IMPACT OF LIVESTOCK KEEPING ON MALARIA TRANSMISSION RISKS IN RURAL SOUTH-EASTERN TANZANIA

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DEDICATION

I dedicate this work to my family members for their support and encouragement during my study period. My father, Anosisye Mwalugelo, and my lovely mom, Elika Kibona, as well as all my brothers and sisters.

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ABSTRACT

IMPACT OF LIVESTOCK KEEPING ON MALARIA TRANSMISSION RISKS IN RURAL SOUTH-EASTERNTANZANIA

Livestock keeping is one of the potential factors related to malaria transmission. To date, the impact of livestock keeping on malaria transmission remains inconclusive, as some studies suggest a zooprophylactic effect while others indicate a zoopotentiation effect. This study assessed the impact of livestock keeping on malaria transmission risks. Additionally, the study explored the knowledge and perceptions of residents about the relationships between livestock keeping and malaria transmission risks in a Minepa village in Ulanga district, south-eastern Tanzania. A longitudinal entomological study was conducted in Minepa village, south-eastern Tanzania. Forty households were randomly selected, of which 20 had livestock and the other 20 had no livestock. Weekly mosquito collection was performed from January to April 2023. The CDC-Light traps and prokopack aspirators were used for indoor mosquito collections, while human-baited double-net traps and resting buckets were used for outdoor collections. A sub-sample of mosquitoes collected was subjected to polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) for mosquito sibling species identification and detection of blood meals, respectively. Negative binomial generalized linear mixed models (GLMMs) were used to assess the influence of livestock on mosquito density. Also, in-depth interviews (IDIs) were conducted to explore community knowledge and perceptions on the relationships between livestock keeping and malaria transmission risks. A total of 48,677 female *Anopheles* mosquitoes were collected. Out of these, 89% were *An. gambiae* s.l., while other species were *An. funestus, An. pharoensis*, *An. coustani,* and *An. squamosus*. Compared to houses with no livestock, there was a significant increase in the overall mean number of *An. funestus* mosquitoes indoors (RR=2.866, 95%CI: 1.471 – 5.582, p=0.002) and outdoors (RR= 1.579,95%CI: 1.080 – 2.865, p=0.023) in households with livestock. The presence of livestock was positively associated with the indoor density of *An. coustani, An. pharoensis,* and *An. squamosus.* The findings revealed a statistically significant increase in the overall number of *An. gambiae* s.l. outdoors (RR=1.181, 95%CI: 1.050 – 1.862, p=0.043) but not indoors. The presence of livestock at varying distances from the household did not show any impact on the densities of major *Anopheles* mosquitoes except for *An. coustani,* where the increase in such distance resulted in a decrease in *An. coustani* mosquito densities (RR = 0.113, 95% CI: 0.022–0.588, p = 0.010). The human blood index in mosquitoes collected from houses with livestock was less than that from houses that had no livestock (OR=0.149, 95%CI: 0.110 – 0.178, p<0.001). In a qualitative assessment, the majority of participants observed the high density of mosquitoes in houses with livestock and suggested that community members living in livestock-keeping communities should be well provided with awareness on how to effectively manage animals alongside malaria and vector control. Despite the potential for zooprophylaxis, this study indicates a higher malaria transmission risk in livestock-keeping communities. It is crucial to prioritise and implement targeted interventions to control vector populations within these communities. Furthermore, it is important to enhance community education and awareness regarding covariates such as livestock keeping that influence malaria transmission.

TABLE OF CONTENTS

DECLARATION AND APPROVAL ii
DEDICATION iii
ACKNOWLEDGEMENT iv
ABSTRACT v
LIST OF TABLES x
LIST OF FIGURES xi
ABREVIATIONS xii
CHAPTER ONE: INTRODUCTION 1
1.1 Background Information 1
1.2 Statement of the Problem 4
1.3 Objectives of the study 5
1.3.1 Main objective 5
1.3.2 Specific objectives 5
1.4 Research Questions 5
1.5 Significance of the study 5
1.6 Scope of the study 6
1.7 Limitation of the study 6
1.8 Definition of Terms 7
CHAPTER TWO: LITERATURE REVIEW 10
2.1 Host seeking behaviour of malaria vectors and its importance to malaria control 10
2.2 Epidemiological relevance of livestock keeping and malaria transmission 10
2.3 Differential attractiveness of hosts to mosquitoes 11
2.4 Livestock keeping as a spatial targeted malaria intervention 11
2.5 Controlling vector density using livestock-based vector control options 12
2.6 Community engagements and malaria control 13
2.7 Livestock and potential for other mosquito borne diseases transmission 14
2.8 Conceptual framework 15
CHAPTER THREE: METHODOLOGY 16
3.1 Study area 16

3.2 Study design 17
3.3 Population...17
3.4 Sample size determination and sampling techniques 17
3,4.1 Sample size determintion..17
3.4.2 Sampling techniques...18
3.5 Inclusion and exclusion criteria...18
3.6 Reliability 18
3.7 Validity 19
3.8 Ethical considerations...19
3.9 Data collection 20
3.10 Data analysis..26
CHAPTER FOUR: RESULTS 29
4.1 Results for objective 1: Impact of livestock keeping on distribution and densities of malaria vectors 29
4.1.1 Summary of All Mosquitoes Collected 29
4.1.2 Molecular identification of mosquito species...30
4.1.3 Common livestock found in the study area 30
4.1.4 Abundance of Host-Seeking Mosquitoes in Houses with and Without Livestock 31
4.1.5 Abundance of Resting *Anopheles* Mosquitoes in Houses with and Without Livestock 33
4.1.6 Impact of Livestock on *Anopheles gambiae* s.l Density 34
4.1.7 Impact of livestock on *Anopheles funestus* density.......................................35
4.1.8 Impact of livestock on *Anopheles coustani* density.......................................37
4.1.9. Impact of livestock on *Anopheles pharoensis* density 39
4.1.10 Impact of livestock on *Anopheles squamosus* mosquitoes' density 41
4.1.11 Distance between houses and livestock pens and mosquito density 43
4.1.12 Contribution of other variables in mediating the association between livestock and malaria vector density...43
4.2 Results for objective 2: Host preference of mosquitoes in livestock-keeping and non-livestock keeping households..45

4.2.1 Blood Meal Sources in Livestock-Keeping and Non-Livestock-Keeping Households 45
4.2.2 Impact of livestock on mosquitoes' ability to acquire human blood............. 46
4.3 Results for objective 3: Knowledge and perception of community members towards the relationship between livestock keeping and malaria transmission...... 47
4.3.1 Demographic description of participants.. 47
4.3.2 Knowledge about mosquitoes and diseases they transmit............................. 48
4.3.3 Knowledge on mosquito biting behaviour and mosquito control 48
4.3.4 Livestock-keeping practices and distance between houses and livestock enclosures 49
4.3.5 Treating Animals with pesticides 49
4.3.6 Relationship between malaria transmission and livestock keeping............... 49
4.3.7 Knowledge on the impact of livestock keeping on malaria transmission 50
CHAPTER FIVE: DISCUSSION 54
5.1 Summary results 54
5.2 Impact of livestock keeping and mosquito distribution and density 54
5.3 Mosquito host preference 56
5.4 Knowledge and perception of community members towards livestock keeping and malaria transmission 57
CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS 60
6.1 Conclusion 60
6.2 Recommendations 60
6.2.1 Recommendations for practice 60
6.2.1 Recommendation for research 61
REFERENCES 62
APPENDICES 74
Appendix 1: Informed Consent Agreement for household's owners who will participate by allowing mosquito collection for field experiments by volunteers from the selected households from Ulanga district, Morogoro region, Tanzania 74
Appendix 2: Informed Consent Agreement for volunteers who will participate in the field experiment including those who will be collecting adult mosquitoes from the

selected households from Ulanga district, Morogoro region, Tanzania.................80

Appendix 3: Informed Consent Agreement for participants of In-depth Interview to assess the knowledge and perception of community members about the relationship between livestock management and malaria transmission in Ulanga district, Morogoro region, Tanzania 86

Appendix 4: Household characterization form...89

Appendix 5: Form for records the design of experiments collecting mosquitoes in the field..93

Appendix 6: Records the process by which a field collection of mosquitoes is sorted into pre-defined subgroups of taxon, sex and abdominal status(Kiware et al., 2016) ....:95

Appendix 7: In-depth Interview guide...96

Appendix 8: Institutionla clearance certificate for conducting health research from the Ifakara Health Institute 101

Appendix 9: Approval to conduct research from the Jaramogi Oginga Odinga University of Science and Technology...102

LIST OF TABLES

Table 4.1: Summary of total number of mosquitoes collected using different traps 30
Table 4.2: Number of livestock present in selected households understudy 31
Table 4.3: Statistical significance of the impact of the number of livestock on the number of An. gambiae s.l mosquitoes in houses with and without livestock indoors and outdoors 35
Table 4.4: Statistical significance of the impact of the number of livestock on the number of An. funestus mosquitoes in houses with and without livestock indoors and outdoors 36
Table 4.5: Statistical significance of the impact of the number of livestock on the number of An. coustani mosquitoes in houses with and without livestock indoor and outdoor 38
Table 4.6: Statistical significance of the impact of the number of livestock on the number of of An. pharoensis mosquitoes in houses with and without livestock indoors and outdoos . 40
Table 4.7: Statistical significance of the impact of the number of livestock on the number of An. squamosus mosquitoes in houses with and without livestock indoors and outdoors 42
Table 4.8: The effect of distance between livestock pens and houses to the indoor density of Anopheles mosquitoes 43
Table 4.9: Impact of Household type and household occupants on malaria vector density 44
Table 4.10: Blood indices of different hosts from Anopheles arabiensis mosquitoes 45
Table 4.11: Impact of household's livestock status and location on An. arabiensis mosquito ability to feed on human 47
Table 4.12: Demographic information of the study participants 48
Table 4.13: List of themes extracted from the qualitative analysis and their respective quotes 50

LIST OF FIGURES

Figure 2.1: Conceptual framework 15
Figure 3.1: A map of a study area and distribution of households under study 16
Figure 3.2: Showing (a) Livestock pen in close proximity to homesteads (b) Cattle are corralled outside homesteads (c) Some sheep which are kept in a study area (d) Sample houses that are found in a study area 20
Figure 3.3: Mosquito traps which were used for mosquito collections; (a) a person collecting resting mosquitoes from resting bucket using prokopack aspirator near cattle shed (b) a volunteer collecting resting mosquitoes indoor using a prokopack aspirator (c) a double net trap (d) a CDC-light trap. 22
Figure 4.1: Density of host-seeking malaria vectors in houses with and without livestock 32
Figure. 4.2: Trend of mosquito density in houses with and without livestock in different months during the rainy season; a period of high malaria transmission...................................... 33
Figure 4.3: Density of resting malaria vectors in houses with and without livestock 34
Figure 4.4: Proportion of blood meal detected from blood fed An. arabiensisl indoors and outdoors.. 46

ABREVIATIONS

ACTs:	Artemisinin-based Combination Therapies
AIC	Akaike Information Criterion
CDC:	Centre for Disease Control and Prevention
ELISA:	Enzyme-linked Immunosorbent Assay
GLMM:	Generalized Linear Mixed Model
HBI:	Human Blood Index
HBR	Human Biting Rate
IHI:	Ifakara Health Institute
IRS:	Indoor Residual Sprays
IRNs	Insecticidal treated Nets
LLINs:	Long lasting Insecticide-treated Nets
MBDs:	Mosquito-Borne Diseases
MoH:	Ministry of Health
NBS:	National Bureau of Statistics
NGOs:	Non-Governmental Organization
NMCP:	National Malaria Control Program
PCR:	Polymerase Chain Reaction
rDNA	Ribosomal Deoxyribonucleic Acid
RVF:	Rift Valley Fever
WHO:	World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Background Information

In the past two decades, malaria has become one of the most challenging vector-borne diseases worldwide, especially in sub-Saharan Africa. The use of insecticidal-treated nets (ITNs), indoor residual sprays (IRS), larval source management (LSM), and case management have been the cornerstones for the reduction of malaria cases and mortality (WHO, 2022). According to WHO. (2022), the use of ITNs and IRS has contributed to the significant decline of malaria case incidence (cases per 1000 population at risk) from 80 in 2000 to 57 in 2019, before increasing again to 59 in 2020. Also, the malaria mortality rate (deaths per 100,000 population at risk) has been reduced from 30 in 2000 to 13 in 2019 before increasing to 15 in 2020 due to the COVID-19 pandemic (Weiss et al., 2021). Other drawbacks to malaria control are malaria parasite resistance to anti-malaria drugs (Corey et al., 2016) and increased insecticide resistance by mosquitoes (Wipf et al., 2022), with the latter being reported in more than 88 countries in 2020 (WHO, 2022). The *Anopheles. gambiae* complex and *Anopheles funestus* group comprise the major and most efficient malaria vectors in sub-Saharan Africa (Sinka et al., 2012; WHO, 2022).

According to the World Health Organization (WHO), Tanzania was one among the top ten countries in 2021 for the number of malaria cases and related deaths (WHO, 2022). It accounted for 4.1% of all cases and 3.1% of all deaths recorded worldwide (WHO, 2022). Additionally, Tanzania was responsible for 12.8% of the malaria cases in east and southern Africa in 2020. The incidence of cases increased by 2.1% over the course of a year, between 2020 and 2021, going from 123 to 126 cases per 1000 people at risk. In contrast, over the same period, the mortality rate saw a slight decline of 1.7%, falling from 0.41 to 0.40 deaths per 1000 people at risk (WHO, 2022). Different measures and interventions have been adopted in the country by the National Malaria Control Program (NMCP) under the Ministry of Health (MoH) in collaboration with Non-Governmental Organizations (NGOs) and partners to control and reduce the prevalence and incidence rates of the disease. Different interventions include larval source management and indoor residual sprays, infection prevention; mass distribution of long-lasting insecticidal nets (LLINs) (Mosha et al., 2022), housing improvements (Bofu et al., 2023; Lwetoijera et al., 2013) and improved case management using Artemisinin-based Combination Therapies (ACTs) (Maheu-Giroux & Castro, 2013). Despite the use of these interventions, still there are some malaria cases and mortality that have been reported each year due to various probable reasons, such as changes in the host preference and seeking behaviours of major malaria vectors and the increased insecticide resistance of mosquitoes (Kisinza et al., 2017; Kreppel et al., 2020). Also, Minepa village in the Kilombero valley is among the villages with high malaria prevalence above the national average (Mshani et al, Unpublished). Therefore, it is important to study other factors including livestock keeping to ensure development of complementary tools that would be used to suppress the effects posed by malaria.

Apart from feeding on sugary plant liquids, female mosquitoes also need blood meal from vertebrates for the development and nourishment of their eggs to increase the generations of mosquitoes (Akogbéto et al., 2018; Takken & Verhulst, 2013). The blood of vertebrates has protein nutrients that aid in the development of eggs in the mosquito abdomen (de Carvalho et al., 2014). Extensive studies have been conducted on the host preference of anopheline mosquitoes in different localities. For instance, it has been found that some mosquito species, for example, *An funestus* s.s, *An. coluzzii* and *An. gambiae s.s* prefer human blood meal over other hosts (Ferguson et al., 2010; Takken & Verhulst, 2013), while other mosquito species, such as *An. arabiensis* and *An. stephensi*, partially feed on either animal (like goats, dogs, cattle, donkeys, and birds) blood or human (Ferguson et al., 2010; Mburu et al., 2021) depending on the availability and abundance of hosts in a particular geographical area (Chaves et al., 2010; Thiemann et al., 2011).

In the process of taking a blood meal, mosquitoes can transmit malaria *Plasmodium* parasites from an infected person to another person who is uninfected (Mathenge et al., 2001; Muriu et al., 2008). The blood-feeding preference of anopheline mosquitoes is a significant parameter in the transmission of malaria parasites. The right identification of a preferred host by malaria vectors is important, for it determines the frequency at which the vector population feeds on humans (Mwangangi et al., 2003). This is the measure of human-vector contact and it is useful in the estimation of vectorial capacity. In sub–Saharan Africa, the main dominant species of *Plasmodium* parasites that affect a large population are *Plasmodium falciparum* and *Plasmodium vivax*, which account for more than 95% of all infections (Loy et al., 2017; Ollomo et al., 2009; Prugnolle et al., 2011; Twohig et al., 2019).

Tanzania is one of the countries with the largest number of livestock in sub-Saharan Africa, comprising approximately 33.8 million cattle (98% of which are indigenous breeds), 24.5 million goats, 8.5 million sheep, 3.2 million pigs, and 87.7million chickens (NBS, 2021). Livestock keeping is one of the economic activities that plays an important role in poverty alleviation, food security enhancement, employment creation, and environmental conservation, particularly, in village settings (Herrero et al., 2013). For a long period of time, it has been proposed to establish interventions to control malaria by using the available livestock in societies by diverting malaria vector biting from humans, an intervention known as zooprophylaxis (Asale et al., 2017; Donnelly et al., 2015). In the process of assessing the effectiveness of livestock-based malaria interventions, there are still contradictions in the number of studies that have been conducted in different parts of the world. Some studies support a zooprophylaxis approach, while others go against it whereby there are studies that indicated that livestock act as the risk factor for malaria transmission when they are corralled in close proximity to the households, for instance the case of Indonesia (Hasyim et al., 2018), Pakistan (Hewitt et al., 1994), Kenya (Smit et al., 2016) and Ethiopia (Massebo et al., 2015; Tirados et al., 2011; Zeru et al., 2020).

It is clear that the impact of livestock on malaria transmission, distribution, and densities of potential malaria vectors is a complex issue that needs more investigation in different settings. This is shown by number of studies in which even the same species of livestock happen to have different impacts on malaria transmission and vector densities in different settings. For instance, a study conducted by Yamamoto et al. (2009) in Burkina Faso found that cattle have no significant protective effects on malaria transmission, while elsewhere (Mayagaya et al., 2015; Mburu et al., 2021), the presence of cattle showed a significant reduction in *An. arabiensis* mosquitoes.

Diseases, including malaria, do not occur randomly in a population; rather, they occur due to risk factors that are not randomly distributed in a population (Gordis, 2013). In order to achieve malaria elimination, it is necessary to identify hotspots of malaria transmission in order to effectively allocate scarce and limited resources to the hotspots of malaria transmission, not only at a large scale but also at a fine scale, such as villages and at the household level (Bousema et al., 2012), and it is more important to target the areas with a high risk of malaria transmission so that malaria elimination can be achieved (Hagenlocher & Castro, 2015). Enough understanding of how variables like livestock keeping affect malaria transmission is essential in determining where and how to prioritize malaria interventions.

Moreover, in order to achieve significant results in the fight against malaria and other mosquito-borne diseases, community participation is an important component to be taken into consideration (Ng’ang’a et al., 2021). There are a limited number of studies on the assessment of the knowledge and perception of community members about the relationship between livestock keeping and malaria transmission. For example, Nguyen-Tien et al. (2021), conducted a study to assess the knowledge and practices on the prevention of mosquito-borne diseases (MBDs) in livestock-keeping and non-livestock-keeping communities. The results showed that people in livestock-keeping communities had less knowledge of practices and prevention against MBDs than non-livestock-keeping communities. However, even the study did not assess the knowledge and perception of community members on the relationship between livestock keeping and malaria transmission. In south-eastern Tanzania, there are a limited number of studies focusing on the impact of livestock on the distribution and densities of malaria vectors. Therefore, this study investigated the impact of livestock management on malaria transmission risks. It further assessed the knowledge and perception of community members towards the relationship between livestock keeping and malaria transmission.

1.2 Statement of the Problem

Despite numerous studies on malaria hotspots, it's unclear if the presence of livestock at the household level affects malaria transmission risk. There are some studies that show that livestock keeping confers a protective measure against malaria transmission whereby mosquitoes are diverted towards livestock (dead-end hosts) such as cattle, which are not infected by *Plasmodium* malaria parasites, and consequently, reduce malaria transmission (Iwashita et al., 2014; Mayagaya et al., 2015; Mburu et al., 2021). However, on the other hand, some studies postulate that livestock keeping is a potential risk factor for malaria transmissions because it offers an alternative source of blood and breeding habitats, which increases vector survival and, subsequently, increases vector densities around households (Hasyim et al., 2018; Zeru et al., 2020). As Minepa, one of the villages which harbours both livestock keeper and rice farmers, it is necessary to investigate the relationship between livestock keeping and malaria transmission risks in order to provide information on further development of effective malaria control interventions. Moreover, in order to achieve significant results in the fight against malaria, community participation is an important component to be taken into consideration. Also, in a malaria-endemic areas including south-eastern Tanzania, there are a limited number of studies focusing on the impact of livestock on the distribution and densities of malaria vectors and assessing the knowledge and perception of the community towards the problem in question. Therefore, this study investigated the impact of livestock keeping on malaria transmission risks and explored the knowledge and perception of the community members towards the relationship between livestock keeping and malaria transmission.

1.3 Objectives of the study

1.3.1 Main objective

To assess the impact of livestock keeping on malaria transmission risks in rural south-eastern Tanzania

1.3.2 Specific objectives

1. To assess the impact of livestock keeping on the distribution and densities of malaria vectors.
2. To determine the host preference of mosquitoes in livestock-keeping and non-livestock-keeping households.
3. To explore the knowledge and perception of community members towards the relationship between livestock keeping and malaria transmission.

1.4 Research Questions

1. What is the impact of livestock keeping on the distribution and densities of malaria vectors?
2. What types of hosts are preferred by mosquitoes in livestock-keeping and non-livestock-keeping households?
3. How do community members understand and perceive the relationship between livestock keeping and malaria transmission?

1.5 Significance of the study

The study provides information that helps to clearly understand about the relationship between livestock keeping and mosquito distribution and densities, and addressing whether keeping livestock protects people or enhances malaria transmission in a study area. This information is helpful in designing and implementing suitable control interventions against the endemicity of malaria in a study area. Also, this study assessed the influence of other factors that contribute to the impact of livestock on malaria transmission such as distance between household and livestock holdings which is not clear in the body of knowledge. Furthermore, the study provides the need for knowledge and education to the community members in order to ensure better participation in malaria and other mosquito-borne diseases.

1.6 Scope of the study

This study was conducted in Ulanga district, Morogoro region, Tanzania. Forty village households were randomly selected: those with livestock and those without livestock. Data on mosquitoes were collected over a four-month period during the rainy season to evaluate the distribution and densities of mosquitoes in households with and without livestock. This assessment occurred during a period of high malaria transmission in a selected village in the Kilombero Valley so that the required measures should be taken to reduce malaria transmission risks at household level. Also, mosquito blood meal was tested to assess their preference towards different kind of hosts available around the homesteads This study did not include data on the prevalence of malaria but only focused on the risks of malaria transmission and the knowledge and perception of the community members on the impact of livestock on malaria transmission risks using in-depth interviews.

1.7 Limitation of the study

In this study, frequent rainfalls brought challenges, especially during the nights when volunteers were required to collect mosquitoes outdoors using human-baited double-net traps. To mitigate this issue, nylon pieces were provided to minimize the impact of rain on mosquito collections.

This study was conducted in the rainy season only. This is because the timeline for submitting a report is short compared to the necessary time that is required for collecting data. Therefore, it would be important to conduct this study in a dry season in order to assess the impact of livestock mosquito density during the dry season.

Given that agriculture is the main economic activity of the community members, it was not easy to find enough participants for the qualitative study assessing the knowledge of people on the relationship between livestock keeping and malaria transmission risks. This is because most of the community members in the study area spend most of their time in rice fields, and some migrate to rice fields for effective cultivation. Thus, an extended period of conducting interviews was necessary to allow all intended respondents to fully participate in the study.

The study did not assess the epidemiologic aspect of the impact of livestock on malaria transmission, but rather, it assessed the entomological part of it. Also, it did not assess the sporozoite status of the mosquitoes collected.

1.8 Definition of Terms

1.8.1 Zooprophylaxis

Zooprophylaxis is the use of wild or domestic animals such as cattle, which are not the reservoir host of a given disease (dead-end or decoy hosts), to divert the blood-seeking malaria vectors from human hosts of that disease (Asale et al., 2017). This is the method of malaria control that is used to reduce the risk of malaria transmission, mostly in livestock-keeping communities and in places where the main malaria vector is zoophilic (Franco et al., 2006). In practice, zooprophylaxis can be used in combination with other malaria control interventions, such as the use of LLINs and IRS. As a method of malaria control, zooprophylaxis can be used in active, passive, or sometimes integrated form, combined with chemical insecticides used in public health (Kaburi et al., 2009).

1.8.2 Zoopotentiation

Zoopotentiation is a phenomenon that occurs in contrast to zooprophylaxis. It actually occurs when livestock increase malaria transmission risks by attracting mosquitoes through their odors, creating an additional blood meal source for female mosquitoes, and hence increasing their survival rate and fecundity (Asale et al., 2017), but also through cattle puddles for drinking water and hoof prints, especially during the rainy season, become potential breeding habitats for malaria vectors. Zoopotentiation has been evidenced by a number of studies, such as in the Gambia (Bøgh et al., 2001), Indonesia (Hasyim et al., 2018), and Ethiopia (Zeru et al., 2020).

1.8.3 Human Blood Index (HBI)

This is the proportion of female mosquitoes sampled that are positive for human blood; usually, it is calculated for a given mosquito species that was verified to have human blood in their abdomens (Williams & Pinto, 2012). It is an index that is often used to assess mosquito host preference in a given area in order to formulate proper control interventions that will target a large population of mosquitoes depending on their host preference (Takken & Verhulst, 2013). HBI is estimated by sampling female mosquitoes indoors and outdoors and identifying the source of their food by using molecular methods such as enzyme-linked immunosorbent assays (ELISA) (Beier et al., 1987; Chow et al., 1993; Mwangangi et al., 2003).

1.8.4 Human Biting Rate (HBR)

This is the number of mosquito bites per person per night (Tangena et al., 2015). It is calculated by dividing the number of mosquitoes caught using various methods, such as CDC-Light traps and Prokopack aspirators, by the number of houses, location (indoor or outdoor), and nights. It is used to compare the risk of malaria transmission by showing the mean number of female mosquitoes per house per night in different places (Brugman et al., 2017; Smith et al., 2021).

1.8.5 Host seeking

The primary purpose of a mosquito's bite is to feed on blood from its host. For the development of their eggs, female mosquitoes need the nutrients in blood. Body heat, body odour, carbon dioxide, and specific chemicals released by the skin are among the cues that draw them to their hosts (Takken & Verhulst, 2013; Thiemann et al., 2011). These cues may cause certain mosquito species to be drawn to particular hosts. Nevertheless, nectar and plant fluids are usually the primary sources of food for male mosquitoes, which do not need blood to reproduce. It's important to note that while seeking a blood meal, mosquitoes can also transmit diseases such as malaria, dengue fever, Zika virus, rift valley fever, and others, making them vectors for various pathogens.

1.8.6 Zoophily

Zoophily refers to the behavior of insects, particularly mosquitoes, in seeking a blood meal from animals (Takken & Verhulst, 2013). Mosquitoes that exhibit zoophily prefer to feed on animals rather than humans. This behavior is in contrast to anthropophily, where mosquitoes primarily seek blood from humans (WHO, 2013).

1.8.7 Exophily

Mosquitoes that prefer to rest and hide outside, away from populated areas, are said to exhibit exophily. Exophilous mosquitoes prefer to rest outdoors, in bushes, trees, and other natural habitats, as opposed to indoors, like in houses (WHO, 2013). This behaviour affects mosquito control interventions, particularly those aimed at diseases that mosquitoes transmit. For example, bed nets and indoor residual spraying may not be as effective against exophilic mosquitoes because these species spend little time indoors, where these treatments are usually used. Understanding mosquito behaviour, including resting habits such as exophily, is essential to putting into practice efficient vector control strategies and lowering the spread of diseases carried by mosquitoes.

1.8.8 Differential attractiveness

Because of a variety of circumstances, mosquitoes may display differential attractiveness, which makes them more drawn to particular people or animals. A host's appeal to mosquitoes is influenced by a number of factors, including body colour, body heat, carbon dioxide emission, skin chemicals, genetic factors, and clothing colour (Takken & Verhulst, 2013)

CHAPTER TWO: LITERATURE REVIEW

2.1 Host seeking behaviour of malaria vectors and its importance to malaria control

Vector ecology is one of the most important factors that should be well investigated in order to achieve the goal of the Global Malaria Eradication Program, which was launched in the middle of the last century (Ferguson et al., 2010). This takes into consideration the host-seeking behaviour of potential malaria vectors when designing vector control interventions. The knowledge of the behaviours of the main malaria vectors in a particular geographical area is regarded as the foundation of victory over malaria vectors and transmission (Ferguson et al., 2010). Epidemiologically, when the mode of transmission of a disease or condition and vector ecology are known, it encourages the design of novel control interventions to reduce malaria transmission as host-seeking behaviour differs among malaria vectors. Some mosquito species, such as *An. funestus,* feed mostly on humans, while other species, such as *An. arabiensis,* obtain their blood meal partially from humans and other vertebrates (Ferguson et al., 2010; Takken & Verhulst, 2013). Mosquitoes behave differently in various environments and geographical areas depending on other factors such as climatic conditions and vector compositions (Pates & Curtis, 2005). Thus, it necessitates an in-depth investigation to reveal the true host-seeking behaviour of malaria vectors and achieve victory over life-threatening malaria disease.

2.2 Epidemiological relevance of livestock keeping and malaria transmission

Two key messages are consistently presented in various published studies investigating the relationship between livestock keeping and malaria transmission. First, the distance between livestock pens and human dwellings is an important factor in malaria transmission risks. It is reported that livestock reduce the risk of malaria transmission when they are kept far from human dwellings, while the risk of malaria transmission is higher when livestock are within or near human dwellings (Mburu et al., 2021). Secondly, the feeding preference of mosquito vectors in a given area is an important parameter for malaria transmission. It is frequently mentioned that in areas where mosquitoes prefer to feed on livestock, keeping livestock can provide a protective effect, unlike in areas where the local mosquito population prefers to feed on humans (Takken & Verhulst, 2013). With regards to the distance between houses and livestock pens, however, it is not known which optimal distance is accepted between human dwellings and livestock shelters so as to observe the protective efficacy of livestock against malaria transmission. Bouma and Rowland (1995) suggested that livestock should be kept between human dwellings and breeding habitats, which sometimes becomes difficult because some mosquito breeding habitats cannot be easily identified and some may be in close proximity to households.

2.3 Differential attractiveness of hosts to mosquitoes

The differential attractiveness of different animal types to mosquitoes needs to be well established. An observation from different studies was variations in attractiveness to mosquitoes by different animal types. Some mosquitoes are more attracted to cattle than humans, and as such, they are associated with reduced malaria transmission risks (Mayagaya et al., 2015; Mburu et al., 2021). A study done in Burkina Faso found that donkeys, pigs, and rabbits had protective effects against malaria transmission risks (Yamamoto et al., 2009). However, they could not find any association between either a reduced or increased risk of malaria transmission for other animal types, even when animals were either kept indoors or in close proximity to human dwellings. Furthermore, other studies found that keeping goats increased the risk of malaria transmission, while keeping cows reduced the risk of malaria transmission (Semakula et al., 2015). In this observation, it was shown that mosquitoes behave differently in different localities due to variations in climatic conditions, urbanization, host availability, and abundance. Future studies are needed to explore the behaviour of local mosquitoes (Takken & Verhulst, 2013) in different malaria endemic zones for effective malaria elimination (Hagenlocher & Castro, 2015).

In order to optimize the effectiveness of interventions in plummeting malaria transmission, it is also important in malaria endemic zones to investigate the behaviours of the main local malaria vectors so that appropriate intervention should be employed with respect to the mosquito's varying behaviour. Zoophilic mosquitoes are important for malaria transmission. This might seem counterintuitive since *Plasmodium* parasites that infect humans cannot infect cattle, which reduces the opportunity for vectors to acquire or transmit *Plasmodium* (Waite et al., 2017). However, the degree of zoophily and exophily vary from one mosquito species to another and from one geographical area to another.

2.4 Livestock keeping as a spatial targeted malaria intervention

Malaria hotspots are important for sustaining transmission even in areas where transmission is seasonal; thus, targeting these areas is expected to achieve a significant reduction in malaria transmission (Bousema et al., 2012). Similarly, mapping of the hotspots would help predict transmission patterns in villages and therefore improve planning for interventions. With such approaches, ongoing malaria prevention strategies could be greatly enhanced. Various studies on hotspot analysis postulate that their occurrence is associated with environmental factors such as proximity to mosquito breeding habitats (Kelly-Hope et al., 2009). However, even though hotspots can occur at different levels, including household level, important factors such as the presence of livestock are not often considered in models used to map spatial malaria patterns in communities. For instance, a study conducted in south-eastern Tanzania provided evidence that the spatial distribution of mosquitoes is influenced by the distribution of household occupants, such that houses with a high number of occupants appear to attract more mosquitoes compared to houses with fewer occupants (Kaindoa et al., 2016).

As malaria control moves towards elimination, it is necessary to begin focusing the limited financial and human resources on hotspots identifiable not only at large-scale landscape levels but also at fine scales within villages (Hardy et al., 2015). A greater understanding of how variables such as livestock keeping influence malaria transmission is essential in determining where and how to prioritize malaria interventions. It should be noted that in assessing the impacts of livestock, other factors such as social, economic, environmental, and anthropogenic factors should be considered. The optimal distances from human dwellings to livestock sheds should be established so as to maximize the impact of targeting livestock for malaria control.

2.5 Controlling vector density using livestock-based vector control options

There are several livestock-based malaria control interventions. They include the use of ivermectin (Khaligh et al., 2021) and spraying cows with insecticides such as deltamethrin (Pooda et al., 2015). For instance, the study conducted in Ethiopia (Franco et al., 2014) found that, in order to apply livestock-based interventions, three conditions must be considered: (1) high treatment coverage in the possible livestock population; (2) using insecticides with stronger or longer-lasting insecticidal effects; and (3) assessing whether there is increased attractiveness of the livestock to the vectors or not. Therefore, the development of optimal control interventions depends on an understanding of the specific feeding and resting behaviours of the species complexes within a specific geographical location. However, there are two concerns about spraying cows with insecticides. First, mosquitoes in many places have developed resistance against these chemical-based interventions (Lyimo et al., 2017). Secondly, chemicals might have repellent effects, hence diverting mosquitoes from houses with livestock to nearby houses without livestock and increasing the risk of disease transmission.

Thus, the choice and application of chemicals for vector control should consider these two possible effects. However, current evidence suggests that the use of endectocides such as ivermectin has been effective against malaria vectors and may be used to complement LLINs and IRS (Mahande et al., 2007). Different studies in Tanzania (Hemingway & Ranson, 2000), Kenya (Slater et al., 2020), Uganda (Smit et al., 2016) and elsewhere (Ashton et al., 2011) have proven that the use of cattle-administered ivermectin for controlling vectors could improve malaria control outcomes as it reduces survival rates and egg production of mosquitoes, hence crushing the vector population.

Using livestock-based interventions does not mean that other malaria interventions are left behind. However, it has been documented that the integration of livestock-based interventions and other malaria interventions in village settings, such as LLINs and IRS, has greater and more significant effects on reducing malaria transmissions (Iwashita et al., 2014). The study by Iwashita et al, (2014) reveals that high coverage of LLINs and IRS will push indoor host-seeking and resting mosquitoes outdoors for blood meal-seeking where they will be pulled by livestock such as cattle because there could be no other blood meal source other than livestock, which are dead-end or decoy hosts This is also revealed by Kaburi et al. (2009) in Kenya, where the use of LLINs and livestock keeping reduced malaria transmission risks (reduction of HBI) when there were less than four cattle within the compound. Thus, LLINs and IRS can act as push factors while livestock act as pull factors to attract mosquitoes, which results in a reduction in human-vector contact and, subsequently, a reduction in malaria transmission. Other interventions, such as repellent lotion (Maia et al., 2013) and treated eave ribbons (Kaindoa et al., 2021; Mwanga et al., 2019) may be used alongside LLINs and IRS to reduce early bites at dusk or dawn and push host-seeking and resting mosquitoes from indoors to outdoors, where they can divert to livestock.

2.6 Community engagements and malaria control

It should be noted that whether using endectocides or developing other livestock-based vector control options, community engagement is an important key (Adhikari et al., 2017). This is due to the fact that livestock are owned and kept by community members, and their acceptance and adoption of the interventions are dependent on their understanding of their importance and any risks. Associated with the interventions. There is limited information on the knowledge, attitudes, and practices of livestock-keeping communities on the relationship between livestock and malaria transmission in their settings. Nguyen-Tien et al. (2021) assessed the knowledge, attitude, and practice related to the prevention and control of mosquito-borne diseases (MBDs) in livestock-keeping and non-livestock-keeping communities and further explored how health workers perceive MBDs and livestock keeping. Surprisingly, knowledge about malaria control and prevention practices was very high among households without livestock compared to households with livestock, and the most mentioned diseases were dengue and malaria. With regards to risk factors for malaria transmission, most participants mentioned the presence of stagnant water, weather, house type, awareness levels, and the presence of livestock. (Nguyen-Tien et al., 2021). Despite assessing the effect of livestock on the prevalence of malaria, Hasyim et al. (2018) did not capture the attitude and perception of community members about how they know and perceive the problem. Whether livestock has zooprophylaxis or zoopotentiation, the community must be aware of the situation, and a proper understanding of the situation will encourage better malaria control strategies among the community members. Further studies are needed to assess the coping strategies among livestock-keeping communities. This necessitates understanding how community members in this area perceive livestock keeping and malaria transmission. Livestock-keeping communities should be engaged to allow high participation in vector control, especially with vector control tools that directly involve cattle as a means of delivering interventions. Also, due to the fact that livestock keeping is one of the most important economic activities, especially in rural settings, community participation will contribute to the management of these activities while minimizing the transmission of MBDs and maximizing living standards and public health.

2.7 Livestock and potential for other mosquito borne diseases transmission

Though this research focused on malaria, there are several diseases associated with mosquitoes and livestock. Rift Valley Fever (RVF), for example, affects both livestock and humans (Kawaguchi et al., 2004). Though the disease is transmitted by infected mosquitoes, it can also be transmitted through contact with infected animal tissues. Livestock keepers are among the riskiest groups. While designing interventions to protect livestock-keeping communities against malaria transmission, other mosquito-borne diseases associated with livestock should also be considered. This calls for multisector approaches, which include the health sector, veterinary sector, housing and settlement sector, as well as the environmental sector, so as to design a consolidated approach against mosquito-borne diseases. As stated in one health approach, the health of humans, animals, and ecosystems is very closely interlinked. The close link between human, animal, and environmental health requires very close coordination, communication, and collaboration between these three potential sectors. These collaborative efforts will help to prevent all diseases affecting humans and animals at large.

2.8 Conceptual framework

The conceptual framework shows the relationship between the independent, dependent, and intervening variables under study. In this study, vectors species composition and density, and blood meal composition were regardes as the response variables. The predictor or independent variable was livestock keeping which was divided into number and species of livestock and the distance between houses and livestock pens. The relationship between the independent and dependent variables was sought to be affected by the modifying variables such as the number of household occupants and the household characteristics

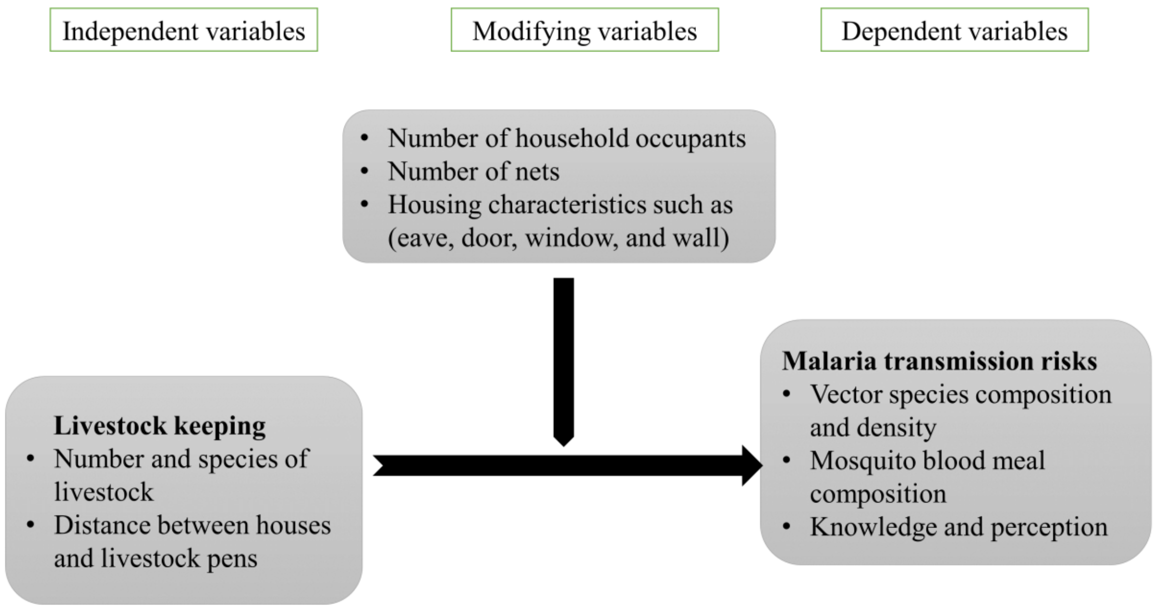

Figure 2.1: Conceptual framework

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CHAPTER THREE: METHODOLOGY

3.1 Study area

This study was conducted in Minepa village (8.21°S to 8.29°S, 36.67°E to 36.71°E), Ulanga district, which is found in Kilombero Valley in south-eastern Tanzania (Figure 3.1). The annual rainfall and temperature vary from 1300 to 3600 mm and 15 to 35 °C, respectively (Urio et al., 2022). Most of the residents are small-scale farmers and engage in livestock husbandry, while others engage in small businesses (Kato, 2007; Matowo et al., 2020). Common livestock that are kept are cattle, goats, sheep, dogs, pigs, and chickens. The principal malaria vectors in the study area are *Anopheles arabiensis* and *Anopheles funestus*, which contribute to more than 80% of contemporary malaria transmission (Kaindoa et al., 2019; Lwetoijera et al., 2014). Other *Anopheles* mosquitoes are found in this area, such as *An. coustani, An. pharoensis, An. squamosus, An. ziemanni,* and *An. wellcomei,* as well as other culicine mosquito species such as *Mansonia*, *Culex,* and *Aedes* (Kaindoa et al., 2019). The main malaria control intervention in the area is LLINs (Renggli et al., 2013). According to the 2022 population census, a village has more than 1786 homesteads.

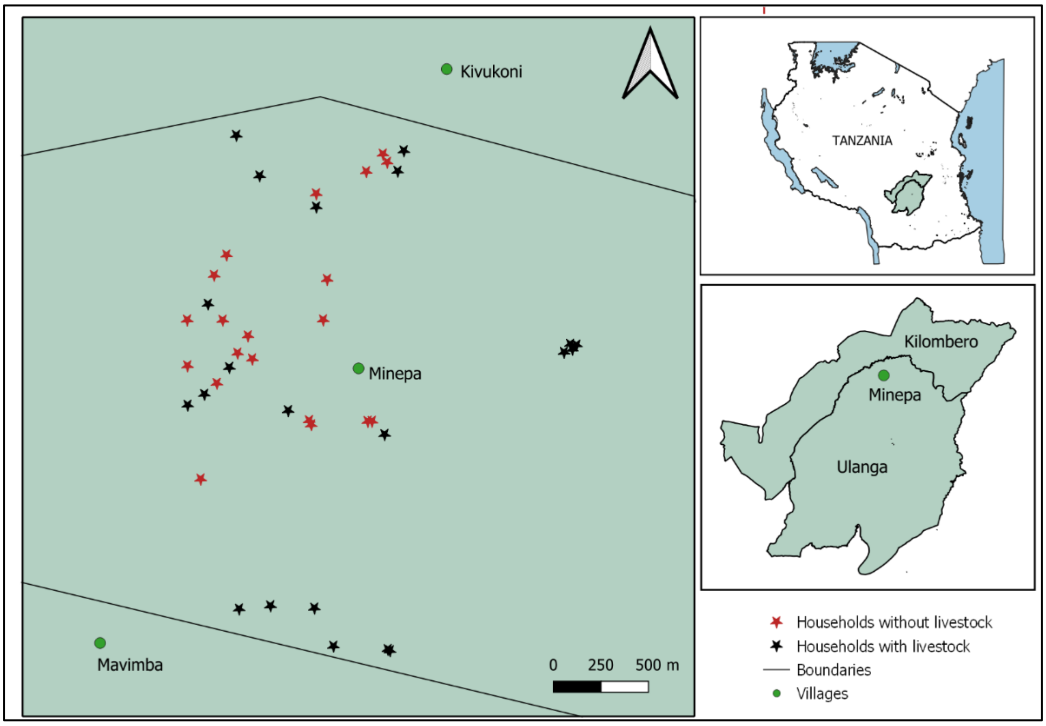

Figure 3.1: A map of a study area and distribution of households under study

3.2 Study design

In this study, a mixed-methods study design was conducted to investigate the impact of livestock keeping on malaria transmission risks. A quantitative study was conducted to assess mosquito species densities, abundance, and blood meal preference, while a qualitative study was conducted to investigate the knowledge and perception of the community members about the relationship between livestock keeping and malaria transmission risks.

3.3 Population

In the quantitative study, a target population is all houses, livestock, and all *Anopheles* mosquitoes, while in the qualitative assessment, a target population is all household heads in Ulanga district, south-eastern Tanzania.

3.4 Sample size determination and sampling techniques

3,4.1 Sample size determintion

The sample size for this study was determined by the idea of power calculation for generalized linear models developed by Cohen (1988). A '*pwr.f2.test()'* function from *pwr* package (Champely et al., 2018) in R, open-source statistical software version 4.2.1 was used to estimate a minimum sample size in each arm of the study. The sample size was calculated assuming a 50% reduction in the number of mosquitoes between houses without livestock (control) and houses with livestock. To achieve 80% power with a 5% significance level, A total of 16 sample houses was reached, however it was approximated to 20 sample houses in each arm of the study to increase accuracy of the results. Therefore, we simulated that a minimum of 20 houses were needed for each arm of the study, and therefore, a total of 40 sample houses were used in this study (20 with livestock and 20 without livestock) in whcich mosquito collection was done repeatedly every week in a duration of 4 months. Therefore, 40 households were randomly selected within astudy area

However, in qualitative interviews, data were collected until saturation was reached. Saturation is the point at which additional interviews would not yield new information on the main subjects of interest (Guest et al., 2006; Hennink & Kaiser, 2022; Saunders et al., 2018). Thus, in this study, the interviewers stopped at the 20^{th} respondent, when there was no new information added from the respondents. Also, this was done in the relatively homogeneous population

3.4.2 Sampling techniques

The random sampling was used for household selection for the quantitative part of this study. For the qualitative part, a purposive sampling of the household heads was used where it involved those whose houses were used for mosquito collection during that period

3.5 Inclusion and exclusion criteria

The inclusion criteria for the houses include; Houses selected must have eave space between wall and roof so that mosquito can be free to enter inside even if all doors are closed, Houses must have unscreened windows or have holes to allow mosquitoes. Also, the participants should be those whose houses are used for mosquito collections This is because they are the ones that witnessed all the processes of mosquito collections in thir houses.

All the houses that have no eave spaces were excluded in the study. Houses with screened windows were also not included in the study for they do not allow mosquitoes to enter when all dores are closed. The study did not allow in-depth interview for the community members whose houses were not recruited for the mosquito collections.

3.6 Reliability

To ensure the reliability of the study results during the experiments, standard methods for mosquito collection were used. For indoor collections, a commonly used CDC-light trap was used to collect host-seeking mosquitoes, while a standard prokopack aspirator was used to collect resting mosquitoes, as described in Activity 1.2.1. For outdoor mosquito collections, human-baited double nets and resting buckets were used to sample host-seeking and resting mosquitoes, respectively.

A pilot study was conducted to test the in-depth interview (IDI) guide used in the qualitative study prior to commencing the field data collection. A sample of four participants (10% of intended participants) from Kivukoni, another village in Ulanga district were recruited. The village residents engage in agricultural activities and livestock keeping. This was done to assess and verify the tool to ensure that it captured basic themes as planned and to expose and train the research assistants. Aftter recruitment of 4 participants (2 male and 2 females) we conducted the IDIs with them separately and all questions were asked and there were no issues came from the tool used because it captured every information needed for our specific objective. The interviews took between 35 to 50 minutes All research assistants used in the study had prior experience and exposure to conducting qualitative studies in rural settings.

3.7 Validity

All mosquito traps were tested for their ability to catch mosquitoes before going to the field for data collection. This was done by by connecting the traps from manufacturers (CDC-Light traps and Prokopack aspirator) to the 12V. Also, additional traps for mosquito collection were kept as reserves to replace those that failed to function during the ongoing mosquito collection process.

An IDI guide was subjected to review by social research scientists, and it went through a series of amendments before being applied to the field. A high-quality IDI guide for field data collection was developed and used as required.

3.8 Ethical considerations

Permission to conduct this study was obtained from the Jaramogi Oginga Odinga University of Science and Technology ethical review committee (JOOUST-ERC) (Ref: JOOUST/DVC-RIO/ERC/E4) and from the Ifakara Health Institute's institutional review board (IHI-IRB) (Ref: IHI/IRB/No:41-2022). Before conducting the study, permission was sought from the district medical officers and local leaders through an introduction letter (Ref No. UDC/ADM/A.10/207/124). Permission to publish part of this thesis was obtained from National Institute for Medical Research (Ref. no.BD. 242/437/01B/19)

Additionally, consent to conduct the study was sought at both the communal and individual levels. Communal consent came from a face-to-face discussion with the local leader about the study and his request to conduct it in his village. Individual consent was obtained by discussing with each participant the study procedures and their implications and importance, followed by a request to participate. Those who agreed to participate were given written consent forms to fill in before participating in this study (Appendices 1, 2 and 3). Participants of the in-depth interviews were not allowed to mention their names and all data collected from them were confidentially kept. Participations were interviewed in their homesteads to give them chance to feel free and comfortable during the interviews and no one else was allowed to interfere with the interviews.

3.9 Data collection

3.9.1 Objective 1: To assess the impact of livestock on the distribution and densities ofmalaria vectors

Activity 1.1: A total of 40 houses (20 with livestock and 20 without livestock), as shown in Figure 3.1 and Figure 3.2, were randomly selected and visited once per week for a period of 4 months. The livestock survey was conducted in the selected households, and the following variables were investigated: (1) the presence and number of livestock, which were classified as (i) large-sized livestock such as cattle (Figure 3.2, b), calves, and donkeys; (ii) medium-sized livestock such as pigs and sheep (Figure 3.2, c), goats, and dogs; and (iii) small-sized livestock such as rabbits, cats, and poultry such as chickens, as described by Hasyim et al. (2018). (2) Distance from the household to where livestock are kept if they are kept outdoors; (3) Number of household occupants; (4) Housing characteristics such as eaves status, window status, wall status, door status, roof status, and floor status; and (5) usage and number of LLINs in use per person per night.

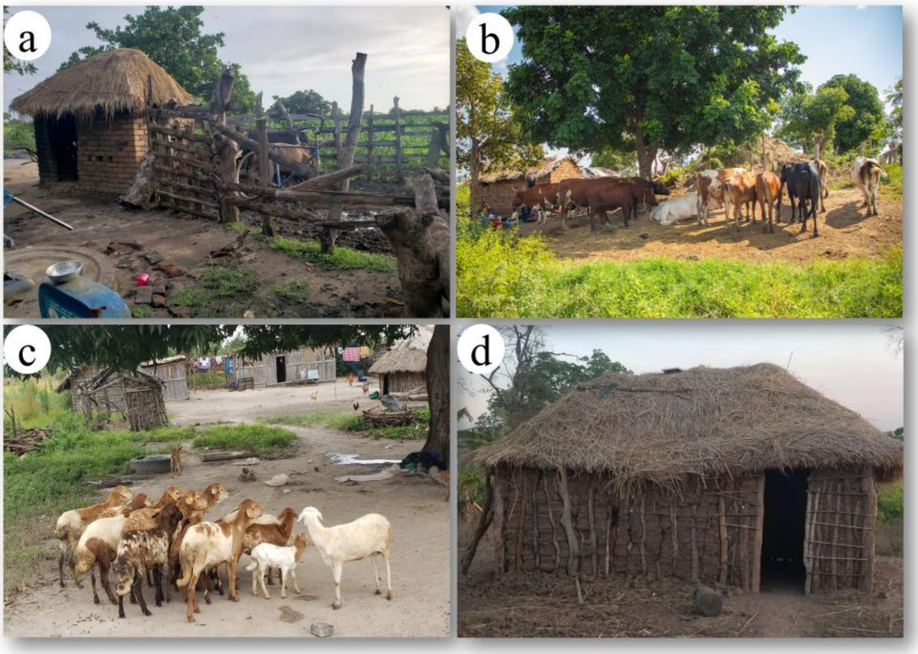

Figure 3.2: Showing **(a)** Livestock pen in close proximity to homesteads **(b)** Cattle are corralled outside homesteads **(c)** Some sheep which are kept in a study area **(d)** Sample houses that are found in a study area

<u>Activity 1.2.1</u>: Mosquito collection

Mosquito collection was done indoors and outdoors. Indoor mosquito collection of host-seeking mosquitoes was done using Centers for Disease Control and Prevention (CDC)-Light traps, model 512, John W. Hock Company, Gainesville, FL, USA (Mboera et al., 1998) as shown in figure 3.3(d). Resting mosquitoes were collected using a Prokopack aspirator (Figure 3.3,b), model 1419, John W. Hock Company, Gainesville, FL, USA (Maia et al., 2011; Vazquez-Prokopec et al., 2009). CDC-Light traps were set from 18:00 hours to 06:00, approximately 1.5 meters from the ground adjacent to the bed where the household occupant slept. To allow mosquito solicitation, the CDC-Light traps were connected to 12 V batteries. Aspirations were done using Prokopack aspirators, which were connected to 12 V batteries. On some occasions, aspirations were not possible to be conducted indoors because households' owners were not available to grant permission to do aspirations indoors; they left their houses very early in the morning for agricultural activities. Indoor aspirations were done from 06:00 hours to around 08:00. The period of aspiration per household lasted for up to 10 minutes, depending on the size of the rooms and houses where aspiration was done. Outdoor collection of hosts seeking mosquitoes was done by well-trained volunteers using human-baited double net traps (Figure 3.3,c) and traditionally-made syphons (Limwagu et al., 2019). Outdoor resting mosquitoes were collected using resting buckets. The double net traps were set outside each selected house from 18:00 to 06:00 hours by two volunteers, one from 18:00 hours to mid-night and the other from mid-night to 06:00 hours. All mosquitoes collected from double-net traps were kept in paper cups covered with a small cloth with small holes. The double net traps were used as an alternative to the human landing catch (HLC) (Tangena et al., 2015) because they protect volunteers from mosquito bites, which may accelerate malaria transmission. Resting buckets (20-liter volume) covered with black cloth inside were placed 5 to 10 meters away from selected houses from 18:00 hours to 06:00 hours to allow mosquitoes to rest after nighttime activities. In the morning, from 06:00 hours to around 08:00 hours, resting mosquitoes were collected from resting buckets using a prokopack aspirator. One resting bucket was placed in each selected house during the night of collection, but in houses with livestock, one additional bucket was placed around the livestock sheds (Figure 3.3, a). The resting buckets were laid on their sides and left open during the night of collection.

Figure 3.3: Mosquito traps which were used for mosquito collections; **(a)** a person collecting resting mosquitoes from resting bucket using prokopack aspirator near cattle shed **(b)** a volunteer collecting resting mosquitoes indoor using a prokopack aspirator **(c)** a double net trap (d) a CDC-light trap.

Activity 1.2.2: Mosquito identification

All mosquitoes collected were killed using petroleum fumes. Female *Anopheles* mosquitoes collected were morphologically identified by taxa and sex levels using key to the females of Afrotropical *Anopheles* mosquitoes (Coetzee, 2020) then classified according to their abdominal status as unfed, partly fed, fed, and gravid. *Anopheles* mosquitoes were kept individually or pooled in 1.5 ml Eppendorf containing silica gel desiccant. Every tube was assigned a unique identification number and placed inside storage boxes that included details such as the village name, house number, trap location, species name, and date. These samples were prepared for further laboratory analysis. A sub-sample of *Anopheles gambiae* s.l was submitted to the Ifakara Health Institute (IHI) laboratory for polymerase chain reaction (PCR) for identification of sibling species using a protocol developed by Scott et al. (1993). Additionally, *An. funestus* group mosquitoes were examined for mosquito identification by PCR using a technique developed by Koekemoer et al. (2002). The procedures were conducted as follows:

(i) DNA Extraction: DNA was extracted from a mosquito sample, typically using a process that breaks open the mosquito cells and releases their genetic material. Mosquito DNA from the *An. gambiae complex* and the *An. funestus group was* extracted from the adult mosquito legs (two legs per mosquito). The two legs of individual mosquitoes were placed separately in a 1.5-ml microcentrifuge tube, followed by the addition of 20µl of TE buffer (Tris-EDTA), then incubated at 95ºC for 15 minutes in the heating block. The tubes were vortexed for 2 minutes, and the DNA-containing supernatant was separated by centrifuging at 12,000 rpm at room temperature for 1 minute.

(ii) Target DNA Selection: Specific genetic markers or regions of DNA that are unique to the mosquito species of interest selected as the target for amplification. These markers are often chosen because they vary between different mosquito species.

For the *An. gambiae complex*, the PCR amplification is based on the species-specific nucleotide sequence of the ribosomal DNA (rDNA) intergenic spacer regions (IGS), as described by *Scott et al*. The IGS region of the rDNA was amplified in a 25µl reaction volume PCR master mix. The mixture contained 12.5 µl of *One Taq Quick Load 2X* master mix (containing 10X PCR buffer, $MgCl_2$, dNTPs, Taq DNA polymerase, and loading dye), 10 µM of each primer (*An. gambiae*, *An. rabiensis*, *An. merus,* and *An. quadrannulatus* primers), and 3 µl of DNA template overlaid by a drop of mineral oil.

For the *An. funestus group,* PCR amplification is based on the species-specific primers in the non-coding region called Internal Transcribed Spacer 2 (ITS2) on the rDNA. The ITS2A region of the rDNA was amplified in a 25µl reaction volume as developed by Koekmoer *et al. to* detect five members of the *Anopheles funestus* group. The PCR master mix contained 12.5 µl of *One Taq Quick Load 2X* master mix (containing 10X PCR buffer, $MgCl_2$, dNTPs, Taq DNA polymerase, and loading dye), 10 µM of each primer (universal, *An. vanedeen, An. funestus, An. rivulorum, An. parensis,* and *An. leesoni* primers), and 3 µl of DNA template over-lied by a drop of mineral oil.

(iii) Thermocycling conditions (PCR itself): The extracted DNA was heated to a high temperature to separate its double-stranded structure into two single strands (denaturation). Short DNA sequences, called primers, are added to the DNA sample. These primers are designed to bind specifically to the target DNA region of the mosquito species being identified (annealing).

(iv) DNA Amplification: DNA polymerase enzyme was added, and the reaction mixture cycled through a series of temperature changes. This process involves repeatedly heating and cooling the sample. During each cycle, DNA polymerase creates new DNA strands by copying the target region using the primers as a starting point. This results in the exponential amplification of the target DNA.

For the *An. gambiae complex,* the PCR conditions included an initial denaturation step at 94^{o}C for 5 minutes, followed by 30 cycles at 94^{o}C for 30 seconds, 50^{o}C for 30 seconds, and 72^{o}C for 30 seconds, with a final extension at 72^{o}C for 3 minutes. One negative control was included, which contained all contents of the PCR mixture except DNA. Two positive controls were also included: *An. gambiae* and *An. arabiensis,* from previous successful amplified samples or known insectary spp.

For the *An. funestus group,* PCR conditions included an initial denaturation step at 94^{o}C for 5 minutes, followed by 30 cycles at 94^{o}C for 30 seconds, 50^{o}C for 30 seconds, and 72^{o}C for 30 seconds, with a final extension at 72^{o}C for 3 minutes. One negative control was included, which contained all substances in the PCR mixture except DNA. Three positive controls were also included: *An. funestus, An. rivulorum, and An. leesoni,* from previous successful amplified samples.

(v) Species detection: After amplification, 10 µl of the PCR products were analyzed by electrophoresis on a 2.5% agarose gel stained with Classic View and a 100-bp DNA ladder included in the gel. DNA bands were revealed and photographed under ultraviolet light using the Kodac Gel Logic 100 imaging system. Mosquito species were determined by comparing the pattern or size of the DNA fragments produced to known standards or controls

3.9.2 Objective 2: To assess mosquito host preference in livestock-keeping and non-livestock-keeping households

Activity 2.1: Blood meal analysis was conducted for all fed mosquitoes collected in activity 1.2. This was done in the laboratory by using an ELISA test, in which a subsample of blood-fed mosquitoes was used to assess the host preference of female *Anopheles* mosquitoes collected indoors and outdoors in activity 1.2. This laboratory analysis was conducted to assess the presence of human, goat, bovine, chicken, or dog blood in the midgut or abdomen of blood-fed *Anopheles* mosquitoes. The procedure was done as follows:

Engorged *Anopheles gambiae* s.l. mosquitoes were analyzed for host bloodmeal identification by the antibody-sandwich ELISA to detect human, bovine, dog, goat, and chicken immunoglobulin G (IgG) antibodies as described by Chow et al. (1993). Mosquito abdomens to be tested were put in 100 µl of phosphate-buffered saline (PBS) at pH 7.4. Samples were incubated at room temperature for 30 minutes. After incubation, each abdomen was homogenized in PBS using a sterile pestle. All ground samples were stored at -20˚ C until time of use. 50 µl of the captured monoclonal antibodies were bound to the plate. After 30 minutes, the well contents were aspirated, and the remaining binding sites were blocked with 200 µl of blocking buffer (0.5% Casein from bovine milk and 0.1N NaOH in PBS, pH 7.4) and incubated at room temperature for 1 hour. After incubation, the blocking buffer was aspirated. This was followed by the addition of 45 µl of blocking buffer and 5 µl of homogenized samples, which were incubated at room temperature for 2 hours. Positive controls prepared from host sera and negative controls (laboratory-reared, non-fed female *Anopheles* mosquitoes prepared in the same way as the test samples) were tested for each plate. After an incubation of two hours at room temperature, the mosquito homogenate was aspirated, and the wells were washed twice with washing buffer (PBS/Tween 20) and dried by blotting each plate on paper towels. Peroxidase-labeled monocolonal antibodies (mAbs) were then added to the wells. After 30 minutes, the well contents were aspirated, washed four times, and 100 µl of the peroxidase substrate solution was added and incubated for 30 minutes at room temperature. After 30 minutes, the ELISA results were read visually and scored as negative if there was no colour change and positive if there was a colour change comparable to the positive control (Chow et al., 1993).

3.9.3 Objective 3: To explore the knowledge of community members towards the relationship between livestock keeping and malaria transmission in the study communities

<u>Activity 3.1:</u> A qualitative data collection was conducted in the village where mosquito collections were conducted. This was done by carrying out in-depth interviews (IDIs) with the household heads where mosquito collections were conducted to assess the knowledge and perception of the community members on the relationship that exists between livestock management practices and malaria transmission. An in-depth interview guide (Appendix 7) was prepared to capture the following areas of interest: (i) mosquito control and malaria transmission; (ii) livestock keeping practices; (iii) types of pesticides used to treat animals; (iv) distance between houses and livestock sheds; (v) relationship between malaria transmission and livestock keeping; (vi) impact of livestock keeping on malaria transmission. These IDIs included both households with and without livestock and included male and female participants. Every interview session lasted for 25 to 50 minutes. All sessions were done in local or village primary school buildings, and on some occasions, the sessions were done within participants' compounds.

3.10 Data analysis

For objectives 1 and 2, data were entered in Microsoft Excel from the Microsoft Office package of 2021, exported, and analysed using R statistical software version 4.2.1 (R, 2022). Data for objective 3 were transcribed, translated, and analyzed using a qualitative analysis tool, Nvivo software version 13 (Dhakal, 2022).

3.10.1 Data analysis for objective 1: Descriptive statistics were calculated on the mean number of mosquitoes collected in livestock-keeping and non-livestock-keeping households and presented in tables, charts, and graphs. All graphs were plotted using the *ggplot2* package (Wickham et al., 2016). Generalized Linear Mixed Models (GLMMs) following negative binomial distribution were used to model the mean number of female *Anopheles* mosquitoes as a response variable while the explanatory variables were the presence and number of different categories of livestock, distance between household and livestock sheds as fixed variables while Household ID and collection date were added as random variables to take into account of variations of the number of mosquitoes which wasexpected to be different in different households and different collection dates. The rate ratios (RRs) were used to assess the association between livestock keeping and malaria transmission risks. Also, the model was used to investigate the effect of the intervening variables (housing characteristics such as eave status, window status, door status, wall status, roof status, and floor status) on the number of malaria vectors collected. The household ID and collection date were used as random variables. All models were implemented using the *glmmTMB* package. (Brooks et al., 2023; Brooks et al., 2017).

The association between livestock and mosquito bite risk was also assumed to be mediated by other variables such as the number of household occupants and household characteristics such as window, roof, wall, eave space, and door status. Almost all houses shared similar characteristics, but not the same wall type or roof type. Both houses selected had an eave space between the wall and the roof, blocked windows and doors, and some spaces that allowed mosquitoes to enter indoors. In this case, the household type was categorized into four categories, namely: (i) thatched and mud wall; (ii) thatched and brick wall; (iii) plastered wall and iron sheet; and (iv) unplastered wall and iron sheet. When these intervening variables were adjusted together with the number of livestock, all the results were not statistically significant. Thus, the variables were used in a separate model to assess their effects on malaria vector density.

3.10.2 Data analysis for objective 2: Blood indices for different hosts (human, bovine, goat, dog, and chicken) were calculated and compared for mosquitoes collected from houses with and without livestock. The hosts' blood indices were obtained using the following formula:

$$Host's\ blood\ index = \frac{Total\ mosquitoes\ with\ host'sblood\ meal}{Total\ mosquitoes\ examined} * 100$$

Also, a multinomial logistic regression model was used to assess the influence of livestock and location on mosquitos' ability to get human blood. In this model, blood meal with three levels (human, other hosts, and mixed blood meal) was used as a response variable, while a household's livestock status and location (indoors or outdoors) were used as predictor variables. This model was implemented using the *multinom()* function from the *nnet* package (Ripley et al., 2016). The akaike information criterion (AIC) was used to select the best model that fit well the data (Portet, 2020). In this case, the first model included only presence of livestock as an independent variable, the second model included only location as an independent variable while the last model included both presence of livestock and loation as independent variable. The model with the lowest AIC value was selected tio be the best model for the interpretation of the results. Odds ratios (OR) and the 95% confidence intervals were estimated to show the effect of the presence of livestock and location upon mosquito ability to obtain blood meal of either human or non human hosts.

3.10.3 Data analysis for objective 3: All audio data recordings from IDIs were transcribed and then translated from Swahili to English. Notes taken during the interviews were incorporated into the written transcripts. The transcripts were then imported into NVIVO software version 13 (Dhakal, 2022) for coding. Findings were presented using the integration principles and practices in mixed methods designs as described by Fetters et al. (2013). Weaving approach was used, in which both qualitative and quantitative findings were reported together based on the relevant themes obtained from the codes identified. Direct quotations from the IDIs participants were reported in some selected cases to further describe the themes.

CHAPTER FOUR: RESULTS

4.1 Results for objective 1: Impact of livestock keeping on distribution and densities of malaria vectors

4.1.1 Summary of All Mosquitoes Collected

A total of 155,752 female mosquitoes were collected. Out of these, 86,491 were collected indoors and 69,261 were collected outdoors. Among mosquito traps, the CDC-Light trap captured the highest number of mosquitoes collecting 76,344 mosquitoes, followed by the double net trap, which collected 64,656. Resting buckets near the livestock pen collected the least number of mosquitoes (1,733) among the traps. As indicated in Table 4.1, 31.3% (48,676) were anophelines, and the remaining were culicine mosquitoes' species. Among the anopheline mosquitoes, *An. gambiae* sensu lato (s.l) was the most abundant, comprising 43,105 mosquitoes (27.7% of all female mosquitoes collected); others were *An. squamosus* (1972), *An. coustani* (1,705), *An. pharoensis* (1,686), and *An. funestus* (209). Near the livestock pens, *An. gambiae* s.l. was the most abundant among the *Anopheles* mosquitoes (95% of all Anopheles mosquitoes, n = 981), followed by *An. funestus* (2%, n = 33). There were no *An. squamosus* resting around the animal pens. *Culex* species was the most abundant among culicine mosquitoes, comprising 105,093 mosquitoes (67.5% of all female mosquitoes collected); others were *Mansonia* 1,758 (1.1%), *Aedes* 177 (0.1%), and *Coquilletidia* species 47 (0.03%). This information is well described in Table 4.1.

Table 4.1*: Summary of total number of mosquitoes collected using different traps*

Mosquito species	CDC-LT	DN-Trap	Prokopack	Resting bucket-NH	Resting Bucket-NL	Total (%)
An. gambiae s.l.	22674	17143	1079	1228	981	43,105 (27.7)
An. funestus	142	85	18	31	33	309 (0.2)
An. pharoensis	613	1060	11	1	1	1686 (1.1)
An. coustani	1045	637	7	5	11	1,705 (1.1)
An. squamosus	1242	625	4	0	1	1,872 (1.2)
Total anophelines	25,716	19550	1,119	1,265	1,027	48,677 (31.3)
Culex spp.	49871	43966	9008	1554	694	105,093 (67.5)
Mansonia spp.	658	1033	16	40	11	1,758 (1.1)
Aedes spp.	91	76	4	5	1	177 (0.1)
Coquilletidia spp.	9	31	0	6	1	47 (0.03)
Total culicines	50,629	45,106	9,028	1,605	707	107,075 (68.7)
Overall	76,344	64,656	10,147	2,870	1,734	155,752 (100)

CDC-LT=Centre for Disease Control and Prevention-Light trap, DN=Double net, NH=Near Houses, NL=Near Livestock pens

4.1.2 Molecular identification of mosquito species

A total of 4,068 mosquitoes were submitted to the laboratory for mosquito identification of sibling species. Among all *Anopheles gambiae* s.l. examined for sibling species identification, 98% (n = 3,991) of these samples were successfully amplified. Almost all verified mosquitoes were *Anopheles arabiensis*, except for one sample that was verified to be *Anopheles quadriannulatus* collected from a house with livestock. A total of 81 *An. funestus* mosquitoes were analyzed for sibling species composition, out of which 87% (n = 67) were amplified. Out of these, 43 were identified as *An. revulorum*, 21 were *An. funestus* sensu stricto (s.s.), and 3 were *An. leesoni*.

4.1.3 Common livestock found in the study area

The moment this study was conducted, most of the livestock corralled in the selected households were poultry (267), followed by medium-sized animals (234) which include sheep, goats, dogs, and pigs, and large-sized animals, specifically cattle (115). This is shown in table 4.2. Small animals were not very common.

Table 4.2: Number of livestock present in selected households understudy

Category	Animals' types	Total
Large size	Cattle	115
Medium size	Sheep	65
	Goats	78
	Dogs	30
	Pigs	58
Small size	Cats	8
Poultry	Chicken	267
Total		661

4.1.4 Abundance of Host-Seeking Mosquitoes in Houses with and Without Livestock

The mean number of *An. gambiae s.l.* host-seeking mosquitoes collected by CDC-light traps indoors in houses with livestock was 38.9 ±2.32 SE, while in houses without livestock, the mean catches were 35.3 ± 2.54 SE (Figure 4.1). The mean catches for indoor collection of *An. funestus* host-seeking mosquitoes in houses with livestock were 0.354 ±0.048 SE, while in houses with no livestock, the mean catch was 0.144 ± 0.029 SE (Figure 4.1).

For outdoor collection, the mean number of *An. gambiae* collected in houses with livestock was 24.6 ± 1.73 SE, while in houses without livestock, the mean catch was 31.9 ± 2.33 SE (Figure 4.1). The outdoor mean catch of *An. funestus* in houses with livestock was 0.228 ± 0.038 SE, while for houses without livestock, the mean catch was 0.129 ± 0.028 SE (Figure 4.1).

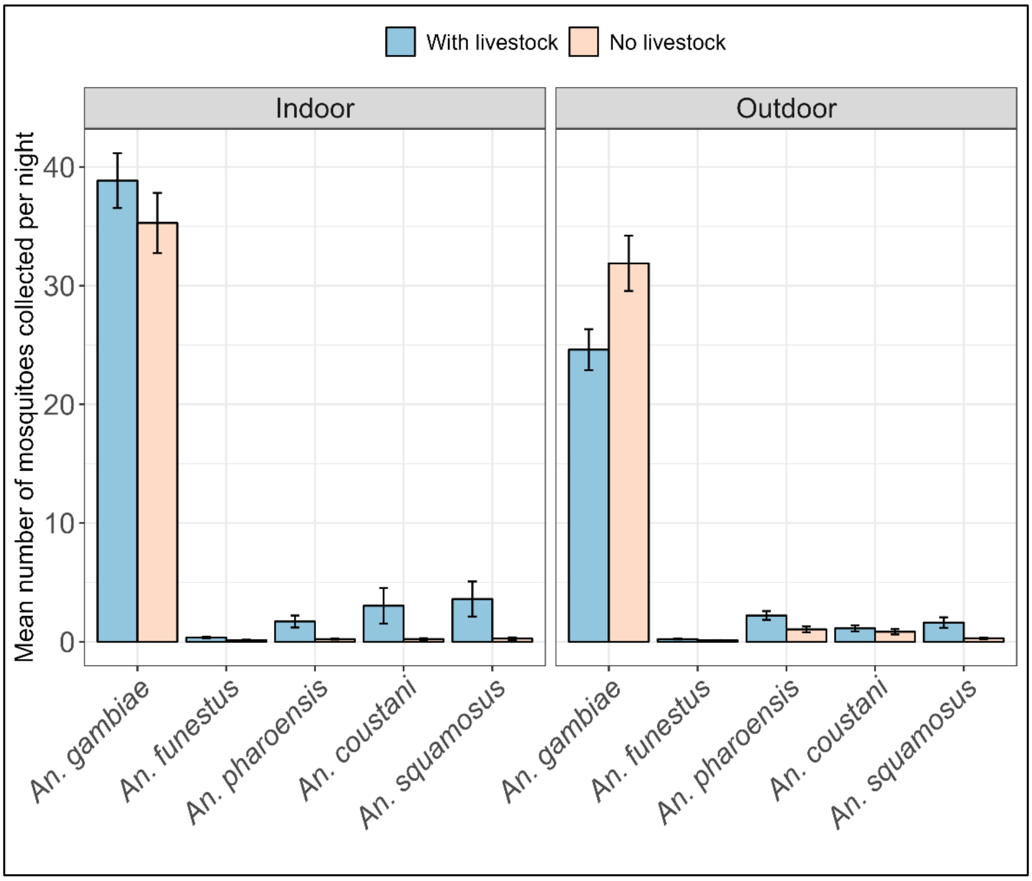

Figure 4.1: Density of host-seeking malaria vectors in houses with and without livestock

There was a slight increase in the mean number of *An. gambiae* s.l. mosquitoes in houses with livestock from January to April, but it was somehow constant in houses without livestock. However, for the other *Anopheles* species, an increase in mosquito density was observed in houses with and without livestock between January and April (Figure 4.2).

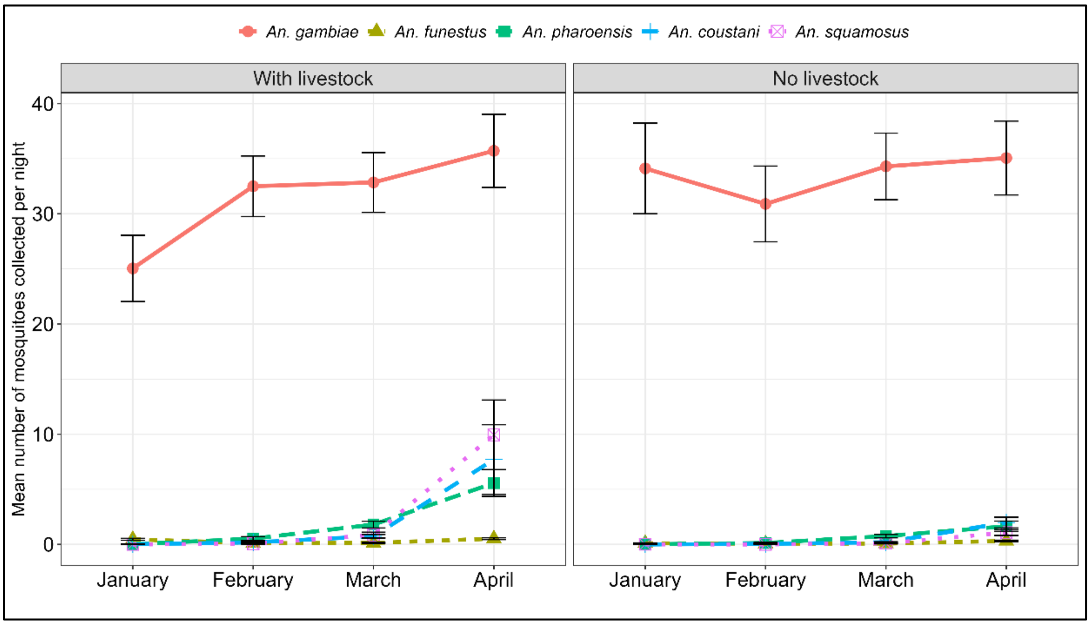

Figure. 4.2: Trend of mosquito density in houses with and without livestock in different months during the rainy season; a period of high malaria transmission.

4.1.5 Abundance of Resting *Anopheles* Mosquitoes in Houses with and Without Livestock

More resting mosquitoes were collected indoors and outdoors from houses with livestock compared to houses with no livestock. The mean number of *An. gambiae* s.l. mosquitoes collected indoors from houses with livestock was 2.50 ± 0.416 SE (Figure 4.3), while in houses without livestock, the mean number of *An. gambiae* s.l. mosquitoes were 1.11 ± 0.243 SE. For outdoor collections, the mean number of *An. gambiae* s.l. mosquitoes collected from houses with livestock was 2.87 ± 0.355 SE, while in houses without livestock, the mean number of *An. gambiae s.l.* mosquitoes were 1.06 ± 0.194 SE (Figure 4.3). The mean numbers of other resting malaria vectors, such as *An. funestus, An. coustani, An. pharoensis, and An. squamosus,* were marginally less than zero indoors and outdoors. Thus, *An. gambiae* s.l. mosquitoes were the most abundant malaria vectors indoors and outdoors. As it is shown in Figure 4.3, the indoor and outdoor collections of *An. gambiae* s.l. and *An. funestus* show that there were more mosquitoes resting in households with livestock than in houses with no livestock.

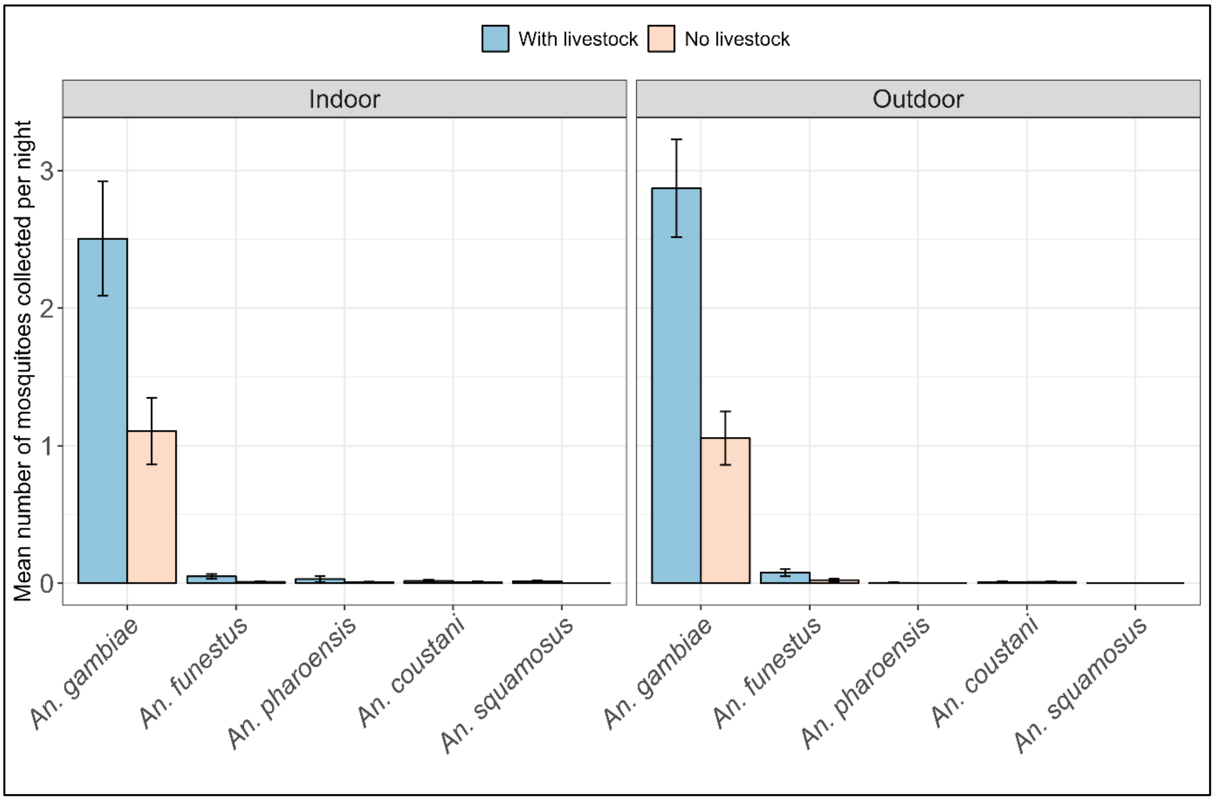

Figure 4.3: Density of resting malaria vectors in houses with and without livestock

4.1.6 Impact of Livestock on *Anopheles gambiae* s.l Density

The indoor density of *An. gambiae* s.l. increased significantly in households with 11–15 cows (RR = 2.5300, 95% CI: 1.225–5.244, *p = 0.012)*, more than 5 goats (RR = 2.656, 95% CI: 1.066–6.619, *p = 0.001)*, 3–4 dogs (RR = 2.086, 95% CI: 1.268–3.432, *p = 0.004)*, 11–20 chickens (RR = 2.18, 95% CI: 1.250–3.803, p = *0.006)*, and more than 20 chickens (RR = 1.9214, 95% CI: 1.0344–3.5689, *p = 0.039)* (Table 4.3). A decrease in *An. gambiae* s.l. catches was revealed to be associated with the presence of 1 to 3 pigs (RR = 0.3444, 95% CI: 0.1909–0.9886, *p = 0.047)* and more than 10 pigs (RR = 0.3344, 95% CI: 0.1164-0.9495, *p = 0.040)* (Table 4.3).

The outdoor density of *An. gambiae* s.l. was 2 times higher in houses with 11 to 15 cows than in houses with no cows (RR = 2.059, 95% CI: 1.056–4.015, *p = 0.034).* The presence of more than 5 sheep increased the number of *An. gambiae* s.l. mosquitoes approximately 2 times more than households with no sheep (RR = 1.840, 95% CI: 1.091–3.100, *p = 0.022).* Likewise, a significant increase in *An. gambiae* s.l. density was observed when there were more than 2 dogs; the mosquito density increased almost 2 times more than households with no dogs (RR

= 1.610, 95% CI: 1.017–2.550, *p = 0.042).* Thus, in outdoor collections, only cattle, sheep, and dogs were significantly associated with the increase in the number of *An. gambiae* s.l. mosquitoes, but not goats, pigs, or chickens, which had an impact on indoor density (Table 4.3).

Table 4.3*: Statistical significance of the impact of the number of livestock on the number of An. gambiae s.l mosquitoes in houses with and without livestock indoors and outdoors*

Livestock type	Number of livestock	Indoor			Outdoor		
		RR	CI	*p*	RR	CI	*p*
Cattle	No cattle	1			1		
	1-5	1.377	0.640- 2.962	0.414	0.523	0.274 - 1.001	1.050
	6-10	1.296	0.756-2.219	0.346	1.283	0.800 - 2.059	0.301
	11-15	2.535	1.225-5.244	**0.012**	2.059	1.056 - 4.015	**0.034**
Sheep	No sheep	1			1		
	1-5	2.548	0.608- 0.687	0.201	3.091	0.888- 10.758	0.076
	> 5	2.508	1.424-4.415	**0.001**	1.840	1.091- 3.100	**0.022**
Goat	No goat	1			1		
	1-5	1.414	0.659- 3.031	0.374	1.149	0.615-2.146	0.663
	> 5	2.656	1.066- 6.619	**0.036**	1.554	0.646-3.741	0.325
Pig	No pig	1			1		
	1-5	0.434	0.191- 0.989	**0.047**	0.520	0.267-1.014	0.055
	6-10	1.612	0.510-5.090	0.416	0.5314	0.178-1.587	0.257
	> 10	0.332	0.116-0.950	**0.040**	0.5518	0.196-1.556	0.261
Dog	No dog	1			1		
	1-2	1.420	0.613-3.286	0.413	0.791	0.368-1.698	0.547
	3-4	2.086	1.268-3.432	**0.004**	1.610	1.017-2.550	**0.042**
Chicken	No chicken	1			1		
	1-10	1.018	0.355-2.923	0.974	0.564	0.232-1.373	0.205
	11-20	2.180	1.250-3.803	**0.006**	0.931	0.561-1.546	0.783
	> 20	1.921	1.034-3.569	**0.039**	1.632	0.970-2.747	0.065

Rate ratio, CI=95% confidence interval, p=p-value, Ref=Reference category

4.1.7 Impact of livestock on *Anopheles funestus* density

The indoor density of *An. funestus* mosquitoes increased significantly when there were 1 to 5 cows (RR = 3.438, 95% CI: 1.3418–8.8098, *p = 0.010)* and 11 to 15 cows (RR = 2.257, 95% CI: 1.623–11.590, *p = 0.004)* (Table 4.4). Likewise, the presence of more than 10 chickens, for instance, 11 to 15 (RR = 6.003, 95% CI: 2.227–16.180, *p*<0.001) and more than 20 chickens (RR = 3.055, 95% CI: 1.188–7.555, *p = 0.021)*, increased indoor *An. funestus* densities (Table 4.4). The number of sheep, goats, and pigs did not have a significant impact on the number of *An. funestus* collected indoors (*p* > 0.05).

The outdoor collection number of *An. funestus* mosquitoes increased when there were 11 to 15 cows (RR = 3.279, 95% CI: 1.404–7.660, $p = 0.006$), more than 5 sheep (RR = 3.001, 95% CI: 1.582–5.692, $p = 0.002$), 3 to 4 dogs (RR = 2.277, 95% CI: 1.273–4.073, $p = 0.006$), and above 20 chickens (RR = 2.541, 95% CI: 1.378–4.687, $p = 0.003$). The number of goats and pigs did not significantly influence the number of *An. funestus* mosquitoes outdoors (p > 0.05) (Table 4.4).

Table 4.4: Statistical significance of the impact of the number of livestock on the number of *An. funestus* mosquitoes in houses with and without livestock indoors and outdoors

			Indoor			Outdoor	
Livestock composition	Number of livestock	RR	CI	*p*	RR	CI	*p*
Cattle	No cattle	1			1		
	1-5	3.438	1.342- 8.810	**0.010**	1.498	0.651- 3.446	0.342
	6-10	1.201	0.526-2.746	0.664	1.420	0.716-2.795	0.310
	11-15	2.257	1.623-11.590	**0.004**	3.279	1.404-7.660	**0.006**
Sheep	No sheep	1			1		
	1-5	4.650	0.664- 32.555	0.122	2.742	0.705-10.656	0.145
	> 5	2.490	0.939-5.637	0.081	3.001	1.582-5.692	**0.002**
Goat	No goat	1			1		
	1-5	1.562	0.487- 5.014	0.454	1.401	0.621-3.163	0.417
	> 5	1.322	0.267-6.532	0.732	1.439	0.429-4.828	0.554
Pig	No pig	1			1		
	1-5	0.876	0.206-3.735	0.858	0.904	0.322-2.542	0.849
	6-10	8.261	1.143-9.691	**0.036**	1.414	0.320-3.240	0.648
	> 10	0.604	0.086-4.250	0.612	1.053	0.243-4.558	0.945
Dog	No dog	1			1		
	1-2	2.863	0.902-9.090	0.074	0.942	0.332-2.672	0.911
	3-4	1.983	0.927-4.241	0.078	2.277	1.273-4.073	**0.006**
Chicken	No chicken	1			1		
	1-10	1.823	0.358-9.272	0.469	0.394	0.079-1.982	0.259
	11-20	6.003	2.227-16.180	**<0.001**	1.108	0.487-2.518	0.807
	> 20	3.055	1.188-7.855	**0.021**	2.541	1.378-4.687	**0.003**

RR=Rate ratio, CI=95% confidence interval, p=p-value

4.1.8 Impact of livestock on *Anopheles coustani* density

The mean number of *An. coustani* indoors was higher when there were an increased number of cattle compared to households without cattle. For instance, when there were 6 to 10 cows (RR = 4.846, 95% CI: 1.177–19.945, *p* = 0.029), (Table 4.5) The increase in *An. coustani* mosquitoes indoors when there were 11 to 115 cows was marginally significant (*p* = 0.05). No significant increase was observed when there were 1 to 5 cows (*p* = 0.081) indoors. The number of cows did not have an impact on the outdoor collection of *An. coustani* (*p* > 0.05). The number of *An. coustani* was observed to be very high in households with sheep compared to households with no cattle indoors or outdoors. For instance, when there were 1 to 5 sheep indoors (*p* = 0.003) and outdoors (*p* = 0.004) and when there were more than 5 sheep indoors (*p* = 0.017) and outdoors (*p* = 0.036), An almost similar number of *An. coustani* mosquitoes were observed in houses with 1–5 goats and those with no goats indoors (*p* = 0.052), but there was an increase in the number of *An. coustani mosquitoes* where there were more than 5 goats (RR = 13.446, 95% CI: 1.206–149.929, *p = 0.035)* (Table 4.5). The presence of 1 or 2 dogs did not have an impact on the number of *An. coustani mosquitoes* indoors (*p* = 0.052), but where there were 3 or 4 dogs, the number of *An. coustani* mosquitoes increased 13 times more than households without dogs indoors (RR = 12.188, 95% CI: 3.633–40.886, *p*<0.001) and outdoors (RR = 1.341, 95% CI: 1.771–10.645, *p = 0.001).* Also, the presence of less than or equal to 10 chickens did not have an impact on the number of *An. coustani* mosquitoes indoors (*p* = 0.157) or outdoors (*p* = 0.821), but an increase to 20 chickens or more than 20 chickens significantly increased the number of *An. coustani* mosquitoes indoors (RR = 4.990, 95% CI: 1.211–20.566, *p<0.001,* and RR = 3.866, 95% CI: 1.365–10.955, *p = 0.026,* respectively). The number of pigs did not significantly affect the number of *An. coustani* indoors as well as outdoors (Table 4.5).

Table 4.5: Statistical significance of the impact of the number of livestock on the number of *An. coustani* mosquitoes in houses with and without livestock indoor and outdoor

Livestock composition	Number of livestock	Indoor			Outdoor		
		RR	CI	p	RR	CI	p
Cattle	No Cattle	1			1		
	1-5	5.001	0.821 – 30.445	0.081	1.059	0.278 – 4.037	0.933
	6-10	4.846	1.177 – 19.945	**0.029**	2.236	0.820 – 6.094	0.116
	11-15	8.144	0.999 – 66.384	0.050	3.812	0.829 – 17.524	0.086
Sheep	No sheep	1			1		
	1-5	97.361	4.840 – 195.654	**0.003**	23.838	2.822 – 201.383	**0.004**
	Above 5	5.873	1.379 – 25.017	**0.017**	2.980	1.076 – 8.255	**0.036**
Goat	No goat	1			1		
	1-5	6.245	0.986 – 39.538	0.052	3.513	0.976 – 12.652	0.055
	Above 5	13.446	1.206 – 149.929	**0.035**	2.286	0.419 – 12.462	0.339
Pig	No pig	1			1		
	1-5	0.948	0.093 – 9.627	0.964	0.432	0.094 – 1.982	0.280
	6-10	0.479	0.009 – 26.120	0.719	0.563	0.044 – 7.256	0.660
	Above 10	2.549	0.077 – 83.968	0.600	2.363	0.239 – 23.333	0.462
Dog	No dog	1			1		
	1-2	4.454	0.656 – 30.216	0.126	1.452	0.372 – 5.674	0.592
	3-4	12.188	3.633 – 40.886	**<0.001**	4.341	1.771 – 10.645	**0.001**
Chicken	No chicken	1			1		
	1-10	4.882	0.543 – 43.917	0.157	1.224	0.212 – 7.068	0.821
	11-20	14.760	3.565 – 61.109	**<0.001**	2.572	0.842 – 7.862	0.097
	Above 20	4.990	1.211 – 20.566	**0.026**	3.866	1.365 – 10.955	**0.01**

RR=Rate ratio, CI=95% confidence interval, p=p-value

4.1.9. Impact of livestock on *Anopheles pharoensis* density

The impact of the number of cows did not significantly affect the density of An. pharoensis indoors and outdoors ($p > 0.05$) (Table 4.6). When the number of sheep was 1 to 5, the number of *An. pharoensis* was 84 times more than houses without sheep indoors (RR = 84.409, 95% CI: 3.427–207.096, $p = 0.007$) and 24 times more outdoors ($p = 0.035$). Also, when the number of sheep was more than 5, the number of *An. pharoensis* mosquitoes indoors increased slightly 5 times more than in houses without sheep (RR = 4.687, 95% CI: 1.039–21.152, $p = 0.045$), but not outdoors. The presence of less than 3 dogs had no significant impact on the number of *An. pharoensis* mosquitoes indoors and outdoors ($p > 0.05$), but when there were 3 to 4 dogs, the number of *An. pharoensis* mosquitoes increased indoors (RR = 10.498, 95% CI: 3.006–36.667, $p<0.001$) and outdoors (RR = 6.722, 95% CI: 1.039–21.152, $p = 0.001$). As shown in Table 4.6, the effect of chickens was seen when there were more than 20 chickens outdoors, where the number of *An. pharoensis* mosquitoes increased four times more than households without chicken (RR = 4.316, 95% CI: 1.062–16.126, $p = 0.041$). However, other animals such as cows, goats, and pigs did not have any impact on the number of *An. pharoensis* mosquitoes either indoors or outdoors.

Table 4.6: *Statistical significance of the impact of the number of livestock on the number of of An. pharoensis mosquitoes in houses with and without livestock indoors and outdoos*

Livestock composition	Number of Livestock	Indoor			Outdoor		
		RR	CI	p	RR	CI	p
	No Cattle	1			1		
Cattle	1-5	2.402	0.405 – 14.245	0.335	1.469	0.256 – 8.422	0.666
	6-10	2.461	0.544 – 11.125	0.242	2.346	0.618 – 8.909	0.211
	111-15	7.582	0.996 – 57.732	0.050	3.489	0.534 – 22.810	0.192
Sheep	No Sheep	1			1		
	1-5	84.409	3.427 – 207.096	**0.007**	24.809	1.256 – 49.090	**0.035**
	Above 5	4.687	1.039 – 21.152	**0.045**	3.591	0.922 – 13.993	0.065
Goat	No Goat	1			1		
	1-5	3.972	0.560 – 28.191	0.168	3.209	0.577 – 17.847	0.183
	Above 5	9.590	0.767 – 119.988	0.079	3.212	0.351 – 29.427	0.302
Pig	No Pig	1			1		
	1-5	0.723	0.067 – 7.831	0.789	0.366	0.053 – 2.507	0.306
	6-10	1.207	0.024 – 59.953	0.925	1.051	0.050 – 22.284	0.974
	Above 10	2.218	0.084 – 58.291	0.633	10.953	0.766 – 15.652	0.078
Dog	No Dog	1			1		
	1-2	2.819	0.475 – 16.725	0.254	2.819	0.475 – 16.725	0.254
	3-4	10.498	3.006 – 36.667	**<0.001**	6.722	2.094 – 21.584	**0.001**
Chicken	No Chicken	1			1		
	11-10	2.988	0.284 – 31.454	0.362	0.735	0.070 – 7.723	0.797
	Above 20	3.621	0.940 – 13.947	0.061	4.138	1.062 – 16.126	**0.041**

RR=Rate ratio, CI=95% confidence interval, p=p-value

4.1.10 Impact of livestock on *Anopheles squamosus* mosquitoes' density

The presence of a different number of cows significantly affected the indoor density of *An. squamosus*. For instance, the presence of 1 to 5 cows increased the number of *An. squamosus* mosquitoes more than 8 times the number in houses without cows (RR = 8.944, 95% CI: 1.468–54.482, *p = 0.017).* The increase of *An. squamosus* mosquitoes was also observed with 6 to 10 and 11 to 15 cows (RR = 6.662, 95% CI: 1.597–27.455*, p = 0.009*, and RR = 21.599, 95% CI: 2.564–181.918, *p = 0.005*, respectively). The presence of 1–5 sheep and more than 5 sheep significantly increased the number of *An. squamosus* mosquitoes indoors and outdoors (Table 4.7). The presence of 1 to 5 goats in a household increased the number of *An. squamosus* mosquitoes indoors (11.174, 95%CI: 1.579–79.089, *p = 0.016)* and outdoors (RR = 9.569, 95%CI: 1.248–73.357, *p = 0.030).* In households with more than 2 dogs, there was an increase in the number of *An. squamosus* indoors and outdoors (RR = 20, 95% CI: 5.880–71.660, *p<0.001,* and RR = 19.931, 95% CI: 5.385–73.774, *p*<0.001). The increase in the number of chickens significantly increased the number of *An. squamosus* mosquitoes indoors. For instance, there were 1 to 10 chickens (RR = 10.473, 95% CI: 1.049–104.586, *p = 0.045),* 11 to 10 chickens (RR = 11.048, 95% CI: 3.060–64.223, *p = 0.001),* and more than 20 chickens (RR = 12.539, 95% CI: 2.925–53.748, *p = 0.001).* For outdoor collections, there was no significant difference between households with less than 10 chickens and houses with no chickens in their compounds (*p* = 0.730). However, there was a significant increase in *An. squamosus* mosquitoes observed when the number of chickens increased, such as from 11 to 20 (RR = 8.314, 95% CI: 1.447–47.753, *p = 0.018)* and more than 20 chickens (RR = 10.774, 95% CI: 2.041–56.863, *p = 0.005).* More information is found in Table 4.7.

Table 4.7: *Statistical significance of the impact of the number of livestock on the number of An. squamosus mosquitoes in houses with and without livestock indoors and outdoors*

Livestock composition	Number of livestock	Indoor			Outdoor		
		RR	CI	*p*	RR	CI	*p*
Cattle	No Cattle	1			1		
	1-5	8.944	1.468 – 54.482	**0.017**	2.018	0.251 – 16.246	0.509
	6-10	6.622	1.597 – 27.455	**0.009**	4.248	0.868 – 20.788	0.074
	11-15	21.599	2.564 – 181.918	**0.005**	16.819	1.472 – 192.154	**0.023**
Sheep	No Sheep	1			1		
	1-5	153.737	6.062 – 389.979	**0.002**	339.587	14.391 – 801.278	**<0.001**
	Above 5	11.991	2.539 – 56.640	**0.002**	10.128	2.346 – 43.730	**0.002**
Goat	No Goat	1			1		
	1-5	11.174	1.579 – 79.089	**0.016**	9.569	1.248 – 73.357	**0.030**
	Above 5	17.300	1.286 – 232.831	**0.032**	10.015	0.722 – 138.896	0.086
Pig	No Pig	1			1		
	1-5	0.726	0.060 – 8.786	0.802	0.356	0.027 – 4.618	0.430
	6-10	0.000	0.000 – Inf	0.997	0.189	0.002 – 22.561	0.495
	Above 10	2.600	0.060 – 112.823	0.619	0.593	0.011 – 30.969	0.796
Dog	No Dog	1			1		
	1-2	4.722	0.572 – 39.010	0.150	2.207	0.286 – 17.028	0.448
	3-4	20.528	5.880 – 71.660	**<0.001**	19.931	5.385 – 73.774	**<0.001**
Chicken	No Chicken	1			1		
	1-10	10.473	1.049 – 104.586	**0.045**	3.476	0.229 – 52.859	0.370
	11-20	14.018	3.060 – 64.223	**0.001**	8.314	1.447 – 47.753	**0.018**
	Above 20	12.539	2.925 – 53.748	**0.001**	10.774	2.041 – 56.863	**0.005**

4.1.11 Distance between houses and livestock pens and mosquito density

The distance between livestock pens and houses did not have an impact on the mosquito density in all species except for *An. coustani,* where the mosquito density at a distance of 30 meters was significantly less than the density at a distance of less than 11 meters (Table 4.8). This means that there was a high density of mosquitoes in houses where the distance between houses and livestock pens was less than or equal to 10 compared to 11–20 meters (RR = 0.113, 95% CI: 0.022–0.588, $p = 0.010$) (Table 4.8).

Table 4.8: *The effect of distance between livestock pens and houses to the indoor density of Anopheles mosquitoes*

Species	Distance (meters)	RR	CI	*p*
An. gambiae	1 - 10	1		
	11 – 20	0.703	0.361 – 1.372	0.302
	21 – 30	1.374	0.549 – 3.442	0.497
	Above 30	0.608	0.137 – 2.701	0.514
An. funestus	1-10	1		
	11 – 20	0.688	0.332 – 1.426	0.315
	21 – 30	1.126	0.440 – 2.879	0.804
	Above 30	0.361	0.061 – 2.134	0.261
An. pharoensis	1-10	1		
	11 – 20	0.185	0.030 – 1.147	0.070
	21 – 30	0.934	0.095 – 9.185	0.953
	Above 30	0.178	0.004 – 8.172	0.376
An. coustani	1-10	1		
	11 – 20	0.113	0.022 – 0.588	**0.010**
	21 – 30	0.208	0.024 – 1.813	0.155
	Above 30	0.098	0.003 – 3.151	0.189
An. squamosus	1-10	1		
	11 – 20	0.262	0.038 – 1.811	0.174
	21 – 30	0.268	0.019 – 3.829	0.332
	Above 30	0.553	0.012 – 24.516	0.760

RR=Rate Ratio, CI=95% Confidence Interval, p= p-value

4.1.12 Contribution of other variables in mediating the association between livestock and malaria vector density

The results showed that improved houses with plastered walls and iron sheets had less *An. gambiae* s.l. indoor mosquito density compared to households with thatched roofs and mud walls (RR = 0.427, 95% CI: 0.137–0.445, $p<0.001$) (Table 4.9), when adjusted to the number of household occupants. Also, in houses with unplastered walls with iron sheets, fewer *An. gambiae* s.l. mosquitoes were collected compared to houses with thatched roofs and mud walls when adjusted to the number of household occupants (RR = 0.553, 95% CI: 0.355–0.861, *p = 0.009).* The unit increase in household occupants increased mosquito density by 1.050 times when adjusted to the household type, but it was not statistically significant (p = 0.442). However, in other *Anopheles* mosquitoes such as *An. funestus, An. coustani, An. pharoensis,* and *An. squamosus,* the impact of household type and number of household occupants was not statistically significant (Table 4.9).

Table 4.9: *Impact of Household type and household occupants on malaria vector density*

	Predictor variables	RR	CI	*p*
An. gambiae s.l	Household type			
	Thatched and mud walls	1		
	Thatched and brick wall	1.652	0.836 - 3.262	0.148
	Plastered and iron sheet	0.247	0.137- 0.445	**<0.001**
	Unblistered and iron sheets	0.553	0.355 - 0.861	**0.009**
	Number of household occupants	1.030	0.955-1.111	0.442
An. funestus	Household type			
	Thatched and mud walls	1		
	Thatched and brick wall	1.160	0.379 - 3.552	0.795
	Plastered and iron sheet	0.743	0.251- 2.197	0.591
	Unblistered and iron sheets	0.951	0.456 – 1.983	0.893
	Number of household occupants	1.935	0.816-1.072	0.335
An. coustani	Household type			
	Thatched and mud walls	1		
	Thatched and brick wall	1.156	0.386 – 3.458	0.795
	Plastered and iron sheet	0.825	0.286- 2.378	0.722
	Unblistered and iron sheets	0.573	0.256 – 1.282	0.175
	Number of household occupants	0.963	0.837-1.108	0.595
An. pharoensis	Household type			
	Thatched and mud walls	1		
	Thatched and brick wall	1.303	0.378 – 4.497	0.675
	Plastered and iron sheet	0.561	0.159- 1.983	0.369
	Unblistered and iron sheets	0.408	0.161 – 1.029	0.058
	Number of household occupants	1.921	0.782-1.085	0.325
An. squamosus	Household type			
	Thatched and mud walls	1		
	Thatched and brick wall	1.181	0.336 – 4.156	0.795
	Plastered and iron sheet	1.060	0.331- 1.390	0.922
	Unblistered and iron sheets	0.575	0.235 – 1.408	0.226
	Number of household occupants	1.006	0.862-1.175	0.935

RR= Rate ratios, CI=95%Confidence interval, p= p-values

4.2 Results for objective 2: Host preference of mosquitoes in livestock-keeping and non-livestock keeping households

4.2.1 Blood Meal Sources in Livestock-Keeping and Non-Livestock-Keeping Households

A sub-sample of 2066 female blood-fed *An. gambiae* s.l. mosquitoes which were all confirmed to be *An. arabiensis* was submitted for ELISA blood meal analysis. The overall identification for blood meal was 76.6% (n = 1583). In houses with livestock, 747 (71.6%) *An. arabiensis*. mosquitoes were positive for bovine blood, 225 (21.6%) for human blood only, 1 for goat, 1 for chicken, 45 for mixed blood meal for human and bovine, 4 for mixed blood meal for human, chicken, and bovine, 1 for chicken blood, 4 for mixed blood meals for human and goat, and 4 for mixed blood meal for human and chicken (Table 4.10). In houses with no livestock, 363 *An. arabiensis* mosquitoes amplified positive for human blood and 159 for bovine blood. Only 11 mosquitoes had a mixture of human and bovine blood; 2 mosquitoes had a mixed blood meal of human and goat; 2 mosquitoes had chicken blood; and 1 mosquito had a mixture of human, bovine, and chicken blood (Table 4.10).

Table 4.10: *Blood indices of different hosts from Anopheles arabiensis mosquitoes*

Hosts Blood	With Livestock (n=1044)	Without Livestock (n = 539)	Overall (n=1583)
Bovine	747 (71.6%)	159 (29.5%)	906 (57.2%)
Bovine+Chicken	2 (0.2%)	1 (0.2%)	3 (0.2%)
Chicken	1 (0.1%)	2 (0.4%)	3 (0.2%)
Goat	13 (1.2%)	0 (0%)	13 (0.8%)
Human	225 (21.6%)	363 (67.3%)	588 (37.1%)
Human+Bovine	45 (4.3%)	11 (2.0%)	56 (3.5%)
Human+Bovine+Chicken	4 (0.4%)	1 (0.2%)	5 (0.3%)
Human+Chicken	4 (0.4%)	0 (0%)	4 (0.3%)
Human+Goat	3 (0.3%)	2 (0.4%)	5 (0.3%)

Indoors, more than 85% of mosquitoes collected indoors in households that have livestock have bovine blood, suggesting that they feed on cows and come indoors to rest (Figure 4.4). Also, in households without livestock, more than 60% of mosquitoes have human blood meal, suggesting that the mosquitoes feed on humans only when there are no animals. This suggests zooprophylaxis. In the outdoors, the majority of the mosquitoes (approximately 75%) in households with livestock had bovine blood meal, suggesting that the livestock attract the mosquitoes outside. In households without livestock, the majority of the mosquitoes had human blood meal, as shown in figure 4.4.

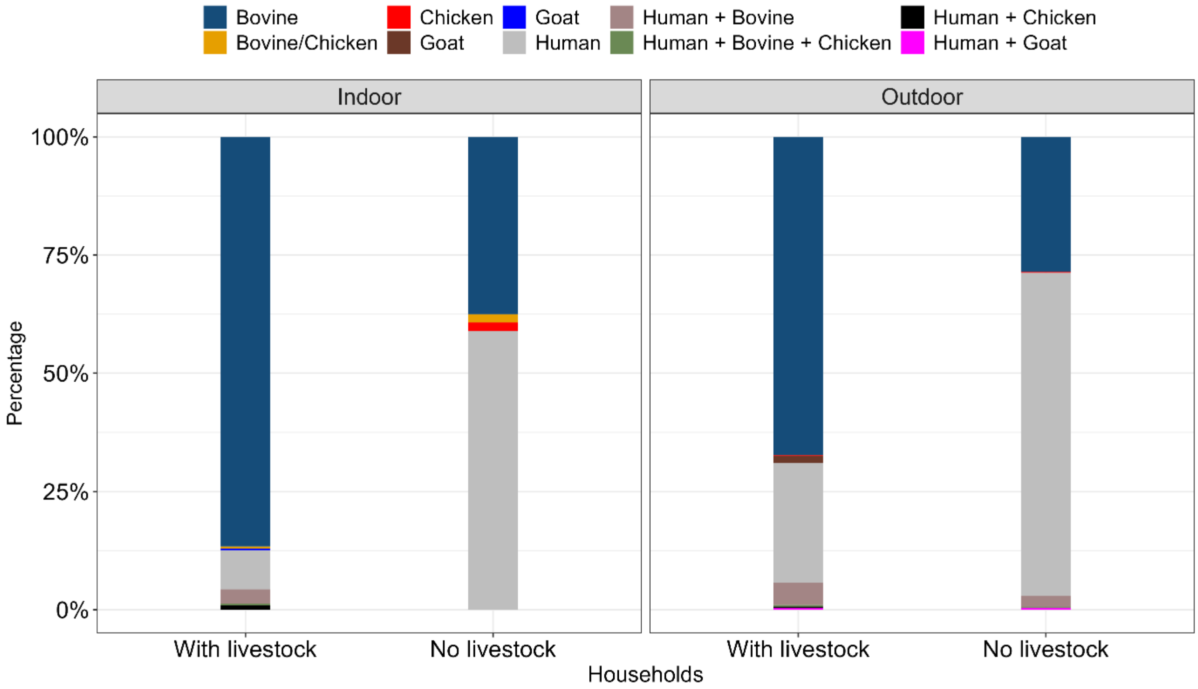

Figure 4.4: Proportion of blood meal detected from blood fed An. arabiensisl indoors and outdoors

4.2.2 Impact of livestock on mosquitoes' ability to acquire human blood

Also, the presence of livestock increased the chance of *An. arabiensis* mosquitoes to feed from other hosts to 7 times more (OR = 7.145, 95% CI: 5.618–9.086, p<0.001) than obtaining human blood (Table 4.11), while mosquitoes were able to obtain a mixed blood of humans from other hosts 6 (OR = 6.350, 95% CI: 3.449–11.694, p<0.001) times more than human blood in houses with livestock than houses with no livestock when adjusted to location. This indicates that in households with livestock, there is a high chance of mosquitoes feeding on other hosts other than human blood. In the outdoors, mosquitoes were less likely to obtain other hosts' blood than human blood (OR = 0.369, 95% CI: 0.260–0.522, p<0.001) when adjusted to the household's livestock status (Table 4.11). This indicates that mosquitoes were able to obtain more human blood meals indoors than outdoors.

Table 4.11: *Impact of household's livestock status and location on An. arabiensis mosquito ability to feed on human*

Variable	Category	Human blood as a reference					
		Other hosts			Mixed (Human + Other)		
		OR	CI	*p*	OR	CI	*p*
Livestock status	No livestock		1		1		
	With livestock	7.145	5.618 – 9.086	<**0.001**	6.350	3.449 – 11.694	<**0.001**
Location	Indoor		1		1		
	Outdoor	0.369	0.260 – 0.522	<**0.001**	0.710	0.339 – 1.490	0.365

OR= Odds ratios, CI=95%Confidence interval, p= p-value

4.3 Results for objective 3: Knowledge and perception of community members towards the relationship between livestock keeping and malaria transmission.

4.3.1 Demographic description of participants

A total of 20 household representatives participated in the IDIs. The demographic characteristics of the participants are provided in Table 4.12.

Table 4.12: Demographic information of the study participants

Variable	% (n)
Gender	
Female	60 (12)
Male	40 (8)
Age group	
18 - 29 Years	40 (8)
30-39 Years	40 (6)
40-49 Years	15 (3)
50 Years and above	15 (3)
Marital status	
Married/Cohabited	60 (12)
Unmarried	35 (7)
Widowed	5 (1)
Educational status	
No formal education	25 (5)
Primary	50 (10)
Secondary and above	25(5)
Main occupation	
Farmer	95 (19)
Business	5 (1)
Household size	
1-3 people	30(6)
4-6 people	35 (7)
Above 6 people	35 (7)

Values are reported as %(n)

4.3.2 Knowledge about mosquitoes and diseases they transmit

The majority of the participants in the IDIs understood about mosquitoes (90%) and their habitats (85%) but could not identify them by species level (100%). However, all respondents were able to differentiate them by looking at their physical appearance and colours. The participants knew some of the diseases transmitted by mosquitoes and how those diseases are transmitted from mosquitoes to humans. Malaria was the most mentioned disease by all the participants among all mosquito-borne diseases. The majority of participants sought services in health care centers (85%), but a few participants reported self-medicating (15%). This is shown by the participants below:

4.3.3 Knowledge on mosquito biting behaviour and mosquito control

The majority of the participants reported staying outside before going to sleep (90%) since some of their houses were so small that they only used them for sleeping. Also, all participants responded that they were being bitten by mosquitoes both indoors and outdoors, especially from 7 to 10 p.m. They indicated that they usually sleep around 9:00 p.m. and wake up around 5:00 a.m. They also said there is a large mosquito density outdoors compared to indoors (60%). Almost all participants (95%) reported that malaria incidences keep decreasing every year due to the use of mosquito control interventions, particularly bed nets. They also reported that other vector control tools should be added in line with mosquito bed nets which they always use regardless of the season to maximize protection against mosquito-borne diseases. Examples of these responses are well illustrated by the participants below:

4.3.4 Livestock-keeping practices and distance between houses and livestock enclosures

The majority of participants (75%) reported that livestock such as cows, pigs, and goats were kept outside the houses, while most chickens were kept inside the houses, sometimes sleeping in the same rooms with people. The distances from the houses and livestock enclosure were reported to range between 5 and 30 meters. The number of livestock ranges between 3 and 50 per household, depending on the category of animals kept, as illustrated by the following participants:

4.3.5 Treating Animals with pesticides

All participants reported to clean places where they keep their livestock often, and they also reported to clean their animals using pesticides. They reported the use of pesticides to protect their animals against animal diseases and also added that even the rest of the community do the same. They usually treat their animals at least once every two weeks; unfortunately, most of them (70%) fail to mention the name of the pesticide they usually use, and few participants (25%) mentioned '*paranex'* as their priority among the pesticides. They also reported that treating the animals with pesticides reduced mosquito density in a few days (55%). This is illustrated by the participants as follows:

4.3.6 Relationship between malaria transmission and livestock keeping

This study found that some of the participants understood the relationship between animal keeping and malaria transmission. They also responded that having many livestock increases the population density of mosquitoes and, hence, increases transmission. They mentioned some of the livestock that can contribute to the increase in mosquito density, such as cattle, chickens, and goats.

4.3.7 Knowledge on the impact of livestock keeping on malaria transmission

Most participants (95%) stated that knowledge of the impact of livestock keeping on malaria transmission is important to their community. Among the participants, 55% of them reported they didn't get this information anywhere, but they were wondering what would happen with that large mosquito population density. They reported that they think the rest of the community members lack this information, so it is important to consider providing this information to the community. This is well explained by the participants below:

Table 4.13: List of themes extracted from the qualitative analysis and their respective quotes

Themes	Quotes
Knowledge about mosquitoes	"What *I know about mosquitoes is that these are insects that transmit diseases such as malaria and lymphatic filariasis."* (Female, 34 years) *"I have heard that there is a mosquito called Anopheles that is responsible for malaria transmission."* (Female, 44 years) *"Mosquitoes are found in a variety of habitats; they first lay their eggs in a wet environment, and when they learn to fly, I believe they migrate to populated areas in search of blood."* (Female, 28 years)
Lnowledge on mosquito biting behaviour	"*Usually, there are a lot of mosquitoes when I'm outside, and even when I'm inside, there are a lot of mosquitoes as well. As you can see, because of the numerous openings throughout our house, mosquitoes can easily get inside."* (Female, 28 years)
Knowledge about malaria	"The *challenge is that, this disease (malaria) is re-emerging because mosquitoes are always here. This causes people to be unable*

	to perform their activities, especially when they are ill and sleeping. For us, finding a daily income and cover hospital expenses is very challenging." (Female, 62 years)
Malaria and its vector control	*"We only use bed nets because we don't have the ability to buy other interventions to protect ourselves from mosquitoes. We sleep very early because our houses are small; if you stay outside, there are many mosquitoes."* (Female, 23 years) *"Depending on the tasks we have to complete, we occasionally go to bed early and occasionally stay up late. This causes a change in our sleeping patterns from day to day. The majority of the time, if we can get to bed early, we sleep at nine o'clock; however, if we are late, we sleep at twelve."* (Female, 24 years) *"Malaria cases have been decreasing to a large extent because significant efforts have been made to ensure that every family member sleeps under a bed net."* (Male, 31 years)
Distance between household and livestock pens	"In *this village, animals like cows, goats, pigs, and sheep are normally kept outdoors, but chickens are kept indoors because they are stolen by thieves during the night, especially in the rainy season."* (Male, 27 years) *"From livestock sheds to houses, it's like 5 meters; if livestock sheds are very far from*

	houses, it is difficult to hear thieves when they wish to steal our animals, and that is a basic reason why we keep animals near homesteads." (Female, 44 years)
Treating anmals with insecticides	*"Animals like cows are brought to the pasture and led through a mixture of water and insecticides, but we also occasionally spray them with pesticides right here on the farm. We normally spray with insecticides every week or every twoo weeks.”* (Female, 44 years) *"Once the insecticide is sprayed on the day we spray for mosquitoes, the insects truly vanish. For about three days, there won't be any mosquito activity, and even if you remain outside, you'll be able to see that there are none. I believe another contributing factor is the smell of the pesticides. The strength of the pesticides seems to weaken the mosquitoes on the day of the spraying, but once it wears off, they return in the same manner.”* (Female, 49 years)
Malaria transmission risks and livestock keeping	*"For instance, mosquitoes frequently attack our coworkers who go into the forest to herd cattle. There are typically a lot of mosquitoes where there are herds of cattle.”* (Male, 48 years) *"The truth is, in areas where there are a large number of animals, the mosquito density becomes very high. I think if these mosquitoes are infected with malaria*

	parasites, the community members will get infected." (Female, 49 years)
Need for education about the role of livestock keeping on malaria transmission	*“It is very important for the community to receive education about the effects of animal keeping and malaria transmission, because I am not sure if this community of livestock keepers and farmers completely understands that. I believe it will be better if the specialists can collaborate with the village government to organize community meetings and pass the knowledge on to us as well.”* (Male, 63 years) *“I believe the community needs more education on unregulated livestock farming; many herders do not follow proper livestock keeping practices, which is why they contribute to the excessive breeding habitats of mosquitoes, especially during the rainy season. Additionally, many of them do not always clean the cattle sheds, resulting in increased mosquito breeding near homesteads. They should be provided with education to bring about a desired change."* (Male, 31 years)

CHAPTER FIVE: DISCUSSION

5.1 Summary results

The study results show that houses with a high number of livestock, especially cattle, sheep, dogs, goats, and chickens, experience a higher density of *Anopheles* mosquitoes indoors and outdoors than houses with no livestock. The *Anopheles* mosquitoes encountered were especially *An. gambiae* s.l. (particularly *An. arabiensis*), *An. funestus, An. pharoensis, An. coustani,* and *An. squamosus*. Subsequently, houses with livestock have an increased risk of malaria transmission. Although the presence of livestock reduced the proportion of *An. gambiae* s.l. mosquitoes with human blood among houses with livestock compared to houses with no livestock, there were some mixed bloods from different hosts, including humans, cattle, goats, and chickens. In houses with livestock, cattle were the most preferred host (71.6%), while in houses with no livestock, humans were the most preferred host (67.3%) by *An. gambiae* s.l. mosquitoes. The effect of distance between houses and livestock shades on the density of mosquitoes collected indoors was not statistically significant except for *An. coustani,* where there was a significant decrease in mosquito collection when livestock were coraled between 11 and 20 meters ($p = 0.010$). A higher density of *Anopheles* mosquitoes was observed in houses with mud walls and thatched roofs than in houses with bricks and iron sheets.

5.2 Impact of livestock keeping and mosquito distribution and density

The increase in mosquito density in houses with livestock was hypothesized to be due to various possible reasons: (i) Mosquitoes are attracted to odors produced by livestock such as cattle, goats, and others (Takken & Knols, 1999; Takken & Verhulst, 2013), (iil) livestock offers an alternative blood meal source to host-seeking mosquitoes. This is because normally livestock are not protected against mosquitoes like humans, especially during nights when the animals are not sprayed with insecticides and provide an open alternative blood source to mosquitoes (Takken & Verhulst, 2013). This was also revealed by the detection of bovine and other hosts' blood DNA from *An. gambiae* s.l. mosquitoes. (iii) Cattle urine has been shown to attract primary and secondary malaria vectors in different settings (Dawit et al., 2022; Katusi et al., 2023; Kweka et al., 2011) as malaria mosquitoes acquire and allocate cattle urine to enhance life cycle traits (Dawit et al., 2022) which might be one of the reasons for the increased mosquito catches in houses with livestock, particularly cattle. Due to that, further studies should be conducted to assess other livestock's urine and other products that might contribute to the increase in mosquito density. The increase in mosquito density in houses with livestock was also revealed in other studies conducted in different countries, such as Pakistan (Hewitt et al., 1994) where there was an increase in human biting rate (HBR) in mosquitoes in the presence of cattle and two goats. In Kenya, Minakawa et al. (2002) showed that the ratio of human density to cow density was positively correlated with the relative abundance of *An. gambiae* larvae in the late rainy period. Furthermore, in Ethiopia, two studies revealed that the presence of cattle in proximity to human dwellings increases the HBR of *An. pharoensis* compared to houses with no livestock (Seyoum et al., 2002; Zeru et al., 2020). The results of this study are contrary to studies that have shown that the presence of livestock, such as cattle, was associated with a significant reduction of *An. arabiensis* mosquitoes indoors and outdoors (Mayagaya et al., 2015; Mburu et al., 2021; Tirados et al., 2011). These studies did not take into account the number and size of livestock corralled. Therefore, it is important to carefully verify these study results in other settings because *Anopheles* mosquitoes seem to behave differently in different geographical areas depending on other covariates, including climatic conditions such as temperature, humidity, and rainfall, and the availability of hosts (Ayanlade et al., 2013; Balls et al., 2004; Caminade et al., 2014; Tanser et al., 2003; Thomas et al., 2013).

In this study, the number of cattle, dogs, and chickens had a positive impact on increasing *Anopheles pharoensis* and *An. squamosus* mosquito densities. The results of these mosquito species were much affected by relatively wider confidence intervals which might brings other issues such as increased uncertainty and less precision of the effect sizes. These issues might be attributed by low number of mosquitoes collected in both houses with and without livestock. Thus, it is essential to consider further studies to be conducted to assess the relationship between livestock keeping and these mosquito species in other settings. Other studies in Ethiopia revealed similar results where the presence of cattle with humans inside increased the number of *An. pharoensis* by 42%, and when cattle were kept outside at a distance of at least 1 meter, the density of these particular mosquitoes increased by 46% in the tent compared to no cattle outside the tent (Zeru et al., 2020). *Anopheles pharoensis* was found to be significantly more prevalent when a calf was present, either inside or adjacent to a tent, relative to a tent without a calf present (Zeru et al., 2020). These results show that there is a need for further studies of long-term surveillance, especially across the seasons of different years, in order to deeply explore and get a clear picture of what is happening in livestock-keeping communities where the risk of malaria transmissions seems to be high. In this study, the number of resting mosquitoes in houses with livestock was much higher in households with livestock than houses with no livestock. This correlates with the results of host-seeking mosquitoes, where *Anopheles* mosquitoes were higher in households with livestock. The high density of host-seeking mosquitoes was probably a reason for the high number of resting mosquitoes, most of whom were fed by gravid mosquitoes. This shows similar findings to those obtained by Mayagara et al. (2015) who observed that the number of outdoor resting *An. gambiae* s.l. was higher in houses with livestock compared to households without, but differed in indoor collections where the number of *An. gambiae* s.l. and *An. funestus* was lower in households with livestock indoors compared to households with no livestock (Mayagaya et al., 2015). In a study conducted in southern Malawi (Mburu et al., 2021) reported that the number of indoor resting *An. gambiae s.l.* and *An. funestus* mosquitoes in houses with cattle did not differ from houses without cattle. Undoubtedly, further studies are required to assess the resting behaviour of *Anopheles* mosquitoes in livestock-keeping communities in order to well design the best and most efficient vector control interventions to control resting mosquitoes.

The presence of livestock at different distances did not have an impact on the densities of malaria vectors except for *An. coustani,* whereby a decrease in mosquito density was observed when livestock were kept between 11 and 20 meters from the house. This indicates that the presence of livestock in close proximity to human dwellings reduces *An. coustani* mosquitoes indoors, probably because mosquitoes are more attracted to animals than humans, so the animals pull them indoors, a situation that was clearly described by Iwashita et al. (2014). A study done in Malawi by Mburu et al. (2021) showed that the presence of cattle at a variety of distances between houses and cattle sheds reduced the density of *An. funestus* mosquitoes when cattle were kept between 1 and 15 meters compared to households without cattle. This shows that there is a need for further studies to be done to assess the effect of various distances between homesteads in order to assess the optimal distance where livestock will be kept to reduce mosquito density around the homesteads.

5.3 Mosquito host preference

In the current study, bovine blood was the most preferred blood source among any other hosts, especially for mosquitoes collected in houses with livestock. This confirms that *An. gambiae* mosquitoes, specifically *An. arabiensis,* which was the most abundant species among the *An. gambiae* complex group, are an opportunistic malaria vector that mostly prefers to feed on cattle’s blood (Ferguson et al., 2010; Takken & Verhulst, 2013). This tells us that the presence of livestock reduces the human blood index, as described by Mayagaya et al. (2015) in which the proportion of human blood index of *An. arabiensis* and *An. funestus* was approximately

50% lower in houses with livestock than those without. This further shows that the presence of livestock offers an alternative blood meal source to mosquitoes, which reduces the risk of malaria transmission due to the fact that livestock are dead-end or decoy hosts because malaria parasites cannot develop in cattle, hence zooprophylaxis. Mahande et al. (2007) also showed that the HBI in *An. araboensis* was lower in households with cattle than those without cattle. This portrays the zoophilic behaviour of these mosquitoes as it was observed in the current study. In this regard, despite having a higher mosquito density in households with livestock, the HBI is much lower than in households without livestock. Surprising results showed that even in houses without livestock, there were mosquitoes with bovine blood and some mixed-blood meals of humans and bovine. This might be due to various possible reasons, such as animals not being zero-grazed and just staying outside the homesteads or the possible flight of mosquitoes between households with livestock and those without. It has been previously reported that blood-fed *An. gambiae* mosquitoes can fly up to 10 kilometers (Kaufmann & Briegel, 2004). Only 76% of blood meals were positively identified, according to laboratory tests, so not all blood meals were detected. This might be caused by a variety of reasons, such as mosquitoes feeding on other vertebrates whose antibodies were not present. Similar results have been found in other studies (Mayagaya et al., 2015; Mburu et al., 2021), showing that *An. gambiae* mosquitoes only amplify to a limited extent during blood meal examinations.

5.4 Knowledge and perception of community members towards livestock keeping and malaria transmission

A qualitative part of this study shows that most community members observe their home environments clearly regarding the issue of malaria transmission. Some of the participants were able to identify mosquitoes based on their physical appearance and colours. This knowledge can also be used for mosquito surveillance using a citizen science approach (Carney et al., 2022), where community members may be professionally trained and can be used to continuously monitor species diversity and densities (Sousa et al., 2022). This will be useful to track trends in dominant mosquito species and be able to detect invasive species that might come into our societies (Palmer et al., 2017; Pernat et al., 2021). Mwangungulu et al. (2016) showed community knowledge and experiences to be used as a crowdsourcing vector surveillance strategy for identifying areas with a high density of mosquitoes instead of conducting large-scale surveillance.

During the qualitative assessment, it was revealed that the majority of community members spend their earlyevenings or nights outdoors engaging in different activities such as cooking, relaxing, and playing before going to sleep. This exposes them to early-biting mosquitoes. Similar findings were reported by a study conducted to link human behaviours and malaria vector biting risks, where most of the activities done by community members before bedtime exposed them to malaria transmission risks (Finda et al., 2019). Therefore, there should be vector control interventions focusing on controlling early biting, such as the use of repellants (Kaindoa et al., 2021; Masalu et al., 2020).

The use of pesticides on animals was one of the key aspects that were observed during IDIs, where most of the participants acknowledged using or seeing others use pesticides to treat animals against diseases and mosquito disturbances. This might increase mosquito resistance against those pesticides, some of which contain pyrethroids. Studies should be conducted to assess the susceptibility status of mosquitoes in livestock-keeping households, like what was done in rural Tanzania to assess the effect of agricultural pesticides on the susceptibility and fitness of major malaria vectors (Urio et al., 2022).

According to community members' observations of their environments and ecosystems, houses with livestock around their homesteads seemed to have more mosquito abundance than those with no livestock. This information was corroborated by the mosquito sampling activities, which revealed the same scenario for malaria vectors. Thus, this shows that the knowledge and experience of the community members are important baseline information for conducting further studies regarding the relationship between malaria transmission and other underlying variables, as well as before applying vector control tools.

Another study showed that livestock-keeping communities have less knowledge and practices on preventing mosquito-mediated diseases than non-livestock-keeping communities (Nguyen-Tien et al., 2021). Therefore, community engagement plays an important role in implementing community-based control interventions against various health issues. Providing and mobilizing knowledge about malaria and its risk factors, such as livestock, will assist in reducing malaria transmissions in our settings (Awasthi et al., 2021; Baltzell et al., 2019). This can be done using different approaches, such as the use of drama (Lim et al., 2017) to convey information to the public.

Despite achieving study results, this study underwent some limitations. First, the study was conducted in the rainy season only and therefore lacks information on the dry season. Thus, it is important for future studies to incorporate seasonality covariates from different consecutive years in order to draw general conclusions. Secondly, the study did not take into account the micro-climatic factors such as temperature and humidity, which also play an important role in the ecology of malaria vectors (Agyekum et al., 2022; Balls et al., 2004; Blanford et al., 2013; Caminade et al., 2014; Yamana & Eltahir, 2013). Thirdly, the sporozoite infection status of the collected female *Anopheles* mosquitoes was not assessed. This lacks confirmation on where exactly malaria transmission is high, despite the presence and absence of livestock. Fourthly, the study did not assess the malaria prevalence of malaria in households with and without livestock. Lastly, the study focused on malaria vectors only, though in the study area, non-malaria vectors coexist with malaria vectors. It is important for other studies to be conducted to assess the impact of livestock on other non-anopheline mosquitoes.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of this study conclude the following: (i) The presence of livestock in close proximity to households increases the density of malaria vectors indoors and outdoors. Animals such as cattle, sheep, goats, dogs, and chickens have been identified to attract mosquitoes, as many participants declared during IDIs. This increases the risk of malaria transmission if no additional malaria control interventions are introduced and implemented. (ii) The presence of livestock in the homesteads reduces the human blood index for *Anopheles arabiensis* mosquitoes; this malaria vector has been regarded as opportunistic host-seeking behaviour. Therefore, keeping livestock, especially cattle, will reduce mosquito bites from humans and hence zooprophylaxis. (iii) Community education of the interactions in the ecosystem is necessary for the prevention of various vector-borne diseases. This will encourage community participation in the early detection of signs and symptoms of the presence of a disease.

6.2 Recommendations

6.2.1 Recommendations for practice

This study suggests several actions to be considered: (i) Though livestock keeping poses a malaria transmission risk, it also has zooprophylaxis potential by diverting mosquitoes from biting humans. To mitigate malaria transmission risks in communities that keep livestock, it is essential to strongly advocate for the adoption of additional mosquito control measures. This includes the use of tools like bed nets and indoor residual sprays, as demonstrated in a study conducted in Ethiopia, which effectively encourage mosquitoes to stay indoors (Iwashita et al., 2014). (ii) Regarding the An. arabiensis behaviour of obtaining blood meal from other non-human hosts, livestock-based interventions such as spraying animals with insecticides and the use of ivemectin should be applied in livestock-keeping communities where *Anopheles arabiensis* is a dominant malaria vector, as the results show its feeding behaviour on cattle’s blood (iii) In order to achieve malaria elimination, community engagement is one of the key aspects to be considered for achieving a significant result against ongoing malaria transmission. This study recommends the provision of education to the community on malaria control practices. This is because when community members have knowledge of malaria control practices, it will be one step towards malaria elimination. is a necessary aspect of malaria control.

6.2.1 Recommendation for research

In order to develop effective interventions against mosquito-borne diseases, further studies should be conducted to assess mosquitoes' behaviour in livestock-keeping communities across all seasons and in different periods of time. Also, studies focusing on the other non-anopheline mosquitoes should be emcouraged in order to develop control interventions targeting all the mosquito species including non-anophelines which transmit malaria and other MBDs in livestock keeping communities.

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APPENDICES

Appendix 1: Informed Consent Agreement for household's owners who will participate by allowing mosquito collection for field experiments by volunteers from the selected households from Ulanga district, Morogoro region, Tanzania

Impact of livestock management on malaria transmission dynamics in rural Tanzania.

Informed Consent Agreement

Background and purpose: According to World Health Organization (WHO), more than a half World's population is at risk of getting infected with malaria. In Tanzania, approximately >95% of the population live in malaria transmission areas. Despite the use of various malaria control interventions such as Insecticide treated nets (ITNs) and Indoor Residual Sprays (IRS), still malaria remains a health problem especially in sub-Saharan Africa. Animals play an important role in transmission of various diseases in which they can either act as reservoirs for disease pathogens or a source of blood meal for insects including mosquitoes which are primary vectors of malaria. Clear understanding of how livestock are managed in relation to malaria transmission risks is an important aspect which might be used to design livestock-based malaria control interventions. This study will help to understand well the relationship that exist between livestock management and malaria transmission risks and this knowledge will be used to design alternative malaria control interventions in livestock keeping communities and encourage community engagement in malaria control programs

Aim of the study: To assess the impact of livestock management on malaria transmission risks in rural Tanzania.

Household owner's role: You will be asked to allow volunteers to trap adult mosquitoes from your household once per week. Mosquito collection will be conducted indoor and outdoor as well as in livestock shelter if available.

Household owner's participation: Your participation in this study is voluntary. We ask your help, but also you can decide to withdraw from the study later and you will not be required to provide any explanation.

Risk: There are no effects associated with this study in terms of health or physical well-being, but in case of any problem, we will be ready and available to help.

Benefits: Information that will be obtained from this study will help to provide information on the impact of livestock management on malaria transmission risks which will be helpful in designing malaria control interventions areas where livestock keeping is the primary economic activities. Furthermore, the study will be used to assess how community engagement can be used as an important aspect in malaria control.

Confidentiality: The information that we collect from this research will be kept confidential.

Sharing of the Results: The knowledge that we get from this study will be shared through publicationand will help interested people to learn from the findings. Also, the feedback from the results will be communicated to the community members. Furthermore, the results will be shared to the Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDEC).

Compensation: The participation is voluntary and no compensation will be issued during study period.

Withdrawal from participation: If you agree to take part you are free to end your participation in this study at any time you want without having to give reasons. Withdrawing will not affect us from working with you later.

Additional Information: If you have any question or concern about this study, please feel free to contact the following researcher for help: **Yohana Mwalugelo**; +255623535841 and **Dr. Emmanuel Kaindoa**; +255787430307

Ifakara Health Institute Review Board **Dr. Mwifadhi Mrisho** Tel: +255655766675/ mmrisho@ihi.or.tz

Informed consent record:

Please read this statement carefully before you sign and if you agree on participating, please fill freeto sign this form below to affirm your consent.

I,_________________________, clearly understand the aims of the study titled "*To assess the impact of livestock management on malaria transmission risks in rural Tanzania.*" which has been explained to me. I have been given the chance to ask questions and I am satisfied with the answers to all of my questions and I agree to participate in the study. I understand that I may revoke my consent and leave the study at any stage, if I wish so, with no negative consequences.

Participant's name: ____

Participant's signature or thumbprint:________________________Date:______________

Witness Name (As appropriate): ____

Witness signature (As appropriate):_______________________Date: _________________

Study team member's statement:

I, the undersigned, have explained to the participant in a language that s/he understands; the procedures to be followed in the study, the risks and benefits involved, and the obligations of the study team.

Name____

Signature____

Date___________________________________

Makubaliano ya ridhio la ushiriki kwa wamiliki wa nyumba ambao wataruhusu ukusanywaji wa mbu kwenye nyumba zao zilizochaguliwa katika wilaya ya Ulanga. Mkoa wa Morogoro, Tanzania.

Athari za usimamizi wa mifugo katika mienendo ya maambukizi ya ugonjwa wa malaria maeneo ya vijijini nchini Tanzania.

Kulingana na shirika la afya duniani (WHO), Zaidi ya nusu ya nusu ya idadi ya watu duniani wapo katika hatari kuambukizwa ugonjwa wa malaria. Nchini Tanzania, Zaidi ya asilimia 95 ya idadi ya watu wanaishi katika maeneo yenye maambukizi ya ugonjwa wa malaria. Licha ya utumiaji wan jia mbalimbali za kudhibiti ugonjwa huo kama vile utumiaji wa vyandarua vyenye dawa na upuliziwaji waw a dawa ndani ya nyumba, bado malaria umebaki kuwa tatizo la kiafya hasa nchi za kusini mwa jangwa la Sahara. Wanyama wana umuhimu mkubwa kwenye maambukizi ya magonjwa mbalimbali ambapo wanaweza kuwa hifadhi ya vimelea vya magonjwa au kuwa chanzo cha damu kama chakula cha wadudu mbalimbali ikiwemo mbu ambao hueneza ugonjwa wa malaria. Uelewa wa wazi kuhusu usimamizi wa mifugo katika uhusiano na hatari ya maambukizi ya ugonjwa wa malaria ni kipengele muhimu kinachoweza kutumiwa kutengeneza njia za kudhibiti malaria zinazohusisha wanyama. Utafiti huu utasaidia kuelewa vizuri uhusiano uliopo kati ya usimamizi wa mifugo na hatari ya maambukizi ya malaria.na maarifa haya yatasaidia kutengeneza njia mbadala za kudhibiti malaria katika jamii za wafugaji na kuhimiza uhusishwaji wa jamii katika programu za kudhibiti ugonjwa wa malaria.

Lengo la utafiti; Kutathmini athari za usimamizi wa mifugo katika hatari ya maambukizi ya malaria maeneo ya vijijini nchini Tanzania.

Jukumu la mmiliki wa nyumba: Utaombwa kuruhusu washiriki kukusanya mbu kwenye nyumba yako mara moja kwa wiki

Ushiriki wa hiari: Ushiriki wako katika utafiti huu ni wa hiari. Tunahitaji msaada wako, lakini pia unaweza ukaamua kujitoa katika utafiti baadae na hutahitajika kutoa maelezo yoyote. Kujitoa kwako hakutaathiri sisi kufanya kazi na wewe baadae.

Madhara ya ushiriki: Hakuna madhara yoyote ya kiafya yanayotokana na utafiti huu,lakini kukiwa na tatizo lolote, tutakuwa tayari na kupatikana ili kusaidia.

Manufaa: Taarifa zitakazopatikana kutokana na utafiti huu zitasaidia kutoa taarifa ya uhusiano uliopo kati ya usimamizi wa mifugo na hatari ya maambukizi ya ugonjwa wa malaria ambayo itasaidia kutengeneza njia au zana za kudhibiti ugonjwa wa malaria kwenye maeneo yenye shughuli za ufugaji. Aidha, utafiti huu utatumika kutathmini namna uhusishwaji wa jamii unavyoweza kutumika kama kipengele muhimu katika kudhibiti ugonjwa wa malaria.

Usiri: Taarifa zitakazo kusanywa wakati wa utafiti huu zitahifadhiwa kwa siri.

Ushirikishaji wa matokeo: Maarifa tutakayoyapata katika utafiti huu yatachapishwa kwa ajili ya watu wengine kujifunza. Maoni kutoka kwenye utafiti huu yatawasilishwa kwa wanajamii. Pia, tutawasilisha matokeo Wizara ya Afya, Maendeleo ya Jamii, Jinsia, Wazee na Watoto.

Fidia: Ushiriki utakuwa jambo la hiari na hauna malipo yoyote.

Ukitaka kujitoa: Kama unakubali kushiriki katika utafiti huu na hauko tayari kuendelea kushiriki, uko huru kuacha ushiriki wakati wowote unaotaka bila kutoa sababu.

Taarifa za Ziada: Ikiwa una swali au wasiwasi wowote kuhusu utafiti huu tafadhali jisikie huru kuwasiliana na watafiti wafuatao kwa usaidizi: **Yohana Mwalugelo**; +255623535841 na **Dr. Emanuel Kaindoa**; +255787430307.

Bodi ya taasisi ya kupitia tafiti: **Dr. Mwifadhi Mrisho** Tel: +255 655 766 675/ mmrisho@ihi.or.tz

Tafadhali soma maelezo haya kwa makini kabla ya kusaini na kama unakubali kushiriki, tafadhali jisikie huru kusaini chini ya fomu hii kuthibitisha ridhaa yako.

Mimi___________________________________naelewa vizuri malengo ya utafiti uitwao "*Kutathmini athari za usimamizi wa wanyama kwenye hatari ya maambukizi ya malaria, Tanzania vijijini"* kama ambayo nimeelezewa. Nimepewa nafasi ya kuuliza maswali na nimeridhishwa na majibu kwa maswali yangu yote na ninakubali kushiriki katika utafiti huu. Najua kwamba kama nitaamua kushiriki katika utafiti huu, naweza kuacha ushiriki katika hatua yoyote kama nikitaka hivyo.

Jina la mshiriki: ____

Sahihi ya mshiriki au dole gumba:________________________Tarehe: ______________________

Jina la shahidi (Kama inavyotakiwa): ____

Sahihi ya shahidi (Kama inavyotakiwa):_______________________Tarehe: ____

Maelezo ya Timu ya Utafifi:

Mimi, mwenye sahihi hapo chini, nimemuelezea mshiriki katika lugha anayoelewa, hatua zitakazofuatwa katika utafiti, madhara na faida za kushiriki na majukumu ya timu ya utafiti.

Jina: ____________________________________ Sahihi: ___________________ Tarehe: _______________

Appendix 2: Informed Consent Agreement for volunteers who will participate in the field experiment including those who will be collecting adult mosquitoes from the selected households from Ulanga district, Morogoro region, Tanzania

Impact of livestock management on malaria transmission dynamics in rural Tanzania.

Informed Consent Agreement

Background and purpose: According to World Health Organization (WHO), more than a half World's population is at risk of getting infected with malaria. In Tanzania, approximately >95% of the population live in malaria transmission areas. Despite the use of various malaria control interventions such as Insecticide treated nets (ITNs) and Indoor Residual Sprays (IRS), still malaria remains a health problem especially in sub-Saharan Africa. Animals play an important role in transmission of various diseases in which they can either act as reservoirs for disease pathogens or a source of blood meal for insects including mosquitoes which are primary vectors of malaria. Clear understanding of how livestock are managed in relation to malaria transmission risks is an important aspect which might be used to design livestock-based malaria control interventions. This study will help to understand well the relationship that exist between livestock management and malaria transmission risks and this knowledge will be used to design alternative malaria control interventions in livestock keeping communities and encourage community engagement in malaria control programs

Aim of the study: To assess the impact of livestock management on malaria transmission risks in rural Tanzania.

Volunteers' role: You will be asked to collect adult mosquitoes from selected households once per week, sorting and packing of mosquito samples.

Voluntary participation: Your participation in this study is voluntary. We ask your help, but also youcan decide to withdraw from the study later and you will not be required to provide any explanation.

Risk: There are no effects associated with this study in terms of health or physical well-being, but in case of any problem, we will be ready and available to help.

Benefits: Information that will be obtained from this study will help to provide information on the impact of livestock management on malaria transmission risks which will be helpful in designing malaria control interventions areas where livestock keeping is the primary economic activities. Furthermore, the study will be used to assess how community engagement can be used as an important aspect on malaria elimination.

Confidentiality: The information that we collect from this research will be kept confidential.

Sharing of the Results: The knowledge that we get from this study will be shared through publicationand will help interested people to learn from the findings. Also, the feedback from the results will be communicated to the community members. Furthermore, the results will be shared to the Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDEC).

Compensation: The participation is voluntary but a small amount will be provided to compensate your time, we will provide 10,000/=TSh per working day.

Withdrawal from participation: If you agree to take part you are free to end your participation in this study at any time you want without having to give reasons. Withdrawing will not affect us from working with you later.

Additional Information: If you have any question or concern about this study, please feel free to contact the following researcher for help: **Yohana Mwalugelo**; +255623535841 and **Dr. Emmanuel Kaindoa**; +255787430307

Ifakara Health Institute Review Board **Dr. Mwifadhi Mrisho** Tel: +255655766675/ mmrisho@ihi.or.tz

Informed consent record:

Please read this statement carefully before you sign and if you agree on participating, please fill freeto sign this form below to affirm your consent.

I,________________________, clearly understand the aims of the study titled "*Impact of livestock management on malaria transmission risks in rural Tanzania.*" which has been explained to me. I have been given the chance to ask questions and I am satisfied with the answers to all of my questions and I agree to participate in the study. I understand that I may revoke my consent and leave the study at any stage, if I wish so, with no negative consequences.

Participant's name: ____

Participant's signature or thumbprint:________________________Date:______________

Witness Name (As appropriate): ____

Witness signature (As appropriate):_______________________Date: _________________

Study team member's statement:

I, the undersigned, have explained to the participant in a language that s/he understands; the procedures to be followed in the study, the risks and benefits involved, and the obligations of the study team.

Name____

Signature____

Date____________________________________

Makubaliano ya ridhio la ushiriki kwa watu ambao watakuwa wananashiriki kukusanya mbu wakubwa kwenye nyumba zilizochaguliwa katika wilaya ya Ulanga. Mkoa wa Morogoro, Tanzania.

Athari za usimamizi wa mifugo katika mienendo ya maambukizi ya ugonjwa wa malaria maeneo ya vijijini nchini Tanzania.

Kulingana na shirika la afya duniani (WHO), Zaidi ya nusu ya nusu ya idadi ya watu duniani wapo katika hatari kuambukizwa ugonjwa wa malaria. Nchini Tanzania,Zaidi ya asilimia 95 ya idadi ya watu wanaishi katika maeneo yenye maambukizi ya ugonjwa wa malaria. Licha ya utumiaji wan jia mbalimbali za kudhibiti ugonjwa huo kama vile utumiaji wa vyandarua vyenye dawa na upuliziwaji waw a dawa ndani ya nyumba, bado malaria umebaki kuwa tatizo la kiafya hasa nchi za kusini mwa jangwa la Sahara. Wanyama wana umuhimu mkubwa kwenye maambukizi ya magonjwa mbalimbali ambapo wanaweza kuwa hifadhi ya vimelea vya magonjwa au kuwa chanzo cha damu kama chakula cha wadudu mbalimbali ikiwemo mbu ambao hueneza ugonjwa wa malaria. Uelewa wa wazi kuhusu usimamizi wa mifugo katika uhusiano na hatari ya maambukizi ya ugonjwa wa malaria ni kipengele muhimu kinachoweza kutumiwa kutengeneza njia za kudhibiti malaria zinazohusisha wanyama. Utafiti huu utasaidia kuelewa vizuri uhusiano uliopo kati ya usimamizi wa mifugo na hatari ya maambukizi ya malaria.na maarifa haya yatasaidia kutengeneza njia mbadala za kudhibiti malaria katika jamii za wafugaji na kuhimiza uhusishwaji wa jamii katika programu za kudhibiti ugonjwa wa malaria.

Lengo la utafiti; Kutathmini athari za usimamizi wa mifugo katika hatari ya maambukizi ya malaria maeneo ya vijijini nchini Tanzania.

Jukumu la Watu wa Kujitolea: Utaombwa kukusanya mbu kwenye nyumba zilizochaguliwa mara moja kwa wiki, kuchagua na kupanga Pamoja na kufunga sampuli za mbu waliokusanywa.

Ushiriki wa hiari: Ushiriki wako katika utafiti huu ni wa hiari. Tunahitaji msaada wako, lakini pia unaweza ukaamua kujitoa katika utafiti baadae na hutahitajika kutoa maelezo yoyote. Kujitoa kwako hakutaathiri sisi kufanya kazi na wewe baadae.

Madhara ya ushiriki: Hakuna madhara yoyote ya kiafya yanayotokana na utafiti huu, lakini kukiwa na tatizo lolote, tutakuwa tayari na kupatikana ili kusaidia.

Manufaa: Taarifa zitakazopatikana kutokana na utafiti huu zitasaidia kukabiliana na changamoto zinazohusiana na afua/ njia za sasa za kemikali kama vile kustahimili mbu kwa kutengeneza zana mpya za kibaolojia, zenye gharama nafuu na rafiki wa mazingira ili kudhibiti maambukizi ya malaria katika maeneo hayo janga ikiwa ni pamoja na jamii yako.

Usiri: Taarifa zitakazo kusanywa wakati wa utafiti huu zitahifadhiwa kwa siri.

Ushirikishaji wa matokeo: Maarifa tutakayoyapata katika utafiti huu yatachapishwa kwa ajili ya watu wengine kujifunza. Maoni kutoka kwenye utafiti huu yatawasilishwa kwa wanajamii. Pia, tutawasilisha matokeo Wizara ya Afya, Maendeleo ya Jamii, Jinsia, Wazee na Watoto.

Fidia: Ushiriki utakuwa jambo la hiari na tutakupa kiasi cha sh. 10,000/= kwa siku ili kufidia muda wako uliotumia kushiriki kwenye utafiti huu.

Ukitaka kujitoa: Kama unakubali kushiriki katika utafiti huu na hauko tayari kuendelea kushiriki, uko huru kuacha ushiriki wakati wowote unaotaka bila kutoa sababu.

Taarifa za Ziada: Ikiwa una swali au wasiwasi wowote kuhusu utafiti huu tafadhali jisikie huru kuwasiliana na watafiti wafuatao kwa usaidizi: **Yohana Mwalugelo**; +255623535841 na **Dr. Emanuel Kaindoa**; +255787430307.

Bodi ya taasisi ya kupitia tafiti: **Dr. Mwifadhi Mrisho** Tel: +255 655 766 675/ mmrisho@ihi.or.tz

Tafadhali soma maelezo haya kwa makini kabla ya kusaini na kama unakubali kushiriki, tafadhali jisikie huru kusaini chini ya fomu hii kuthibitisha ridhaa yako.

Mimi___________________________________naelewa vizuri malengo ya utafiti uitwao "*Kutathmini athari za usimamizi wa wanyama kwenye hatari ya maambukizi ya malaria, Tanzania vijijini'*" kama ambayo nimeelezewa. Nimepewa nafasi ya kuuliza maswali na nimeridhishwa na majibu kwa maswali yangu yote na ninakubali kushiriki katika utafiti huu. Najua kwamba kama nitaamua kushiriki katika utafiti huu, naweza kuacha ushiriki katika hatua yoyote kama nikitaka hivyo.

Jina la mshiriki: ____

Sahihi ya mshiriki au dole gumba:________________________Tarehe:___________________

Jina la shahidi (Kama inavyotakiwa): ____

Sahihi ya shahidi (Kama inavyotakiwa):_______________________Tarehe:
____

Maelezo ya Timu ya Utafifi:

Mimi, mwenye sahihi hapo chini, nimemuelezea mshiriki katika lugha anayoelewa, hatua zitakazofuatwa katika utafiti, madhara na faida za kushiriki na majukumu ya timu ya utafiti.

Jina: _____________________________ Sahihi: _______________ Tarehe: ______________

Appendix 3: Informed Consent Agreement for participants of In-depth Interview to assess the knowledge and perception of community members about the relationship between livestock management and malaria transmission in Ulanga district, Morogoro region, Tanzania

Impact of livestock management on malaria transmission dynamics in rural Tanzania.

Background and purpose: According to World Health Organization (WHO), more than a half World's population is at risk of getting infected with malaria. In Tanzania, approximately >95% of the population live in malaria transmission areas. Despite the use of various malaria control interventions such as Insecticide treated nets (ITNs) and Indoor Residual Sprays (IRS), still malaria remains a health problem especially in sub-Saharan Africa. Animals play an important role in transmission of various diseases in which they can either act as reservoirs for disease pathogens or a source of blood meal for insects including mosquitoes which are primary vectors of malaria. Clear understanding of how livestock are managed in relation to malaria transmission risks is an important aspect which might be used to design livestock-based malaria control interventions. This study will help to understand well the relationship that exist between livestock management and malaria transmission risks and this knowledge will be used to design alternative malaria control interventions in livestock keeping communities and encourage community engagement in malaria control programs.

Aim of the study: To assess the impact of livestock management on malaria transmission risks in rural Tanzania.

Household owner's role: You will be asked to allow volunteers to trap adult mosquitoes from your household once per week. Mosquito collection will be conducted indoor and outdoor as well as in livestock shelter if available.

Household owner's participation: Your participation in this study is voluntary. We ask your help, but also you can decide to withdraw from the study later and you will not be required to provide any explanation.

Risk: There are no effects associated with this study in terms of health or physical well-being, but in case of any problem, we will be ready and available to help.

Benefits: Information that will be obtained from this study will help to provide information on the impact of livestock management on malaria transmission risks which will be helpful in designing malaria control interventions areas where livestock keeping is the primary economic activities. Furthermore, the study will be used to assess how community engagement can be used as an important aspect in malaria control.

Confidentiality: The information that we collect from this research will be kept confidential.

Sharing of the Results: The knowledge that we get from this study will be shared through publicationand will help interested people to learn from the findings. Also, the feedback from the results will be communicated to the community members. Furthermore, the results will be shared to the Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDEC).

Compensation: The participation is voluntary but a small amount will be provided to compensate your time, we will provide 10,000/=Tsh per working day.

Withdrawal from participation: If you agree to take part you are free to end your participation in this study at any time you want without having to give reasons. Withdrawing will not affect us from working with you later.

Additional Information: If you have any question or concern about this study, please feel free to contact the following researcher for help: **Yohana Mwalugelo**; +255623535841 and **Dr. Emmanuel Kaindoa**; +255787430307

Ifakara Health Institute Review Board **Dr. Mwifadhi Mrisho** Tel: +255655766675/ mmrisho@ihi.or.tz

Informed consent record:

Please read this statement carefully before you sign and if you agree on participating, please fill freeto sign this form below to affirm your consent.

I,________________________,_clearly understand the aims of the study titled "*Impact of livestock management on malaria transmission risks in rural Tanzania."* which has been explained to me. I have been given the chance to ask questions and I am satisfied with the answers to all of my questions and I agree to participate in the study. I understand that I may revoke my consent and leave the study at any stage, if I wish so, with no negative consequences.

Participant's name: ____

Participant's signature or thumbprint:________________________Date:______________

Witness Name (As appropriate): ____

Witness signature (As appropriate):________________________Date: _________________

Study team member's statement:

I, the undersigned, have explained to the participant in a language that s/he understands; the procedures to be followed in the study, the risks and benefits involved, and the obligations of the study team.

Name____

Signature____

Date______________________________________

Appendix 4: Household characterization form

Form serial number (*Namba ya fomu*) : |___|___|___|___|	Date (*Tarehe*):	Day (Siku) ☐☐ Month (Mwezi) ☐☐ Year (Mwaka) ☐☐☐☐
Household head (*Mkuu wa kaya*):		
Household ID (*Namba ya kaya*): |__|__|__|__| - |__|__|__|__|	ID of fieldworker (*Kitambulisho cha mafanyakazi*): |__|__|__|	
District (*Wilaya*): |______________|	Ward (*Kata*): |______________|	
Village (*Kijiji*): |______________|	Sub-village (*Kitongoji*): |______________|	
Latitude (*Latitudo*): |______________|	Longitude (*Longitudo*): |______________|	
No. of household occupants (*Idadi ya wanakaya*): |__|__|		
Male under 5 (*Wakiume chini ya miaka 5*) |__|__|	Female under 5 (*Wakike chini ya miaka 5*) |__|__|	
Male between 5-15 (*Wakiume kati ya miaka 5-15*) |__|__|	Female between 5-15 (*Wakike kati ya miaka 5-15*) |__|__|	

Eave/ Mtambaa panya		
1. What is the status of eave present in the house?/ Je ipi ni hali ya mtambaa panya?		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
Open (*Upo wazi*)	01	|___|___|
Partly screened/ blocked (*Umewekwa wavu au kuzibwa kwa baadhi ya maeneo tu*)	02	
Screened (*Umewekewa wavu wote*)	03	
Blocked (*Umezibwa wote*)	04	
Others (*Nyinginezo*)	99	
1.1. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		

Roof/ Paa		
2. What materials were used to make the roof?/ Ni vifaa gani vilivyotumika kutengeneza paa?		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
Grass or thatched (*Nyasi*)	01	|___|___|
Metal (*Chuma/ Bati*)	02	
Others (*Nyinginezo*)	99	
2.1. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		

Ceiling		
3. Does the house have a ceiling?/ (*Je, dari la nyumba limezibwa?*)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
No	01	|___|___|
Yes	02	
Others	99	
3.1. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		

Windows on collection room/ Madirisha kwenye chumba cha kukusanyia mbu		
4. How many windows are on the collection room? (*Je, kuna madirisha mangapi kwenye chumba cha kukusanyia mbu?*)		|___|___|
4.1. What is the status of windows on the collection room?		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
Open (*Yapo wazi*)	01	|___|___|
Blocked (*Yamezibwa*)	02	
Screened (*Yana wavu wa kuzuia mbu*)	03	
Others (*Nyinginezo*)	99	

4.2. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		
Windows on the other rooms (*Madirisha kwenye vyumba vingine*)		
5. How many windows are on the other rooms? (Je, kuna madirisha mangapi kwenye vyumba vingine?)		|___|___|
5.1. What is the status of windows on the other rooms? (Je hali ya madririsha kwenye vyumba nzima ipoje?)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
Open (*Yapo wazi*)	01	|___|___|
Blocked (*Yamezibwa*)	02	
Screened (*Yana wavu wa kuzuia mbu*)	03	
Others (*Nyinginezo*)	99	
5.2. If others, specify (Kama ni nyinginezo, tafadhari taja)		
Status of the doors/ Hali ya milango		
6. How many entry doors are on the house? (Je kuna milango mingapi ya kuingilia kwenye nyumba?)		|___|___|
6.1. What is the status of the house entry doors? (Je, hali ya milango ya kuingilia kwenye nyumba ikoje?)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
Open (*Ipo wazi, hakuna paneli*)	1	|___|___|
Door panel with gaps (*Paneli ya mlango ina nafasi za kuruhusu mbu*)	2	
Tightly fitted door panel (*Paneli ya mlango iliyofungwa vizuri nahairuhusu mbu*)	3	
Others (*Nyinginezo*)	99	
6.2. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		
6.3. What is the status of the door on the mosquito collection room? (*Je, hali ya mlango kwenye chumba cha kukusanyia mbu ikoje?*)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
Open (*Ipo wazi, hakuna paneli*)	1	|___|___|
Door panel with gaps (*Paneli ya mlango ina nafasi za kuruhusu mbu*)	2	
Tightly fitted door panel (*Paneli ya mlango iliyofungwa vizuri nahairuhusu mbu*)	3	
Others (*Nyinginezo*)	99	
6.4. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		

Walls (materials)/ Kuta (Vifaa)		
7. What materials are making the walls? (*Ni vifaa gani vilivyotumika kutengeneza kuta?*)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
Mud (*Matope*)	1	|___|___|
Brick (*Tofali za kuchoma*)	2	
Others (*Nyinginezo*)	99	
7.1. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		

Walls (status of interior side of the walls)/ Kuta (Hali ya upande wa ndani wa kuta)		
8. Is the interior wall of the house plastered? (*Je, ukuta wa ndani wa nyumba umesakafiwa?*)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
No (*Hapana*)	1	|___|___|
Yes, with cement (*Ndio, kwa saruji/ simenti*)	2	
Yes, with mud (*Ndio, kwa matope*)	3	
Others (*Nyinginezo*)	99	
8.1. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		

Floor/ Sakafu		
9. Is the floor of the house plastered? (*Je, sakafu ya nyumba imepigwa plasta?*)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
Dusty/ Muddy (*Mavumbi/ Matope*)	1	
Plastered with cement (*Imesakafiwa kwa saruji/ simenti*)	2	

Others (*Nyinginezo*)	99	|___|___|
9.1. If others, specify (*Kama ni nyinginezo, tafadhari taja*)		
Latrine/ Choo		
10. Is the latrine inside the house? (*Je, choo kiko ndani ya nyumba?*)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
No (*Hapana*)	1	|___|___|
Yes (*Ndio*)	2	
10.1. If no, specify distance to the nearest latrine (in metres) (*Ikiwa hapana, taja umbaliwa choo cha karibu (katika mita)*)		|___|___|

Bed nets/ Vyandarua	
11. How many bed nets are there (used) in the house? (Je, kuna vyandarua vingapi (vinavyotumika) ndani ya nyumba?)	|___|___|
Of all bed nets, how many are: (K*ati ya vyandarua vyote, vingapi ni:*)	
Treated bed nets (*Vyandarua vilivyowekwa dawa*)	|___|___|
Untreated bed nets (*Vyandarua visivyokuwa na dawa*)	|___|___|
Un-identified (*Havijatambuliwa kuwa na dawa au la.*)	|___|___|

Animal shed/ Mabanda ya Wanyama		
12. Is there animal shed within 100 m? (*Je kuna banda la wanyama ndani ya mita 100?*)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
No (*Hapana*)	1	|___|___|
Yes (*Ndio*)	2	|___|___|
12.1. If yes, specify animal kept (*Kama ndiyo, taja wanyama a wanaofugwa*)		

Other interventions used/ Afua nyingine zilizotumika		
13. Was there any vector intervention used in the night of collection?		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
No (*Hapana*)	1	|___|___|
Yes (*Ndio*)	2	
13.1. If yes, specify intervention used (*Kama ndiyo, taja afua zilizotumika*)		

Poultry inside houses		
14. Are chicken or other poultry kept inside the house? (*Je, kuku au ndege wengine hufugwa ndani ya nyumba?*)		
Response options (*Chagua kati ya haya*)	Code (*Namba*)	Response (Majibu)
No (*Hapana*)	1	|___|___|
Yes (*Ndio*)	2	

Sleeping time/ Muda wa kulala		
15. At what time did the first person go to inside the collection room last night. (*mtu wa kwanza aliingia saa ngapi ndani ya chumba cha kukusanyiambu jana usiku?*)	HH (SS)	**MM (DD)**

Number of participants who slept in the collection room/ *Idadi ya washiriki waliolala kwenye chumba cha kukusanya mbu*			
16. Number of participants who slept in the collection room (*Idadi ya washiriki waliolala kwenye chumba cha kukusanyia mbu*)			|___|___|
Male under 5 (*Wakiume chini ya miaka 5*)	|___|___|	Female under 5 (*Wakike chini ya miaka 5*)	|___|___|
Male between 5-15 (*Wakiume kati ya miaka 5-15*)	|___|___|	Female between 5-15 (*Wakike kati ya miaka 5-15*)	|___|___|
Male above 15 (*Wakiume juu ya miaka 15*)	|___|___|	Female above 15 (*Wakike juu ya miaka 15*)	|___|___|

Other comments/ descriptions

HH_Char_MosCol_V1_01122021

Appendix 5: Form for records the design of experiments collecting mosquitoes in the field

Experimental design: Field Collections	PROJECT CODE (PC):	EXPERIMENT NO (EN):	SERIAL NO (SEN):
	FORM TYPE (FT): ED1	SITE (SI):	

		COLLECTION ATTRUBUTES																						
		GENERIC										EXPERIMENT SPECIFIC												
Form Row (FR)	Date of Collection (DT)	Enumeration area (EA)	Cluster (CR)	Compound or Plot (CP)	Household (HH)	Structure ID (SID)	Method (ME)	Indoor/Outdoor (IN) (1 = in, 2 = out)	Habitat type (HT)	Start Time (ST)	Finish Time (FT)	Holding Period (HP)	Round (RND)	Block (BLK)	House/Hut (SHH)	Station (ST)	Volunteer initials (VI)	Treatment (TR)	Experimental Day (DY)	Humidity (H)	Temperature(T)	Rain(R)	Valid Catch (VC): 1=yes, 2=missing data	SS1 Form Serial No. (DSEN)
		MARK X FOR ALL VARIABLES WHICH MUST BE RECORDED																						
							X	X		X	X													
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T		U		V	W
01																								
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20																								

EXPERIMENT SUPERVISORS INITIALS (ESI):	AND SIGNATURE:
RESPONSIBLE SCIENTISTS INITIALS (RSI:)	AND SIGNATURE:

Method (ME): 01 = HLC; 02 = ITT; 03 = CDC LT; 04 = Resting box; 05 = Aspirator resting sites; 06 = Floor; 07 = Entry window; 08 = Entry eave; 09 = Entry; 10 = Exit window; 11 = Exit eave; 12 = Exit; 13 = PSC; 14 = Dip; 15 = Substrate; 16 = Emergence trap; 17 = Ovitrap; 18 = Electric Grid(EG)

Habitat type (HT): 01 = Puddles & tire tracks; 02 = Swampy areas; 03 = Mangrove swamp/saltwater marsh; 04 = Drain/ditch; 05 = Construction pit/foundations/man-made hole; 06 = Water storage container; 07 = Rice paddy; 08 = Raised bed agriculture; 09 = Other agriculture; 10 = Stream/river bed; 11 = Pond; 12 = Other (describe)

ED1_EHES_V1_25/01/201

Sample Sorting: Adult Field Collection	Project Code (PC):	Experiment No. (EN):	Serial No. (SEN):
Form Type (FT): SS1	ED Form Serial No. (SSEN):	ED Form Row (SFR):	

Body Form (BF): 01

SS1 Form Row (FR)	Taxon (TX)	Taxon Description	Sex & abdominal status (SAS)	Sex and abdominal status description	Number Caught (N)	Notes and observations	Sample Label Code (SLC)	Number of Individuals	Number of batches	Number in Batch 1 (SID = 01)	Number in Batch 2 (SID = 02)	Number in Batch 3 (SID = 03)	Number in Batch 4 (SID = 04)	Number in Batch 5 (SID = 05)	Number in Batch 6 (SID = 06)	Number in Batch 7 (SID = 07)	Number Discarded
								Sample type variables									
								Sample type (ST):									
A	B	C	D	E	F	G	H	I	K	L	M	N	O	P	Q	R	S
ANOPHELES SPECIES								1		2	2	2	2	2	2	2	
01	01	An. gambiae s.l.	1	Total Male													
02			2	Unfed													
03			3	Partly Fed													
04			4	Fed													
05			5	Gravid/semigravid													
06			6	Total Female													
07	02	An. funestus	1	Total Male													
8			2	Unfed													
9			3	Partly Fed													
10			4	Fed													
11			5	Gravid/semigravid													
12			6	Total Female													
13			1	Total Male													
14			2	Unfed													
15			3	Partly Fed													
16			4	Fed													
17			5	Gravid/semigravid													
18			6	Total Female													
19			1	Total Male													
20			2	Unfed													
21			3	Partly Fed													
22			4	Fed													
23			5	Gravid/semigravid													
24			6	Total Female													
25			1	Total Male													
26			2	Unfed													
27			3	Partly Fed													
28			4	Fed													
29			5	Gravid/semigravid													
30			6	Total Female													
31			1	Total Male													
32			2	Unfed													
33			3	Partly Fed													
34			4	Fed													
35			5	Gravid/semigravid													
36			6	Total Female													
CULICINES																	
37	50	Culex sp.	1	Total Male													
38			6	Total Female													
39	60	Mansonia sp.	1	Total Male													
40			6	Total Female													
41	70	Aedes sp.	1	Total Male													
42			6	Total Female													
43	80	Coquilettidia sp.	1	Total Male													
44			6	Total Female													
45			1	Total Male													
46			6	Total Female													

Taxon (TX): 03 = An. coustani; 04 = An. pharoensis; 05 = An. squamosus; 06 = An. maculipalpis; 07 = An. pretoriensis; 08 = An. paludis; 09 = An. wellcomei; 10 = An. ziemanni

Labelling details: First line: FT.SEN.FR
SLC.BP.ST.SID

Appendix 6: Records the process by which a field collection of mosquitoes is sorted into pre-defined subgroups of taxon, sex and abdominal status(Kiware et al., 2016) :

Appendix 7: In-depth Interview guide

IN-DEPTH INTERVIEW GUIDE

MWONGOZO WA MAHOJIANO YA KINA

Community knowledge and perception about the relationship between livestock keeping and malaria transmission in Ulanga district, South-eastern Tanzania

Ufahamu na mtazamo wa wanajamii kuhusu uhusiano kati ya ufugaji wa wanyama na maambukizi ya ugonjwa wa malaria wilaya ya Ulanga, Kusini mashariki mwa Tanzania

Section 1: Demographic information

Sex/Jinsi	**Education**/Elimu	**Occupation**/Kazi	**Age**/Umri	#of year in particular village/ Miaka ndani	Marital status/Hali ya ndoa	# of children/ Idadi ya watoto

Section 2: Knowledge and perception of the community about mosquito and malaria transmission / Uelewa na mtazamo wa jamii kuhusu mbu na maambukizi ya malaria

1. Please, tell me what do you understand about mosquitoes/ Tafadhari, niambie unaelewa nini kuhusu mbu?
   i. where are they found in your environment? / Mbu wanapatikana wapi kwenye mazingira yako ya kawaida?
   ii. What species of mosquitoes do you know? / Je, unajua aina gani ya mbu?
   iii. What diseases are they transmitting? / Je, mbu wanaeneza magonjwa gan?
   iv. Which mosquito species can transmit malaria from one person to another? / Je, mbu wa aina gani wanaeneza malaria kutoka kwa mtu Kwenda kwa mwingine?
2. Please tell me what do you understand about malaria / Tafadhari, unaelewa nini kuhusu malaria?
   i. To your understanding how is malaria transmitted from person to another? / Je, ni kwa namna gani malaria inaenezwa kutoka kwa mtu mmoja Kwenda kwa mwingine?

ii. Who is at risk of getting malaria? and how are they at risk? / Je, ni ni mtu wa aina gani yupo kwenye hatari ya kuambukizwa malaria?

iii. How often have you/member of the family gotten malaria? When was the last time you/member of the family got it? / NI kwa mara ngapi wewe au mwanafamilia hupata malaria? Ni lini mara ya mwisho kwa wewe au mwanafamilia kuambukizwa malaria?

iv. How is it treated? How do you treat it? Challenge for treating it? / J e, malaria inatibiwa kwa namna gani? Je ulitibiwaje ulipoumwa malaria? Nini changamoto ulizopata kutibu ugonjwa wa malaria

v. How much does it cost to treat malaria per one person? / Je, inagharimu pesa kiasi gani kutibu ugonjwa wa malaria?

3. Please tell me about mosquito biting behaviour / Tafadhari, unaelewa nini kuhusu tabia ya ung'ataji wa mbu?

i. Which places do you always stay most in the evenings is it outside the house or inside the house? / Ni maeneo gani ambayo huwa unakuwepo majira ya jiono? Je, ni ndani au nje ya nyumba?

ii. Which place do you always bitten by mosquitoes, is it inside the house or outside the houses? / Ni maeneo gan ambayo mara nyingi unayong'atwa na mbu? Je, ni ndani au nje y anyumba?

iii. If its inside/outside, why do you stay? / Kama ni nje kwanini unakaa nje wakati unang'atwa na mbu?

iv. At what time do you usually go to sleep? / Je, ni muda gani ambao huwa unaenda kulala wakati wa usiku?

v. At what time do you usually weak up? / Je, ni muda gani ambao huwa unaamka asubuhi?

vi. What is the density of mosquito when you stay indoor compared to outdoor? / Je, Ukikaa ndani nan je ya nyumba, sehemu gani inakua na idadi ya mbu wengi Zaidi?

vii. How do you see the trend of malaria transmission in your community? Is it increasing or decreasing? / Je, unaonaje mwenendo wa maambukizi ya malaria katika jamii yako? Je, yanaongezeka au yanapungua?

viii. If it is increasing/decreasing which factors contribute to increase/decrease? / Kama yanaongezeka au kupungua, mambo gani yanayopelekea kuongezeka au kupungua?

Section 3: Knowledge and perception of community members on Malaria and mosquito control / Uelewa na mtazamo wa wanajamii kuhusu ugonjwa wa malaria na udhibiti wa mbu

1. Do you think mosquitoes can be controlled? / Je, unafikiri mbu wanaweza kudhibitiwa?

i. If yes how mosquito can be controlled? What control tools are available to you? What do you use? What do other people use? Where did you get these tools? Kama ndiyo, kwa namna gani mbu wanaweza kudhibitiwa? Je, ni zana zipi zilizopo unazotumia kudhibiti mbu? Je watu wengine wanatumia zana zipi kudhibiti mbu? Ulipata wapi hizo zana unazotumia kudhibiti mbu?

ii. If no, what are the challenges for controlling mosquitoes? / Kama siyo, je, kuna changamoto gani katika kudhibiti mbu?

iii. Are these control tools sufficient enough to control mosquitoes? / Je, zana za kudhibiti mbu zilizopo zinatosha katika kudhibiti mbu?

iv. Do you think the community need extra tools for mosquito control? If yes which tools do the community need for mosquito control? / Je unafikiri jamii inahitaji zana za ziada za kudhibiti mbu? Kama ndiyo, je ni zana gani za ziada amabazo jamii inazihitaji ili kudhibiti mbu?

Section 4: Knowledge and perception of community members on relationship between malaria transmission and livestock keeping / Uelewa na mtazamo wa wanajamii kuhusu uhusiano kati ya maambukizi ya ugonjwa wa malaria na ufugaji wa wanyama.

1. What are the common livestock kept in this village? / Katika Kijiji hiki, ni aina gani hasa ya wanyama wanaofugwa?

i. How do you keep your livestock? How does other member of the community keep their livestock? Inside or outside houses? / Je, ni kwa namna gani unafuga wanyama wako? Je, wanajamii wengine wanafugaje wanyama wao? Ni ndani au nje ya nyumba?

ii. If livestock are kept outside, what is the average distance between livestock and house? / Kama wanyama wanafugwa nje ya nyumba, je ni umbali kiasi gani ambacho wanyama huwekwa kutoka kwenye nyumba?

iii. If livestock are kept inside the houses, are they kept together with humans? / kama wanyama wanafugwa ndani ya nyumba, je, wanawekwa pamoja na watu?

iv. How many livestock do you have in this house? Mention their categories / je, unao wanyama wangapi wa kufugwa nyumbani kwako? Yataji majina na aina ya wanyama hao.

v. Do you clean where you keep your livestock? How often? / Je unasafisha mahali ambapo wanyama wako huwekwa

vi. Do you use insecticide to treat your livestock against diseases? What about the rest of the community member are they using insecticides to treat their livestock? / Je, unatumia dawa ya wadudu katika kutibu wanyama wakodhidi ya magonjwa? Je, wanajamii wengine hutumia dawa za wadudu kutibu wanyama dhidi ya magonjwa?

vii. How often do you/community use those insecticides? Can you mention the name of insecticide that you usually use? / Je, ni mara ngapi wewe/ wanajamii hutumia dawa za wadudu kutibu wanyama? Je, unaweza kutaja majina ya dawa hizo?

2. Do you think there is relationship between livestock keeping and malaria transmission? / Je, unafikiri kuna uhusiano wowote kati ya ufugaji wanyama na maambukizi ya

ugonjwa wa malaria?

i. If yes, how livestock keeping is related to malaria transmission? / Kama ndiyo, ni kwa namna gani ufugaji wanyama unahusiana na maambukizi ya ugonjwa wa malaria?
ii. In your perspective, do you think livestock keeping contributes to the increase or decrease of malaria transmission? / Kwa mtazamo wako, unafikiri ufugaji wanyama unaongeza au kupunguza maambukizi ya ugonjwa wa malaria?
iii. If it contributes to the increase in malaria transmission, how? Kama ufugaji unaongeza maambukizi ya malaria, ni kwa namna gani?
iv. If it contributes to the decrease in malaria transmission, how? / Kama ufugaji unapunguza maambukizi ya malaria, je, ni kwa namna gani?
v. Do you think livestock increase or decrease mosquito density in the area? If yes/ no why? / Je unafikiri wanyama wanaongeza au kupunguza idadi ya mbu katika eneo lako au jamii yako? Kama ndiyo au Hapana, kwanini?
vi. What are the common livestock that increases/decrease mosquito density inside and outside the houses? / Je, ni wanyama gani ambao
vii. Do you think the use of insecticides to treat animals has any impact on the ability of mosquitoes to transmit malaria? If yes/no why? / Je, unafikiri matumizi ya dawa za kuua wadudu kutibu wanyama dhidi ya magonjwa kuna athari zozote kwenye uwezo wa mbu kusambaza ugonjwa wa malaria?

3. **Do you have an understanding about the impact of livestock keeping on malaria transmission?** / Je, una ufahamu wowote kuhusu athari za ufugaji wanyama kwenye maambukizi ya ugonjwa wa malaria?

i. If yes, where did you get such information? / Kama ndiyo, Je, ulipata wapi taarifa hizo?
ii. How do you use the knowledge on the impact of livestock keeping on malaria transmission to protect yourself and your family from malaria? / Je, Unatumiaje maarifa na ufahamu wa athari za ufugaji kwenye maambukizi ya malaria kujilinda wewe Pamoja na familia yako dhidi ya maambukizi ya malaria?
iii. If no, pause and explain to them. / Kama siyo, sitisha kidogo mahojiano na uwaeleze Zaidi.
iv. Do you think the knowledge of the impact of livestock keeping on malaria transmission is important to the community? / Unafikiri maarifa na ufahamu kuhusu athari za ufugaji mifugo kwenye maambukizi ya malaria ni muhimu kwenye jamiia?

v. What is the reason for your answer (iv) above / N nini sababu ya jibu lako hapo juu?

vi. Do you think the community need education on the impact of livestock keeping on malaria transmission? And to what extent? / Je, unafikiri jamii inahitaji elimu ya athari za ufugaji wanyama kwenye maambukizi ya ugonjwa wa malaria? Na ni kwa kiasigani jamii inahitaji elimu hiyo?

vii. In your perspective, what are the possible barriers in getting the knowledge on the impact of livestock keeping on malaria transmission to the community? / Kwa mtazamo wako, ni vikwazo au changamoto gani zinazojitokeza kwenye katika kupata elimu kuhusu athari za ufugaji wanyama kwenye maambukizi ya ugonjwa wa malaria?

viii. Please, can you tell us what should be done to increase the knowledge of the community members about the impact of livestock keeping on malaria transmission? / Tafadhali, tuambie ni nini kifanyike kuongeza maarifa na uelewa wa wanajamii kuhusu athari za ufugaji wanyama kwenye maambukizi ya ugonjwa wa malaria?

Conclusion / Hitimisho

Thank you for taking the time to participate today. Your feedback will be collected and included in a report. Again, thank you. / Asanteni sana kwa kutoa muda wenu kushiriki mahojiano ya leo. Maoni yenu yatakusanywa na kujumuishwa kwenye ripoti. Kwa mara nyingine, asanteni sana.

Appendix 8: Institutionla clearance certificate for conducting health research from the Ifakara Health Institute

F120-ILH-v20.0

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IFAKARA HEALTH INSTITUTE
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INSTITUTIONAL REVIEW BOARD

November 1, 2022

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P O Box 9653
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Yohana Mwalugelo
Ifakara Health Institute
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IHI/IRB/No: 41-2022

INSTITUTIONAL CLEARANCE CERTIFICATE FOR CONDUCTING HEALTH RESEARCH

On 28th October **2022**, the Ifakara Health Institute Review Board (IHI-IRB) reviewed from study titled: ***"Impact of livestock management on malaria transmission risks in rural Tanzania"*** submitted by the study Principal Investigator Yohana Mwalugelo. The study has been approved for implementation after IRB consensus. This certificate thus indicates that; the above- mentioned study has been granted an Institutional Ethics Clearance to conduct this study in **Ulanga district**.

The Principal Investigator of the study must ensure that, the following conditions are fulfilled during or after the implementation of the study:

1. PI should submit a six-month progress report and the final report at the end of the project
2. Any amendment, which will be done after the approval of the protocol, must be communicated as soon as possible to the IRB for another approval
3. All research must stop after the project expiration date, unless there is prior information and justification to the IRB.
4. There should be plans to give feedback to the community on the findings
5. The PI should seek permission to publish findings from NIMR
6. The approval is valid until **28th October 2023**

The following documents were reviewed and approved:

1. Cover letter from the principal investigator **2.** Completed application form for ethical clearance (IRB-01) **3.** Summary of the research protocol **4.** Detailed research protocol **5.** Budget and budget justification **6.** Informed consent forms (English and Kiswahili Versions) 7. Investigators' CVs

The IRB reserves the right to undertake field inspections to check on the protocol compliance

Chairperson
Prof. Dr Esther Mwaikambo

IRB Secretary
Dr Mwifadhi Mrisho

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Appendix 9: Approval to conduct research from the Jaramogi Oginga Odinga University of Science and Technology

JARAMOGI OGINGA ODINGA
UNIVERSITY OF SCIENCE AND TECHNOLOGY

DIVISION OF RESEARCH, INNOVATION AND OUTREACH
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BONDO

OUR REF: JOOUST/DVC-RIO/ERC/E4

10th January, 2023

Yohana Mwalughelo
SHS
JOOUST

Dear Mr. Mwalughelo,

RE: APPROVAL TO CONDUCT RESEARCH TITLED "IMPACT OF LIVESTOCK MANAGEMENT ON MALARIA TRANSMISSION DYNAMICS IN RURAL TANZANIA"

This is to inform you that JOOUST ERC has reviewed and approved your above research proposal. Your application approval number is **ERC 35/12/23-15/04.** The approval period is from 10th January, 2023– 09th January, 2024.

This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations and violations) are submitted for review and approval by JOOUST IERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to NACOSTI IERC within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks of affected safety or welfare of study participants and others or affect the integrity of the research must be reported to NACOSTI IERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JOOUST IERC.

Prior to commencing your study, you will be expected to obtain a research permit from National Commission for Science, Technology and Innovation (NACOSTI) https:oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

For
Prof. Francis Anga'wa
Chairman, JOOUST ERC

Copy to: Deputy Vice-Chancellor, RIO   Director, BPS   DEAN, SHS