only a negligible number of the *Tabanids* could even show complete engorgement success as observed in previous studies (Muzari *et al.,* 2010a). The interrupted feeding adaptive feature of *Tabanus* species makes them potential mechanical vector of trypanosomiasis (Muzari *et al.,* 2010a). Partial meals made by most of the observed *Tabanus* spp. to reach engorgement on cattle host could be due to interruption from defensive behaviours exhibited by the cattle. Affinity for the head and neck region for the stomoxyines indicate the possibility of *S. niger* abundance in the fly population as compared to *S. calcitrans* which preferred the limbs. Mean adult longevity have generally been observed more in *S. niger* in comparison to *S. calcitrans* in hot weathers (Schofield and Torr, 2002).

Indicators such as vegetation changes, generic and intrinsic factors, climatic conditions, availability of food (host) may reduce tsetse distribution in affected areas (Torr and Hargrove, 1998; Albert *et al.,* 2015). The discrete location of tsetse flies around forest edges and low approach to cattle host in the study areas could explain these variations. Habitat loss and vector fly reproductive ability of tsetse are other major factors affecting its distribution. *Tabanids* distribution and abundance varies due to vegetational changes observed. The considerable increased feeding duration in tsetse flies in comparison to stomoxyine from this study is contrary to previously reported study (Scofield and Torr 2002), this could be attributed to type of tsetse species, host breed and vegetation type. The thick skin of adult cattle compared to the calves could explain the lower host defenses exhibited by the adult

during stomoxyine annoyance from feeding. The short feeding duration reported in *Tabanus rubidus*, *T. biguttatus*, *T. par*, *T. pertinens* was because of its intermittent feeding habit, which confirmed them as effective mechanical vectors. No *Tabanus* species could achieve 100% engorgement success, due to its freight response. This is probably because the high level of annoyance of cattle-host at its strong bite. The landing principle could follow insectidal treatment plan with only 20% representing the most landed body parts for the vector flies. This concept tends to improve treatment by optimizing the use of insecticides on vector control, stabilizing the threat emanating from natural fauna and reducing environmental pollution (Torr *et al.,* 2007). Although, species like *Tabanus par* preference may need further investigation.

5.4.2. Host defensive mechanisms

This study showed that vector flies assessed were influenced by the behaviour of the host. *Glossina* and *Tabanus* species are more affected by host defensive behaviour compared to stomoxyine. Generic variations between feeding periods, showed greater affinity of stomoxyine flies to their host compared to *Tabanus* and tsetse despite host disturbances. Invariably, it was also discovered that the returning period to the host is quicker with stomoxyine in comparison to *Tabanus*, while tsetse may not return in most cases, this could be as a result of variations to fly behaviour to host response. However, for *Glossina*, the feeding success is related to nutritional status (unfed, partial or fed), in which case hungry *Glossina* species response less to host behaviours (Torr and Hargrove, 1998). Increased energy expended on scaring off vector flies could be attributed to the reduced productivity of most cattle produced in the country. For example, the irritation caused by *Stomoxys calcitrans* have been reported to affect the grazing yearling weight gain (Campbell *et al.*, 2001). On other instances, reduced host responses to biting during acute trypanosomosis infection has been identified because of immune depression, therefore the early stages of chronic and acute infections may exacerbate trypanosome transmission through vector bites (Foil, 1989).

The prevalent *Stomoxys* species were *Stomoxys calcitrans* and *Stomoxys niger* and it has been reported that *Stomoxys calcitrans* which need 60 days to complete its lifecycle at low temperature could complete in less than 12 days at high temperatures (> 30°C) (Lysyk, 1995; Mavoungou *et al.,* 2012). The threat created by *Stomoxys* spp. seems to be highest in southwest Nigeria with greater host reactions. This study showed disease transmission is

increased during increased host switching and feeding frequency of biting flies (Torr and Mangwiro, 2000). Incessant AAT infections and outbreaks in tsetse free areas could be attributed to reinvasion of few infected *Glossina* species and predominance of *Tabanids* and stomoxyine flies. *Glossina* species remain the effective biological vector of the disease, and the biting rate and engorgement success suggest its effectiveness. These behaviours from vector flies could provide greater insights into effective control measures to boost livestock production in the country.

5.5.1. Cypermethrin application protocols

Many cattle owners control trypanosomiasis mainly with prophylactic and curative drugs. However, the sustainability of this approach is becoming difficult due to the problems of resistance to trypanocides (Geerts *et al.,* 2001). Hence, the alternative approach is integrating vector control methods to reduce the impact of transmission (Vreysen *et al.,* 2013). Back linning of pour-on insecticides has produced better results compared to hand spraying of insecticides (Thompson *et al.,* 1991). Pour-on is well-appreciated among livestock keepers in Nigeria due to its body persistence.

The use of RAP on cattle shows prolonged persistence with variations (~3 and 21 days) which are based on several factors such as application timing and methods, expertise and prevailing weather. (Torr *et al.,* 2007). In this study, body persistence was shortest for the FAA which was mainly due to adulterated products available in the market and the factors earlier stated. Climatic factors such as high temperature and rainfall have negative effects on insecticidal persistence (Torr *et al.,* 2007). The RAP figures revealed higher persistence compared to FAA all through the studies which could to developed resistance to available insecticides with the conventional application strategy. Reports on sub-standard veterinary products across the country has been reported (Kingsley, 2015). Similar reports of adulterated veterinary products have been reported in other African countries (Teko-Agbo, 2008). Notably, the application methods by most herdsmen are faulty with the reconsititution strategy and the use of a singular insecticides throughout a breeding season could lead to emergence of resistance.

The importance of RAP is to achieve a better result with insecticidal application restriction to most landed body parts (Torr *et al.,* 2007). Regardless of insecticidal concentration on first application, tsetse flies and Tabanids disappeared unlike stomoxyines and were not observed

till the end of the experiment. Hence, the used insecticides could readily prevent challenges from heavy bites in most cattle settlements in the study area. However, stomoxyine tends to persist regardless of dosage which could suggest developed resistance from constant exposure to these preparations. The stomoxyine *in vivo* and *in vitro* results showed that some species of stomoxyine flies are resistant to the commonly available cypermethrin in the market. Dispensed cypermethrin and cypermethrin-mix (Fulani concoction) were often encountered on the field because they seem affordable to the livestock owners (Kingsley, 2015).

The bioassay showed a low survival rate of stomoxyine exposed to cypermethrin-Pantex®, hence the use of approved veterinary preparations for field use could prevent the emergence of resistance in the cattle farms. The evaluated cypermethrin dispensed in most of the markets showed that half of the stomoxyines survival at recommended concentration indicated the presence of resistance. The concoction mixed (synthetic insecticides + ethnomedicine) by the Fulani herdsmen could exacerbate resistance. Contributing factors to resistance include application methods and qualities of the insecticides. Stomoxyine persistence experiment and the *in vitro* results showed that commonly used cypermethrin are resistant to stomoxyine flies. Hence, there is a need to effectively standardise veterinary products and engage veterinary doctors to control transmitting vectors of bovine trypanosomosis.

5.6.1. Reasons for Fulani migration in relation to bovine trypanosomosis

Cattle production has been the occupation of the Fulani since before the creation of Nigeria. However, their activities only took place in northern Nigeria because of its favourable vegetation that prevents tsetse flies from thriving in large areas. Migration activities started when transhumance to guinea savannah became the solution to feeding and watering problems during the dry season (Majekodunmi *et al.,* 2013a), amongst other socioecological factors such as land clearance, increased demand for livestock products, Sahelian drought of early 1970s and reduction in wildlife population (Akinwunmi and Ikpi, 1985; Swallow and Jabbar, 1994). The guinea savannah contains areas of woodland vegetation with little tree patches along the river course. This has been the system over the years until the 1980s when tsetse flies were observed in some of the savannah areas. Malnutrition of livestock due to increased stock population and heavy traffic of transhumance in grazing reserves by pastoralists was already a problem in northern Nigeria by the 1970s (Pulan, 1978, 1980a,

1980b; Majekodunmi *et al.,* 2013a) and since then land use conflict from pastoralist marginalization and contraction of agricultural activities have been witnessed.

Effective control measures for AAT becomes more difficult because of transhumance pattern in Nigeria. There is grass and water throughout the year in southwestern Nigeria, the marketing of livestock is mouth-watering compared to the north with a single disadvantage of fly abundance in this derived savannah. Soon, a larger percentage of the Fulani migrants became settlers in southern Nigeria and resulted to sedentary methods of rearing livestock depending more on trypanocides and insecticides. Azuwike and Enwerem (2010) reported that an animal that cost US$ 124 in the north is sold for US$ 355 in the south. Profit margin per animal in this study revealed a sum of ≥ US$ 35 – ≥ US$ 355 depending on the size and market structure. There is significant increase ($\alpha < 0.05$) in profit margin as the size of the animal increases. Due to the flexibility of marketing structures in the south, northerners could penetrate the markets.

Fulanis no longer practice nomadism alone but rather engage in other activities to increase their indirect profit from livestock production. This study revealed that practices to cope with fly abundance like use of insecticides, smoking to generate heat energy, engaging large numbers of animal stock around market environs and wet season transhumance resulted in changes in activities among livestock owners. Veterinary services were generally not consulted and there were poor measures to prevent drug abuse. Trypanosome species (*T. vivax* and *T. congolense*) have been observed in the field to be resistant to trypanocides in Nigeria, which could be as a result of drug misuse (Ilemobade, 1979). Observations on the combined practices indicated that not much profit has been reaped from it. This study revealed that cattle keepers understood the significance of seasonal effect and how it correlates with AAT and its transmitting vectors which was also reported in Ethiopia (Seyoum *et al.,* 2013). However, both biting flies (mechanical transmission) and more importantly tsetse flies (biological transmission) were seen as threats but only a few could acknowledge them as vectors of trypanosomosis, hence the epidemiology of the disease is not properly understood by most farmers despite the long-age awareness. BT peaks were observed during the rainy season. The abundance of the transmitting vectors during this season and quick development of tsetse fly puparium could be the possible explanation.

5.6.2. Practices and livelihood

During this study it was noticed that there was utilization of animal manures in exchange for crop residues, but the use of animal traction for mechanized farming was not of interest to livestock owners in southwestern Nigeria compared to the keepers in the north. Raw materials such as horns, hoof, bones, fur, manure and leather were no longer maximized by local industries as they were found littering some of the abattoir areas for months in Oyo and Osun states. From this study, several social factors have suggested an altered pattern of transhumance conducted by Fulani herdsmen. Community elite in southern Nigeria have also engaged in livestock keeping, engaging some of these herdsmen in rearing their animals using a semi-intensive system. However, many more herders have turned farmers becoming sedentary and in conflict with poor resource farmers (Azuwike and Enwerem, 2010). The rearing system coupled with the changing environmental factors have been observed to affect the transmitting vectors of bovine trypanosomosis.

The change in fly habitat poses both positive and negative impacts on livestock production. Only 13.4% (95%CI: 9.43 – 18.68%) practiced cattle migration in the rainy season and a large number concentrate on both crop cultivation and livestock rearing. This contrasts with 73.4% of livestock keepers who practiced same in northern Nigeria (Majekodunmi *et al.,* 2013a). The families of sedentary farmers feed on farm produce throughout the year; hence little is spent on upkeep. Factors like desertification, high temperature (≥ 38°C), poor market value on animal product e.g. milk and low annual rainfall (up to < 400mm) has reduced productivity over the years in the northern Nigeria. In the southwestern part, environmental surrogates favoured crop cultivation and livestock rearing throughout the year.

General assessment revealed that the high number of butchers was because of general demand for meat due to the increased human population in recent times. Some of the livestock keepers from the north were also involved in butchering, fellow household members from the north provide them with cattle to trade and slaughter in the south and hence a change of role has evolved over the years. The impact of this role has increased the prevalence of trypanosomosis due to reduced transhumance from the south to the north and increase migration of nomads from the north to the south. This study also revealed that 37.8% (95%CI: 31.50 – 44.54%) of livestock owners were involved in combined activities. Diversification was again noticed in their practices as 21.1% (95%CI: 16.07 – 27.08%) were involved in combined role. A larger percentage of reared cattle in northern Nigeria, Niger

Republic and Chad were sold in southwestern Nigeria; hence a large number of herders stay in the region during the rainy season. Majority of the Fulanis have farm settlement where they practice mixed farming, pastoralism and marketing by mixing up with traders and butchers of the Yoruba origin.

5.6.3. The struggle with bovine trypanosomosis continues

Interestingly, FMD (Foot and mouth disease) was the most mentioned disease followed by trypanosomosis among the focus group. This could be because of obvious clinical signs of FMD compared to that of trypanosomosis. However, deaths recorded were highest in trypanosomosis. Treatment strategies evaluation used by cattle keepers revealed self-treatment of animals with different drug combinations, of which the trypanocide cost was estimated at US$ 8.4 million in southwest Nigeria. Although, integrated control measures in the past have involved trypanocides, feeding of supplements and use of anthelminthic (Holmes *et al.*, 2004) because of the established trypanosome-helminthes synergy (Kaufmann *et al.*, 1992), yet treatment strategies observed in southwestern Nigeria by the resource livestock keepers were skewed.

Only diminazene aceturate is the widely known chemotherapeutic substance effective against *Trypanosoma congolense*, *Trypanosoma brucei* and *Trypanosoma vivax*. All these species have also been reported to have developed resistance against available trypanocides in at least one region in Nigeria (Greets *et al.*, 2011). Unfortunately, some trypanocides like quinapyramine, isometamidium chloride and homidium bromide which confer three months prophylaxis against certain species of trypanosomes in the past now hardly give any effective protection (Delespaux *et al.*, 2008). Resistance to trypanocidal drugs have been reported in Africa (Geerts *et al.*, 2001; Murilla *et al.*, 2002; Sinyangwe *et al.*, 2004; Delespaux *et al.*, 2008), and most of the farmers reported that after several treatments, animals that do not recover are either slaughtered or sold immediately regardless of the drug residue. Culling of animal who did not respond to trypanocides were observed in 77.5% (CI: 71.4 – 82.7%) of cattle keepers, this could be due to resistance to the drug.

Administration of drugs by livestock keepers need urgent evaluation as their dosing system patterns were not professional as observed in this study. When the herdsmen struggle with the persistence of these flies, they sometimes change to another market product which has similar metabolic pathways for detoxification which tend to increase resistance. Also, drug residues

in animal products could be dangerous to both animal and human health. This resistance could increase more because of the hostility of livestock keepers, failure to embrace veterinary services and use of multiple trypanocides. Most of these drugs were introduced decades ago to combat trypanosomosis, and currently they are still in use, enhancing resistance and making control difficult (Geerts *et al.,* 2001). Drug resistance coupled with AAT persistence and fly vector abundance, threatens livestock production in Nigeria.

5.7.1. AAT prevalence in Nigeria

Despite continuous efforts to combat AAT using different governmental institutions such as NITR and PATTEC, the country still battles with the disease even with several years of control (Putt and Shaw, 1982; Vreysen *et al.,* 2000). To our knowledge, the study prevalence from this study projects the first of its kind since Nigeria's independence. This analysis is important in developing control measures of AAT in the country, while all field-related AAT studies were evaluated. The status of AAT has reported in this study suggest that AAT is present in the country despite several programme in the past. Previous study reported that a quarter to half of livestock population are lost to AAT and with 0.2 elasticity, the gross domestic product (GDP) can be decreased by up to 10% due to the persistence of AAT (Isaac *et al.,* 2016). This current assessment revealed variations in prevalence across the states with most studied areas having veterinary faculties.

5.7.2. Regional subgroup analysis of AAT

AAT prevalence reported in northern and southern Nigeria have show no statistical association, although significantly higher studies have been done in northern Nigeria compared to the south. Notably, there are more cattle pastoralists in the savannah areas of the north with not less than 75% of total livestock in Nigeria (Majekodunmi *et al.,* 2013a). Since, southern Nigeria is highly industralised with a tropical rainforest vegetation and falls around the tsetse-belt, pastoralist keep their animals by administering trypanocides continuously. The prevalence results from both regions indicates that every cattle area is at risk of AAT. There was a decline in the prevalence of AAT after governmental control efforts in the north were implemented (Onyiah, 1997), this had a corresponding effect in southern Nigeria (Opasina and Ekwuruke, 1987; Ikede *et al.,* 1987). The negligence to sustain past efforts have led to AAT resurgence, since risk factors such as Fulani migration are on the increase (Oluwafemi *et al.,* 2007).

5.7.3. Tsetse-trypanosome infection rates

Glossina species are present across the country (Isaac *et al.,* 2016). About 196,000 km^2 landmass was cleared of tsetse flies to boast cattle production (ILCA/NAPRI, 1984) and additional 1,500 km^2 in north-central region in 1987 (Leak, 1998). However, the presence of trypanosomes in *Glossina* species trapped from these cleared areas suggests re-invasion of infected flies (Oluwafemi *et al.,* 2007). There is heterogeneity across the regional studies in the country, hence; it is of no doubt that the southern region of the country is still known to be infested with *Glossina* species, because of favourable vegetation. Notably, most of these studies were before the 21st century shows that *G. tachinoides* was mostly trapped for assessment while *G. morsitans* was mostly infected with trypanosomes. However, in recent evaluation boosted with modern techniques, *G. palpalis* was mostly trapped and infected. The changes in abundance, distribution and infection could be attributed to human activities and improved diagnostic approach (Isaac *et al.,* 2016).

5.7.4. Sub-group AAT analyses of livestock

Bovine trypanosomosis have the highest number of searches in literature because of the importance attached to animal production and source of income to pastoralists. AAT prevalence is significantly affected by diagnostic approach. The prevalence of AAT in southwest Nigeria is high and could be attributed to the vegetational status. Southwest Nigeria is known to be in tsetse-belt and the examined tsetse flies showed high trypanosomes even with dissection diagnostic technique. The active component of cattle production, presence of wildlife, thick vegetation, favourable environmental factors could be responsible for this prevalence (Albert *et al.,* 2015).

5.7.5. Heterogeneity of AAT distribution

The characteristic animal group sampled for AAT evaluation among other factors such as seasonal variations, regional effects, sampling time and diagnostic approach could affect the prevalence report. Concise heterogeneity among studies conducted was significant. Incidentally, important risk factors of AAT such as breed-type, husbandary and management, transhumance activities, sampling methodology, migration and settlement activities, frequency of insecticides and trypanocides were often omitted in most of the assessed studies in the country, these could impact the basic understanding of the disease. The increasing trend of AAT prevalence in livestock could be correlated with the continuous *Glossina*

trypanosome infection rate. Also, reports have shown that biting flies could maintain the infection in cattle herd (Abdi *et al.,* 2017; Anene and Ezekwe, 1995).

5.7.6. Variations in AAT prevalence: Effect of diagnostic techniques on analysis

The analysed decadal bar chart of AAT studies revealed a decline in prevalence from 1960s through to 1990s. The low prevalence in the 1990s could be connected to the government response to oust the vector flies by intensifying the aerial spraying of insecticides in 1967 to boost cattle production (Leak, 1998). Between 1979 and 1987, there was also BICOT project (Oluwafemi *et al.,* 2007), to solidify the control approach.

The increasing prevalence noticed from 2000s, with more assessed numbers of animals could mean that there is lack of sustainability in the control measures. The variations in prevalence was influenced by diagnostic approach with PCR > ELISA > Microscopy, except in pigs which ELISA revealed the highest prevalence. The porcine report could be attributed to the limited recovered studies. Overall, PCR shows to be better in terms of specificity and sensitivity for trypanosome identification compared to other techniques (Takeet *et al.,* 2013; Wastling and Welburn, 2011).

Arguably, microscopy revealed *T. vivax* readily compared to other species while serology and PCR reported *T. congolense* readily. It could be that *T. vivax* was seemingly recognisable or confused with other species under the microscope. For PCR, the ITS primers make a wide range of *Trypanosoma* species to be identified (Njiru *et al.,* 2005). In all the techniques, *T. brucei* was the least detected. Factors responsible for variability includes fly abundance and dispersion, presence of wildlife reservoirs, trypanotolerant species present, infection stage in fly vectors, farmers-herdsmen conflict among many others (Majekodunmi *et al.,* 2013a).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Proper identification of vectors is important in the surveillance of arthropod-borne diseases. *Glossina* species have been reported to transmit trypanosomes in Nigeria. It is well-known that biting flies are mechanical vectors for bovine trypanosomosis, but its abundance and distribution has been poorly reported in Nigeria due to identification difficulties. The abundance of stomoxyine flies is high from this study, coupled with the high vectorial capacity, their role in transmission of trypanosomes in cattle herd has been under-rated before now. The abundance of these vector flies were observed to increase in areas with dense vegetation and decrease in areas with high human population. These could restrict the infection to certain areas across the country, complicating the epidemiology of the disease.

The detrimental effects of these transmitting-vectors especially in the identification of pathogenic species in both tsetse and biting flies could make elimination of AAT difficult in Nigeria. Despite the low trapping of the biological vector, the abundance of biting flies (stomoxyine and *Tabanus*), especially in the dry months could sustain infection in livestock herd. The high *T. vivax* in dry months suggests overwhelming presence of biting flies in the study sites. Hence, effective elimination programmes need to consider all the transmitting-vectors as identified in this study. Concurrent trypanosome isolates identified in both vertebrate and invertebrate hosts indicate that more research into transmission dynamics and vectoral capacity in southwest Nigeria is needful.

There could be a potential asymptomatic human African trypanosomosis in southwest Nigeria considering the current species (Palpalis group) of identified tsetse and the high human bloodmeal from the vector flies. Hence, further studies on transmission dynamics of trypanosomes from these vectors to livestock and humans will be important to understanding the epidemiology of the disease. Assessment of vector competence will be a great insight into AAT and HAT in Nigeria. There is an urgent need to examine human for HAT to prevent silent presence of the disease in Nigeria.

The present study confirms the spectrum of trypanosomes in *Glossina*, *Tabanus* and stomoxyine flies. The overwhelming preference for human blood in all the vectors makes livestock owners vulnerable to potential HAT. The potential role of human as reservoir host in the epidemiology of AAT is now known. Improved control measures need to be adopted considering the distribution and types *Trypanosoma* species observed both in the fly vectors and vertebrate host.

With high incidence of bovine trypanosomosis in southwest states of Nigeria from this study and high prevalence of trypanosomes in its transmitting vectors, there is a need for regional effort to oust the vectors for improved livestock production in Nigeria. Literature searches revealed that flies have not been collected and examined for trypanosome identification using molecular methods in cattle farms from southwest Nigeria. African animal trypanosomosis is a major problem of livestock in southwest Nigeria and it has been well reported in cattle. Nigeria relied so much on cattle as a major protein source, due to the high demand for beef and diary products. The high morbidity and mortality rate associated to bovine trypanosomosis is a drawback to sustainable livestock production. Hence, improved diagnostic kits for field assessment of bovine trypanosomiasis is essential to improving production and economic livelihoods of livestock keepers. The use of microscopy in detecting the presence of trypanosomes may not help in the elimination campaign. Most of the investigated studies on tsetse and AAT infections in Nigeria were widely conducted with microscopy. Study methodology and risk factor assessment is necessary to validate research output.

The interrupted feeding common in stomoxyine and tabanids correlated with the frequency of host defensive behaviour largely caused by stomoxyine flies, could lead to continuous transmission of AAT. Meanwhile, bovine trypanosomosis in areas free of tsetse flies could be attributed to the abundant presence of these biting flies. Fly vectors engorgement successes suggest their effectiveness in transmitting trypanosomosis. These behaviours can be properly monitored to provide further understanding on the epidemiology of trypanosomosis in livestock industry. Also, RAP method and chemotherapy can be used as integrated control approach in eliminating bovine trypanosomosis in Nigeria.

Emphasis on this study has been related to the general socioeconomic impact of trypanosomosis, a disease that affect every aspect of livestock activity in Nigeria. Holistic evaluation of socioeconomic and production impact of bovine trypanosomosis is important in

the disease epidemiology. Factors including seasonal variation, market structures, practices, treatment and government impact were all considered. If this disease can be properly controlled studying the epidemiology, bovine population can be doubled with consequent increase in standard of living, food security and national income.

In southwest Nigeria alone, we estimated US$ 8.4 m has money loss to trypanocide annually frome this study. Hence, greater unimaginable losses from indirect costs could put the livestock industry at risk. The agent-based model constructed indicated that elimination of the disease is possible in Nigeria provided that all the aetiological agents are targeted with holistic trypanocidal and insecticidal approach illustrated in this study. The modelled biting flies as contaminated, provided a better understanding to the natural field approach on the control measures. The constant losses to bovine trypanosomosis and low productivity have discouraged the farmers and the government to involve monetary approach over time. Overall losses can be diverted to the economy and a well-organised cattle trade migrating routes should be monitored. National policies could be reviewed, deliberate efforts to oust transmitting vectors and ensuring other control measures discussed are implemented.

Further research is required to develop quick molecular diagnostic kits at low cost to be used on the field for effective treatment of infected animal. Screening other animals such as small ruminants and pigs is important to reduce its trypanosome prevalence in cattle herd. Besides, researches on trypanosomosis prevalence in wild vertebrates and humans is necessary to reduce the disease effects on livestock industry. Transgenetic researches targeting the evolutionary genes of endosymbionts in the susceptibility of trypanosomes in tsetse flies is important for future control.

CONTRIBUTIONS TO KNOWLEDGE

- Stomoxyine flies contributed highest apparent density, followed by *Tabanus* and *Glossina* in southwest Nigeria.

- Fly vectors increased with vegetation density and decreased in areas with high human population density.

- Biting flies (*Tabanus* and *Stomoxys*) are as important as biological vector (*Glossina*) in the transmission of bovine trypanosomosis in southwest Nigeria.

- *Glossina* and biting flies coupled with the trypanosomes harboured were detected molecularly in southwest Nigeria. The isolates deposited in the GenBank include *Tabanus* species (MF448236, MF448237, MF448238, MF448239, MF448240 & MF448241)

- Detection of eight vertebrate blood meal sources (human, cattle and wild animals) of fly vector in Nigeria. A total of 25 nucleotide sequences of vector bloodmeal (MH884483 – MH884507) have been deposited in GenBank.

- Assessment of FAA and RAP methods against fly vectors in Nigeria indicates that insecticidal resistance on stomoxyine flies are dependent on application approaches.

- Asymptomatic AAT in southwest Nigeria considering the species of identified tsetse (Palpalis group) and the high human bloodmeal from the vector flies.

REFERENCES

Abebe, G. and Jobre, Y. 1996. Trypanosomosis; A Threat to Cattle Production in Ethiopia. *Revue d'elevage et de Medecine Veterinaire des Pays Tropicaux* 147: 897–902.

Abenga, J.N., Enwezor, F.N.G., Lawani, F.G., Osue, H.O., Ikemereh, E.C.D. 2004. Trypanosome prevalence in cattle in Lere area in Kaduna State, North central Nigeria. *Revue d'elevage et de Medecine Veterinaire des Pays Tropicaux* 57 (1– 2): 45–48.

Abubakar, A., Sabo, H.M., Abdulkadir, S., Halliru, S.N., Umar, R.B., Abdulkadir, D.A. 2016. Epidemiology of trypanosomosis in derived savannah of Nigeria. *Journal of Pharmacy and Biological Sciences* 11: 51–54.

Ahmed, A.B. 2004. A peridomestic population of the tsetse fly *Glossina palpalis palpalis* Robineau-Desvoidy, 1830 (Diptera: Glossinidae) at Kontagora town, Niger state, Nigeria. *Entomology and Vectors* 11(4): 599–610.

Ahmed, A.B., Okiwelu, S.N., Samdi, S.M. 2005. Species diversity, abundance and seasonal occurrence of some biting flies in southern Kaduna, Nigeria. *African Journal of Biomedical Research* 8: 113–118.

Ahmed, H.A., MacLeod, E.T., Hide, G., Welburn, S.C., Picozzi, K. 2011. The best practice for preparation of samples from FTA® cards for diagnosis of blood borne infections using African trypanosomes as a model system. *Parasites & Vectors* 4: 68.

Akinwunmi, J.A. and Ikpi, T.E. 1985. Trypanotolerant cattle production in Southern Nigeria. Ibadan: International Livestock Centre for Africa.

Albert, M., Wardrop, N.A., Atkinson, P.M., Torr, S.J., Welburn, S.C. 2015. Tsetse fly (*G. f. fuscipes*) Distribution in the Lake Victoria Basin of Uganda. *PLoS Neglected Tropical Disease* 9(4): e0003705.

Alsford, S., Turner, D.J., Obado, S.O., Sanchez-Flores, A., Glover, L., Berriman, M. et al. 2011. High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome. *Genome Research* 21: 915-924.

Anene, B.M. and Ezekwe, A.G. 1995. Trypanosomosis in intensively reared Muturu calves in Nigeria. *Tropical Animal Health Production* 27: 229–230.

Anene, B.M., Chima, A.B., Jibike, G.I., Anika, S.M. 1991. Prevalence of trypanosomosis in Zebu at Obudu ranch- a tsetse free zone in Nigeria. *Preventive Veterinary Medicine* 10: 257–260.

Austen, E.E. 1909. Illustrations of African blood sucking flies other than mosquito and tsetse flies.

Azuwike, O.D. and Enwerem, E. 2010. Nigeria's changing environment and pastoral nomadism: redistribution of pains and gains. In Proceedings of the Berlin Conferences on Human Dimensions of Global Environmental Change Berlin, 2010.

Baldry, D.A.T., Riordan, K. 1967. A review of 50 years entomology of insect-borne disease of veterinary importance in Nigeria, with special reference to tsetse. *Proceeding of Entomological Society in Nigeria* 1967: 43–55.

Banerjee, D., Kumar, V., Maity, A., Ghosh, B., Tyagi, K., Singha, D., Kundu, S., Laskar, B. A. and Naskar, A. 2015. Identification through DNA barcoding of Tabanidae (Diptera) vectors of surra disease in India. *Acta Tropica* 150: 52–58.

Barros, A.T. 2001. Seasonality and relative abundance of Tabanidae (Diptera) captured on horses in the Pantanal, Brazil. *Memorias do Instituto Oswaldo Cruz* 96: 917–923.

Becker, S., Franco, J.R., Simarro, P.P., Stich, A., Abel, P.M., Steverding, D. 2004. Real-time PCR for detection of *Trypanosoma brucei* in human blood samples. *Diagnostic Microbiology and Infectious Diseases* 50: 193–199.

Bitome-Essono, P.Y., Dechaume-Moncharmont, F-X., Mavoungou, J., Mba, R.O., Duvallet, G., Bretagnolle, F. 2015. Distribution and abundance of haematopagous flies (Glossinidae, *Stomoxys* and Tabanidae) in two national parks of Gabon. *Parasite* (22-23): 1–11.

Campbell, J.B., Skoda, S.R., Berkebile, D.R., Boxler, D.J., Thomas, G.D., Adams, D.C., Davis, R. 2001. Effects of stable flies (Diptera: Muscidae) on weight gain of grazing yearling cattle. *Journal of Economical Entomology* 94: 780–783.

Cayla, M., Rojas, F., Silvester, E., Venter, F. and Matthews, K.R. 2019. African trypanosomes. *Parasites and Vectors* 12: 190.

Challier, A., Laveissière, C. 1973. Un nouveau piège pour la capture des glossines (*Glossina*: Diptera, Muscidae) description et essais sur le terrain. *Cahiers de l'Office de la Recherche*

Scientifique et Technique Outre-Mer Serie Entomologie Medecine Parasitologie 11: 251–260.

Cheng, Q., Ruel, T. D., Zhou, W., Moloo, S. K., Majiwa, P., O'Neill, S. L., et al. 2000. Tissue distribution and prevalence of *Wolbachia* infections in tsetse flies, *Glossina* spp. *Medical and Veterinary Entomology* 14: 44–50.

Chilonda, P. and Van Huylenbroeck, G. 2001. Attitude towards and uptake of veterinary services by small-scale cattle farmers in Eastern province, Zambia. *Outlook Agriculture* 30: 213-218.

Claes, F., Radwanska, M., Urakawa, T., Majiwa, P.A.O., Godderis, B., Buscher, P. 2004. Variable surface glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. *Kinetoplastid Biology and Disease* 3: 3.

Crosskey, R.W., Crosskey, M.E. 1954. The transactions of the Royal Entomological Society of London: The horse-flies (Diptera: Tabanidae) of Nigeria and the British Cameroons.

Dagnachew, S., Terefe, G., Abebe, G., Barry, D., McCulloch, R., Goddeeris, B. 2015. *In vivo* experimental drug resistance study in *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. *Acta Tropica* 146: 95–100.

D'Amico, F., Gouteux, J. P., Le Gall, F., Cuisance, D. 1996. Are stable fly (Diptera: Stomoxyinae) vectors of *Trypanosoma vivax* in the Central African Republic. *Veterinary Researh* 27: 161–170.

Davis, H. 1977. Tsetse flies of Nigeria. A handbook for junior control staff (3rd edition): Oxford University Press, Ibadan.

Delafosse, A., Thébaud, E., Desquesnes, M., Michaux, Y. 2006. Epidemiology of *Trypanosoma vivax* infection in cattle in the tse-tse free area of Lake Chad. *Preventive Veterinary Medicine* 74: 108–119.

Delespaux, V., Geysen, D., Van den Bossche, P., Geerts, S. 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. *Trends in Parasitology* 24: 236–242.

Desquesnes, M. and Dia, M.L. 2003. *Trypanosoma vivax*: mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. *Experimental Parasitology* 103: 35–43.

Dipeolu, O.O. 1974. Studies on the developmment of *T. congolense* in tsetse flies (Glossinidae: Diptera) and factors affecting it. *Acta Protozoologica* 14: 241–251.

Dipeolu, O.O. 1977. Studies on the incidence, distribution and prevalence of flies of veterinary importance in Nigeria. *Bulletin of Animal Health and Production in Africa* 25(1): 33–39.

Dsouli-Aymes, N., Michaux, J., De Stordeur, E., Couloux, A., Veuille, M., Duvallet G. 2011. Global population structure of the stable fly (*Stomoxys calcitrans*) inferred by mitochondrial and nuclear sequence data. *Infection, Genetics and Evolution* 11: 334–342.

Ducrotoy, M.J., Majekodunmi, A.O., Shaw, A.P.M., Bagulo, H., Musa, U.B., Bertu, W.J., Gusi, A.M., Ocholi, R.A., Bryssinckx, W., Welburn, S.C. 2016. Fulani cattle productivity and management in the Kachia Grazing Reserve, Nigeria. *Pastoralism: Research, Policy & Practices* 6: 25.

Ebhodaghe, F., Isaac, C. and Ohiolei, J.A. 2018. A meta-analysis of the prevalence of bovine trypanosomiasis in some African countries from 200 to 2018. *Preventive Veterinary Medicine* 160: 35-46.

Engel, A. and Okidegbe, N. 1997. Summary report of an International workshop on sector investment Programme (SIP) in Agriculture, GTZ/World Bank

Erhun, W.O., Babalola, O.O., Erhun, M.O. 2001. Drug regulation and control in Nigeria: The challenge of counterfeit drugs. *Journal of Health & Population for Developing Countries* 4(2): 23–34.

Ezeh, I.O., Iheagwam, C.N., Ikeoha, L.L., Obi, F.C., Ezeokonkwo, R.C. 2014. The effects of different storage temperature conditions on the infectivity of *Trypanosoma brucei* in mice in southeastern Nigeria. *Comparative Clinical Pathology* 23: 91–95.

Fetehanegest, D., Berhanu, A., Fentahun, T. and Chanie, M. 2012. Occurrence of bovine trypanosomosis, in the Blue Nile river basin, Northwest Ethiopia. *European Journal of Applied Sciences* 4: 129-135

Foil, L. 1989. Tabanids as vectors of disease agents. *Parasitology Today* 5: 88–96.

Food and Agriculture Organisation of the United Nations (FAO) 2008. A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis.

Food and Agriculture Organisation of the United Nations (FAO) 2014. FAOSTAT: Statistics Division. Production / Live animals Nigeria. http://faostat3.fao.org/browse/Q/QA/E. Accessed 2017.

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

Geerts, S., Holmes, P.H. 1998. Drug management and parasite resistance in bovine trypanosomosis in Africa. PAAT Technical Scientific Series 1. FAO.

Holmes, P.H., Eisler, M.C., Geerts, S. 2004. Current chemotherapy of animal trypanosomosis. In: I. Maudlin, P.H. Holmes and M.A. Miles, Editors, The Trypanosomosis, pp. 431–444. CABI Publishing, Wallingford. England.

Holt, H.R., Selny, R., Mumba, C., Napier, G.B. and Guitian, J. 2016. Assessment of animal African trypanosomiais (AAT) vulnerability in cattle-owing communities of sub-Saharan Africa. *Parasites and Vectors* 9: 53

Hoppenheit, A., Murugaiyan, J., Bauer, B., Clausen, P-H., Roesler, U. 2014. Analysis of *Glossina palpalis gambiensis* and *Glossina tachinoides* from two distant locations in Burkina Faso using MALDI TOF MS. *Parasitology Research* 113: 723–726.

Ijagbone, I.F., Esuruoso, G.O., Agbede, S.A. 2004. Comparative application of antigen detection Enzyme-linked immunosorbent assay and buffy coat parasitological technique for diagnosis of bovine trypanosomosis in Nigeria. *Nigeria Veterinary Journal* 25:13–18.

Ikede, B.O., Reynolds, L., Ogunsanmi, A.O., Fawumi, M.K., Ekwuruke, J.O., Taiwo, V.O. 1987. The epizootiology of bovine trypanosomosis in the derived savannah zone of Nigeria: a preliminary report. In: 19th meeting of the *International Science Council for Trypanosomiasis Research. & Control* Lome, Togo.

Ilemobade, A.A. 1979. Drug sensitivity of mouse –infective *Trypanosoma vivax* isolates in cattle and sheep. International Scientific Council for Trypanosomosis Research and control (ISCTRC), pp. 251-253. 16th Meeting, Yaounde, Cameroon.

Isaac, C., Ciosi, M., Hamilton, A., Scullion, K.M., Dede, P., Igbinosa, I.B., Nmorsi, O.P. G., Masiga, D., Turner, C.M.R. 2016. Molecular identification of different trypanosome species and subspecies in tsetse flies of northern Nigeria. *Parasites and Vectors* 9: 301.

Jordan, A.M. 1986. Trypanosomosis control and African rural development. pp 357 Longman, London.

Jordan, A.M. 1993. Tsetse-flies (Glossinidae). In R.P. Lane and R.W. Crosskey (Eds.), Medical Insects and Arachnids, pp: 333 – 338. London: Chapman and Hall.

Kalu, A.U. 1996. Current status of tsetse fly and animal trypanosomosis on the Jos Plateau, Nigera. *Preventive Veterinary Medicine* 27: 107–113.

Karshima, S.N., Ajogi, I., Lawal, A.I., Mohammed, G., Okubanjo, O.O. 2012. Detection of *Trypanosoma brucei gambiense* specific LiTat 1.3 antibodies in humans and cattle in Taraba State, North-Eastern Nigeria. *Journal of Veterinary Advances* 2(12): 580–585.

Kaufmann, J., Dwinger, R.H., Hallebeek, A., van Dijk, B., Pfister, K. 1992. The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in trypanotolerant N'dama cattle. *Veterinary Parasitology* 43: 157–170.

Kernaghan, R.J. and Johnston, M.R.L. 1962. A method of determining insecticide persistence in tsetse fly control operations. *Bulletin of the World Health Organisation* 26: 139–141.

Kilgour, V. and Godfrey, D.G. 1978. The influence of lorry transport on the *Trypanosoma vivax* infection rate in Nigerian trade cattle. *Tropical Animal Health & Production* 10: 145–148.

King, H.H. 1910. Some observations on the bionomics of *Tabanus par,* Walker, and *Tabanus taeniola,* Pal. de Beauv. *Bulletin of Entomological Research* 1(2): 99–105

Kingsley, P. 2015. Inscrutable medicines and marginal markets: tackling substandard veterinary drugs in Nigeria. *Pastoralism: Research, Policy & Practice* 5: 2.

Lane, R.S. 1976. Density and diversity of immature *Tabanidae* (Diptera) in relation to habitat type in Mendocino County, California. *Journal of Medical Entomology* 12: 683–691.

Leak, S.G.A. 1998. Tsetse biology and ecology: their role in the epidemiology and control of trypanosomosis. Published in association with the International Livestock Research institute. CABI Publishing, CABI International, Wallingford: United Kingdom.

Lehane, M.J. 2005. Biology of blood-sucking in insects, second edition, pp 321. Cambridge University Press, United Kingdom.

Lessard, B.D., Cameron, S.L., Bayless, K.M., Wiegmann, B.M., Yeates, D.K. 2013. The evolution and biogeography of the austral horse fly tribe Scionini (Diptera: Tabanidae: Pangoniinae) inferred from multiple mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 68: 516–540.

Leta, S., Alemayehu, G., Seyoum, Z. and Bezie, M. 2016. Prevalence of bovine trypanosomosis in Ethiopia: a meta-analysis. *Parasites and Vectors* 9: 139

Lopez, M.A., Saada, E.A. and Hill, K.L. 2015. Insect stage-specific adenylate cyclases regulate social motility in African trypanosomes. *Eukaryotic Cell* 14: 104-112.

Lysyk, T.J. 1995. Temperature and population density effects on feeding activity of *Stomoxys calcitrans* (Diptera: Muscidae) on cattle. *Journal of Medical Entomology* 32: 508–514.

Machila, N., Fèvre, E.M., Maudlin, I., Eisler, M.C. 2008. Farmer estimation of live bodyweight of cattle: Implications for veterinary drug dosing in East Africa. *Preventive Veterinary Medicine* 87(3): 394–403.

Machila, N., Wanyangu, S.W., McDermott, J., Welburn, S.C., Maudlin, I. and Eisler, M.C. 2003. Cattle owners' perceptions of African bovine trypanosomosis and its control in Busia and Kwale districts of Kenya. *Acta Tropica* 86:25–34.

MacLennan, K.J.R. 1956. The Nigerian Tsetse and Trypanosomosis Unit, Annual Report, 1955/1956

Majekodunmi, A.O., Fajinmi, A., Dongkum, C., Picozzi, K., MacLeod, E., Thrusfield, M.V., Shaw, A.P.M., Welburn, S.C. 2013a. Social factors affecting seasonal variation in bovine trypanosomosis on the Jos Plateau, Nigeria. *Parasites & Vectors* 6: 293.

Majekodunmi, A.O., Fajinmi, A., Dongkum, C., Picozzi, K., Thrusfield, M.V., Welburn, S.C. 2013b. A longitudinal survey of African animal trypanosomosis in domestic cattle on the Jos Plateau, Nigeria: prevalence, distribution and risk factor. *Parasites & Vectors* 6: 239.

Majekodunmi, A.O., Fajinmi, A., Dongkum, C., Shaw, A.P.M., Welburn, S.C. 2014. Pastoral livelihoods of the Fulani on the Jos Plateau of Nigeria. *Pastoralism* 4: 20.

Mamoudou, A., Njanloga, A., Hayatou, A., Suh, P.F., Achukwi, M.D. 2016. Animal trypanosomosis in clinically healthy cattle of north Cameroon: epidemiological implications. *Parasites & Vectors* 9: 206.

Masiga, D.K., Tait, A., Turner C.M.R. 2000. Amplified restriction fragment length polymorphism in parasite genetics. *Parasitology Today* 16: 350–353.

Masmeatathip, R., Gilles, J., Ketavan, C., Duvallet, G. 2006. First survey of seasonal abundance and daily activity of Stomoxys s pp. (Diptera: Muscidae) in Kamphaengsaen campus, Nakornpathom Province Thailand. *Parasite* 13: 245–250.

Matovu, E., Iten, M., Enyaru, J.C.K., Schmid, C., Lubega, G.W., Brun, R., Kaminsky, R. 1997. Susceptibility of Ugandan *Trypanosoma brucei rhodesiense* isolated from man and animal reservoirs to diminazene, isometamidium and melarsoprol. *Tropical Medicine & International Health* 2: 13–18.

Matthew, C. Z. 2007. Biological and Molecular Aspects of *Sodalis glossinidius*, PhD Thesis. The University of Edinburgh, College of Medicine and Veterinary Medicine; 2007.

Maudlin, I., Holmes, P.H., Miles, M.A. 2004. Trypanosomes. In: The trypanosomiases. CABI International Wallingford, UK: 2004 p. 1-25.

Mavoungou, J.F., Picard, N., Kohagne, L.T., Batchi, B.M., Gilles, J., Duvallet, G. 2012. Spatio-temporal variation of biting flies, *Stomoxys* spp. (Diptera: Muscidae), along a man-made disturbance gradient, from primary forest to the city of Makokou (North-East, Gabon). *Medical and Veterinary Entomology* 27: 339–345.

McNamara, J.J., Mohammed, G., Gibson, W.C. 1994. *Trypanosoma* (Nannomonas) *godfeyi* sp. nov. from tsetse flies in The Gambia: biological and biochemical characterization. *Parasitology* 109: 497–509.

Mekata, H., Konnai, S., Simuunza, M., Chembensofu, M., Kano, R., Witola, W.H., Tembo, M.E., Chitambo, H., Inoue, N., Onuma, M., Ohashi, K. 2008. Prevalence and source of

trypanosome infections in field captured vector flies (*Glossina pallidipes*) in south eastern Zambia. *Journal of Veterinary Medical Science* 70: 923-928.

Mihok, S. 2002. The development of a multipurpose trap (the Nzi) for tsetse and other biting flies. *Bulletin of Entomological Research* 92: 385–403.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., Group, T.P. 2010. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *International Journal of Surgery* 8: 336–341.

Moloo, S.K. and Gray, M.A. 1989. New observations on cyclical development of *Trypanosoma vivax* in *Glossina*. *Acta Tropica* 46: 167–172.

Moloo, S.K., Olubayo, R.O., Kabata, J.M., Okumu, I.O. 1992. A comparison of African buffalo, N'dama and Boran cattle as reservoirs of *Trypanosoma congolense* for different *Glossina* species. *Medical Veterinary Entomology* 6(3): 225–230.

Morita, S.I., Bayless, K.M., Yeates, D.K., Wiegmann, B.M. 2016. Molecular phylogeny of the horse flies: a framework for renewing tabanid taxonomy. *Systematic Entomology* 41: 56–72.

Mullens, G. 2002. Horse flies and deer flies (Tabanidae). In: Mullen, G., Durden, L. (Eds.), *Medical and Veterinary Entomology*. Academic Press, San Diego, pp. 263–277.

Murilla, G.A., Peregrine, A.S., Ndungu, J.M., Holmes, P., Eisler, M. 2002. The effects of drug-sensitive and drug-resistant *Trypanosoma congolense* infections on the pharmacokinetics of homidium in Boran cattle. *Acta Tropica* 81(3): 185–195.

Muturi, C.N., Ouma, J.O., Malele, I.I., Ngure, R.M., Rutto, J.J., Mithofer, K.M., Enyaru, J., Masiga, D.K. 2011. Tracking the feeding patterns of tsetse flies (*Glossina* genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS One* 6: e17284.

Muzari, M.O., Burgess, G.W., Skerratt, L.F., Jones, R.E., Duran, T.l. 2010a. Host preferences of *Tabanid* flies based on identification of bloodmeals by ELISA. *Veterinary Parasitology* 174: 191–198.

Muzari, M.O., Skerratt, L.F., Jones, R.E., Duran, T.I. 2010b. Alighting and feeding behaviour of tabanids on horses, kangaroos and pigs. *Veterinary Parasitology* 170: 104–111.

Mwandiringana, E., Gori, E., Nyengerai, T., Chidzwondo F. 2012. Polymerase chain reaction (PCR) detection of mixed trypanosome infection and bloodmeal origin in field-captured tsetse flies from Zambia. *African Journal of Biotechnology* 11: 14490 - 97.

Nigerian Institute for Trypanosomosis Research (NITR) 1983. Annual report of Nigerian institute for Trypanosomosis Research, pp 177, Nigeria.

Njiokou, F., Nimpaye, H., Simo, G., Njitchouang, G.R., Asonganyi, T., Cuny, G., Herder, S. 2010. Domestic animals as potential reservoir hosts of *Trypanosoma brucei gambiense* in sleeping sickness foci in Cameroon. *Parasite* 17(1): 61–66.

Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C.A., Davila, A.M.R. 2005. The use of ITS1 rDNA PCR in detecting African trypanosomes. *Parasitology Research* 95: 186–192.

Oberholzer, M., Lopez, M.A., McLelland, B.T. and Hill, K.L. 2010. Social motility in African trypanosomes. *PLoS Pathogens* 6: e1000739.

Oberholzer, M., Saada, E.A. and Hill, K.L. 2015. Cyclic AMP regulates social behaviour in African trypanosomes. *mBio* 6: e01954.

Ode, S., Adamu, M., Taioe, M., Thekisoe, O., Adamu, S., Saror, D. 2017. Molecular occurence of trypanosomes, erythrocyte and serum sialic acid concentrations of Muturu and Bunaji cattle in Benue state, Nigeria. *Veterinary Parasitology* 242: 10–13.

Office International des Epizooties (OIE) 2008. Standardized techniques for the diagnosis of tsetse transmitted trypanosomosis. Terrestrial Manual, OIE, Rome.

Office International des Epizooties (OIE) 2013. Office International des Epizooties. Trypanosomosis (tsetse fly transmitted). Terrestial manuscript.

Oluwafemi, R.A., Ilemobade, A.A., Laseinde, E.A.O. 2007. The impact of African animal trypanosomosis and tsetse on the livelihood and wellbeing of cattle and their owners in the BICOT study area of Nigeria. *Science Research Essay* 2(9): 380–383

Omoogun, G.A. 1994. The design and construction of the Nitse trap. *Insect Science and its Application* 15: 535–539.

Onyiah, J.A. 1997. African animal trypanosomosis: an overview of current status in Nigeria. *Tropica Veterinarian* 15: 111–116.

Onyiah, J.A., Naisa, B.K., Riodan, K., Gregory, W. 1983. Tsetse distribution in Nigeria. *Bulletin of Animal Health & Production in Africa* 3(2): 141–150.

Opasina, B.A. and Ekwuruke, J.O. 1987. Trypanosomosis in Nigerian trade cattle. *Tropical Animal Health & Production* 19: 251–252.

Pape, T. and Evenhuis, N.L. 2013. Systema Dipterorum, Version1.5. http://www.diptera.org/

Peacock, L., Cook, S., Ferris, V., Bailey, M., Gibson, W. 2012. The life cycle of *Trypanosoma* (Nannomonas) *congolense* in the tsetse fly. *Parasites & Vectors* 5: 109.

Picozzi, K., Tilley, A., Fevre, E.M., Coleman, P.G., Magona, J.W., Oditt, M., Eisler, M.C., Welburn, S.C. 2002. The diagnosis of trypanosome infections: applications of novel technology for reducing disease risk. *African Journal Biotechology* 1(2): 39–45.

Putt, S.N.H and Shaw, A.P.M. 1982. The socio-economic effects of the control of tsetse transmitted trypanosomosis in Nigeria. Proceedings of the 3rd International Symposium on Veterinary Epidemiology and Economics. Available at www.sciquest.org.nz

Radwanska, M., Claes, F., Magez, S., Magnus, E., Perez-Morga, D., Pays, E., Buscher, P. 2002. Novel primer sequences for polymerase chain reaction-based detection of Trypanosoma brucei gambiense. *American Journal of Tropical Medicine and Hygiene* 67: 289–295.

Reichard, R.E. 2002. Area-wide biological control of disease vectors and agents affecting wildlife. *Revue scientifique et technique (International Office of Epizootics)* 21(1): 179–185.

Reid, R.S., Kruska, R.L., Deichmann, U., Thornton, P.K., Leak, S.G.A. 2000. Human population growth and the extinction of the tsetse fly. *Agriculture, Ecosystems and Environment* 77: 227–236.

Roskov, Y., Kunze, T., Paglinawan, L., Abucay, L., Orrell, T., Nicolson, D., Culham, A., Bailly, N., Kirk, P., Bourgoin, T., Baillargeon, G., Decock, W., De Wever, A., Didz ˇiulis, V. 2013. Species 2000 and ITIS Catalogue of Life, 10th December 2013. Species 2000, Naturalis, Leiden, The Netherlands. Digital resource at www.catalogueoflife.org/col

Salim, B., Bakheit, M.A., Kamau, J., Nakamura, I., Sugimoto, C. 2011. Molecular epidemiology of camel trypanosomosis based on ITS1 rDNA and RoTat 1.2 VSG gene in the Sudan. *Parasites & Vectors* 4: 31.

Sam-Wobo, S.O., Igenezoa, A.J., Idowu, O.A., Otesile, E.B., Ekpo, U.F., Kehinde, O.O. 2010. Bovine trypanosomosis and its impact on cattle in derived savanna areas of Ogun state, Nigeria. *Journal of Public Health Epidemiology* 1(3): 43–47.

Schofield, S. and Torr, S.J. 2002. Feeding behaviour of *Stomoxys* and tsetse. *Medical and Veterinary Entomology* 16: 177–185.

Seyoum, Z., Terefe, G., Ashenafi, H. 2013. Farmers' perception of impacts of bovine trypanosomosis and tsetse fly in selected districts in Baro-Akobo and Gojeb river basins, Southwestern Ethiopia. *Veterinary Research* 9: 214

Shaw, A.P.M. 2004. Economics Impacts of African Trypanosomosis. In: Trypanosomiasis. Edited by Maudlin, *et al.*, Walling ford, UK: CAB International Publishing, pp: 369–402.

Silvester, E., Young, J., Ivens, A. and Matthews, K.R. 2017. Interspecies quorum sensing in co-infections can manipulate trypanosome transmission potential. *Nature Micriobiology* 2: 1471-1479.

Simo, G., Asonganyi, T., Nkinin, S.W., Njiokou, F., Herder, S. 2006. High prevalence of *Trypanosoma brucei gambiense* group 1 in pigs from the Fontem sleeping sickness focus in Cameroon. *Veterinary Parasiotology* 139(1-3): 57–66.

Sinshaw, A., Abebe, G., Desquesnes, M. and Yoni, W. 2006. Biting flies and Trypanosoma vivax infection in three highland districts bordering Lake Tana, Ethiopia. *Veterinary Parasitology* 142(1-2): 35-46.

Sinyangwe L., Delespaux, V., Brandt, J., Geerts, S., Mubanga, J., Machila, N., Holmes, P.H., Eisler., M.C. 2004. Trypanocidal drug resistance in Eastern province of Zambia, *Veterinary Parasitology* 119: 125–135.

Solano, P., Ravel, S., Bouyer, J., Camara, M., Kagbadouno, M. S., Dyer, N., Gardes, L., Herault, D., Donnelly, M. J. and De Meeus, T. 2009. The population structure of *Glossina palpalis*

gambiensis from Island and continental locations in coastal Guinea. *PLoS Neglected Tropical Diseases* 3(3): e392.

Soumana, I. H., Simo, G., Njiokou, F., Tchicaya, B., Abd-Alla, A. M., Cuny, G., et al. 2013. The bacterial flora of tsetse fly midgut and its effect on trypanosome transmission. *Journal of Invertebrate Pathology* 112 (Suppl.): S89–S93.

Stephen, L. 1986. Trypanosomiasis: A Veterinary Perspective. New York: Pergamon Press, pp 351-420.

Steuber, S., Abdel-Rady, A., Clausen, P-H. 2005. PCR-RFLP analysis: a promising technique for host species identification of bloodmeals from tsetse flies (Diptera: Glossinidae). *Parasitology Research* 97: 247–254.

Sumba, A.L., Mihok, S., Oyieke, F.A. 1998. Mechanical transmission of *Trypanosoma evansi* and *T. congolense* by *Stomoxys niger* and *S. taeniatus* in a laboratory mouse model. *Medical and Veterinary Entomology* 12: 417–422.

Swallow, B.M. 2000. Impacts of trypanosomosis on African agriculture. In: *PAAT Technical and Scientific Series 2*, pp: 52. PAAT Information Service Publication, FAO, Rome.

Swallow, B.M. and Jabbar, M.A. 1994. Cattle breed preferences and breeding practices in southern Nigeria. *International Laboratory for Research on Animal Diseases*

Taioe, M.O., Motloang, M.Y., Namangala, B., Chota, A., Molefe, N.I., Musinguzi, S.P., Suganuma, K., Hayes, P., Tsilo, T.J., Chainey, J., Inoue, N., Thekisoe, O.M.M. 2017. Characterization of tabanid flies (Diptera: Tabanidae) in South Africa and Zambia and detection of protozoan parasites they are harbouring. *Parasitology* 144(9): 1162–1178.

Takeet, M.I., Fagbemi, B.O., De Donato, M., Yakubu, A., Rodulfo, H.E., Peters, S.O., Wheto, M., Imumorin, I.G. 2013. Molecular survey of pathogenic trypanosomes in naturally infected Nigerian Cattle. *Research in Veterinary Science,* 94: 555–561.

Takken, W., Oladunmade, M.A., Dengwat, L., Feldmann, H.U., Onah, J.A., Tenabe, S.O., Hamann, H.J. 1986. The eradication of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae) using traps, insecticide-impregnated targets and the sterile insect technique in central Nigeria. *Bulletin of Entomological Research* 76: 275–286.

Taylor, A.W. 1930. Experiments on the mechanical transmission of West African strains of *Trypanosoma brucei* and *T. gambiense* by *Glossina* and other biting flies. *Transactions of the Royal Society of Tropical Medicine & Hygiene* 24: 289–303.

Teko-Agbo, A. 2008. Quality of veterinary medicinal products in circulation in Cameroun and Senegal. OIE Conference on Veterinary Medicinal Products in Africa, 25-27 March, Paris: OIE. Dakar, Senegal.

Thompson, J.W., Mitchell, M., Rees, R.B., Shereni, W., Schoenfeld, A.H., Wilson, A. 1991. Studies on the efficacy of deltamethrin applied to cattle for the control of tsetse flies (*Glossina spp.*) in southern Africa. *Tropical Animal Health and Production* 23: 221–226.

Torr, S.J. 1994. Responses of tsetse flies (Diptera: Glossinidae) to warthog (*Phacochoerus aethiopicus* Pallas). *Bulletin of Entomological Research* 84: 411–419.

Torr, S.J. and Hargrove, J.W. 1998. Factors effecting the landing and feeding responses of the tsetse fly *Glossina pallidipes* to a stationary ox. *Medical and Veterinary Entomology* 12: 196–207.

Torr, S.J. and Mangwiro, T.N.C. 2000. Interactions between cattle and biting flies: effects on the feeding rate of tsetse. *Medical and Veterinary Entomology* 14: 400–409.

Torr, S.J., Prior, A., Wilson, P.J., Schofield, S. 2007. Is there safety in numbers? The effect of cattle herding on biting risk from tsetse flies. *Medical and Veterinary Entomology* 21: 301–311.

United Nations (UN) 2001. The role of the United Nations in support of the efforts of the African countries to achieve sustainable development. Report of the Secretary General. High level segement, substantive session. 2nd – 27th July 2001, Geneva, Switzerland.

Vale, G.A. 1974. New field methods for studying the responses of tsetse flies (Diptera: Glossinidae) to hosts. *Bulletin of Entomological Research* 64: 199–208.

Vale, G.A. 1977. Feeding responses to tsetse flies (Diptera: Glossinidae) to stationary hosts. *Bulletin of Entomological Research* 67: 635–649.

Vale, G.A., Mutika, G., Lovemore, D.F. 1999. Insecticide-treated cattle for controlling tsetse (Diptera: Glossinidae): some questions answered, many posed. *Bulletin of Entomological Research* 89: 567–577.

Van den Abbeele, J., Claes, Y., Bockstaele, D., Ray, D., Cooseman, M. 1999. *Trypanosoma brucei* spp. development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis. *Parasitology* 118: 469–478.

Veer, V., Parashar, B.D., Prakash, S. 2002. *Tabanid* and muscoid haematophagous flies, vectors of trypanosomosis or surra disease in wild animals and livestock in Nandankanan Biological Park, Bhubaneswar, Orissa, India. *Current Science* 82: 500–503.

Vickerman, K., Myler, P.J., Stuart, K. 1993. African trypanosomiasis. In: "Immunology and molecular biology of parasitic infections," Warren, K.S. (Editor). Blackwell Scientific Publications, Chapter 10, pp 223–227.

Vincendeau, P. and Bouteille, B. 2006. Immunology and immunopathology of African trypanosomosis. Anais da Academia Brasileira de Ciências 78: 645–665.

Votypka, J., Radrova, J., Skalicky, T., Jirku, M., JIrsova, D., Mihalca, A.D., D'Amico, G., Petrzelkova, K.J., Modry, D., Lukes, J. 2015. A tsetse and tabanid fly survey of African great ape habitats reveals the presence of a novel trypanosome lineage but the absence of *Trypanososoma brucei*. *International Journal for Parasitology* 45: 741–748.

Vreysen, M.J., Saleh, K.M., Ali, M.Y., Abdulla, A.M., Zhu, Z., Juma, K.G., Dyck, A., Msangi, A.R., Mkonyi, P.A., Feldmann, A.M. 2000. *Glossina austeni* (Diptera: Glossinidae) eradicated on the Island of Unguga, Zanzibar, using the sterile insect technique. *Journal of Economic Entomology* 93: 123–135.

Vreysen, M.J.B., Seck, M.T., Sall, B., Bouyer, J. 2013. Tsetse flies: Their biology and control using area-wide integrated pest management approaches. *Journal of Invertebrate Pathology* 112: S15–S25.

Wastling, S.L. and Welburn, S.C. 2011. Diagnosis of human sleeping sickness: sense and sensitivity. *Trends in Parasitology* 27: 394–402.

Welburn, S.C., Coleman, P.G., Maudlin, I., Fevre, E.M., Odiit, M. and Eisler, M.C. 2006. Crisis, what crisis? control of rhodesian sleeping sickness. *Trends in Parasitology* 22(3): 123–128.

Yesufu, H.M. and Mshelbwala, A.S. 1973. Trypanosomosis survey in cattle and tsetse flies along a trade cattle route in southern Nigeria. *Annals of Tropical Medicine & Parasitology* 67: 307–312.

Zumpt, F. 1973. The Stomoxyinae biting flies of the world. In: Taxonomy, Biology, Economic Importance and Control Measures, pp: 175. Gustav Fischer Verlag, Stuttgart.

Appendix I: Ethical approval

ANIMAL CARE USE AND RESEARCH ETHICS COMMITTEE (ACUREC)

UNIVERSITY OF IBADAN

08176917269

E.mail: animaluseresearch@gmail.com / animaluseresearch@yahoo.com

Our Ref: ..... Your Ref: .....

Date: .....

U.I. ACUREC/OOS/29-12-2016

29th December, 2016

ADEMOLA, Isaiah Oluwafemi
Department of Veterinary Microbiology and Parasitology,
Faculty of Veterinary Medicine,
University of Ibadan,
Ibadan.

NOTICE OF ETHICAL APPROVAL FOR A RESEARCH PROJECT PROPOSAL

On behalf of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC), I write to grant you an ethical approval to carry out your research project work titled: **"BOVINE TRYPANOSOMOSIS: POPULATION AND TRANSMISSION DYNAMICS OF ITS VECTOR AND CONTROL IN SOUTHWESTERN NIGERIA"**

refers: strictly as outlined in your proposal submitted for assessment.

Please quote **UI-ACUREC/App/12/2016/05** as reference for this approval.

You are to note that **UI-ACUREC** reserves the right to monitor and conduct compliance visit to your research site without previous notification.

Thank you.

Prof. S.I.B. Cadmus
Chairman, UI-ACUREC

NB: The committee reserves the right to revoke this approval if there is non-compliance to the approved proposal concerning ACUREC guidelines

Chairman: Professor S. I. B. Cadmus (DVM, Ph.D)
Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Appendix II: Bovine trypanosomosis prevalence of published articles in Nigeria from 1987-2016.

Author	Study location, north-N; south- S	Animal type(s), cattle- C	Total sampled	Total positive (%)	95%CI	Diagnostic method(s)
Opasina *et al.,* 1987	S	C	3387	289 (8.5)	7.6-9.5	BCM
Joshua *et al.,* 1989	N	C	323	31 (9.6)	6.6-13.3	STDM, MI
Kalu, 1991	N	C	629	243 (38.6)	34.8-42.6	BCM, HCT
Onah, 1991	S	C	150	46 (30.7)	23.4-38.7	BCM, HCT
Daniel *et al.,* 1993	N	C	1065	42 (3.9)	2.9-5.3	BCM, HCT
Fakae *et al.,* 1993	S	C	107	14 (13.1)	7.3-21.0	BCM
Anene *et al.,* 1995	S	C	1995	3 (0.2)	0.0-0.4	BCM
Kalu, 1995	N	C	268	24 (9.0)	5.8-13.0	BCM, HCT
Nawathe, 1995	N	C	1014	8 (0.8)	0.3-1.5	BCM
Kalu, 1996	N	C	55	9 (16.4)	7.8-28.8	BCM, HCT
Kalu *et al.,* 1996	N	C	1106	59 (5.3)	4.1-6.8	BCM, HCT
Ogunsanmi *et al.,* 2000b	S	C	189	51 (27.0)	20.8-33.9	HCT, ELISA
Anosike *et al.,* 2003	N	C	200	76 (38.0)	31.2-45.1	STDM
Abenga *et al.,* 2004	N	C	526	48 (9.1)	6.8-11.9	STDM
Ijagbone *et al.,* 2004	N & S	C	911	202 (22.2)	19.5-25.0	BCM, ELISA
Oluwafemi *et al.,* 2007	N	C	400	39 (9.8)	7.0-13.1	BCM, HCT
Ezeani *et al.,* 2008	S	C	264	49 (18.6)	14.1-23.8	ELISA
Qadeer *et al.,* 2008	N	C	1300	180 (13.8)	12.0-15.8	STDM
Enwezor, 2009	N	C	1293	109 (8.4)	7.0-10.1	STDM
Adama *et al.,* 2010	N	C	300	19 (6.3)	3.9-9.7	STDM
Akande *et al.,* 2010	S	C	200	12 (6.0)	3.1-10.2	STDM
Danbimi *et al.,* 2010	N	C	32	13 (40.6)	23.7-59.4	STDM
Sam-Wobo *et al.,* 2010	S	C	133	42 (31.6)	23.8-40.2	BCM, HCT

Enwezor *et al.*, 2011a	N	C	410	60 (14.6)	11.4-18.4	BCM
Enwezor *et al.*, 2011b	N	C	964	212 (22.0)	19.4-24.7	BCM
Fajinmi *et al.*, 2011	N	C	500	9 (1.8)	0.8-3.4	STDM, PCR
Owai, 2011	S	C	6203	405 (6.5)	5.9-7.2	BCM
Samdi *et al.*, 2011	N	C	634	14 (2.2)	1.2-3.7	STDM
Enwezor *et al.*, 2012	N	C	395	15 (3.8)	2.1-6.2	STDM
Karshima *et al.*, 2012	N	C	400	37 (9.3)	6.6-12.5	CATT
Majekodunmi *et al.*, 2013	N	C	7143	3343 (46.8)	45.6-48.0	PCR
Takeet *et al.*, 2013	N & S	C	411	262 (63.7)	58.9-68.4	STDM, PCR
Zubairu *et al.*, 2013	N	C	240	32 (13.3)	9.3-18.3	BCM
Andrew *et al.*, 2014	N	C	96	15 (15.6)	9.0-24.5	BCM, HCT
Fasanmi *et al.*, 2014	S	C	320	16 (5.0)	2.9-8.0	STDM
Okarafor and Nzeako, 2014	S	C	180	7 (3.9)	1.6-7.8	STDM
Abenga *et al.*, 2015	N	C	214	28 (13.1)	8.9-18.4	BCM, HCT
Obaloto *et al.*, 2015	N	C	448	11 (2.5)	1.2-4.4	BCM, HCT,
Yusuf *et al.*, 2015	N	C	118	11 (9.3)	4.7-16.1	PCR
Abubakar *et al.*, 2016	S	C	133	42 (31.6)	23.8-40.2	STDM
Hassan *et al.*, 2016	N	C	150	80 (53.3)	45.0-61.5	BCM, HCT
Pam *et al.*, 2016	N	C	200	9 (4.5)	2.1-8.4	STDM
Muhammad *et al.*, 2016	N	C	150	39 (26.0)	19.2-33.8	STDM, PCR

Abbreviations: BCM – buffy coat method, HCT – haematocrit concentration technique, STDM – standard trypanosome detection technique, CATT – card agglutination trypanosomosis test, ELISA – enzyme-linked immunosorbent assay and PCR – polymerase chain reaction.

Appendix III: Important variables of bovine trypanosomiasis in southwest Nigeria.

Factor	Indices	Number sampled	Number positive	Prevalence	P-value
Species	*T. vivax*	745	86	11.5*	$X^2 = 441.39$, *df* = 10, α < 0.0001
	T. congolense	745	63	8.5*	
	T. b. brucei	745	36	4.8*	
	T. evansi	745	13	1.7	
	T. vivax/T. b. brucei	745	4	0.5	
	T. vivax/T. congolense	745	6	0.8	
	T. congolense/T. b. brucei	745	4	0.5	
	T. congolense/T. evansi	745	2	0.3	
	T. evansi/ T. b. brucei	745	3	0.4	
	T. vivax/T. congolense/T. evansi	745	1	0.1	
	T. congolense/T. b. brucei/ T. evansi	745	1	0.1	
Sources	Abattoirs	306	83	27.1*	α = 0.350; S.E = 0.257; CI: -1.061 – 0.440
	Fulani cattle farms	74	40	54.1*	
	Institutional farms	163	24	14.7	
	Cattle markets	202	30	14.9	
States	Oyo	210	56	26.7	α = 0.495; S.E = 0.081; CI: -0.234 – 0.112
	Ogun	182	45	24.7	
	Lagos	251	55	21.9	

	Ondo	60	9	15.0	
	Osun	29	10	34.5	
	Ekiti	13	2	15.4	
Breeds	White Fulani	480	119	24.8	α = 0.525; S.E = 0.039; CI: -0.145 – 0.085
	Sokoto Gudali	111	24	21.6	
	Red Bororo	121	30	24.8	
	Ambala	8	0	0.0[a]	
	Kuri	14	3	21.4	
	Muturu	10	0	0.0[a]	
	N'dama	1	1[a]	-	
Body scores	Good	124	12	9.7[*]	α = 0.383; S.E = 0.333; CI: -1.620 – 2.591
	Intermediate	257	72	28.0	
	Poor	364	93	25.5	
Age groups	≤ 1 year	69	13	18.8[*]	α = 0.272; S.E = 0.060; CI: -0.249 – 0.513
	≤ 3 years	289	69	23.9	
	> 3 years	387	95	24.5	
Seasons	Wet	480	128	26.7[*]	α = 0.012, X^2 = 6.30, OR = 1.60 (CI: 1.09 – 2.37)
	Dry	265	49	18.5	

Sex	Male	362	79	21.8	$\alpha = 0.228$, $X^2 = 1.45$, OR = 0.81 (CI: 0.57 – 1.16)
	Female	383	98	25.6	

Symbols: (*) indicates pairwise significance ($\alpha < 0.05$) with Tukey multiple comparison test, ([a]) indicates data not included in analysis because of small numbers observed in sampled population.

Appendix IV: Proportion of trypanocide use/strategy among livestock owners

Drug used by cattle keepers	Treatment regimen per season	Dosage plan and administration	Proportions and 95% CI
Diminazene aceturate (DA) only	1. Once- 14% 2. Twice- 78% 3. > Twice- 8%	10g + water = 15 mls of drug/ animal. Drug given intramuscular (i/m).	55% (95%CI: 46.4 –63.4%)
Isometamidium chloride (IC) only	1. Once- 19% 2. Twice- 74% 3. > Twice- 7%	10 g IC reconstituted with water to form 50 ml of drug given 10 mls (i/m) per cattle.	21% (95%CI: 14.8 – 28.7%)
DA + IC	1. Once- 44% 2. Twice- 56%	10 g DA + 10 g IC, reconstituted with water to form 100 ml of drug. Cattle is given 10 mls (i/m).	7% (95%CI: 3.7 – 12.7%)
DA + Antibiotics	1. Once- 64% 2. Twice- 18% 3. > Twice- 18%	10 g of DA with water to form 15 mls + 20 mls of antibiotics given i/m.	8.5% (95%CI: 4.8 – 14.6%)
DA + Antibiotics + Vitamins	1. Once- 14% 2. Twice- 57% 3. > Twice- 29%	10 g of DA reconstitute to form 15 mls + 20 mls of antibiotics + 10 mls of vitamins given to cattle i/m.	5.4% (95%CI: 2.7 – 1.1)
Antibiotics	1. Twice- 25%	20 mls of antibiotics given i/m.	3.1% (CI: 1.2 – 7.7%)

2. > Twice- 75%	
Total	100%

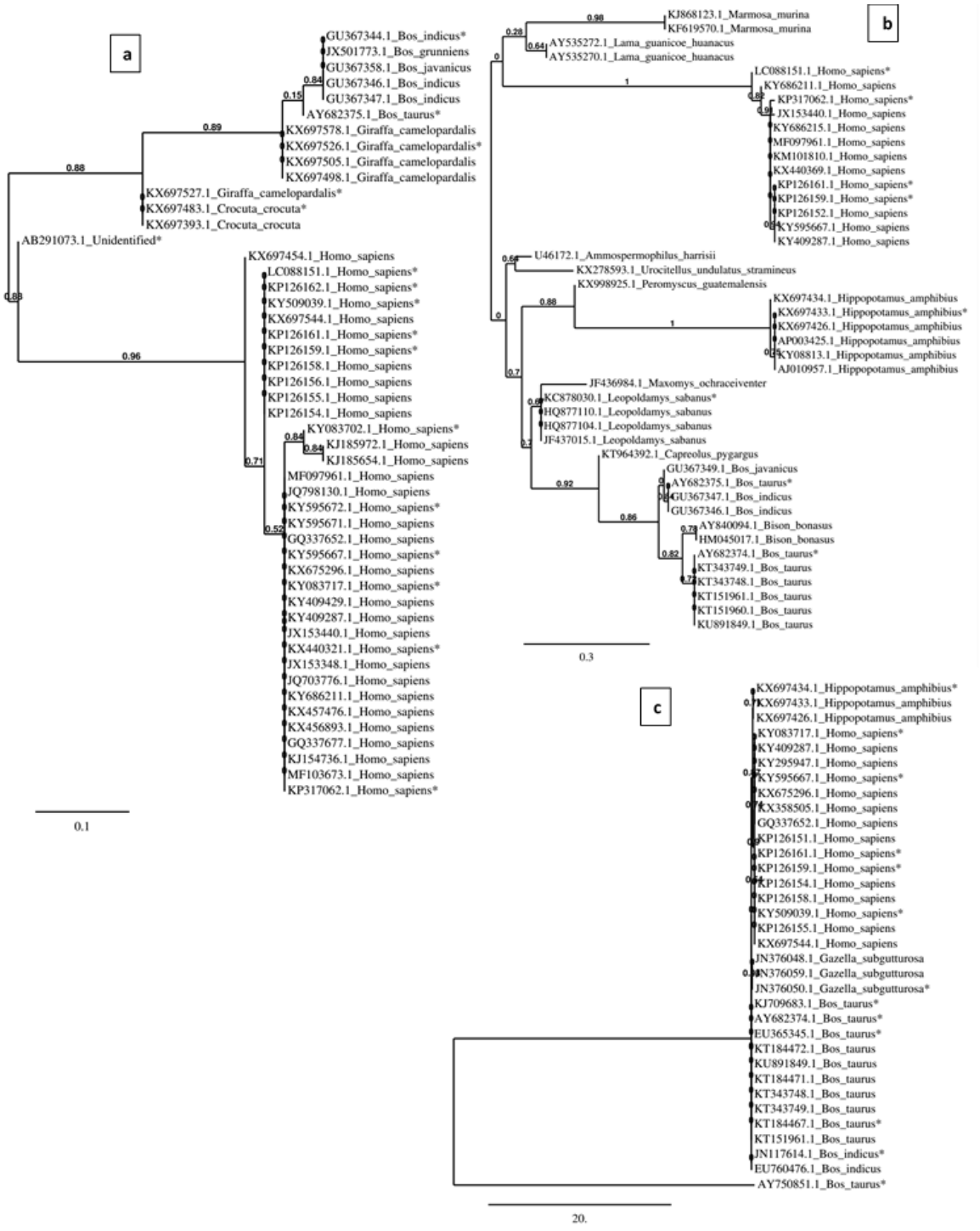
*estimate based on 97.0% that make use of synthetic drugs against trypanosomosis. CI-Confidence interval.

Appendix V: Landing, biting and feeding durations on cow, bull and cattle.

Bovine host	Fly genera	Vector fly species	Landing	Biting	Engorged	Engorgement success (%)	Median feeding durations (s)	Feeding duration ranges (s)
Cow	*Tabanus*	*T. taeniola*	58	39	13	33.3	125	13 – 201
		T. subangustus	72	42	8	19.0	97	11 – 254
		T. biguttatus	32	21	1	4.8	55	5 – 103
		T. gratus	31	19	3	15.8	100	24 – 173
		T. pertinens	7	3	1	33.3	79	22 – 112
		T. par	19	11	2	18.2	26	12 – 207
		T. thoracinus	29	22	9	40.9	81	51 – 101
		T. brucei	22	10	0	0.0	44	7 – 59
		T. rubidus	4	2	0	0.0	12	5 – 19
	Glossina	*G. palpalis*	16	11	7	63.6	133	101 – 197
		G. tachinoides	5	4	3	75.0	97	81 – 131
	Stomoxys	*Stomoxys* spp.	2155	983	94	9.6	54	1 – 81
Bulls	*Tabanus*	*T. subangustus*	69	48	9	18.8	101	21 – 229
		T. gratus	23	15	2	13.3	83	8 – 173
		T. taeniola	57	41	11	26.8	161	30 – 267
		T. brucei	29	18	2	11.1	111	17 – 151
		T. pertinens	17	10	0	0.0	73	7 – 91
		T. thoracinus	18	12	1	8.3	127	10 – 163
	Glossina	*G. palpalis*	12	9	9	100.0	151	144 – 205
		G. tachinoides	15	10	7	70.0	108	94 – 131

	Stomoxys	*Stomoxys* spp.	1836	901	101	11.2	49	3 – 68
Calves	*Tabanus*	*T. par*	8	2	0	0.0	32	3 – 60
		T. thoracinus	9	1	0	0.0	29	29
		T. subangustus	4	1	0	0.0	86	86
		T. taeniola	16	5	1	20.0	74	22 – 109
	Glossina	*G. palpalis*	5	2	2	100.0	123	116 – 129
		G. tachinoides	2	0	0	0.0	0	0
	Stomoxys	*Stomoxys* spp.	817	106	7	6.6	22	1 – 33

Appendix VI: Phylogenetics of vertebrate hosts found in vectors bloodmeal. a. *Glossina* b. *Tabanus* c. stomoxyine



Appendix VII: PRIMSA CHECKLIST

Meta-analysis

Abstract: This is well-structured and it includes: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings.

Introduction: The rationale for the analysis in the context of what is already known has been clearly stated, and an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design.

Methods: Eligibility criteria includes study characteristics and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. All the information sources have been described (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. Electronic search strategies of all database have been provided in supplementary file one including the limits used (1960 - 2017), such that it could be repeated. A figure has been presented inform of a flow chart which illustrates the selection criteria (screening, eligibility, inclusion). Data extraction process, assessment of bias, summary measures and measure of consistency were all included. All additional method of analysis (sub-group) have been clearly stated.

Results: Numbers of study screened, assessed for eligibility and included in the review was clearly stated. For each study, characteristics for which data were extracted (e.g., study size) have been clearly stated with necessary citations. Results of individual study have been presented. complete meta-analysis result was presented with all additional analysis.

Discussion: Findings have been properly summarised with strength of evidence for main outcome. The relevance of the report was also discussed with limitations.

Conclusion: General interpretation of the results in the context of other evidence, and implications for future research concludes the study report.

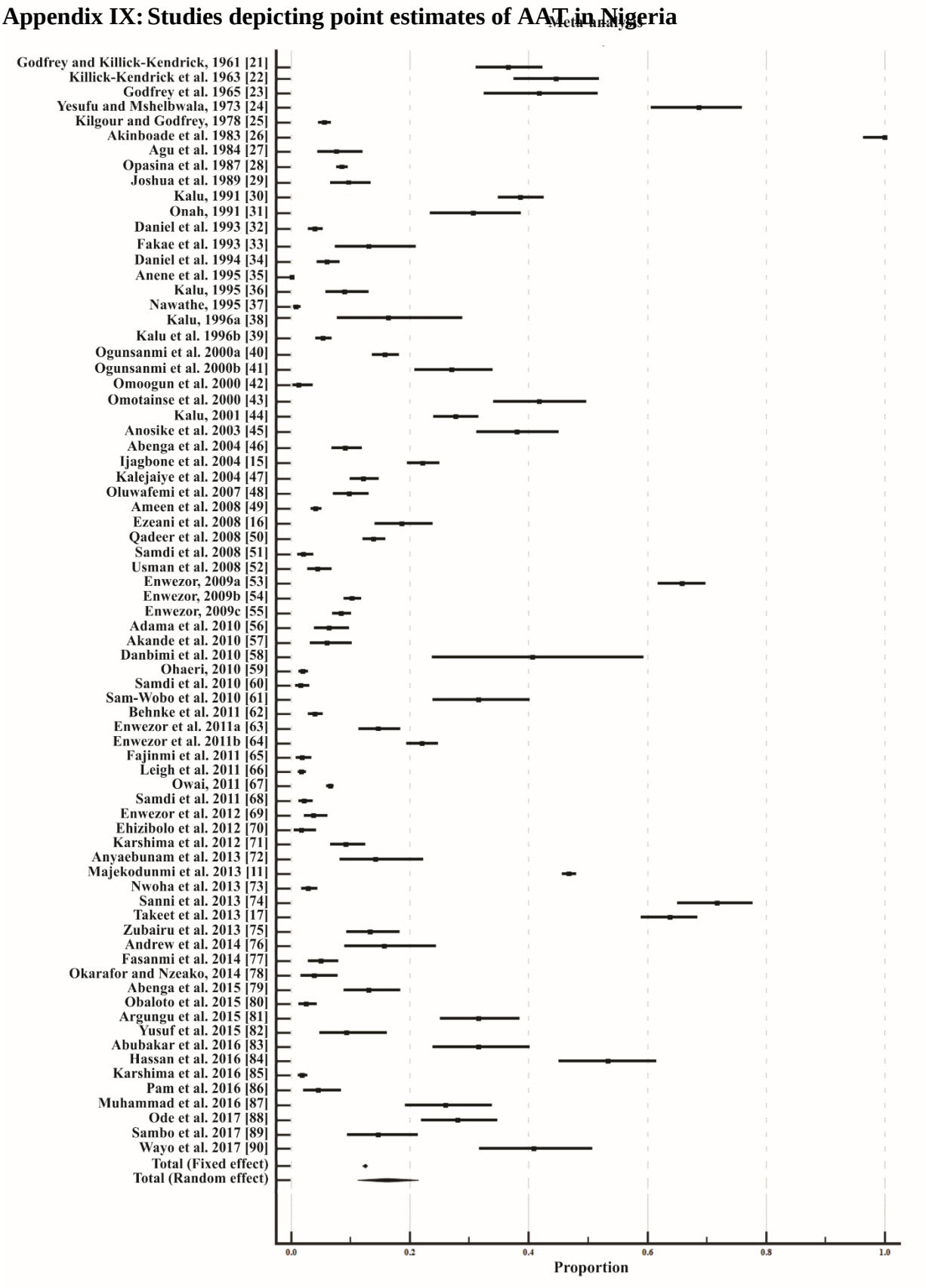
Funding: funding bodies were properly acknowledged.

Appendix VIII: Questionnaire

BOX 4. QUESTIONNAIRE

Name of Respondent_____
Location _____
Profession (Trader, Herdsman, Farmer, Butcher)
How were your animals transported to southwest Nigeria?
_____
Where are your animals from (source)?
_____
Which breed(s) of animal do you rear?
_____
What type of practices? (Zero grazing, nomadism, mixed farming, pastoralism)
How many unskilled labours do you have?
_____
Do extreme weather conditions affect your animals (Yes/No)?
If yes, how _____
When do your animals set out to graze/feed in the case of zero grazing?
_____
How many animals do you have? _____
List the types of animals you rear/trade? (Cattle, Sheep, Goat)
How much do you purchase your animals from source? (only for traders)_____
How many do you sell per annum? _____
Mention the diseases that affect your cattle.
_____
Have you heard of bovine trypanosomosis? (Yes/No)
How do you treat trypanosomosis?
_____
How many of your animals do you lose in transit as a result of trypanosomosis?
_____
How many cattle do you lose to trypanosomosis after arrival?
_____
What do you think is responsible for the transmission of trypanosomosis?
_____
Mention the drugs used to treat trypanosomosis.
_____
How many times do you repeat the drug used?
_____
How much do you spend on treating bovine trypanosomosis/animal per annum?
_____
If the disease symptoms persist, what do you do?
_____
How does it affect the value of the animal?
_____
How often do you request for the services of a veterinarian?
_____
How often do you encounter trypanosomosis?
_____
Which season/month of the year is the prevalence high?
_____
What are the signs/symptoms of bovine trypanosomosis noticed before you commence treatment?
_____
Do you see tsetse/biting flies as threat? (Yes/No)
Do you know they are responsible for the transmission of trypanosomosis? (Yes/No)
When do you encounter tsetse/biting flies most? (rainy, dry, late dry, early rain).
How do you prevent your animals from flies?
_____
Which drug do you use to combat the flies?
_____
How often is it applied?
_____
Do you practice transhumance? (Yes/No)
Why do you engage in migration practices?
_____
How often do you have access to water in dry season? (Frequently, Rarely, Difficult)
How often do you have access to grass in dry season? (Frequently, Rarely, Difficult)
Mortality rate in the course of transhumance
_____
What are the difficulties encountered during transhumance?
_____
Is there any established government intervention programme? (Yes/No)
If yes, mention _____
_____
If No, will you be willing to join, assist and participate in the intervention programme?
_____
What type of free service(s) have you enjoyed in preventing your animals from trypanosomosis?
_____
Any other information
_____

Appendix IX: Studies depicting point estimates of AAT in Nigeria



Appendix X: Nzi trap setting on Akingbite cattle farm

Appendix XI: Onyaerugbulem cattle abattoir in Ondo State

Appendix XII: Livestock owners during interviewing phase.