The prevalence of trypanosome infection in tsetse flies at Rekomichi Research Station, Mana Pools, Zimbabwe.

By

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DECLARATION

I, Leocadia Munyoro, hereby declare that the research project presented here is entirely my original work. I have duly acknowledged all materials and academic sources of information used in this work. Furthermore, this project has not been submitted to any other academic institution for any academic merit.

Signature: L. Munyoro

Date:06/06/2024

DEDICATION

I dedicate this work to my future self, may it serve as a testament to the power of determination, the value of critical thinking, and the transformative potential of scholarly inquiry.

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APPROVAL FORM

The undersigned certify that they have read the dissertation and it is suitable for submission to the Faculty of Science and Engineering and checked for conformity with the faculty, in the partial fulfillment of the requirements for the Bachelor of Science Honors Degree in Biosciences

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Supervisor:  **Date: 05/06/24**

Chairperson:  **Date: 08/10/24**

ABSTRACT

This study investigated prevalence of trypanosome infection in tsetse flies at Rekomechi Research Station, Mana Pools, Zimbabwe, during the period 2022-2023. Tsetse are the primary vectors of trypanosomes, responsible for African Animal Trypanosomiasis (AAT) in animals and Human African Trypanosomiasis (HAT) in humans. The study aimed to determine the prevalence of various trypanosome species across different age categories of tsetse and to evaluate the relationship between fly age and infection status. Fly collection was done using Epsilon traps, and dissections of the proboscis, salivary glands, and midgut were used to identify trypanosome infection. Wing fray analysis and ovarian dissection were employed to determine the age of the flies.

A total of 3,150 flies were dissected, with 5.78% testing positive for trypanosome infection. *Trypanosoma vivax* showed the highest prevalence, particularly in older flies, with infection rates of 1.20% in ovarian category 5. *T. congolense* was the second most common trypanosome, with an overall infection rate of 0.33%. *T. brucei*, the least prevalent species, was detected in only 0.05% of the flies, with the highest infection rates found in older age categories based on wing fray and ovarian analysis. The study found a significant correlation between fly age and infection rates, with older tsetse flies being more susceptible to infection. Statistical analysis confirmed significant differences in infection rates among the various age groups, with flies in wing fray categories 4 and 5 showing the highest prevalence of infection.

Results emphasize the need for continued surveillance, particularly in older fly populations, and the potential for improved molecular diagnostic techniques to better monitor infection dynamics. This study provides crucial insights into the epidemiology of trypanosomiasis in Zimbabwe, highlighting the importance of targeted control measures for managing disease transmission.

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1. INTRODUCTION

1.1 Background of the study

Tsetse flies (*Glossina* spp.) are the prominent vector of African trypanosome protozoan endoparasites (*Trypanosoma* spp.) (Bateta, 2017). Tsetse flies (Diptera: Glossinidae) are found in Sub tropical Africa. Tsetse are vectors of protozoan parasites that feeds exclusively on blood. The most common tsetse species include *Glossina morsitans morsitans, Glossina pallidipes, Glossina brevipalpis,* and *Glossina fuscipes* (Kabaka et al., 2020). They transmit trypanosomes that cause Animal African Trypanosomiasis (AAT) in animals and Human African Trypanosomiasis (HAT) in humans, also known as sleeping sickness in humans and Nagana in animals (Shereni et.al., 2016). In particular, tsetse distribution in Zimbabwe has always been restricted to the north, northwest, northeast and southeast of the country with the central highveld being ecologically unsuitable for the fly (Shereni et.al., 2021). Currently, only two species of tsetse, *Glossina morsitans morsitans* (Gm) and *G. pallidipes* (Gp), are reported to occur in Zimbabwe (Shereni et.al., 2021). Each species has specific ecological preferences and may exhibit variations in infection rates and susceptibility to different trypanosome species. *Trypanosoma brucei, Trypanosoma congolense, and Trypanosoma vivax* are the primary trypanosome species identified in tsetse flies in Zimbabwe (Roditii & Lehane, 2008). *T brucei* is responsible for HAT while *T. congolense and T. vivax* cause (AAT). Trypanosomiasis affects the economy of Zimbabwe by reducing the productivity of livestock, human labour, and animal draught power. Adult male tsetse flies may live for two to three weeks while females can live for 1 to 4 months. The prevalence of trypanosome infection in tsetse flies varies across different regions and tsetse species in Zimbabwe. Studies have reported infection rates ranging from 5% to 40% in various tsetse populations. Factors

such as the availability of suitable hosts, vegetation cover, temperature, humidity, and proximity to water bodies influence the prevalence rates. Several factors contribute to the prevalence of trypanosome infection in tsetse flies. Vegetation cover, such as woodlands or riverine forests, provides suitable habitats for tsetse populations and influences their abundance. Temperature and humidity affect tsetse fly survival and activity (Shereni., 2016). Proximity to water bodies, such as rivers or lakes, is crucial for tsetse fly reproduction and larval development. Livestock movement patterns and host availability also impact trypanosome transmission dynamics. To combat trypanosomiasis, Zimbabwe has implemented various control measures. These include the use of insecticide-treated traps, insecticide-treated livestock (e.g., cattle ear tags), and the sterile insect technique. Insecticide-treated traps attract and kill tsetse flies, reducing their population density. Insecticide-treated livestock reduces the risk of tsetse bites and trypanosome transmission. The sterile insect technique involves the release of sterile male tsetse flies to reduce the overall reproductive capacity of tsetse populations. Despite efforts to control trypanosomiasis, several challenges persist. Limited resources, inadequate surveillance systems, and the emergence of drug-resistant trypanosome strains hinder effective control (Morrison et.al., 2024; Krafsur and Maudlin.,2018). Socio-economic factors, such as poverty and limited access to healthcare, also contribute to the persistence of the disease. Future research should focus on improving surveillance systems, studying the epidemiology and transmission dynamics of trypanosomes, and developing sustainable and innovative control strategies (Shereni, 1990). The prevalence of trypanosome infection in tsetse flies in Zimbabwe highlights the ongoing threat of trypanosomiasis to both human and animal health (Shereni et.al., 2016). Understanding the distribution, prevalence, and factors influencing trypanosome infections in tsetse populations is crucial for implementing effective control measures and reducing the burden of the disease (Shereni et.al., 2021).

1.2 Problem Statement

Tsetse-transmitted animal African trypanosomiasis has caused many farmers to move away from highly productive lands and grazing sites to survive in less productive agricultural areas, which has had a negative impact on their livelihoods and agricultural production (Torr et al., 2005). The disease affects livestock productivity and production by reducing meat quality and milk yield. It directly affects human settlement and reduces the productivity of people affected by the disease. Because the disease is spread by the tsetse fly vector, it is important to understand the dynamics of the infection in the flies.

1.3 Justification of the study

Cyclical transmission of trypanosomiasis by tsetse to both humans and animals has significantly impacted food production, natural resource utilization, as people are restricted from certain areas due to tsetse infestation, and resettlement. Trypanosomiasis is currently being eliminated from the continent (WHO, 2022). People in safari camps are being treated for sleeping sickness, but little is known about the prevalence of trypanosome infection in tsetse flies. (ZimParks Official, pers comm). Therefore, there is a constant need to monitor the distribution and prevalence of infection to inform evidence-based control efforts.

1.4 Aim

The aim was to determine the prevalence of different trypanosome species in tsetse in 2023 at Rekomichi Research Station, Mana Pools.

1.5 Objectives

1. To determine the infection rates in various age categories of tsetse caught in 2023 in comparison with 2022.
2. To establish if a relationship exists between fly age and infection status.

1.6 Research Questions

1. Is there a significant difference in the infection rates of flies caught in 2023 compared to 2022?
2. Is there an association/ relationship between age of fly and infection status?

1.7 Hypothesis

- H_0:, There is no significant difference in infection among the various age categories
- H_1: There is a difference in infection among the various age categories

Chapter 2

2. LITERATURE REVIEW

Zimbabwe several tsetse-infested areas mainly to the northern districts in the Zambezi Valley, including Gokwe, Kanyemba, Kariba, Muzarabani, Hurungwe, and Binga districts (Shereni, 2016; Shereni et al., 2021). These regions have a higher risk of trypanosome transmission due to the presence of suitable habitats for tsetse flies and a high density of livestock. Different species of tsetse flies contribute to the transmission of trypanosomes in Zimbabwe.

2.1 Tsetse Flies

One characteristic that sets tsetse apart from other flies is its forward-projecting, skin-piercing proboscis. In addition to biting humans, a wide range of domesticated and wild mammals, some reptiles, and birds, both men and females can transmit parasites. While some tsetse species exhibit host preferences, all species feed on different kinds of hosts (Ackley et.al., 2017). The larvae of tsetse flies hatch from an egg inside the female uterus and feed on a nutritional fluid generated by paired milk glands on the uterine wall. Tsetse flies are larviparous. The next three phases take roughly nine days. The larva is laid out on the ground, where it burrows into the ground and, in one to five hours, pupates. The moment tsetse flies consume blood from an infected host, they may then transmit this to other humans or animals.

2.2 Trypanosomes

Trypanosomes are a collection of flagellated protozoan endoparasites that can infect both humans and animals. Animals and humans should be considered separately because different diseases affect them. Animals are more susceptible to trypanosome infections than humans. Animal African trypanosomiasis (AAT) is caused mainly by three species of trypanosomes, namely Trypanosoma *vivax, T. congolense,* T. *brucei,* while human African trypanosomiasis (HAT) is caused by two sub-species of T. *brucei* i.e., *T.b rhodesiense* and *T. b gambiense. T vivax* is found in the

mouthparts of the fly, *T. congolense* in the midgut and mouthparts and *T brucei* in the mouthparts, midgut and salivary gland (Ngomtcho et al., 2017). It is typically adequate to determine trypanosome infection rates through dissection for most tsetse surveys related to control or eradication programs. However, if necessary, subsamples could be collected for diagnosis using molecular techniques. Whenever possible, PCR could be performed solely on the tsetse fly's mouthparts to quickly estimate mature infections, saving time and money that would have been spent on identifying trypanosomes in the midgut, which is less informative because many trypanosome infections in the guts of tsetse flies do not mature. Assessing trypanosome infection rates in tsetse flies and identifying trypanosome species will help in developing control plans and conducting surveys for tsetse flies.

2.3 Dissection for Trypanosome Infection

Trypanosome infection rates in tsetse can be determined either by dissection and observation of the parasites in organs of the fly (based on which, an imprecise identification of the trypanosome species subgenera can be made), or using molecular biological diagnostic procedures (DNA probes and polymerase chain reaction (PCR)). There are advantages and disadvantages to both methods. The basic dissection method is not precise enough to identify the specific trypanosome species accurately. Inaccuracies can also occur due to mixed infections, immature infections, and the inability to distinguish between certain trypanosome species within the same group, whether they are pathogenic to domestic livestock or humans. For example, *Trypanosoma simiae* is indistinguishable from *T. congolense*, but it primarily parasitizes warthogs and is not harmful to cattle, while it can be pathogenic to domestic pigs (Abdi et al., 2017). Diagnosis using molecular biological techniques is more specific and sensitive compared to the microscopic technique, and it

can accurately identify the trypanosome species. A disadvantage of PCR is that it needs advanced laboratories, specialized staff, and reagents, making it more costly to carry out. In the past, issues have occurred with trypanosome strains due to the unavailability of primers. For most tsetse surveys related to control or eradication programs, it should be adequate to determine trypanosome infection rates through dissection alone. However, subsamples may be collected for diagnosis using molecular techniques if necessary. When feasible, PCR could be performed on mouthparts only to quickly estimate mature infections and save time and money that would be spent on identifying trypanosomes in the midgut. This is beneficial because a large proportion of trypanosome infections in the guts of tsetse do not mature.

Both male and female tsetse flies can transmit trypanosomes, and it is assumed that all species of Glossina can act as vectors. The pathogenic trypanosomes of mammals such as *T. brucei, T. congolense, T. simiae, T. godfreyi*, and *T. vivax* are transmitted via the saliva during feeding. On the other hand, those with reptilian hosts (e.g. *T. grayi*) and possibly other species of trypanosome (e.g. *T. theileri*) are transmitted via the fecal, contaminative route (Isaac et al., 2016; Ngomtcho et al., 2021). Within the fly, trypanosomes undergo cycles of development and multiplication, involving different parts of the alimentary tract depending on the trypanosome species. In the past, methods for identifying trypanosomes within the fly relied on specific developmental sites (Lloyd and Johnson, 1924) until the advent of more accurate methods based on deoxyribonucleic acid (DNA) (Kukla et al., 1987; Gibson et al., 1988). The complex life cycle in the fly may take from a few days for T. vivax to a few weeks for T. brucei. However, the trypanosome then gains the advantages of (i) amplifying a typically scanty parasitemia in the host, and (ii) the potential transfer of infective forms every time the fly has a chance to bite a new host.

A significant effort has been made to map the distribution of tsetse flies and their infection rates to tackle the problem of trypanosomiasis transmitted by tsetse flies at the continental level. In addition to tracking trypanosome infections in vertebrate hosts, special attention has been given to infections in tsetse flies, although data on these infections are limited. Georeferenced data on trypanosome infections in tsetse flies are crucial to complement AAT and HAT data for a comprehensive assessment of disease risks. This study is part of the continental initiative and provides data on tsetse fly distribution and trypanosome infections in these vectors. Several studies have reported the prevalence of trypanosome infections in tsetse flies in the field (Ford and Leggate, 1961). It has been reported that mature infections become more prevalent with age (Ryan et al., 1982), and various factors may influence the age-prevalence curve, including the infection rate, the developmental period of trypanosomes within the fly, and the effects of infection on fly mortality rate.

A systematic review and meta-analysis carried out by Abdi et al. (2017) aimed to consolidate information on the prevalence of trypanosome in tsetse flies. The key findings are as follows:
Overall Prevalence: The combined prevalence of trypanosome infection in tsetse flies was 10.3% (95% confidence interval [CI] = 8.1%, 12.4%) for field survey data and 31.0% (95% CI = 20.0%, 42.0%) for laboratory experiment data.

Diagnostic Methods: The dissection approach, which involves microscopic analysis of dissected insects, was the most widely used technique for identifying trypanosomes in Tsetse flies. On the other hand, information about specificity was scarce, and alternative diagnostic methods used on dissection-positive samples had low sensitivity ramifications. Field-collected tsetse flies have

varying incidence of trypanosome infection both temporally and spatially. To determine the true risk factors causing this difference, more research is required. For precise prevalence estimation, increasing the sensitivity and figuring out the specificity of diagnostic instruments should be top priorities. Evaluating the success of control or eradication programs, particularly those that employ SIT or aerial spraying, requires determining the physiological age structure of tsetse populations. Any tsetse trapped inside a spray block in the course of successive aerial spraying of non-residual pesticides in the few days after spraying should be young flies that have just emerged from pupae in the ground. Any older flies captured must either have entered the spray block from the neighbouring area indicating problems of re-invasion or must result from some fault in the spraying operation.

2.4 Wing fray analysis

Wing fray analysis is a method used to assess the condition and age of tsetse flies based on the wear and tear of their wings (Hailemariam et al., 2005; Hargrove, 2020; Mweempwa et al., 2021; Hargrove et al., 2023). As tsetse flies age and experience environmental stressors, their wings may become damaged, leading to fraying and deterioration. Wing fray analysis can provide valuable information about the population dynamics, longevity, and overall health of tsetse fly populations. The primary objective of wing fray analysis is to estimate the age structure of tsetse fly populations (Ebhodaghe et al., 2022). By examining the extent of wing damage, researchers can make inferences about the age distribution of the flies within a given population. This information is crucial for understanding the population dynamics, reproductive potential, and survival rates of tsetse flies (Hargrove, 2020). Wing fray analysis involves collecting tsetse flies from the field, typically through trapping methods or direct capture. The collected flies are then examined under

a microscope or photographed to assess the condition of their wings. The degree of wing damage, including fraying, notching, and loss of wing scales, is visually scored and categorized into different age classes. The specific age classes used in wing fray analysis may vary depending on the study design and the tsetse fly species under investigation. Age classes are typically determined based on established correlations between wing damage and the chronological age of the flies. For example, age classes may include "young," "middle-aged," and "old" flies, or they may be further divided into more precise categories. The interpretation of wing fray analysis results requires a calibration process that relates the observed wing damage to the chronological age of the flies. This calibration is typically achieved through laboratory experiments that correlate wing damage with known ages of tsetse flies. Once the calibration is established, the observed wing damage in field-collected flies can be used to estimate the age distribution of the population. Wing fray analysis provides important insights into various aspects of tsetse fly biology and ecology. It can help researchers understand the impact of environmental factors, such as habitat quality or climatic conditions, on the survival and reproductive success of tsetse flies. Additionally, it aids in evaluating the effectiveness of control measures by assessing the age structure of tsetse populations before and after interventions. While wing fray analysis is a valuable tool, it does have limitations. The accuracy of age estimation relies on the calibration between wing damage and chronological age, which may vary among tsetse fly species and populations. Additionally, other factors such as sex, nutritional status, and genetic variability can influence wing damage, potentially affecting the accuracy of age estimates (Lehane and Hargrove., 1988). Wing fray analysis is a useful method for estimating the age structure and assessing the condition of tsetse fly populations. By examining the extent of wing damage, researchers can gain insights into population dynamics, reproductive potential, and environmental stressors that may impact tsetse fly populations. This information is

valuable for understanding the biology, ecology, and control of tsetse flies, which are important vectors of diseases such as trypanosomiasis.

Data on human African trypanosomiasis (HAT) mortalities in Zimbabwe is limited. Only twenty-two cases were reported between 2012 and 2024, with a peak of 9 cases in 2012. Long-term monitoring of HAT in Zimbabwe indicates a very low incidence (Katsidzira and Fana, 2010), making these new cases unusual. HAT is significantly under-reported, as its symptoms are similar to malaria and other fevers (Odiit et al., 2005). Most recorded cases involve tourists, hunters, or those working with the national parks service. For example, in 2012, two tourists and a professional hunter were affected, while a game ranger succumbed to the disease in 2010.

(Opiro., 2021) conducted a study that explored on the influence of tsetse fly age on trypanosome infection rates. It discusses the experimental methods used to determine the infection rates in flies of different ages. The study examines both laboratory-reared and wild-caught tsetse flies to assess the impact of age on infection susceptibility. The findings suggest that younger flies may be more susceptible to trypanosome infections compared to older flies. The study emphasizes the need for considering fly age when designing control strategies targeting tsetse-borne diseases. It provides insights into the experimental procedures used to assess the infection rates and transmission potential of tsetse flies at different ages. The study discusses the implications of age-related variations in infection rates for disease spread and control. The findings suggest that older tsetse flies may have a higher likelihood of transmitting trypanosomes, emphasizing the role of fly age in disease transmission dynamics. It describes the molecular techniques employed to detect and quantify trypanosome infections in flies of different ages. The researchers investigate the

prevalence of different trypanosome strains among different age groups of tsetse flies. The study highlights age-related variations in infection prevalence, potentially indicating differences in susceptibility, exposure, or immune responses among different age categories. It describes the field sampling methods used to collect tsetse flies of different ages and the subsequent analysis of their infection status. The study presents observations of infection rates among different age groups and discusses potential factors influencing the observed patterns. The findings emphasize the need to consider fly age when assessing disease transmission risks and developing control strategies.

Studying the prevalence of trypanosome infection in tsetse flies is of significant as, tsetse flies are the primary vectors for transmitting trypanosomes, which are parasites responsible for causing diseases like African trypanosomiasis or sleeping sickness in humans and nagana in livestock. By understanding the prevalence of trypanosome infection in tsetse flies, researchers and healthcare professionals can assess the risk of disease transmission and design effective control strategies. This knowledge helps in reducing the burden of trypanosomiasis and ultimately eradicating it. By studying the prevalence of trypanosome infection in tsetse flies, researchers can identify high-risk areas where the transmission of trypanosomes is more intense. This information allows for targeted interventions such as insecticide-treated traps, insecticide spraying, or sterile insect technique (SIT) (Woolhouse and Hargrove, 1998). Targeted interventions are more cost-effective and environmentally friendly than broad-scale interventions, as they focus resources on areas with the highest disease burden. Monitoring the prevalence of trypanosome infection in tsetse flies is crucial for evaluating the effectiveness of control measures. By comparing prevalence data before and after implementing interventions, researchers can determine if the measures are reducing the infection rates in tsetse fly populations. This information helps in refining control strategies and

optimizing resource allocation. The prevalence of trypanosome infection in tsetse flies can serve as an early warning system for potential disease outbreaks. By regularly monitoring the infection rates, public health authorities can detect changes in trypanosome transmission patterns and take proactive measures to prevent the spread of the disease. Early detection and response are crucial for controlling outbreaks and minimizing the impact on human and animal health. Studying the prevalence of trypanosome infection in tsetse flies provides valuable insights into the ecology of the disease. It helps in understanding the factors influencing the distribution and abundance of tsetse flies, as well as the dynamics of trypanosome transmission within fly populations. This knowledge is essential for formulating comprehensive strategies that address the ecological complexities of the disease.

Chapter 3

3. MATERIALS AND METHODS

3.1 Study site

The study was conducted at Rekomichi Research Station (16° 18' S & 29° 23' E), Mana Pools Game Park in the Zambezi valley, Zimbabwe. Elephants, buffalo, impala, warthogs, and cheetahs are among the many wild species located in this protected area. Tsetse flies' primary source of blood meals in this habitat is wildlife (Torr et.al., 2012). The two primary species of trees are *Adansonia digitata* (Baobab) and *Colophospermum mopane* (Mopane), a thick thicket of deciduous plants is another feature of the area (Shereni.et.al., 2016).

3.2 Fly Collection

A suitable tsetse habitat was identified and an epsilon trap deployed ensuring that the GPS (global positioning system) location is also recorded. The surrounding vegetation was removed to improve sight, and the trap's legs were lubricated to keep ants from eating the flies that were trapped. Tsetse were most attracted to electric or royal phthalogen blue, according to experiments conducted on their color responses in the 1980s, black was notably more attractive than blue and produced a considerably better landing response. (Green, 1988). As a result, most traps have been made utilizing a combination of these colors, with black encouraging the flies to land on the trap and blue drawing them in. The black portion of the trap is typically inside since the goal is to draw the tsetse flies into it and then capture them to some extent, so that tsetse flies landing inside the trap will go upwards, funneled towards the catching device (cage) rather than just flying out in the same direction that they entered (Albert et al., 2015). Tsetse flies can detect a wider range of the

wavelength spectrum than humans, and are sensitive to wavelengths in the near ultraviolet (UV) part of the spectrum. Tsetse flies are drawn to UV light, particularly for the palpalis group of tsetse flies, a high UV reflectivity promotes a landing response. This is why UV light from fluorescent tubes is frequently employed in appliances to kill tsetse flies in locations where food is produced and consumed. This has been used to create targets for the management of such species. It's crucial to compare color choices under natural light rather than artificial illumination. *Glossina morsitans* flies' settling reflex may be inhibited by shiny surfaces, which could lessen the efficiency of synthetic textiles and plastics (Santer, 2014).

3.3 Deployment of Epsilon trap

The site was cleared of vegetation that could provide a mechanical or visual barrier to the trap. The terrain was leveled and cleaned of any obstacles. The majority of the trap's parts, including the guy ropes, poles, pegs, and cover, came in a single package. A hammer was the other instrument that was utilized. The trap's corners were fitted with three long poles. The flat-ended pole was positioned at the rear. Light bits of dead foliage made it easiest to check the direction of the wind. The trap was set up with the entrance facing downwind. The pegs were hammered into the ground to take the guy ropes to each corner. The guy ropes were attached to the pegs and tightened to support the trap. A cage was attached to retain the tsetse attracted into the trap. The funnel was inserted through the hole in the netting. Fitted the top of the cage onto the funnel. It is crucially important to get the attachment to the netting right. It should be as neat as possible. Attached a polythene bag to the cage. An odour sachet was placed in one of the pockets. The trap was baited with 3-n-propylphenol, octenol, 4- methyl-phenol and acetone (Hargrove & Langley, 1990).

Monitoring and emptying of the trap was done at least once every month for routine operations and for research purposes some were monitored hourly or every 24 hours for 21 days each month.

Plate 3.1: Monitoring and emptying of the trap.

3.4 Species Identification and Recording

Glossina morsitans are colored from dark brown to black. They are in between the smaller and larger tsetse species in size, being medium-sized. *G. morsitans* wings are distinguished by their black edge. The proboscis is shorter than the palps, or mouthparts. The abdomen has a long, thin shape. The environments of savannah and forest are home to *G. morsitans.*

Glossina pallidipes are pale brown or yellowish-brown. They are large-sized, with a robust appearance. They have a strong appearance and are huge in size. Unlike *G. morsitans, G. pallidipes*

wings do not have a dark border. Compared to the proboscis, the palps are shorter. Males have a club-shaped, bulbous belly, whereas females have a thin abdomen. Common habitats for *G. pallidipes* are woodland and riverine areas. Male tsetse flies usually have a bulging, club-shaped abdomen, while female tsetse flies have a slim abdomen, making sex easy to detect with the naked eye (Abdi., 2017). Following species identification, the quantity of tsetse flies captured was noted on a recording data sheet based on the species type and sex.

3.5 Dissection for Trypanosome Infection

3.5.1 Procedure

Using a low magnification dissecting microscope, the fly was placed on its back in the center of a glass slide with a drop of saline solution or phosphate-buffered saline (PBS) accessible. Using one pair of fine forceps to keep the fly's thorax in place, an incision was made on each side of the abdomen, near the thorax, using a pair of fine spring scissors. A second pair was used to hold the base of the abdomen and slowly pull it back, allowing the intestines and other internal contents to spread out without breaking and the "skin" to slowly tear off.

3.5.2 Dissection of the Proboscis

The proboscis was removed for additional analysis. The labium was extracted from the other two mouthparts and the three mouthparts the labium, labrum, and hypopharynx were separated. Starting from the end of the proboscis with the thecal bulb, two mounted needles—one in each hand were used to pull apart the labrum, hypopharynx, and labium. The magnification was increased as needed to make the proboscis clearly visible under the microscope.

It was carefully monitored that the structures were not ripped during the process of not pulling them apart, as this could occasionally lead to some tendon-like organs being mistaken for the hypopharynx. The thick, brown labium was removed because it would obstruct vision and make the coverslip slide improperly over the mouthparts. The labrum and hypopharynx were covered with a cover slip that had a tiny saline drop on it. Under a compound microscope with a 10× eyepiece and 25× objective lens, each organ the labrum, hypopharynx, salivary gland, and midgut—was examined for the presence of trypanosomes, which, if present, were typically seen in motion. The presence of trypanosomes was noted under the appropriate column of the recording sheet. It was reported as negative if trypanosomes were not observed.

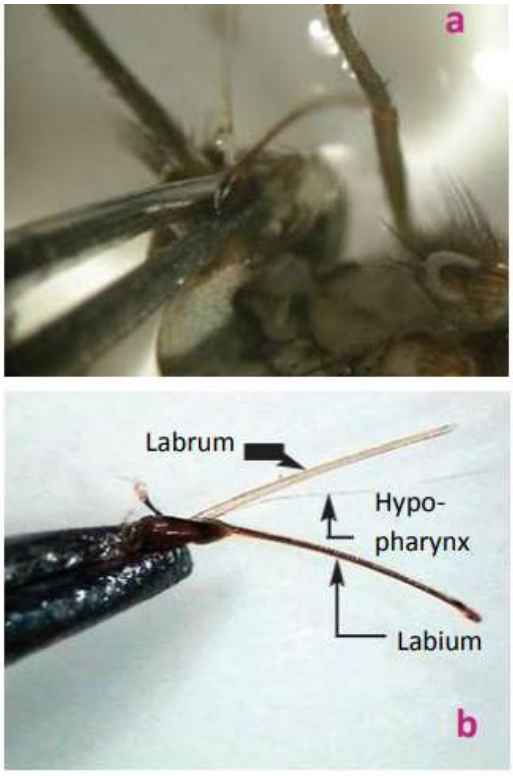

Figure 3.1: Mouthparts (proboscis of a tsetse fly, showing labrum and hypopharynx with trypanosome clusters) (Maudlin.,2018).

3.5.3 Dissection of Salivary glands

The fly was covered with saline water after a thin strip was cut off from the abdomen's left and right borders. A dissecting microscope with either x20 or x30 magnification was utilized. One by one, salivary glands were removed, and then saline water and a cover slip were added. Typically, salivary glands appear as transparent, silvery tubes in the saline solution drop, one on each side of the stomach. Each of these glands was carefully removed from the fly using a single pair of fine forceps, placed on a different area of the slide with a drop of saline, and covered with a cover slip. After removing the head and thorax from the fly and cutting the stomach near the thorax with the flat end of a mounted needle, the abdominal contents were squeezed out of the "skin" and placed in the center of the slide. The skin of the abdomen was discarded. A saline drop and a cover slip were placed over the contents of the gut. Using an x400 magnification, the salivary gland's inside was checked for trypanosomes.

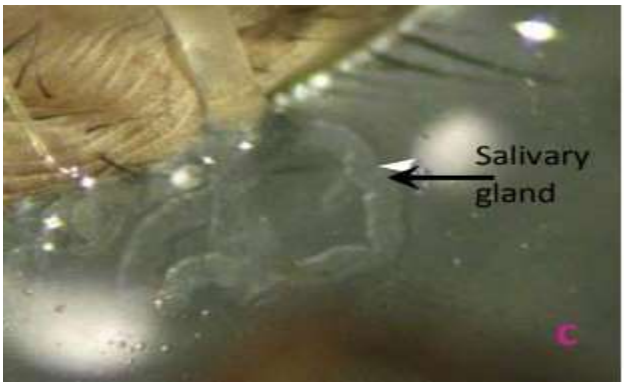

Figure 3.2: Salivary glands (Maudlin.,2018)

Infection rates are determined by dissection of these organs and examining them under a compound microscope. Infection rates depend on the age of the fly.

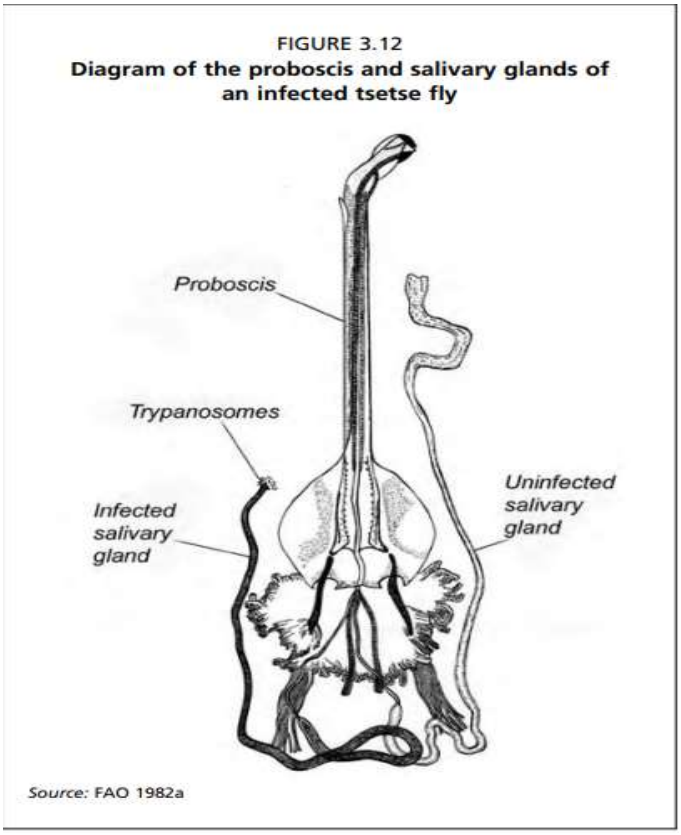

Figure 3.3: Diagram of the proboscis and salivary glands of an infected tsetse fly ((Buxton,1955).

3.5.4 Dissection of the Midgut and the Proventriculus

Following the dissection of the salivary glands, the fat bodies and a portion of the hind intestine can be removed, leaving the remaining contents of the abdomen under a third cover slip. Eliminating the fat bodies and the hind stomach, especially in a freshly fed fly, facilitates investigation because they would otherwise obstruct the vision and make trypanosome detection more difficult. Before examination, lightly push the cover slip to extract the contents of the stomach. Infection rates are determined by dissection of these organs and examining them under a compound microscope. Infection rates depend on the age of the fly.

3.5.5 Determining infection status

he life cycles of trypanosomes vary depending on the species. Trypanosome species can be identified by observing how the parasites travel throughout the organs of the fly following a blood meal on an infected host. *Trypanosoma vivax*: A portion of the parasites that the fly consumes adhere to the walls of the food channel within its proboscis. They reproduce and establish colonies. Once in the hypopharynx, the infectious forms that are generated go there and eventually settle to form rosettes. As a result, these trypanosomes are limited to the oral cavity. *Trypanosoma congolense* parasite grows in the midgut after ingestion and then moves to the proboscis, where it adheres to the wall and multiplies. The infectious forms move to the throat. Consequently, the only places these trypanosomes may be discovered are in the mouth and intestines. Trypanosoma brucei: They have a far more intricate cycle. Following the path traveled by the blood meal, these trypanosomes eventually change into procyclic forms in the midgut, infective metacyclic forms in the salivary glands, and finally migrate to the hypopharynx after being consumed. Some Trypanosomes are therefore found in the midgut, the salivary glands and the mouthparts (Abdi et al., 2017).

3.6 Dissection for Wing Fray Analysis, Size Estimation and Determination of Ovarian Age

3.6.1 Determining age in males: Procedure for wing fray analysis

Using a set of fine (watchmakers) forceps, remove each wing from the fly by pulling from the base, being careful not to harm the trailing edge. Gently but firmly crush one fly to immobilize it. A cover slip was put on top of the wings after they had been placed on a slide with a drop of saline solution (or water). Using a dissecting microscope with a 25× magnification, the wings were inspected. To reduce the possibility of overestimating wing fray (due to damage sustained in the

trap cage), the least damaged wing was chosen. Using the diagram below as a guide, the degree of wing fray was assessed, and the wing fray category was noted along with other fly details (species, sex, trap number, and date) onto the recording sheet.

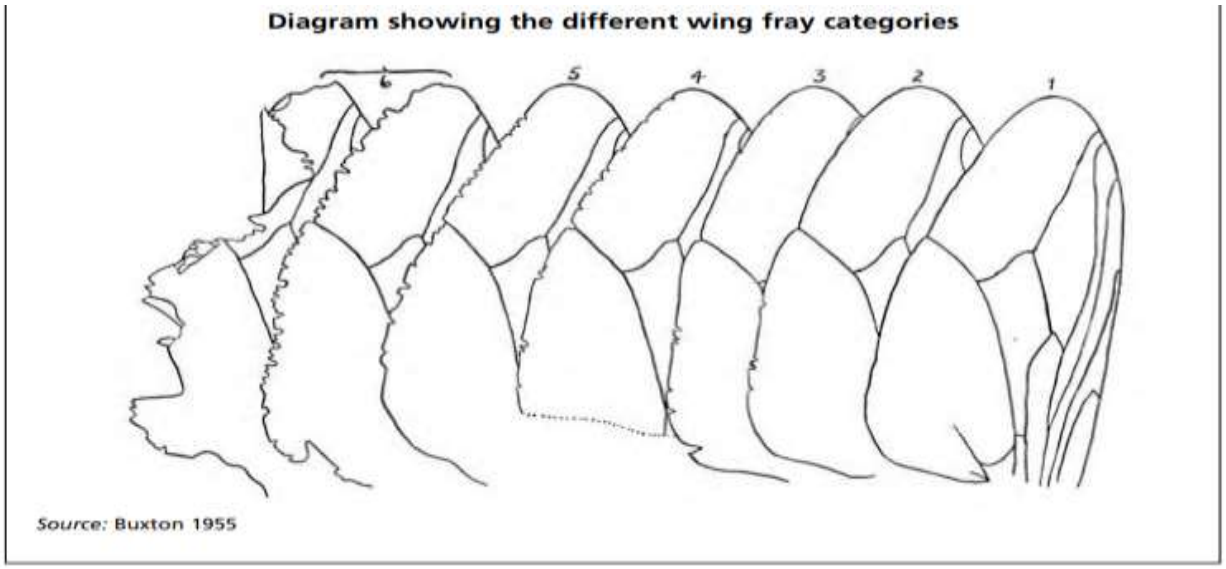

Figure 3.4: Wing fray categories (Buxton., 1955)

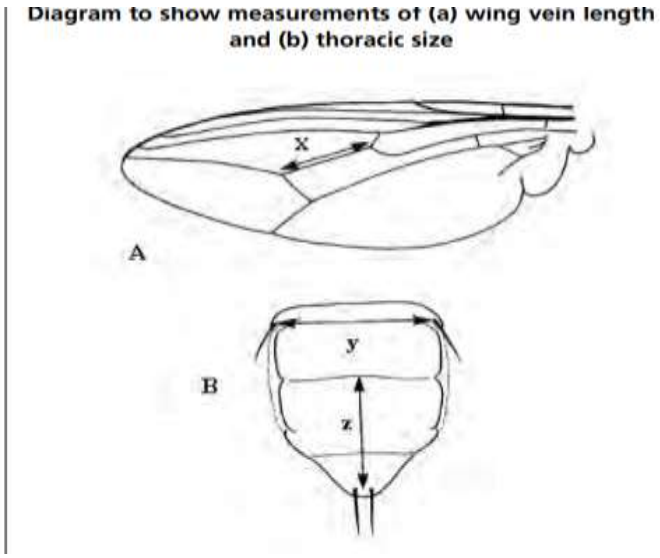

Figure 3.5: Measurement of wing vein length (Buxton,1955)

3.7 Dissection for ovarian ageing

A more accurate method of ageing tsetse, applicable only to female flies, is the determination of their age based upon ovarian dissection to determine the number of eggs produced by the female and the stage in the ovarian cycle at the time of dissection. This dissection technique, obviously for female flies only, requires some practice, ideally with tsetse of a known age before reliable results can be obtained so it is less appropriate for instructions of the sort provided here, it requires demonstration.

3.7.1 Procedure for ovarian dissection

First, using tiny forceps to remove the fly's wings and legs because they might obstruct the dissection, the female tsetse fly was dissected for ovarian aging. Dorsal side up, the fly was positioned on a glass slide that had enough buffered saline in it to keep the dissected organs from drying up. A one-millimeter incision was made on either side of the fifth or sixth tergite, and the abdomen's tip was gently grabbed and pushed. The abdominal wall was torn apart by a side-to-side motion, exposing the internal organs and making the uterus, spermathecae, and ovaries visible as they floated in the phosphate-buffered saline

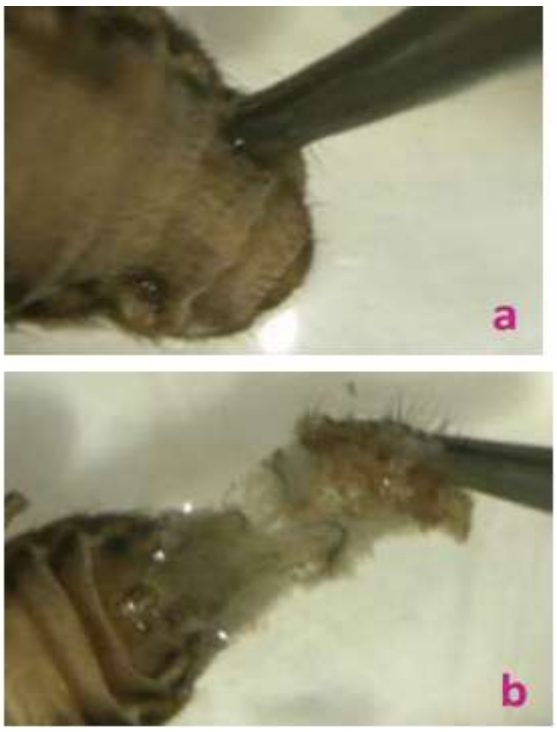

Figure 3.6: Removal of ovaries from the tsetse abdomen (Maudlin, 2018)

The location of the spermathecal duct when it enters the uterus was examined since it shows whether or not the reproductive organs are twisted or turned around. An estimation of the percentage of sperm in the spermathecae was made when the contents of the uterus were seen. Determined whether the most developed ovarioles are on the left inside, left outside, right inside, or right outside after making an initial examination of their configuration. The following size was handled in the same way, and so forth. This made it easier to compare with (fig 3.9) and ascertain the female fly's physiological age.

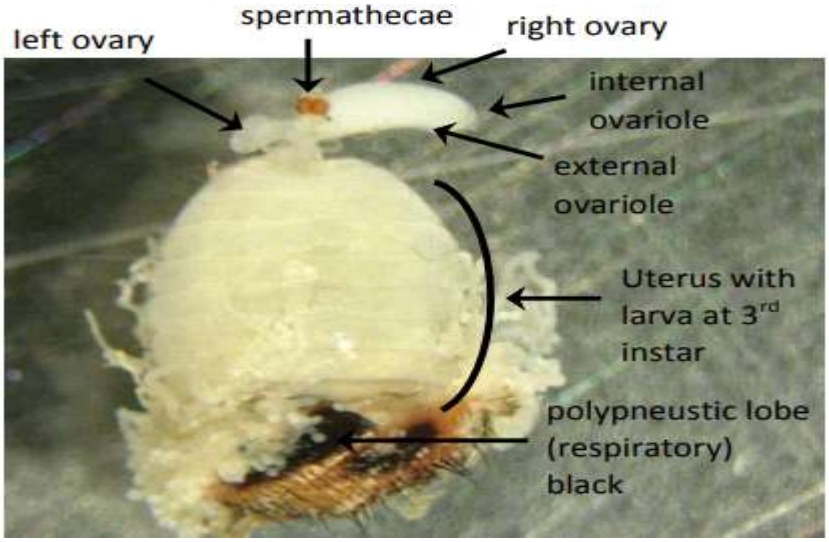

Figure 3.7: Contents of the uterus (Maudlin, 2018)

The first thing to notice is how the ovarioles are arranged, namely which ones are most developed on the left, right, inside, or outside. Until all four were completed, the process was repeated for the following size up. The observations were helpful in determining the female fly's physiological age, which is shown in (fig. 3.9).

Ovarian age category	Configuration	Category / uterine contents	Estimated age (days)	Later category	Age range
0	 Non-ovulated	-	0-8	8a 8b 8c	80-84 84-87 87-90
1	 1-ovulation	1a 1b 1c	8-12 13-16 16-19	9a 9b 9c	90-94 94-97 97-100
2	 2-ovulations	2a 2b 2c	20-24 24-27 27-30	10a 10b 10c	100-104 104-107 107-110
3	 3-ovulations	3a 3b 3c	30-34 34-37 37-40	11a 11b 11c	110-114 114-117 117-120
4	 4,8,12-ovulations	4a 4b 4c	40-44 44-47 47-50	12a 12b 12c	120-124 124-127 127-130
5	 5,9,13-ovulations	5a 5b 5c	50-54 54-57 57-60	13a 13b 13c	130-134 134-137 137-140
6	 6,10,14-ovulations	6a 6b 6c	60-64 64-67 67-70	14a 14b 14c	140-144 144-147 147-150
7	 7,11,15-ovulations	7a 7b 7c	70-74 74-77 77-80	15a 15b 15c	150-154 154-157 157-160

Figure 3.8: Ovarian categories for Glossina spp.

This procedure aided in identifying the ovarioles that would be cut open to look for a follicular remnant. After figuring it out, portions and fat bodies were cut off. The ovarioles' outer membrane was broken using fine-mounted needles or high-quality fine forceps under a higher magnification to release the developing egg. We looked at the eggs to see if there was a follicular remnant. There

may only be one opportunity to search for a relic in particular age combinations, and if the dissection goes wrong for example, because the egg broke there may be no other options. If the initial attempt fails, there may be another opportunity in different setups. The secret to success is maintaining moisture in the tissues, preventing the reproductive system from twisting or moving, and carefully dissecting the organ to prevent puncturing the growing ovaries. They leak white material when they are punctured, making it impossible to accurately monitor the situation. If the right and left ovaries have not twisted, the spermathecal ducts should be visible entering the uterus from above. Once in the proper position, they should allow for the identification of the largest ovariole and its sequence. Additionally, the accuracy of the order's setting can be confirmed. When dissecting, avoid pulling the abdomen back so far that the gut becomes fractured if the stomach is full, as this can hide the reproductive system. The fly's body can then be extracted from the reproductive organs by carefully severing the stomach close to the rectum. Drawbacks to ovarian ageing are that it requires a skilled technician to perform the dissection accurately and that skill requires not only adequate training, with tsetse of known ages available for verification, but also enough practice (Hargrove.,2020).

3.8 Data analysis

Statistical analysis was done using SPSS version 21 software package (IBM SPSS, 2012). Normality tests were conducted on the data following which Analysis of variance (ANOVA) was used to assess the significance of differences in infection rates between different ovarian categories in females and wing fray in males. Regression analysis was also used to determine the relationship between age and infection status of the flies. Chi –square test was used to assess infection status by month and sex since the parameters were categorical.

Chapter 4

4. RESULTS

4.1 Summary Results

In the two species dissected, three trypanosome species were detected for the period under review, 2022-2023, i.e. *T. congolense, T. vivax* and *T. brucei*. From 3 150 dissected tsetse flies the proportions were 4.47%, 3.05%, 37.37% and 55.11% for male *Glossina morsitans*, female *Glossina morsitans*, male *Glossina pallidipes* and female *Glossina pallidipes* respectively. Infection rates in the flies were found to be 10.64%, 12.50%, 5.69% and 5.01% respectively. Infection rates refer to the proportion of infected flies relative to the dissected flies. The overall infection rate for the total dissected flies was 5.78%

Table 4.1: Summary table for dissected flies.

Species	Sex	Sample size (n)	*Trypanosoma brucei*	*Trypanosoma vivax*	*Trypanosoma congolense*	Total infected	Infection rate (%)
Glossina morsitans	M	141 (4.47%)	0	9	6	15	*10.64*
Glossina morsitans	F	96 (3.05%)	0	8	4	12	*12.50*
Glossina pallidipes	M	1177 (37.37%)	0	43	24	67	*5.69*
Glossina pallidipes	F	1736 (55.11%)	1	63	24	88	*5.01*
		3150	1	123	58	*182*	*5.78*

4.1.1 Normality test

Data for wing fray was subjected to a normality test to establish if the data was normally distributed or not.

Table 4.2: Normality tests for ovarian wing fray and category.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Wing_fray	.205	3150	.020	.900	3150	.010

a. Lilliefors Significance Correction

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Ovarian Category	.174	1838	.015	.942	1838	.008

a. Lilliefors Significance Correction

Data for wing fray and ovarian category were subjected to a normality test. Considering that the Sig value for both tests are greater than 0.05, the null hypothesis that data is normally distributed is accepted. Following this result data was processed using tests which assume normal distribution for data.

4.1.2 Trypanosome infection by Ovarian category

Ovarian category is an assigned value which enables one to estimate realistically the age of a female tsetse based on the number of ovulations / eggs deposited. Figure 4.1 shows the prevalence of trypanosomiasis in 1832 female tsetse that were dissected. Highest prevalence was recorded for *T. vivax* with a highest of 1.20% in ovarian category 5 followed by 0.98% in category 4. Generally,

this was the most common trypanosome species that was found in tsetse flies. *T. congolense* prevalence was moderate with a highest of 0.33% infection rate. *T. brucei* was the least prevalent with an infection rate of 0.05% recorded.

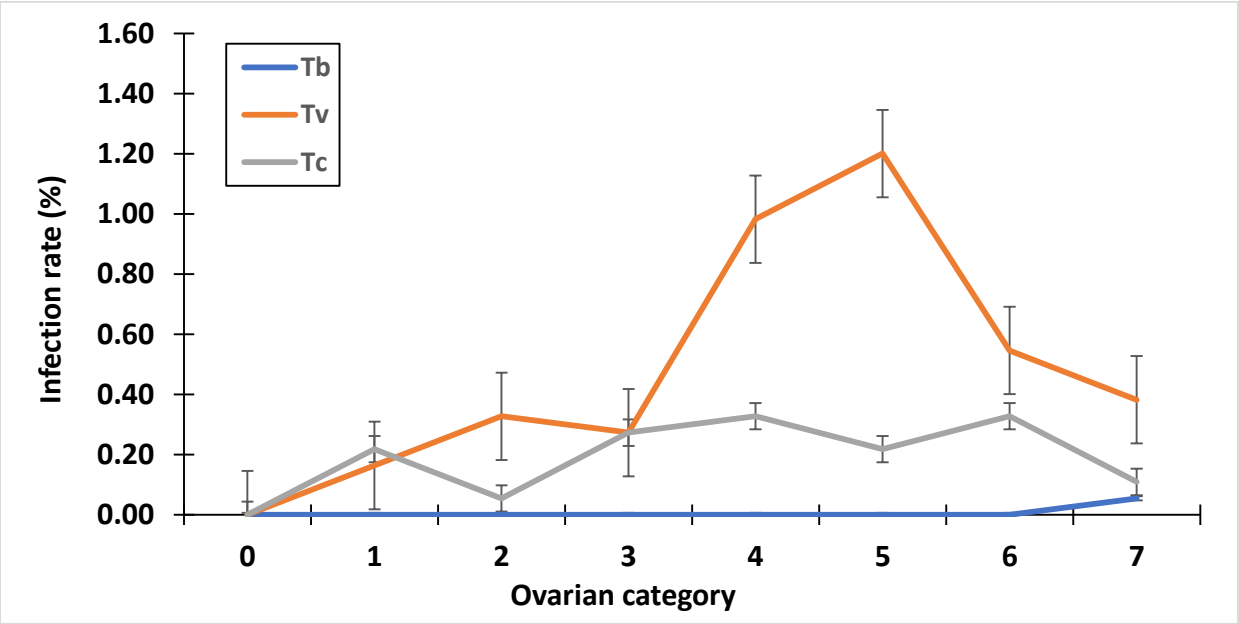

Figure 4.1: Prevalence of trypanosome infection in female tsetse as a function of Ovarian ageing

Analysis of variance tests conducted for trypanosome infection by ovarian category resulted in p=0.162 at 5% significance level as shown in Table 4.3. indicating no statistical significance

Table 4.3: Analysis of variance of infections within ovarian categories for female tsetse flies.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.049	7	.150	1.503	.162
Within Groups	181.721	1823	.100		
Total	182.770	1830			

Results from 96 *Glossina morsitans* female tsetse dissected are shown in Figure 4.2. The most prevalent trypanosome species was *T. vivax* with a high of 2.08% in categories 2, 4, 5 and 6 followed by *T. congolense* with 1.04% in categories 1,3,5 and 6 while no *T. brucei* cases were detected for the 2 year period of study. There was notable significant differences in infection rates between *T. vivax, T. congolense* and *T. brucei* infections in female *G. morsitans* flies. Analysis of variance tests resulted in p=0.086 at 5% significance level implying no statistical significance in differences observed. Differences of means were found to be statistically different for means of ovarian category 4 and 6. The rest were not statistically significant.

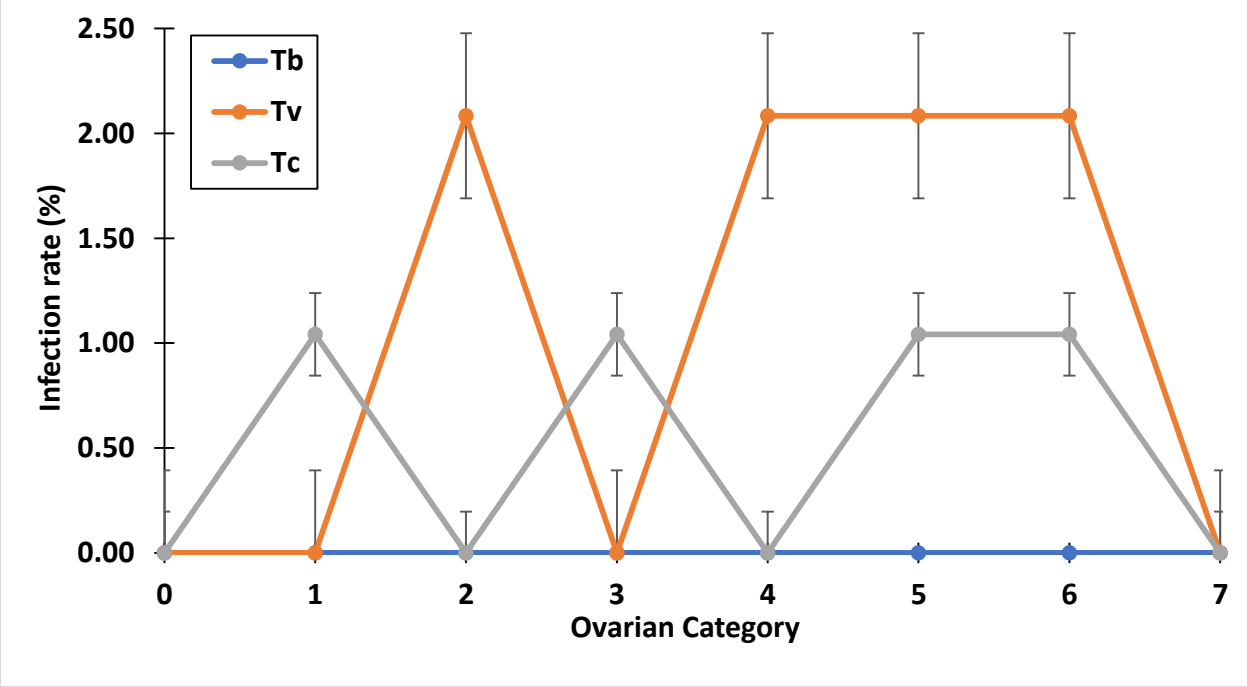

Figure 4.2: Prevalence of *T. vivax, T. congolense* and *T. brucei* in female *G. morsitans* as a function of Ovarian category

In female *G. pallidipes* trypanosome infection rate was observed to increase fast, 0.17-1.15% from categories 1 to 5 then a sharp decline to 0.40% between categories 7 and 8 for *T. vivax* while *Trypanosoma congolese* infection fluctuated below 0.35% from ovarian categories 1-7 with the highest infection of 0.35% in category 4. *T. brucei* recorded 0.06% only in category 7 which are the oldest flies. The results are illustrated in Figure 4.2. A p-value of 0.163 for female *G. pallidipes* was observed after running analysis of variance (ANOVA) tests indicating that there were no statistically significant differences in categories at 5% significance level. Multiple comparisons for differences of means showed that there were no statistically significant differences in the ovarian categories for *G. pallidipes*.

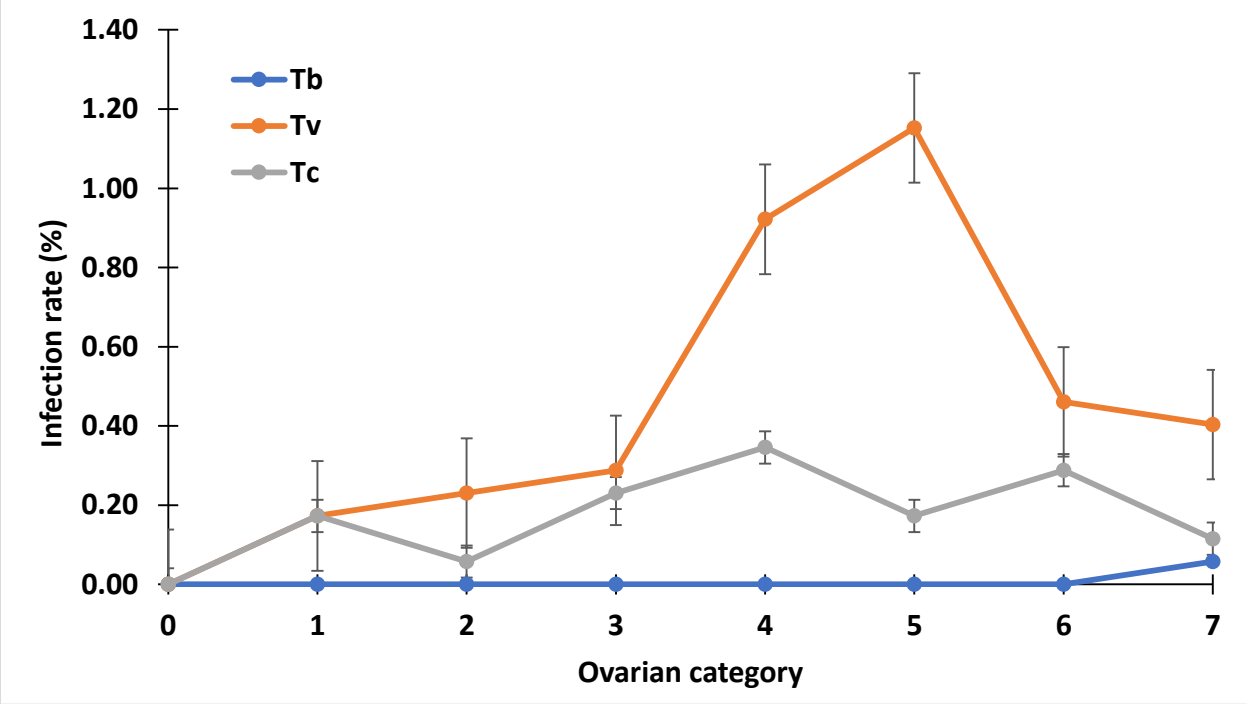

Figure 4.3: Prevalence of *T. vivax, T. congolense* and T. brucei in female *G. pallidipes* as a function of Ovarian category.

4.1.3 Trypanosome infection by wing fray ageing

As wing fray applies to the ageing of both sexes of tsetse, the analysis was conducted on all 3150 tsetse flies captured. 14.5% of flies dissected were found to be in wing fray category 1, 11.3% in category 2, 12% in category 3, 26.3% in category 4, 26.3% in category 5 and 9.5% in category 6. Of the total dissected flies, 182 were found infected with trypanosomes. The proportions of infection where 3.9% *T. vivax*, 1.8% *T. congolense* and 0.0003% *T. brucei*. There was a general increase in *T. vivax* and *T. congolense* infections with age in the flies dissected although prevalence of *T. congolense* infections were lower.

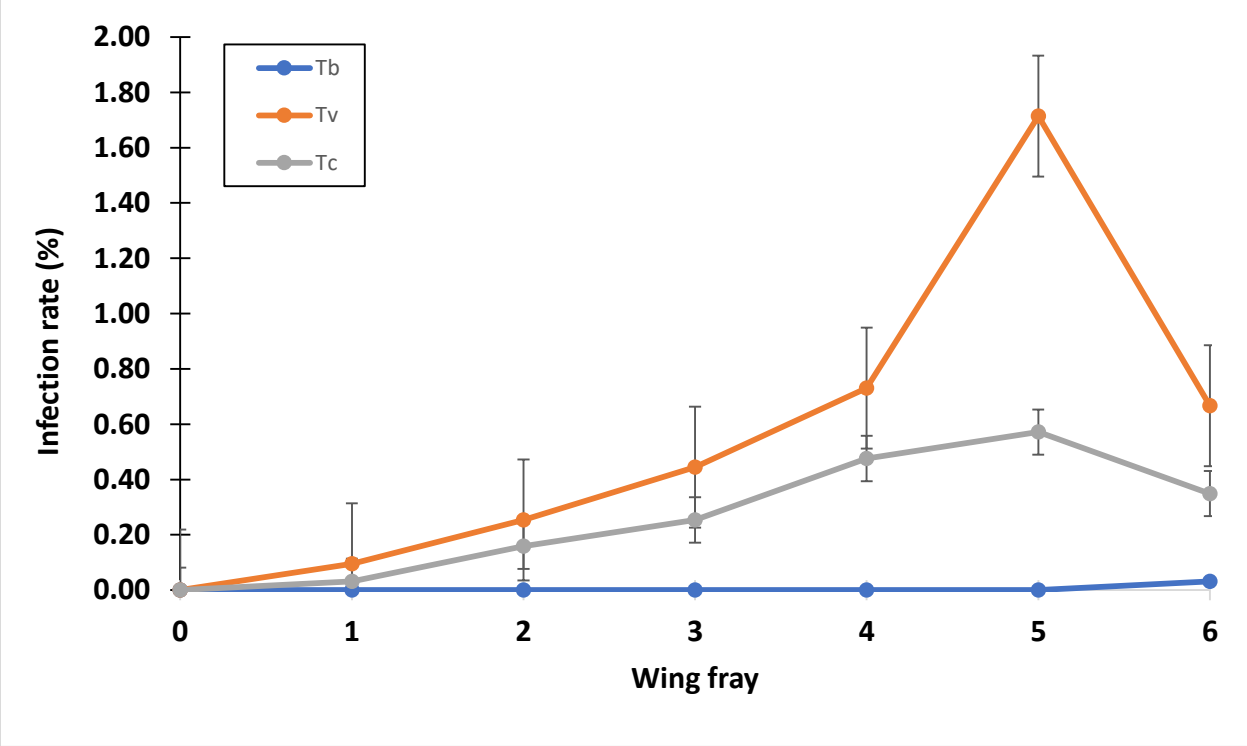

Figure 4.4: Prevalence of trypanosome infection in tsetse as a function of wing fray.

Analysis of variance tests conducted on all dissected tsetse resulted in a p-value of 0.000 at 5% significance level as shown in Table 4.4. This indicated that the was a statistically significant difference within the various wing fray categories. Multiple comparisons within the categories indicated that the mean for wing fray category 1 had statistically significant differences with means for categories 3, 5 and 6. Mean differences were also significant for category 2 and 6 as well as category 4 and 6. Category 3 was also statistically significantly different from means of categories 1 and 6.

Table 4.4: Analysis of variance of trypanosome infections by wing fray analysis for all tsetse flies.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.974	5	.995	9.185	.000
Within Groups	340.428	3143	.108		
Total	345.402	3148			

Analysis of variance conducted based on sex of the flies indicated that infections for wing fray categories in both male and female flies were statistically significant as denoted by p=0.01 and p=0.00 respectively at 5% significance level. Multiple comparisons in females showed that means were significantly different in categories 1, 3, 5 and 6 at 5% significance level.

4.1.4 Determination of association and relationship infection by period of sample collection

Chi square test conducted to determine whether there is an association between year of capture and observed infections indicated that there was no statistically significant difference as denoted by Pearson Chi-square value of p=0.428 at 5% significance level. In 2022, percentage infection

was 4.2, 2.1 and 0.1% whilst in 2023 it was 3.7, 1.7 and 0% for *T. vivax, T. congolense* and *T. brucei* respectively. When the same association was tested at monthly level, it was observed that there was a strong association between month of capture and infection rate in the 2 species as denoted by p=0.00 at 5% significance level.

Table 4.5: Analysis of association between year and infection status of tsetse

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.770[a]	3	.428
Linear-by-Linear Association	1.752	1	.186
N of Valid Cases	3149		

The year 2022 started off with high infections through to around May then a steep decline was noted from June to December while in 2023infection dropped in February but steadily increased as the year progressed. A T-test was conducted to understand if there was a significant difference in infection status of the flies as a function of the year of capture. A p-value = 0.601 at 5% significance level was observed indicating that there was no significant difference in the infection rates for the 2 years under study.

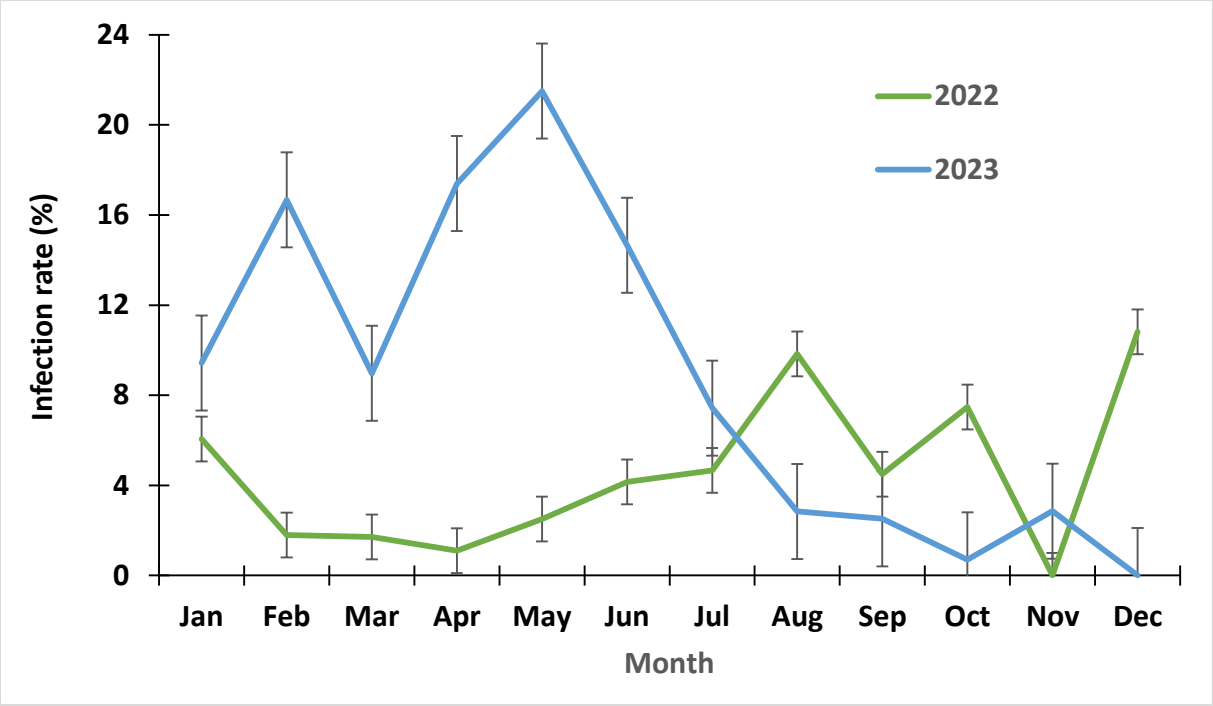

Figure 4.5: Monthly fluctuations of infection rate in 2022 and 2023.

Chapter 5

5. DISCUSSION AND CONCLUSIONS

5.1 Discussion

Trypanosome infections rates were highest for *T. vivax* followed by *T. congolense* and *T.brucei* was the least. *Trypanosoma vivax* was mostly detected in both species of tsetse and this can be attributed to developmental cycle of the parasite which is consistent with previous studies conducted at Rekomichi by (Woolhouse et.al.,1993). The infection rate of 5.78 observed in this study is also consistent with the 1993 study in which the infection rate was found to be 5.5% (3.1 *T. vivax*, and 2.4% *T. congolense*). The high prevalence of *T. vivax* infections can be attributed to their short development lifecycle which is about 10 days and occurs entirely in the fly's proboscis protecting it from anti-trypanosomal factors/ agents in the gut compared to other trypanosomes. *T. brucei's* life cycle is more complicated and takes between 17-45 days of development as the development has to occur in the midgut before migration of trypanosomes to the proboscis (Gibson and Bailey, 2003). Peacock et.al, (2012) further explained that the migration of trypanosomes from midgut to proboscis encounters harsh conditions in the salivary glands leading to low *T. brucei* infections which in our study was 0.11% for the total samples dissected and 0.06% in female *G. pallidipes flies*. Dyer et. al., 2013, provided further evidence suggesting that midgut contains lectins which are able to denature trypanosomes.

The midgut also contains proteolytic digestive enzymes, (Aksoy., 2003), and other molecules which ultimately can prevent maturation of *T. congolense* and *T. brucei* infections as these reach the midgut as part of their development cycle. Most infections were found in ovarian categories and wing fray categories 4 and 5 indicating that these are mature flies that usually harbour the

infections. Extreme categories tend to have less infections as very few reach that age and for young categories, in some instances infection will not have matured yet.

Both sexes of tsetse feed only on blood and therefore have high capability to transmit pathogenic trypanosomes (Peacock et. al., 2012). It has been observed that under natural conditions, female tsetse normally have higher prevalence of infections than males partly due to longer lifespans when compared to males. This ultimately enhances the probability of females feeding on infected hosts and picking infections in the process (Leak., 1999).

Maudlin et. al., (1991) conducted research which concluded that there is a difference in susceptibility between male and female tsetse with males being more susceptible to *T. brucei* than females. This was different from observations made in this study which only yielded one *T. brucei* infection in a female fly. Ryan et al. (1982) and colleagues observed a positive linear relationship between infection rate and tsetse age meaning that with increasing age, infection also goes up. This is to a greater extent consistent with findings from this study.

In analysis conducted for females using ovarian categories, a challenge arises when there are flies in categories between 8-11 and 12-15 which are very old flies, likely to be infected, as these will be included in the categories 4-7. This as a result increase infection for the ovarian categories 4-7. This is attributed to the ovarian dissection key which acknowledges the difficulty in determining the categories beyond 7 (Figure 3.8).

Results showed that there was no significant difference between infection rates observed for 2022 and 2023. Chi-square assessment also revealed that there was no significant association between year and infection rate within the flies. This indicates that conditions in the environment could have been consistent thereby no major deviations were noted. As a matter of fact, the study only corroborated the fact that infections at the research stations range between 5-15% as indicated in various literature including Woolhouse, 1993.

5.2 Conclusion

Results from the study helps to shed light on some complex aspects of tsetse transmitted trypanosomes epidemiology as it gives evidence to support postulated relationships between age and trypanosome infection as well as differences of infections with months. It can be concluded that infection rate increases with age as measured by ovarian category and wing fray index. It was also confirmed that three (3) trypanosome species are in existence around Rekomichi research station namely *T. vivax, T. congolense* and *T. brucei*. The overall infection rate remains generally low and constant at around 5.78% in the Zambezi Valley. *Trypanosoma vivax* type, detected in the mouthparts, was the most dominant trypanosome species detected followed by *T. congolense*. Lowest prevalence was observed for *T. brucei*.

The study also concluded that trypanosome infection generally increased as the year progresses. No significant change was noted between 2022 and 2023 in terms of infection rates.

5.3 Recommendations

In detecting trypanosomes in tsetse flies, molecular techniques should be utilized due to their heightened sensitivity and specificity. While the Veterinary Department currently relies on microscopy for diagnosing trypanosomiasis in tsetse flies, this method can be enhanced by using Polymerase chain reaction which is more specific and sensitive. It is essential to carry out similar studies in other regions of the country where tsetse flies are present, as there is limited data on trypanosome infection rates in Zimbabwe beyond Rekomichi. Following this study, further research could be undertaken to examine the impact of climatic variables and climate change.

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