# Sokoine University of Agriculture

## PhD Thesis

# Ecological assessment of Rodents and Flea populations and Disease awareness in Plague foci, Mbulu District, Northern Tanzania

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# Ecological assessment of Rodents and Flea populations and Disease awareness in Plague foci, Mbulu District, Northen Tanzania

***Thesis submitted to Sokoine University of Agriculture in Fulfilment of the Requirements for the Degree of Doctor of Philosphy***

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# EXTENDED ABSTRACT

Plague, a rodent-borne disease caused by *Yersinia pestis,* continues to be a major public health concern in several African countries. Disease outbreaks are influenced by various factors, including rodent population dynamics, flea infestations, human behaviors and practices and environmental conditions. Conducting comprehensive studies addressing these factors particularly in plague endemic areas is crucial for understanding their involvenment in plague persistence and developing effective preventive and control measures. In this study, several methods were used to assess different factors associated with the continued existence of plague in the plague foci, Mbulu district, Tanzania. The study was conducted between November 2019 to November 2021. To assess rodent species richness, diversity, and community structure in relation to plague persistence in different habitats, seasons and localities, a removel trapping method was applied. Findings indicated that areas with plague persistent had higher rodent abundance and species richness than non-persistent areas. Nortably, species richness was lower in-house premises compared to other habitats. Also, the study revealed three rodent community structures that varied significantly between studied habitat types, suggesting interactions influenced by available resources in the particular habitats.

The population dynamics of *Mastomys natalensis* and *Lophuromys makundii* were assessed using a Capture-Mark-Recapture method. Results showed that the abundance of *M. natalensis* was significantly higher in farms present in plague persistent than those present in non-plague persistent locality. The dry season showed a significant increase of abundance compared to the long rain season and the short rain season. Plague persistent had a significant increase in breeding females compared to non-plague persistent localities, while in the different habitats, farms showed a significant decrease in breeding females compared to forests. Also, dry season was associated with an increase in breeding females compared to the long rain season and the short rain season. Furthermore, the abundance of *L. makundii* and proportion of breeding females showed a significant increase in forest present in plague persistent than non-plague persistent localities. The abundance increased significantly during the dry season and long rain season compared to the short rain season. The breeding females significantly increased during the dry and the short rain season than the long rain season.

For the assessment of flea infestation of rodents, fleas ectoparasites were collected by fur brushing the captured rodents. The study aimed to determine flea infestation prevalence, community structure, and variations between localities, habitats (farn and forest) and seasons. Flea abundance did not show significant differences between localities, habitats and seasons. However, prevalence of flea infestation was significantly positively associated with the plague persistent locality and the short rain season. Also, the study revealed two broad flea community structures based on farm and forest habitats.

Fluctuations in flea abundance thoughout the sampling period and the influence of household behavior (such as mat and sleeping loft) on flea abundance within house were examined using a removal method and light trap. The study found seven flea species within houses with *Ctenocephalides canis* being the most abundant, followed by *Pullex irritans* and *Ctenocephalides felis*. The abundance was significantly higher in dry than long rain season. Houses with sleeping bed, mattress and loft had significantly higher flea abundance than houses with bed and mat alone. Also, households that shared livestock in one roof had significantly more fleas than non-shared households.

Finally, a survey questionnaire consisting of both open and closed ended questions was used to assess peoples' knowledge and awareness on plague disease, risk factors related to flea bite and control measures associated with spread of plague disease in the foci. The Majority of participants were familiar with the plague disease and identified swelling lymph nodes as a common symptom. Most participants claimed to observe human plague cases during the long rain season. The majority of participants reported to experience flea bite in their domestic settings, with most stating that they experienced more flea bites during the dry season. Houses with livestock had a greater likelihood of flea bite compared to houses with no livestock. Furthermore, residents reported using both natural and chemical methods to control rodents and flea inside houses. Most respondents in flea control preferred using natural methods. Respondents stated that the efficacy of flea control methods applied ranged from few days to several months.

The findings from this study highlighted the importance of ecological factors, such as habitat types, seasons, and human behaviors in shaping rodent and flea populations. It also provides valuable information for planning and designing interventions, improving community engagement, and implementing effective flea and rodent control measures in plague foci to reduce the risks of plague transmission.

**Key words:** Rodent population, Flea abundance, household behavior, plague persistence

# IKISIRI KUU

Tauni ni ugonjwa unaosambazwa na panya na kusababishwa na *Yersinia pestis*, unaendelea kuwa tishio kubwa kwa afya ya umma katika nchi kadhaa za Afrika. Mlipuko wa ugonjwa huu unategemea mambo mbalimbali, ikiwa ni pamoja na mabadiliko ya idadi ya panya, uvamizi wa wadudu aina ya viroboto, tabia na matendo ya binadamu, na hali ya mazingira. Kuna haja ya kufanya utafiti wa kina utakao shughulikia sababu hizi juu hasa katika maeneo yenye mlipuko wa tauni. Utafiti huu ni muhimu ili kupata uelewa wa mambo yanayochangia kuendelea kuwepo kwa ugonjwa wa tauni na kutengeneza njia za kuzuia na kudhibiti ugonjwa huu kwa ufanisi. Katika utafiti huu, njia mbalimbali zilitumika kuchunguza mambo mbalimbali yanayohusiana na kuendelea kwa tauni katika eneo la Mbulu, Tanzania. Kwanza, njia ya kukamata na kuua panya ilitumika kutathmini idadi ya panya, tofauti katika spishi, na muundo wa jamii ya panya katika mazingira tofauti, misimu, na maeneo husika kulingana na kuendelea kwa mlipuko wa tauni. Utafiti ulibaini kuwa eneo lenye kuendelea kuwa na mlipuko wa tauni lina idadi kubwa zaidi ya panya na spishi nyingi zaidi kuliko eneo lisilo na mlipuko wa tauni. Pia, makazi ya watu yalionyesha kuwa na spishi chache zaidi ikilinganishwa na mazingira mengine. Aidha, utafiti ulibainisha muundo wa jamii ya panya uligawanyika katika sehemu tatu amabazo zilitofautiana katika mazingira yaliyochunguzwa, ukionyesha kuwa kuna mwingiliano mkubwa kati ya panya na wingi wa rasilimali katika mazingira yaliyochunguzwa.

Pili, njia ya kukamata kuweka alama na kuwarudisha panya eneo walilokamatwa ilitumika kutathmini mabadiliko ya idadi ya panya wa spishi ya *Mastomys natalensis* na *Lophuromys makundii*. Utafiti ulionyesha kuwa idadi ya *M. natalensis* ilikuwa kubwa sana katika mashamba na katika eneo lenye kuendelea kuwa na mlipuko wa tauni kuliko eneo lisilo na mlipuko wa tauni. Msimu wa ukame ulionyesha

ongezeko kubwa la idadi ya panya ikilinganishwa na msimu wa masika na msimu wa mvua za vuli. Eneo lenye kuendelea kuwa na mlipuko wa tauni ulionyesha ongezeko kubwa la panya wa kike wanaozaa ikilinganishwa na eneo lisilokuwa na mlipuko wa tauni. Katika mazingira, mashamba yalionyesha kupungua kwa panya wa kike wanaozaa ikilinganishwa na misitu. Pia, idadi ya *L. makundii* na panya wa kike wanaozaa ilionyesha ongezeko kubwa katika misitu ya eneo lenye kuendelea kuwa na mlipuko wa tauni kuliko eneo lisilo na mlipuko wa tauni. Idadi ya panya iliongezeka sana wakati wa msimu wa ukame na msimu wa mvua za masika ikilinganishwa na msimu wa mvua za vuli. Panya wa kike wanaozaa waliongezeka sana wakati wa msimu wa ukame na msimu wa mvua za vuli ikilinganishwa na msimu wa mvua za masika.

Tatu, wadudu aina ya viroboto walikusanywa kwa kubrashi manyoya ya panya. Panya walikamwatwa katika mazingira ya mashamani na misituni ili kupima idadi na muundo wa jamii ya viroboto waliopo, na sababu zinazoweza kuchochea ongezeko la viroboto katika eneo lenye mlipuko wa tauni na yasiyo na mlipuko wa tauni. Utafiti ulionyesha, hakukuwa na utofauti wa idadi ya viroboto kati ya maeneo, mazingira, na misimu iliyofanyiwa tafiti. Hata hivyo, kuenea kwa viroboto kulihusishwa kwa kiwango kikubwa katika eneo lenye mlipuko wa tauni na wakati wa msimu mvua za vuli. Wingi wa viroboto uliongezeka kadri uzito wa panya ulivyoongezeka. Pia tafiti ilionyesha kuwepo kwa aina kuu mbili za jamii ya viroboto kulingana na mazingira ya mashambani na misituni.

Nne, njia ya kubrashi manyoya ya panya na mitego ya mwanga ilitumika kukusanya na kutathmini utofauti wa wingi wa viroboto katika kipindi cha ukusanyaji pamoja, na athari za tabia na matendo ya binadamu katika kuchangia wingi wa viroboto ndani ya nyumba za vijijini Wilayani Mbulu, Tanzania. Tafiti hii iligundua uwepo wa aina saba za viroboto ndani ya nyumba ambapo aina ya kiroboto *Ctenocephalides canis* ilikuwa aina

yenye idadi kubwa, ikifuatiwa na *Pullex irritans* na *Ctenocephalides felis*. Wingi wa viroboto ulikuwa mkubwa zaidi katika msimu wa ukame kuliko msimu wa mvua za masika. Nyumba zilizokuwa na vitanda, magodoro, na dari zilikuwa na wingi mkubwa wa viroboto kuliko nyumba zilizokuwa na kitanda na mkeka pekee. Pia, kaya zilizoshiriki kuishi na mifugo katika paa moja zilikuwa na wingi mkubwa wa viroboto kuliko kaya ambazo hazikushiriki kuishi na mifugo.

Tano, utafiti ulichunguza maarifa na mitazamo ya wana jamii juu ya ugonjwa wa tauni, sababu zinazochangia uwepo wa kung'atwa na viroboto na njia zilizotumiwa na wakazi wa maeneo yanayoathiriwa na tauni kudhibiti panya na viroboto. Wengi ya washiriki walikuwa wanafahamu ugonjwa wa tauni na kuvimba tezi ilitajwa na wengi kama dalili kubwa ya ugonjwa. Aidha, wengi ya washiriki waliripoti kuumwa na viroboto ndani ya nyumba zao, na wengi wao walisema kuwa walipata kuumwa zaidi na viroboto wakati wa msimu wa ukame. Kuwepo kwa mifugo katika maeneo ya nyumba zao ilionekana kuongeza hatari ya kuumwa na viroboto kuliko nyumba zisizo na mifugo. Mbali na hilo, wakazi waliripoti kutumia njia za asili na kemikali katika udhibiti wa panya na viroboto ndani ya nyumba. Washiriki walieleza kuwa ufanisi wa njia za udhibiti wa voroboto ulikuwa kati ya siku hadi miezi kadhaa.

Matokeo ya tafiti hii yalionyesha umuhimu wa kuelewa ekolojia, kama vile aina za mazingira, misimu, na tabia za binadamu katika uwepo wa panya na viroboto. Pia, utafiti ulionyesha umuhimu wa kuboresha ushiriki wa jamii, kupanga na kubuni mikakati ya kudhibiti viroboto na panya katika maeneo yanayoathiriwa ili kupunguza hatari ya kuambukizwa tauni.

**Maneno muhimu**: Idadi ya panya, wingi wa viroboto, Tabia za wanakaya, Uwepo wa tauni.

# DECLARATION

I, **Stella Thadeus Kessy**, do hereby declare, to the Senate of Sokoine University of Agriculture, that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

____________________ __________________
Stella T. Kessy Date
(**PhD Candidate**)

The above declaration confirmed by:

____________________ __________________
Prof. Apia. A. Massawe Date
(**Supervisor**)

____________________ __________________
Prof. Alphan A. Rija Date
(**Supervisor**)

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# DEDICATION

I dedicate this thesis to my young sister, Neema Mkunde Mosha, with sincere hope that this thesis will serve as a profound source of inspiration for you to pursue your own academic journey with unwavering determination and passion.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACE-IRPM& BTD | African Centre of Excellence for Innovative |
| Rodent | Pest Management and Biosensor Technology Development |
| IPM | Institute of Pest management |
| Ltrap | Light trap |
| SUA | Sokoine University of Agriculture |
| WHO | World Health Organization |

# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION

## 1.1 Plague Background Information

Plague, is a zoonotic disease caused by the bacteria *Yersinia pestis*, whichprimarily circulates among animal reservoir hosts, with rodents playing crucial role as the main reservoirs (Morelli *et al.*, 2010; Ligon, 2006). The transmission of the bacteria occurs through the bite of an infected flea and in some cases, from lice vectors known as *Pediculus humanus* mostly during the disease outbreak (Gage & Kosoy, 2005; Ratovonjato *et al.,* 2014). In addition to flea bite, direct contact with infected materials such as contaminated meat and droplet infection can also lead into pneumonic plague case (Yue *et al.,* 2017; Hinnebusch *et al.,* 2016). Usually, it manifests in three forms; Bubonic plague, Pneumonic plague and Septicaemic plague (Ligon, 2006). The bubonic form results from the bite of an infected flea, Pneumonic form results from inhaling contaminated air droplets and Septicaemic form occurs when bacteria multiply in the blood which mostly occurs when one has untreated bubonic and pneumonic plague (Ibeji, 2011; Stenseth *et al.,* 2008). During a disease epidemic, the diagnosis of plague in humans is mostly based on the assessment of the patient's symptoms, which may include the presence of buboes, coughing with bloody sputum, fever as well as patient's exposure history (Ligon, 2006). However, since these symptoms are similar to those of other diseases, laboratory techniques, such as the isolation and identification of *Y. pestis* from clinical specimens, are used to confirm the diagnosis of plague (Yang, 2018).

Furthermore, plague is widely known as one of the diseases in human history affecting millions of people via three major pandemics (Keim & Wagner 2009; Bramanti *et al.,* 2016; Schaub & Vogel, 2023). These includes, the Justinian plague pandemic that occurred from 541-543AD

(Wagner *et al.,* 2014), the Black Death that occurred between 1347-1351 and the Oriental plague pandemic, that originated in China during the mid-19th century and subsequently spread globally (Stenseth et al., 2008; Harbeck et al., 2013). The disease was prevalent in some regions in Africa including Northern, Eastern, Southern, and Central Africa from the 19th to the 20th centuries. The countries that have been affected include: Libya, Algeria, Tanzania, Uganda, Kenya, Senegal, Republic of South Africa, Zimbabwe, Mozambique, the Democratic Republic of Congo and Madagascar. Of these, the disease is still active in Madagascar, Mozambique, Uganda, Republic of Congo and Tanzania (Stenseth *et al.,* 2008). In 2014-2015 more than 90% of the worldwide cases were reported, whereby Madagascar and Republic of Congo were the highest endemic countries (Lotfy, 2015). In late 2017, Madagascar experienced large outbreak of plague, with a total of 2348 confirmed plague suspects cases and 202 human deaths recorded (WHO, 2017; Mead, 2018). In Tanzania plague can be explained as a re-emerging disease since it has been on and off since precolonial (Msangi, 1969, Kilonzo, 1976; Neerinckx *et al.,* 2010; Ziwa *et al.,* 2013). However, the largest plague outbreak occurred in Lushoto 1980 claiming 640 human lives and persisted for 13 years (Kilonzo *et al.,* 2006; Kilonzo & Mhina, 1982). This was followed by other outbreaks in Karatu and Mbulu District in 1996 (Kilonzo *et al.,* 1997, 2006; Makundi *et al.,* 2008). However, studies have indicated that all of these Tanzanian foci have undergone recurring disease outbreaks, resulting in a substantial number of human cases and case-fatality rates. The Mbulu district, especially, has recently reported a substantial number of human plague cases (Ziwa *et al.,* 2013; Mwalimu *et al.,* 2022).

### 1.1.1 Rodent hosts

Rodents play a role in the dynamics of plague transmission since they serve as amplifying hosts for the bacteria and a source of infection for fleas, which are the primary vectors of *Y. pestis* (Gage & Kosoy, 2005).

Several rodent species including *Ratus rattus* and *Mastomys natalensis* have been involved in plague epidemic (Andrianaivoarimanana *et al*., 2013; Sun *et al*., 2019; Makundi *et al*., 2008). These plague epidemics have been documented in wild rodents, including chipmunks, prairie dogs and ground squirrels (Keeling & Gilligan, 2000; Mahmoundi *et al.,* 2021; Fernandes *et al*., 2021). For instance, In South America, 50 wild rodent species have been identified as plague reservoirs (Bonvicino *et al*., 2015). Additionally, the Nile rats (Arvicanthis niloticus) have been identified as potential rodent reservoirs of *Y. pestis* in a plague-endemic region of Uganda (Eisen *et al*., 2008).

The maintenance of plague in rodent populations relies on the presence of suitable flea vectors and favourable environmental conditions (Kehrmann *et al*., 2020). Fleas, such as *Xenopsylla cheopis* and *Xenopsylla brasiliensis*, acquire *Y. pestis* from infected rodents and transmit the bacteria to other hosts, including humans (Barbieri *et al*., 2020). Other factors that contribute to the transmission cycle include rodent population dynamics, species composition, and habitat suitability (Sun *et al*., 2019; Snäll *et al*., 2008). In fact, Sun *et al*. (2019) reported that the presence of rodent species, particularly those that serve as hosts for plague, are positively associated with the occurrence of human plague. This suggest that a comprehensive understanding of the diversity and distribution of rodent reservoir hosts is essential for assessing plague transmission risk in affected areas.

### 1.1.2 Flea vector and transmission dynamics

Fleas play an important role in the spread of plague, acting as vectors by acquiring *Yersinia pestis* from infected rodents and transmitting it to other hosts including human (Gage & Kosoy,2005; Prentice & Rahalison, 2007). Different flea species have varying levels of competence in transmitting the bacteria, and their interactions with reservoir hosts are essential to the dynamics of plague transmission

(Eisen *et al*., 2009). Cat fleas (*Ctenocephalides felis*), for example, are identified as potential secondary vectors while rodent fleas (*Xenopsylla brasilliensis*) are identified as primary vectors of plague (Eisen *et al*., 2008).

Multiple factors influence the transmission of *Y. pestis* by fleas, including the ability of the bacteria to persist and multiply within the flea, the feeding behaviour of fleas, and the presence of specific genes that enhance transmission (Vadyvaloo *et al*., 2010). The interactions between fleas and reservoir hosts are complex, involving the acquisition and maintenance of the bacteria within the flea population (Vadyvaloo *et al*., 2010). This favors the enzootic cycle which refers to continuous circulation of *Y. pestis* within rodent host population as well as their associated flea vectors (Gage & Kosoy, 2005). Moreover, the emergence of epizootic events may also occur when there are unexpected outbreaks of plague, resulting in increased transmission rates and the possibility of spill over to other species. Epizootics occurs when there is an increase in the number of susceptible hosts, alterations in host behaviour or population dynamics, or changes in flea populations (Walsh & Haseeb, 2015; Holmes *et al*., 2006). Hence, fleas contribute to the persistence of *Y. pestis* by serving as a reservoir for the bacteria and allowing it to survive between host interactions (Bosio *et al*., 2020).

Furthermore, plague bacteria persist in the reservoir host population due to a combination of factors, such as the capacity of *Y. pestis* to colonize and multiply within the host, the development of immune responses in the host, and the presence of competent flea vectors (Gauge & Kosoy, 2005; Buhnerkempe *et al*., 2011). The occurrence and extent of these events are influenced by environmental factors such as climate, habitat disturbance, and resource availability (Walsh & Haseeb, 2015; Holmes *et al*., 2006). This complex ecological interaction between reservoir hosts, flea vectors, and the environment influence the dynamics of plague transmission which can lead into zoonotic cycle. Zoonotic occurs when plague kills the susceptible rodents and their infected fleas leave the carcasses and look for new hosts. This may encourage the increase of fleas moving in search of a new host, and therefore spreading plague pathogens from infected natural hosts. (Figure 1).

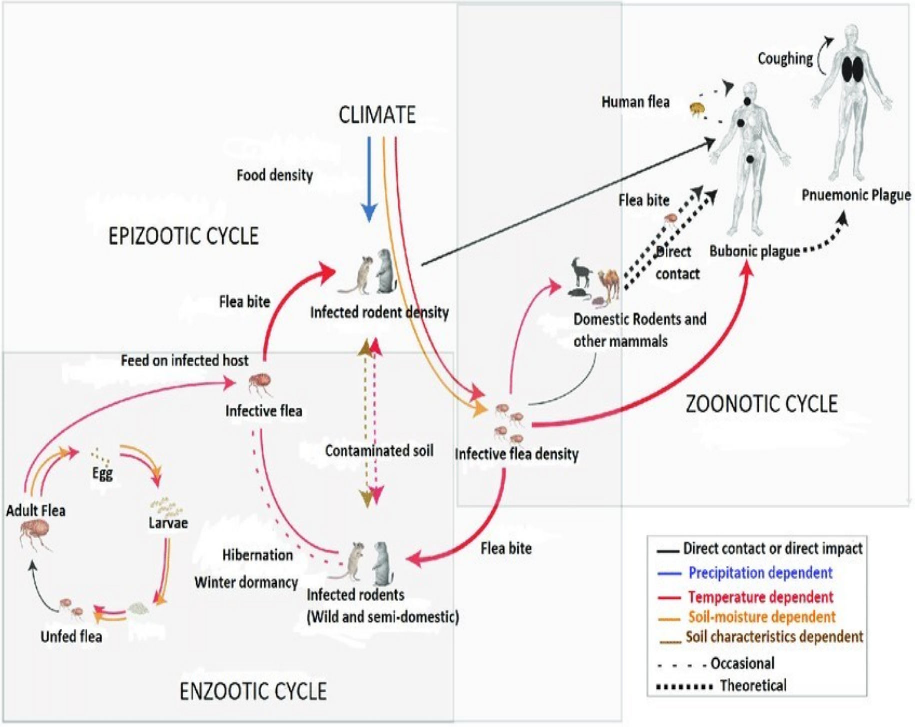

**Figure 1:** Plague transmission cycle in the foci
Source; Ben Ari *et al*., 2011

### 1.1.3 Environmental and social factors in the persistence of plague

Plague persistence is influenced by a combination of environmental and social factors. Environmental factors such as temperature, humidity, rainfall patterns, and habitat characteristics can influence the populations of reservoir hosts and fleas (He *et al.,* 2021; Duplantier *et al.,* 2005; Samia *et al.,* 2011). Warm and humid environments may facilitate flea reproduction and survival, while specific habitats or landscapes may favour the development of certain rodent species (Snäll *et al.,* 2008; Zeppelini *et al.,* 2016; Girard *et al.,* 2004). A study in Thailand, for example, found that changes in habitat diversity, specifically reduced forest cover, increased fragmentation, and rising urbanization, favored the presence of synanthropic rodent species like *Rattus tanezumi*, which were known to cause crop damage and serve as hantaviruses' reservoirs (Morand *et al.,* 2015). Also, Warmer temperatures and higher humidity in Madagascar have been linked to shorter development periods and increased flea survival, resulting in greater flea abundance (Kreppel *et al.,* 2016). Similarly, moister climatic conditions were discovered to improve flea survival and reproduction, potentially increasing plague transmission (Parmenter *et al.,* 1999; Ben Ari *et al.,* 2011).

In addition, social cultural, human behaviour and life style practices within communities contribute to the transmission and persistence of diseases (Nyirenda *et al.,* 2017; Rivière-Cinnamond *et al.,* 2018). The effect of traditional beliefs about the cause of plague and health seeking-behaviour for treatment has been seen in the persistence and recurrence of plague outbreak in Lushoto district, Tanzania, as well as in Zambia and Uganda (Kilonzo *et al.,* 1997; Ngulube *et al.,* 2006; Ogen-Odoi, 1993). Studies conducted by Christie *et al.* (1980) and Saeed *et al.* (2005) In Libya and Kenya, examined a situation where people were infected with the plague due to the consumption of suspected animal carcases of camel and goats. Also, a case study was documented in USA after conducting an autopsy of a dead mountain lion (Wong *et al.,* 2009). Moreover, people who dig rodent burrows may get infection inhaling particulate matter from recently dead rodent burrows that have been affected by plague. It has been reported that *Y. pestis* can survive in soil for quite some time and may also become airborne (Ayyadurai *et al.,* 2008).

### 1.1.4 Surveillance and control strategies

Surveillance programs that monitor plague in both human and animal populations are important for early detection of cases and outbreaks, allowing timely implementation of control measures (Gage & Kosoy, 2005; Andrianaivoarimanana *et al*., 2013). Effective surveillance helps identify areas at risk by monitoring reservoir host populations, and tracking flea activities (Grácio & Grácio, 2017). Plague control strategies include various measures, such as flea control, rodent control, and public health initiative to prevent human exposure to infected fleas and/ or tissue of infected animal (Butler, 2013; Samia *et al*., 2011). Rodent control strategies involve habitat modification to prevent rodent infestation as well as use of rodenticides or traps to control rodent populations (Eisen *et al*., 2014; Boegler *et al*., 2018). Flea control measures include insecticide-treated baits, and the application of insecticide sprays or dusts on their habitats (Poché a *et al*., 2017; Eisen *et al*., 2014). Moreover, public health initiatives involve educational campaigns that raise awareness about the signs and symptoms of plague, the importance of early diagnosis and treatment, as well as the importance of practicing personal hygiene and avoiding contact with rodents and fleas (Butler, 2013; Agrawal *et al*., 2023). Generally, control strategies are recommended to consider connectivity between indoor and outdoor rodent-flea population (Rahelinirina *et al*., 2021).

## 1.2 Statement Problem and Justification of the Study

Plague foci are present in some districts and regions of Tanzania, including Mbulu, Lushoto, Karatu, Singida, Iringa, Same and Hai (Ziwa *et al*., 2013). However, since the 1980s, most of the plague foci in Tanzania have been inactive except in Lushoto, Karatu and Mbulu distrcts. Mbulu ditstrict experienced its plague for the first time in 1996 with 186 clinical cases and 12 deaths (Kilonzo *et al*., 2006). This was followed by another outbreak in 2007 that caused 35 human cases and 6 deaths (Makundi *et al*., 2008). Following this, subsequent human plague cases have been reported in 2010 and 2019 (Makundi *et al*., 2008 and Mwalimu *et al*., 2022).

Moreover, plague persistence and recurrence were connected to socio, environmental and biological factors. Several studies have identified plague pathogen in rodent reservoirs, potential vectors, and reported multiple interactions between host and vectors all of which contributed to the transmission and persistence of plague in the foci (Ziwa *et al*., 2013;Haule *et al.,* 2013; Makundi *et al*., 2015; Haikukutu *et al*., 2022; Mwalimu *et al*., 2022). Flea species, such as *Xenopsylla cheopis* and *Xenopsylla brasilliensis*, were found in domestic environments on *Rattus rattus*, semi domestic on *Mastomys natalensis* and wild environments on *Lemniscomys zebra* (Makundi *et al*., 2015). Furthermore, land use change has been found to influence the abundance and diversity of rodent communities, therefore affecting plague activity in the foci (McCauley *et al*., 2015). Deforestation, for instance, may reduce the availability of natural forested areas, forcing rodents to search alternative habitats in human-modified landscapes leading to increased contact between rodents and humans. Inaddition, some of human behavior and practises, such as sleeping on the ground, sleeping with animals in one house have been linked to an increased risk of plague transmissions. (Ziwa *et al*., 2013). Despite this valuable knowledge, there is lack of information regarding the co-existence of rodent and flea specie between different localities, specifically between villages that have experienced recurrence of human plague (plague persistent localities) and those with no human plague cases (non-plague persistent). In this particular scenario, it is important to investigate the ecology of rodents and flea ectoparasites within the plague foci. This research study aims to understand the population trends, diversity, and distribution of rodents and fleas between plague persistent and non-plague persistent localities, as well as their variations across seasons and within different habitats. Furthermore, it is important to assess human behavior and practices in the foci that may potentially enhance plague persistence. These findings will have several implications; (i) directing pre-planning measures for preventing the recurrence of the bubonic plague, (ii) developing suitable initiatives to reduce flea predisposing causes to lower the chances of repeated plague cases in these rural areas, (iii) helping in the designing of appropriate educational and preventive measures to minimize the risk of plague transmission.

## 1.3 Objectives of the study

### 1.3.1 General objective

To assess the ecology of rodents and flea ectoparasites, as well as human activities and people awareness on plague in Mbulu district, northern Tanzania.

### 1.3.2 Specific objectives

i. To assess species richness, diversity and community structure of rodents and how they vary between plague persistent and non-plague persistent localities in the foci

ii. To assessed the population dynamics of the two-rodent species (*Mastomys. natalensis* and *Lophuromys. makundii*) across seasons and localities (persistent and non-persistent), sampled in different habitats (farm and forest).

iii. To assess flea infestation on rodents, their community structure and how this infestation varies between plague persistent and non-persistent.

iv. To examine the fluctuations of flea abundance across the sampling period and how household behaviour and practices influenced flea abundance within houses
v. To assess peoples' knowledge and awareness on plague disease, the risk factors related to flea bite and control measures related to the spread of plague disease in the foci .

### 1.3.3 Research question

i. What are the patterns of species richness, diversity, and community structure of rodents in the foci, and how do they vary across plague persistent and non-persistent localities?
ii. How is the population dynamics of *Mastomys natalensis* and *Lophuromys makundii* in different habitats (farm and forest) across seasons and localities (plague persistent and non-persistent)?
iii. What is the most current information on flea infestations in the foci, and how does the infestation vary between plague persistent and non-persistent localities?
iv. How do flactuations of fle flea abundance occur across the sampling period and how do household behaviour and practices influence flea abundance within houses?
v. What is the level of knowledge and awareness among people regarding plague disease, the risk factors associated with flea bites and the control measures related to the spread of plague disease in the foci?

### 1.3.4 Organization of the thesis

The thesis consists of eight chapters, beginning with the introduction in Chapter One. This chapter provides a general background information and research problem of the study. The second chapter presents the first manuscript, which focuses on the abundance, diversity, and community structure of rodents within bubonic plague foci. This chapter has been published in the Mammalia Journal. Chapter three presents manuscript two, which assessed the population dynamics of the two-rodent species *Mastomys natalensis* and *Lophuromys makundii* and their implications for the persistence of plague in Mbulu District, Tanzania. This manuscript has been published in European Journal of Wildlife Research. Chapter four presents manuscript three, which focused on the Flea infestation of rodent and their community structure in frequent and non- frequent plague outbreak areas in Mbulu District, Northern Tanzania. The manuscript has been published in the Journal for parasitology: parasites and wildlife. Chapter five includes manuscript four which presents how ecological and environmental factors correlates with flea abundances within human residences. The manuscript has been submitted in the journal PLOS Global Public Health. Chapter six presents manuscript five which provides information on people's knowledge and practices concerning plague in the study area. This has been submitted for publication in the journal PLOS Neglected Tropical Diseases. Chapter seven provides a general discussion and interpretation of the thesis's findings. Finally, Chapter eight presents conclusion and recommendations of the study.

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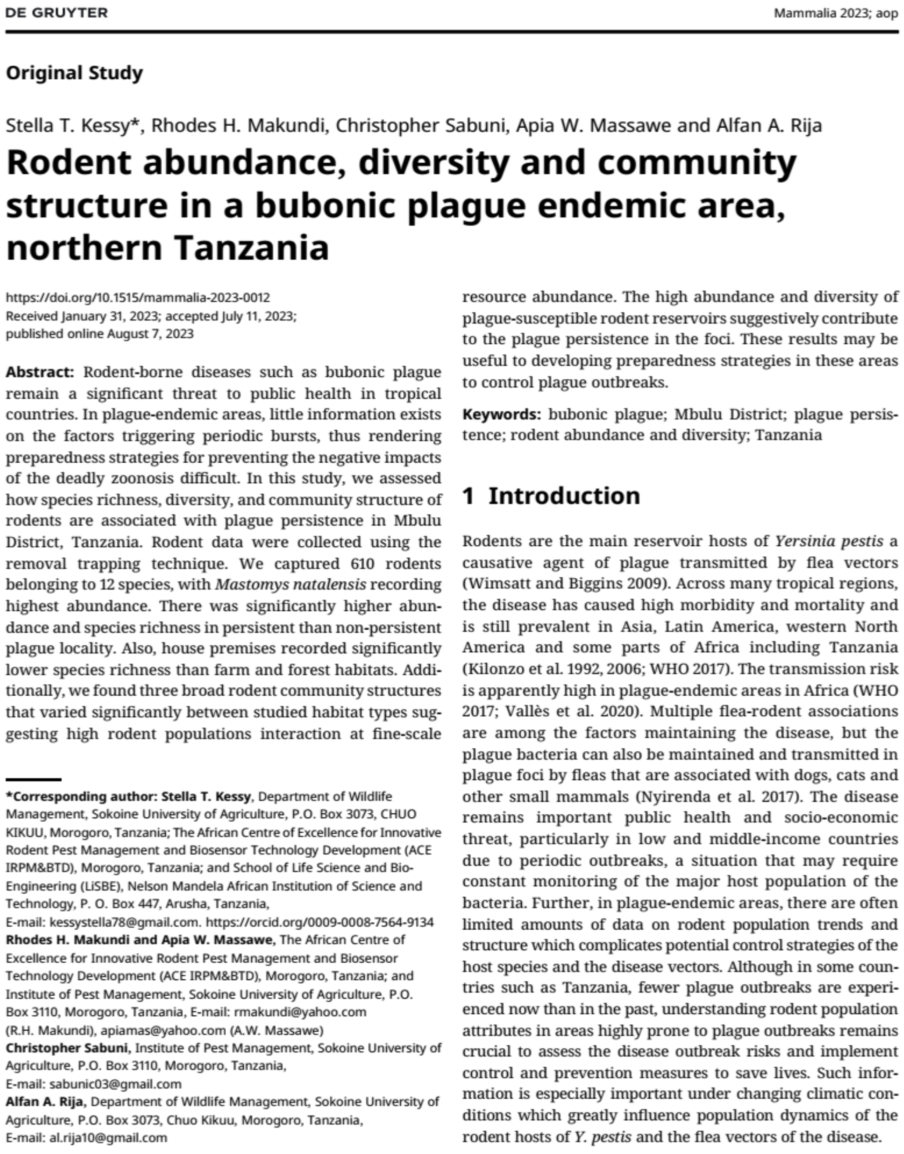
# CHAPTER TWO
# PAPER I



**DE GRUYTER** Mammalia 2023; aop

**Original Study**

Stella T. Kessy*, Rhodes H. Makundi, Christopher Sabuni, Apia W. Massawe and Alfan A. Rija

## Rodent abundance, diversity and community structure in a bubonic plague endemic area, northern Tanzania

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**Abstract:** Rodent-borne diseases such as bubonic plague remain a significant threat to public health in tropical countries. In plague-endemic areas, little information exists on the factors triggering periodic bursts, thus rendering preparedness strategies for preventing the negative impacts of the deadly zoonosis difficult. In this study, we assessed how species richness, diversity, and community structure of rodents are associated with plague persistence in Mbulu District, Tanzania. Rodent data were collected using the removal trapping technique. We captured 610 rodents belonging to 12 species, with *Mastomys natalensis* recording highest abundance. There was significantly higher abundance and species richness in persistent than non-persistent plague locality. Also, house premises recorded significantly lower species richness than farm and forest habitats. Additionally, we found three broad rodent community structures that varied significantly between studied habitat types suggesting high rodent populations interaction at fine-scale resource abundance. The high abundance and diversity of plague-susceptible rodent reservoirs suggestively contribute to the plague persistence in the foci. These results may be useful to developing preparedness strategies in these areas to control plague outbreaks.

**Keywords:** bubonic plague; Mbulu District; plague persistence; rodent abundance and diversity; Tanzania

## 1 Introduction

Rodents are the main reservoir hosts of *Yersinia pestis* a causative agent of plague transmitted by flea vectors (Wimsatt and Biggins 2009). Across many tropical regions, the disease has caused high morbidity and mortality and is still prevalent in Asia, Latin America, western North America and some parts of Africa including Tanzania (Kilonzo et al. 1992, 2006; WHO 2017). The transmission risk is apparently high in plague-endemic areas in Africa (WHO 2017; Vallès et al. 2020). Multiple flea-rodent associations are among the factors maintaining the disease, but the plague bacteria can also be maintained and transmitted in plague foci by fleas that are associated with dogs, cats and other small mammals (Nyirenda et al. 2017). The disease remains important public health and socio-economic threat, particularly in low and middle-income countries due to periodic outbreaks, a situation that may require constant monitoring of the major host population of the bacteria. Further, in plague-endemic areas, there are often limited amounts of data on rodent population trends and structure which complicates potential control strategies of the host species and the disease vectors. Although in some countries such as Tanzania, fewer plague outbreaks are experienced now than in the past, understanding rodent population attributes in areas highly prone to plague outbreaks remains crucial to assess the disease outbreak risks and implement control and prevention measures to save lives. Such information is especially important under changing climatic conditions which greatly influence population dynamics of the rodent hosts of *Y. pestis* and the flea vectors of the disease.

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Disease persistence and transmission are influenced by various factors. Changes in land use patterns, such as clearing forests or woodlands for agriculture, is known to influence rodent and flea populations leading to increased risk of plague outbreak (McCauley et al. 2015). Farming activities for example, provide important food and shelter for rodents. After harvest and land preparation, rodents are severely impacted by reduced shelter and food (Massawe et al. 2005), leading to changes in species distribution and abundance which may result in movement of rodents into domestic areas and houses or away from human activities (Bösing et al. 2014; Morand et al. 2015; Shilereyo et al. 2019). Rodent population density changes and dispersal may influence the distribution and abundance of flea vectors thereby, influencing disease persistence and potential plague transmission pathways (McCauley et al. 2015).

Persistence and frequency of the disease outbreaks in plague foci can be understood better by assessing the rodent population and how rodents interact with humans, which may increase exposure and disease contraction (Ostfeld 2017). Diversity, for instance, can increase or decrease pathogen transmissions. High diversity with susceptible hosts (i.e. species that are more likely to get *Y. pestis* infection) in the population may increase the risk of disease while high diversity with non-susceptible hosts may tend to lower the abundance of susceptible hosts and reduce disease risks, a phenomenon termed as dilution effect (Keesing et al. 2006; Ostfeld and Keesing 2012). Several studies have demonstrated that high diversity of host that are highly susceptible to the disease is related to the emergence of zoonotic diseases (Allen et al. 2017; Keesing et al. 2006; 2010; Ostfeld and Keesing 2012). High species richness of wild rodents increases the likelihood of plague transmission in natural environments, which may also spread directly to bridge rodents and/or other hosts when bridge hosts and wild rodents share habitats. This situation increases the chance for transferring flea vectors to humans living in domestic and peri-domestic areas (Bonvicino et al. 2015). According to Caron et al. (2015) bridge host is capable of spreading the disease by direct contact or sharing environment with the other population. For example, in Yunnan China, a study revealed that domestic animals served as bridge hosts for the spread of plague disease from wild rodents (Mu et al. 2010). Further frequent contacts between hosts and vector maintain enzootic transmission, which may also depend on host and vector abundance in the plague foci (Eisen and Gage 2009). On the other hand increased exposure to the *Y. pestis* bacteria increases the possibility of resistance in susceptible rodent species, which has been shown to increase their survival rates in plague foci (Andrianaivoarimanana et al. 2018). Moreover, fleas infest not just rodent but also other mammalian hosts (Lawrence et al. 2015; Otranto et al. 2017), which make them reservoir of pathogen (Dobler and Pfeffer 2011). It is also considered that human ectoparasites, such as human fleas and lice, contribute to plague epidemics (Laudisoit et al. 2007; Piarroux et al. 2013; Ratovonjato et al. 2014); however, their role in plague outbreaks is not well understood. Furthermore, the contact between mammals and contaminated soil maintains plague in the foci (Malek et al. 2017). For example, when naïve rodents burrow in contaminated soils, they may contract bacterial infection by inhalation and/or ingestion, thereby transmitting the bacteria to the rodent and its flea parasites (Andrianaivoarimanana et al. 2013). However, these mechanisms remain unclear, since prior studies have demonstrated that *Y.pestis* may survive in the soil for at least 24 days under natural conditions (Eisen et al. 2008), while other studies indicate a one year survival (Ayyadurai et al. 2008). Despite this wealth of knowledge, yet information on the local-scale abundance and community structure of rodents and how these vary across areas differing in proneness to plague, habitats and seasonality especially in historically plague-affected regions is still scarce. This gap hampers the possibility for designing effective mitigation measures to prevent potential catastrophic outbreaks of the disease.

In this study, we aimed to understand the species richness, diversity and community structure of rodents in Mbulu District, Tanzania-historically a bubonic plague endemic area. Specifically, we assessed how rodent abundance, species richness and diversity vary with the local-scale factors such as rainfall, habitat types and plague history. Also, we assessed how the rodent community structure vary across the habitat types. We hypothesized that the relative abundance, species richness and diversity of rodents will be higher in areas previously identified as plague foci sites consistent with existing knowledge that high richness and diversity of rodents are associated with bubonic plague. Further, we also hypothesized that local scale factors will have stronger influence on the rodent community structuring and that different rodent communities will show low variation in plague foci due to high species interactions associated with similar resources use such as food and habitats. These data may be useful for informing the pre-planning strategies for controlling the potential re-occurrence of the bubonic plague.

## 2 Materials and methods

### 2.1 Study area

This study was conducted in two villages Mongahay (04° 03′ S, 35° 26′E) and Endesh (04° 03′ S, 35° 27′E) located in Mbulu District, Manyara Region in Northern Tanzania from January to December 2020 (Figure 1). The district lies between 1000 and 2400 m above sea level and is characterized by bimodal rainfall pattern, with a long rainy season between March and May, and a short rainy season between October and January. The economic activities in the area include livestock keeping including cattle, goats and pigs, and crop farming including maize, peas and vegetables. Several studies have documented presence of plague pathogen in the rodent population and there have been reports of human plague in the foci (Haikukutu et al. 2022; Makundi et al. 2008; Ziwa et al. 2013a). The selection of villages with and without human plague cases was purposeful and also based on confirmation of the presence or absence of bubonic plague cases through village health office records in consultation with the village leaders. The plague persistent (Endeshi village) and non-persistent (Mongahay village) localities were defined as localities with and without history of bubonic plague cases.

## 2.2 Rodent trapping

Rodents were live trapped using Sherman traps (LFA 7.6 × 8.9 × 23 cm, H.B. Sherman Trap, Inc., Tallahassee, USA) and locally made wooden box traps (11 cm width, 17 cm length and 16 cm height size) baited with peanut butter mixed with maize flour. Five transect lines with 10 trapping stations set 10 m apart were established in the forest (natural forest) and farmland (mixed farming) habitats in each village. Both plague and non-plague locality had primary regenerating forests which were assumed to be of similar forest structure with closed canopy of tall and medium-sized trees and were both managed by the local villagers. The ground was covered with dense dead leaves and woods. In both forests some human activities, such as collecting firewood and livestock grazing were considered to be encroachment. Further, in both localities mixed farming involved short-cycle crops such as beans often planted in December and harvested in March while long-cycle crops (maize and peas) were planted in December and harvested in July or August. Intercropping is done with maize, beans, and peas while onions, garlic, Irish potatoes, and sweet potatoes are often grown singly. To collect data from inside houses, a random sample of 125 houses was evaluated, representing 10 percent of the households in the villages. A few houses had brick walls but the majority were muddy and thatched. Livestock were kept in sleeping house or corrals constructed near sleeping houses. Harvested crops were also stored inside sleeping houses. All assessed houses were located between a minimum distance of 5–200 m from the

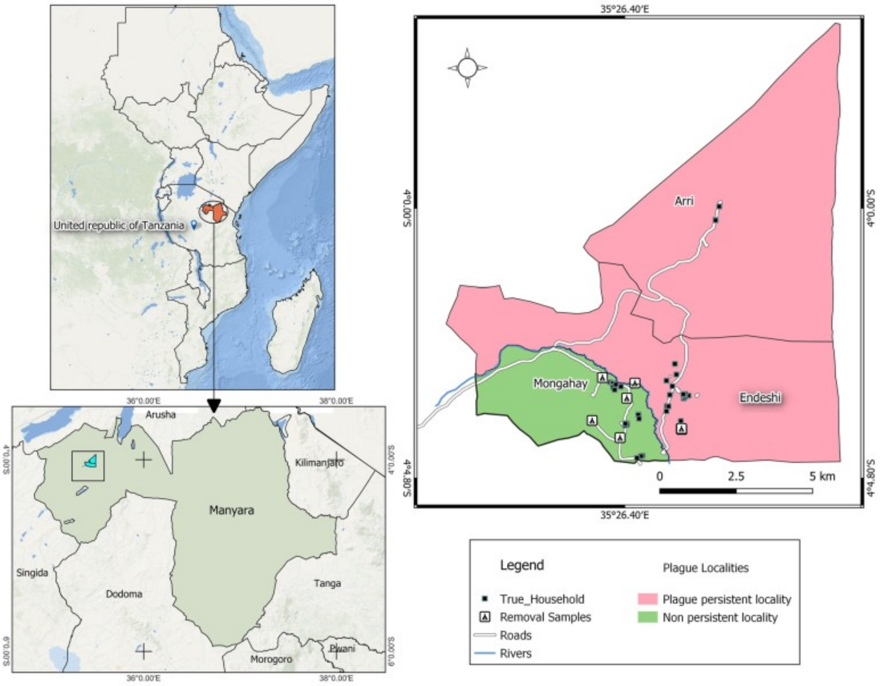

**Figure 1:** Map of Mbulu district in Tanzania showing the locations of the study sites with areas of plague and non-plague history.

farms. Compared to plague persistent locality, non-plague locality had steeper hills and more widely spaced houses. Also, the plague persistence locality had 489 more houses than the non-persistent locality (267). Altogether, there were 726 houses in localities. To collect rodent data from these houses, 44 (from 125 available houses) were selected for rodent trapping using Sherman and box traps (22 houses per locality). Two-three traps were left overnight and inspected each morning for three consecutive days to capture rodents in each selected house. Trapping was conducted every month for 12 months between Jan 2019 and Dec 2019.

Captured animals were anaesthetized with diethyl ether for immobilization following available ethical procedure for handling animals. Animals were removed from the holding bag and carefully brushed in a pan to remove fleas and other ectoparasites (results not reported here). Morphological measurements and other characteristics of each captured animal (weight, head body length, sex and reproductive status) were recorded. Rodents were identified to species level using Happold and Happold (2013) and species names were confirmed by sequencing the mitochondrial cytochrome b gene at the Institute of Vertebrate Biology, Czech Republic. All rodent specimens were labelled and preserved in 10 % formalin for future studies. To understand how local climatic parameters influence rodents in the area, rain data were measured and recorded using ordinary rain gauge with a cylindrical diameter of 12.5 cm and a height of 50 cm (made by Tanzania Meteorological Authority-TMA). The device was installed outside Mongahay village office between Jan 2019 to Dec 2019. Rainfall data were recorded every day, and monthly mean values were calculated for further analysis.

### 2.3 Data analysis

To determine the relative abundance, we used trap success expressed as a percentage using the following formula:

$$\text{Trap sucess} = \frac{N}{N_t \times N_n} \times 100$$

where, $N$ = total number of rodents trapped, $N_t$ = number of traps used per night, $N_n$ = total number of trapping nights. The relative abundance data were further used for multivariate analysis.

Species diversity and species richness were computed in program PRIMER ver.6 using abundance matrix data. We calculated Hill's diversity index which incorporates relative abundance and species richness independently at trap site-level enabling to assess how these indices varied across different environmental and habitat characteristics. Further, we plotted species rarefaction curves using the R package Vegan (Oksanen 2011) to examine the number of individual samples and number of species likely to have been seen at each locality, habitat, and season. To assess how rodent abundance, varied between the study localities, we used the Mann-Whitney-Wilcoxon test and subsequently used the Kruskal-Wallis test on rodent abundance across the seasons and habitat types after confirming non-normal distribution data (Shapiro test $p > 0.05$). Further, we used analysis of variance (ANOVA) to examine difference in the species richness and species diversity across the localities, habitats and seasons.

Furthermore, to evaluate the effect of environmental characteristics on the species richness we built a Poisson generalized linear model (GLM) with a log link function using four independent variables (localities, season, habitat type and rainfall). Since the trap success data (relative abundance) were in proportional, we used GLM with a logit link and the binomial. The GLM model fit was evaluated by deleting non-significant model term in a backward step-wise process, assessing model variance at each step of the modelling using the Wald test (Rija et al. 2020). The best model fitting the data was chosen using the Akaike Information Criterion (AIC) value with 72 % and 82 % cumulative weights for the abundance and species richness models, respectively. Further, we used the predict function implemented in 'Predict package' to assess how each variable in the final best fitting model efficiently predicted rodent abundance and richness. All analyses were performed in R software 4.0.2.

To see how rodent community is structured between localities, habitats and seasons we used cluster analysis based on a Bray-Curtis similarity matrix of grouped variables with the program PRIMER v6. Before formal analysis the abundance data were square root transformed to down-weight high abundance, creating a normally distributed data subsequently creating a resemblance matrix that was used in further analyses. We assessed clustering of the rodent populations using dendrogram plots for the sites, habitats and seasons. We employed the Similarity Profile (SIMPROF) test on a randomly selected samples and 999 permutations to find evidence of genuine clustering in the rodent communities (Clarke et al. 2001; Clarke and Gorley 2006). Further, analysis of similarities (ANOSIM) test was used to find out how each community group was different from the other based on the measured local-scale environmental characteristics (Clarke et al. 2014; Clarke and Gorley 2006). This test provides a global R statistic, which is a measure of distance across rodent assemblages and can range between 0 and 1. When R value is close to 0, it suggests similarity of rodent assemblage between compared sites whereas R value close to 1 indicates a high level of separation between compared sites (Clarke 1993). This test is appropriate for examining likely causes of species interactions at a community-level and has previously been used in rodents (Mwasapi and Rija 2022) and plant community studies elsewhere (Rija et al. 2014).

## 3 Results

### 3.1 Rodent abundance, species richness and diversity

A total of 610 rodents belonging to 12 species were captured in 1800 trap nights with 33.89 % trap success. Total trap nights and trap success in each season for different habitats and localities are summarized in Table 1. Plague persistent locality had the highest abundance ($N = 435$; trap success = 24.17 %) compared to non-persistent locality ($N = 175$; trap success = 9.72 %) (Figure 2a). Also, farm and forest habitats had the highest abundance ($N = 127$; trap success = 12.06 % and $N = 203$; trap success = 11.28 % respectively) compared to the house ($N = 190$; trap success = 10.56 %) (Figure 2b). Short rain season had the highest abundance ($N = 322$; trap success = 53.67 %) compared to dry season ($N = 179$; trap success = 23.87 %) and long rain season ($N = 109$; trap success = 24.22 %) (Figure 2c). Of the captured species, the highest abundant in decreasing order was *Mastomys natalensis* (N = 245; trap success = 13.61 %) followed by *Ratus rattus* (N = 149; trap success = 8.28 %), *Praomys delectorum* (N = 85; trap success = 4.72 %), *Lophuromys makundii* (N = 32; trap success = 1.78 %), *Aethomys kaiseri* (N = 27; trap success = 1.5 %), *Grammomys cf. macmillani* (N = 23;

**Table 1:** Summary of total trap nights, number of rodents captured (*N*) and trap success (%) by habitats and localities for each season.

| | | Non-plague persistent | | | | Plague persistent | | | |
|---|---|---|---|---|---|---|---|---|---|
| Seasons | Trap nights | Farm *N* (%) | Forest *N* (%) | House *N* (%) | Total *N* (%) | Farm *N* (%) | Forest *N* (%) | House *N* (%) | Total *N* (%) |
| Dry | 750 | 18(2.4) | 4(0.53) | 22(2.93) | 44(5.87) | 53(7.07) | 39(5.2) | 43(5.73) | 135(18) |
| Long rain | 450 | 8(1.78) | 6(1.33) | 11(2.44) | 25(5.56) | 31(6.89) | 29(6.44) | 24(5.33) | 84(18.67) |
| Short rain | 600 | 40(6.67) | 34(5.67) | 32(5.33) | 106(17.67) | 67(11.17) | 91(15.17) | 58(9.67) | 216(36) |

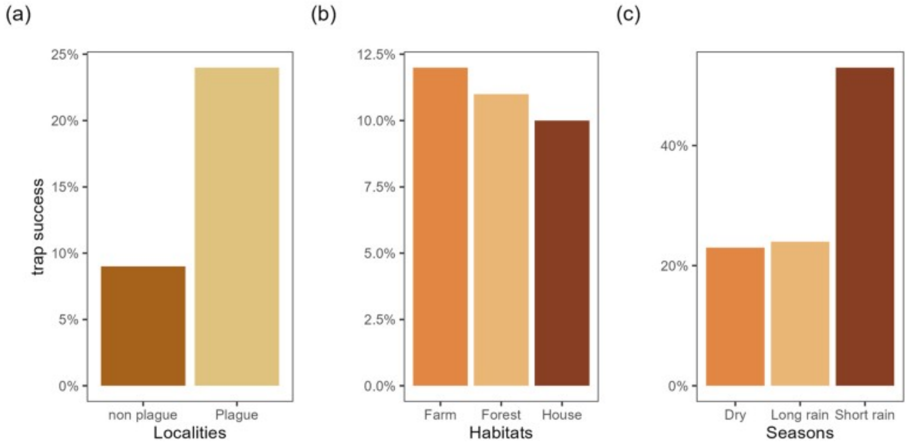

**Figure 2:** Rodent trap success across the (a) localities, (b) habitats, and (c) seasons in the study area.

trap success = 1.28 %), *Lemniscomys striatus* 15 (N = 15; trap success = 0.83 %), *Graphiurus cf. raptor* (N = 9; trap success = 0.5 %), *Mus minutoides* (N = 9; trap success = 0.5 %), *Arvicanthis masai-mara* (N = 8; trap success = 0.44 %), *Lemniscomys zebra* (N = 5; trap success = 0.28 %), and Mus cf.gratus (N = 3; trap success = 0.17 %).

Further, rarefaction curves leveled off for the localities, seasons and habitat types indicating complete sampling in the persistent locality, short rain season and habitat types (Figure 3a–c) but less so in non-persistent locality, long rain and dry seasons. There was a significant difference in rodent abundance between localities (W = 5076, p = 0.03) but not between habitat types ($\chi^2$ = 3.86, df = 2, $p$ = 0.14) or seasons ($\chi^2$ = 5.09, df = 2, $p$ = 0.06).

We found species richness significantly different between localities (df = 1, F value = 40.47, $p < 0.001$; Figure 4a) and habitats (df = 2, F value = 4.24, $p$ = 0.02; Figure 4b) but not between seasons (df = 2, F value = 0.78, $p$ = 0.46; Figure 4c). Across the study sites, plague persistent locality had higher species richness than non-persistent locality and was lower in the house than in the forest and farm fields. Also, species richness was higher during the short rain season than the long rain and dry seasons.

Assessing patterns of rodent diversity in the study area, we found significant variation in Hill's diversity (*N*1) between locality where plague persistent locality had significantly higher diversity than non-persistent locality (df = 1, F value = 7.24, $p$ = 0.02; Figure 5a). However, no significant differences between habitats (df = 2, F value = 3.52, $p$ = 0.06; Figure 5b) and seasons (df = 2, F value = 2.16, $p$ = 0.16; Figure 5c) were observed.

### 3.2 Effect of environmental characteristics on rodent abundance and species richness

The GLM model results indicated persistent locality had significant positive association with the rodent abundance (mean = 2.34, ±1.10SE, $p$ = 0.03). Also, rainfall was negatively

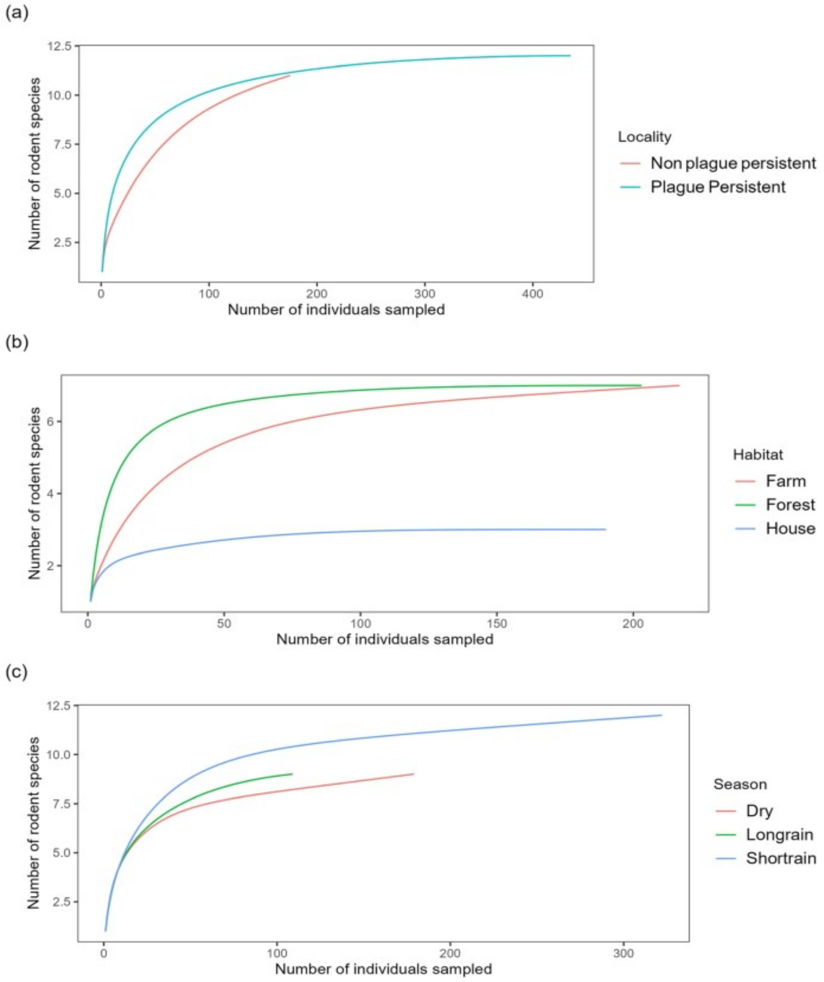

**Figure 3:** Rarefaction plot of sampled population showing the number of species expected to be found for the individuals sampled in the (a) localities, (b) seasons, and (c) habitats. The plot also indicates species richness across these variables.

associated with rodent abundance but not statistically significant (mean −0.002, ±0.001SE, $p = 0.08$; Table 2).

Furthermore, species richness was also significantly positively associated with the plague persistent locality (mean = 0.73, ±0.162SE, $p < 0.001$; Table 3). Across habitats, species richness was negatively associated with domestic premises (houses) but positively associated with forest habitat although not statistically significant.

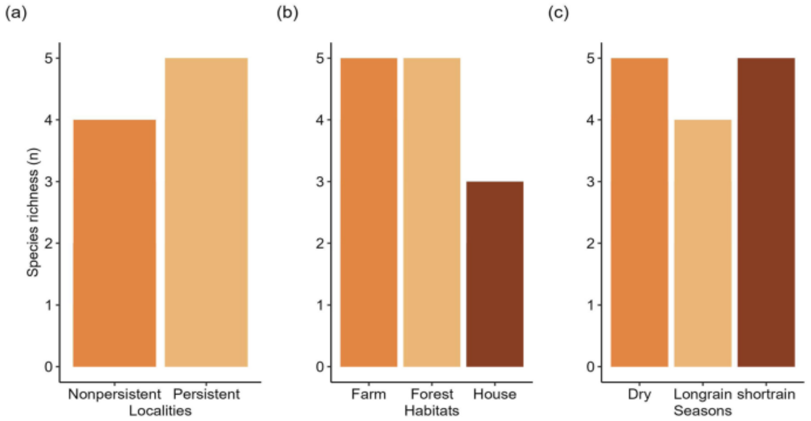

**Figure 4:** Rodent species richness (*n*) across the (a) localities, (b) habitats, and (c) seasons in the study area. The data were analyzed using the R statistical software and visualized using the ggplot package. The x-axis labels indicate the name of each locality, habitat, and season, and the y-axis labels indicate the number of species observed.

### 3.3 Rodent community structure in the localities, habitats and seasons

Analyzing community structure, we found rodents in the study areas were clustered into three broad communities ($R = 0.98$, $p < 0.05$; Figure 6). The assemblages were strongly positively associated with the measured environmental characteristics. Further, a pairwise comparison using ANOSIM test indicated strong significant difference between any two communities. The difference was between forest and house ($R = 1$, $p = 0.01$), forest and farm ($R = 0.98$, $p = 0.001$) and between house and farm habitat ($R = 1$, $p = 0.001$).

## 4 Discussion

The purpose of this study was to understand pattern of rodent population abundance, diversity and rodent community structure across a gradient of environmental characteristics in areas prone to bubonic plague. We found higher rodent abundance, species richness and diversity in plague persistent than non-persistent localities. There was also higher rodent abundance in the short rain season than in other seasons. We also found higher species richness in the forest habitat than in houses with the rodent communities structured in three broader categories based on the measured environmental characteristics.

Species abundance, diversity, and richness appeared to peak in bubonic plague persistence locality, consistent with previous studies conducted in bubonic plague foci elsewhere in South America and China (Bonvicino et al. 2015; Sun et al. 2019). Further, it was expected that the increased diversity would be detected in the non-plague-persistent locality due to a dilution effect, but instead it was recorded in plague persistent locality that has repeated human plague cases. Previous studies elsewhere (Keesing et al. 2006; Ostfeld and Keesing 2012), suggest that, higher diversity of non-susceptible hosts tend to lower abundance of susceptible hosts and reduce the disease risks. Overall, in the current study, *M. natalensis*, *R. rattus* and *P. delectorum* had the highest abundance among all species captured. It is known that these species harbor *Y. pestis* and contribute to the persistence of plague in the foci (Kilonzo et al. 1992; Kilonzo 1999, 2006; Makundi et al. 2008; Njunwa et al. 1989). Furthermore, although, *P. delectorum* is believed to be associated with plague in the foci (Makundi et al. 2008), conclusive empirical studies are still lacking suggesting that the higher diversity contributes to the repeated human plague cases in the plague persistent locality with a high abundance of susceptible rodent species. Other studies however, suggest that when the susceptible host species are exposed to *Y. pestis* bacteria more frequently it increases the likelihood of resistance and survival (Andrianaivoarimanana et al. 2018; Mitchell et al. 2022). This may further suggest the susceptible species in the plague locality were higher in

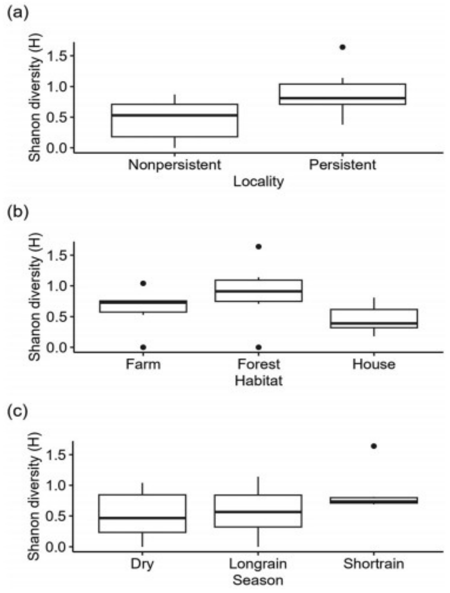

**Figure 5:** Patterns of rodent diversity across the studied sites in northern Tanzania (a–c) indicating significantly different Hill's diversity in the localities, habitats and seasons. Error bars represent a 95 % confidence interval.

**Table 2:** Estimated effect size with standard errors (±SE) of the study site identity and habitats on the rodent abundance as in the final best fitting GLM model.

| Predictors | Mean ± SE | Z-value | P-value |
|---|---|---|---|
| Intercept | 2.05 ± 0.53 | 3.87 | <0.001 |
| Locality: Plague persistent | 2.34 ± 1.10 | 2.12 | **0.03** |
| Rainfall | −0.002 ± 0.001 | −1.71 | 0.08 |

Bold value indicates the statistical significance of the effect, whereby, a P-value less than 0.05 is often used as a threshold for statistical significance.

**Table 3:** Estimated effect size with standard errors (±SE) of the study site identity and habitats on the rodent species richness as in the final best fitting GLM model.

| Predictors | Mean ± SE | Z-value | P-value |
|---|---|---|---|
| Intercept | 0.28 ± 0.17 | 1.68 | 0.09 |
| Locality: Plague persistent | 0.73 ± 0.16 | 4.55 | <0.001 |
| Habitat: Forest | 0.14 ± 0.18 | 0.79 | 0.43 |
| Habitat: House | −0.26 ± 0.19 | −1.34 | 0.18 |

abundance due to higher survival rate that was facilitated by their development of immunity towards bacterial infection. Survival rate saves both rodent host and flea vector; in this case, the repeated human plague cases may be a result of frequent contacts between rodent hosts, flea vector, human and other hosts (e.g. domestic animals) which were mostly observed in the peri domestic settings.

The observed high abundance of rodents during the short rain season could be a result of breeding and reproduction which normally occur during the long rain season (Mlyashimbi et al. 2018; Mulungu et al. 2013) as wet seasons are associated with high food availability, which favors growth and survival of rodents (Clausnitzer et al. 2003; Makundi et al. 2007). Additionally, the rodent population across the plague and non-plague localities show dynamic pattern, where during the dry season (June to September) more active females were captured than during the short rain season (Sept-Dec) whereas during long rain season, more non-actives females became prominent in the captures suggesting that recruitment of youngs into the population occurs during this season. A recent study conducted in Mbulu District showed more rodents tested positive for *Y.pestis* during the short and long rain season (October-April) (Haikukutu et al. 2022), which may suggest potentially higher infection rates in increasing population due to the high recruitment of young. Elsewhere, disease persistence and transmission are well linked with multi-host diversity (Silk and Fefferman 2021; Voinson et al. 2022), which may suggest two possibilities in our study area: That the disease persists with increasing rodent abundance and species richness, and that the disease persists as more rodent hosts become infected with *Y. pestis*. However, we still don't know how the interaction between climatic conditions, human activities and land uses may influence plague epidemiology in the study area.

The structuring of the rodent communities observed in this study may be associated with habitats characteristics. Some recent studies demonstrated how macro- and micro-habitats provide resources to support diverse rodent species in a variety of settings (Byrom et al. 2014; Loggins et al. 2019a,b). For instance, *M. natalensis* and *R. rattus* occurred as a single community, mostly due to the adequate shelter since houses and farms were very close, thus likely supported switching of the habitats due perhaps to resource variability (e.g. food) between the two habitats. This is consistent with a previous study that has demonstrated how these two species coexist with human (Bonwitt et al. 2017). Also, studies conducted in this foci have shown the two rodent species harbour the disease-causing *Y. pestis* (Haikukutu et al. 2022; Ziwa et al. 2013a). Additionally, the two rodent host species were infested with more than two

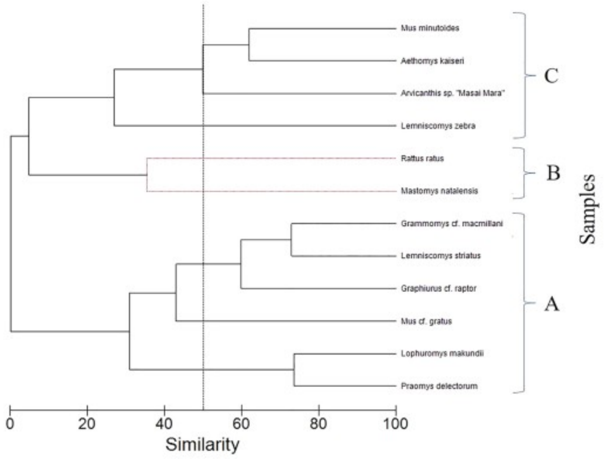

**Figure 6:** Dendrogram of rodent species clustered into three main groups (ABC) based on a Bray-Curtis similarity matrix of grouped variables. The dendrogram was produced using the program PRIMER v6 and the similarity profile (SIMPROF) test was applied to test for significant clustering. The dotted line indicates the similarity level in the study area. There was a significant structuring within individual rodent communities.

flea species, including human flea, *Pullex irritants* (Kessy et al- *in review*). This species has been shown to play role in plague epidemiology in Lushoto (Laudisoit et al. 2007). Other flea vectors such as *Xenopsylla brasiliensis* and *Dinopsyllus sp*, were found infested on these rodent species. These same flea species were confirmed to be potential vector of plague among sylvatic rodents (Ziwa et al. 2013b). The sharing of rodent hosts with multiple flea vectors could indicate an increase in transmission risks and disease persistence, as flea population diversifies within the foci.

Further, although we did not measure critical habitat characteristics such as cover and plant density, however, such characteristics have been reported to influence community structure of rodents in Lulanda Forest reserve in south-eastern Tanzania (Mwasapi and Rija 2022) and other forests elsewhere (DeWalt et al. 2003; Van Breugel et al. 2006). Furthermore, the occurrence of the community comprising species *M. minutoides*, *A. kaiseri*, *Arvicanthis* sp. *"Masai Mara"* and *L. zebra* could have been influenced by grassland characteristics in the forest as these species are associated with grassland habitats (Happold and Happold 2013; Monadjem et al. 2015). Over 80 % of people in the study area worked in agriculture. This allowed for activities such as clearing land near forests and grass areas. Nonetheless, the risk of human bubonic infection increases when human enter a zone containing infected wild rats through activities such as farming and hunting (Zimba et al. 2011). In a recent study in Mbulu for example, *Y.pestis* was found to infect the species *P.delectorum*, *A. kaiseri* and *L.makundii* in the foci (Haikukutu et al. 2022), a situation that may pose risks of the bacteria transmission to humans. This is due to the fact that human and rodents in the study area share habitats due to activities conducted in the wild environment. Certain rodent hosts or other hosts such as livestock, that move between wild environments and peri domestic to domestic settings have the possibility of acting as bridge hosts and increase the likelihood of plague transmissions in the studied landscape.

## 5 Conclusions

The study emphasizes the importance of considering environmental characteristics and species diversity in understanding the persistence and transmission of bubonic plague. The study also suggests the need for further empirical studies to confirm the role of susceptible rodent species in the persistence of bubonic plague and the interaction between climatic conditions, human activities, and land uses in influencing plague epidemiology.

**Research ethics:** Ethical clearance was obtained from Sokoine University of Agriculture Ref. no. DPRTC/R/126/182/38, Manyara region Ref. no. FA.262/347/01/H/247, Mbulu district Ref. no. AB.323/381/01/`B`/9. Animal handling followed the guidelines of the American Society of Mammalogists (ASM) for the use of wild mammals in research and education (Sikes and Animal Care and Use Committee of the American Society of Mammalogists, 2016).

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**Author contributions:** STK designed, conducted field data collection, data analysis and wrote original draft manuscript. AAR analyzed the data and reviewed the original drafts. RHM, AM and CS reviewed the manuscripts. AAR, RHM & AM supervised the research. All authors read and approved the final version of the manuscript for submission.

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**Data availability:** All data used in this analysis can be obtained from the corresponding author upon request.

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# CHAPTER THREE

## PAPER II

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RESEARCH

### Population dynamics of the Multimammate rat (*Mastomys natalensis*) and Makundi's brush fur rat (*Lophuromys makundii*) and their implications in disease persistence in Mbulu District, Tanzania

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**Abstract**
Understanding host population dynamics in connection to disease persistence is important for determining the epizootic risks present in plague foci. We used a capture-mark-recapture method to investigate the population dynamics of *Mastomys natalensis* and *Lophuromys makundii* in an active plague focus, in Mbulu District, Tanzania. We hypothesized higher abundance in plague-persistent locality and between habits and seasons. We found distinct patterns of abundance in *M. natalensis* between farm and forest habitats. The abundance was significantly higher in farms in plague persistent than non-plague persistent areas. The dry season showed a significant increase of abundance compared to the long rain season and the short rain season. A significant increase in breeding females was observed in farms in plague persistent than non-plague persistent localities while farms showed a significant decrease compared to forests. Dry season was associated with an increase in breeding females compared to the long rain season and the short rain season. Furthermore, the abundance of *L. makundii* showed a significant increase in forest in plague persistent than non-plague persistent localities. The abundance increased significantly during the dry season and long rain season compared to the short rain season. The proportion of breeding females was significantly higher in forests in plague persistent than non-plague persistent localities. The breeding females significantly increased during the dry and the short rain season than the long rain season. These findings contribute to our understanding of the ecological factors shaping the population dynamics of these species and their potential roles in plague persistence.

**Keywords** Plague persistent · Rodent host · Population dynamic · Breeding pattern

#### Introduction

Understanding the temporal population dynamics of hosts of *Yersinia pestis*, the causative agent of plague in humans, is of paramount importance in the context of disease persistence in plague outbreak foci. Plague is a severe zoonotic disease that poses significant health risks to humans (Andrianaivoarimanana et al. 2013; Jones et al. 2019; Makundi et al. 2008). Disease hotspots have been identified and linked to factors like mammal biodiversity, seasonal and annual fluctuation of rodent populations and variations in breeding patterns (Biggins and Eads 2019; Davis and Calvet 2005). By investigating the population dynamics of these hosts, we can gain valuable insights into the ecological factors influencing disease persistence and transmission and develop effective strategies to reduce community disease risks, support early detection and response, and inform targeted interventions aimed at reducing the impact of plague outbreaks.

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Disease persistence in active plague foci can be facilitated by presence of multiple hosts, direct transmission between hosts in their burrows, seasonal variation in host breeding patterns, a metapopulation structure of susceptible hosts that slows the recolonization dynamics and spread of the disease, and the potential for *Y. pestis* to persist in soils (Gascuel et al. 2013; Keeling and Gilligan 2000). Several studies have separately explored these factors in connection to their role in plague persistence in the population structure of rodents (Carmichael 2014; Jones et al. 2019; Moore et al. 2015). However, not all rodent species are effective hosts that contribute to plague persistence (Eisen and Gage 2009). In a population, a low proportion of reservoirs can lead to high persistence among animal hosts and lower human infections, while a high proportion infected reservoir leads to a short-lived epizootic and increases in human infections (Ostfeld and Keesing 2012). The presence of susceptible species has important implications for public health in terms of transmission to other rodents and / or animals, as well as the possibility of subsequent plague outbreaks (Collinge et al. 2005; Hagenaars et al. 2004; Stapp et al. 2008).

An understanding of host density and breeding patterns in plague-prone areas can provide valuable information on the nature of the epizootic cycle in different habitats and seasons within the foci. Previous studies have explored rodent population dynamics within the plague foci in Lushoto (Makundi et al. 2007a, b). Although they were not directly linked to disease persistence in the area, still the information is useful for assessing the risks of disease persistence. In Mbulu district, a plague-endemic area in Northern Tanzania, several rodent species, including wild rodents (*Lophuromys makundii*, *Praomys delectorum*, *Graphiurus murinus*, *Lemniscomys striatus*), and domestic and peri-domestic rodents (*Rattus rattus* and *Mastomys natalensis*), were found to have been exposed to *Yersinia pestis*, the causative agent of the plague (Kilonzo et al. 2006; Makundi et al. 2008). Recent studies reported an ongoing circulation of plague bacteria among susceptible rodents in the foci (Haikukutu et al. 2022; Ziwa et al. 2013). Despite the available information, there is still paucity of knowledge regarding rodent population dynamics in the active foci, particularly fluctuations in rodent population density along with breeding patterns of the reservoir hosts of *Y. pestis*.

*Mastomys natalensis* and *L. makundii* are two dominant rodent species which were previously identified as substantial contributors to the transmission and maintenance of *Y. pestis* in the Mbulu plague foci (Makundi et al. 2008). *Mastomys natalensis* is widely distributed in Africa and exhibits its adaptability to various habitats, including agricultural landscapes, human settlements, and sometimes in savanna habitats (Colangelo et al. 2013; Granjon and Duplantier 1993; Mulungu et al. 2013). This adaptability might make it a potential bridge for disease transmission between wildlife and human populations in the foci. It is known to have specific habitat preferences, occupying forested areas and shrubs (Sabuni et al. 2018; Verheyen et al. 2007).

Here, we assessed the pattern of population abundance and the proportion of breeding females for *M. natalensis* and *L. makundii* across seasons and localities (persistent and non-persistent), sampled in different habitats (farm and forest). Additionally, we explored the effects of localities and seasons on the abundance and the proportion of breeding females. We hypothesized that in plague persistence locality, both species would exhibit higher abundance and higher proportion of breeding females compared to non-persistence locality, indicating a potential link between breeding patterns and the persistence of plague. Also, we hypothesized that the abundance of both species would exhibit seasonal variations in both localities, corresponding to the different climatic seasons. Furthermore, we predict that during the short rain season, there would be an increase in the abundance and a higher proportion of breeding females in both species compared to the long rain. We expected these seasonal variations to reflect the influence of environmental factors, such as resource availability (e.g., food) and climatic conditions. These findings will provide valuable insights into population dynamics of these species and its potential implications for disease persistence in the foci.

## Material and methods

### Study area

The study was conducted in Mbulu district, located at 03° 57′ 097″ S, 35° 18′ 39.60″ E in Northern Tanzania (Fig. 1), between November 2018 and January 2020. The area was chosen due to the persistence of plague outbreaks over the last 15 years after a relatively long quiescent period (Kilonzo et al. 2006; Makundi et al. 2015, 2008). Villages were purposefully selected in consultation with village leaders, with some having a history of human plague cases (Endeshi-Arri village, a plague-persistent locality, consisting of plague farm-Pfarm and plague forest-Pforest) and others with no such history (Mongahay village, a non-plague persistent locality, consisting of non-plague farm-NPfarm and non-plague forest-NPforest). The district lies between 1000 and 2400 m above sea level and is characterized by bimodal rainfall pattern. The long rainy season occurs from March to May, while the short rainy season takes place between October and December with a rainy spell in January and February, and a dry season from June to September. The short rainy season is characterized by unpredictable, sporadic, and light rainfall, whereas the long rainy season is accompanied by heavy rainfall. Furthermore, the rainy spell period between January and February was characterized by a

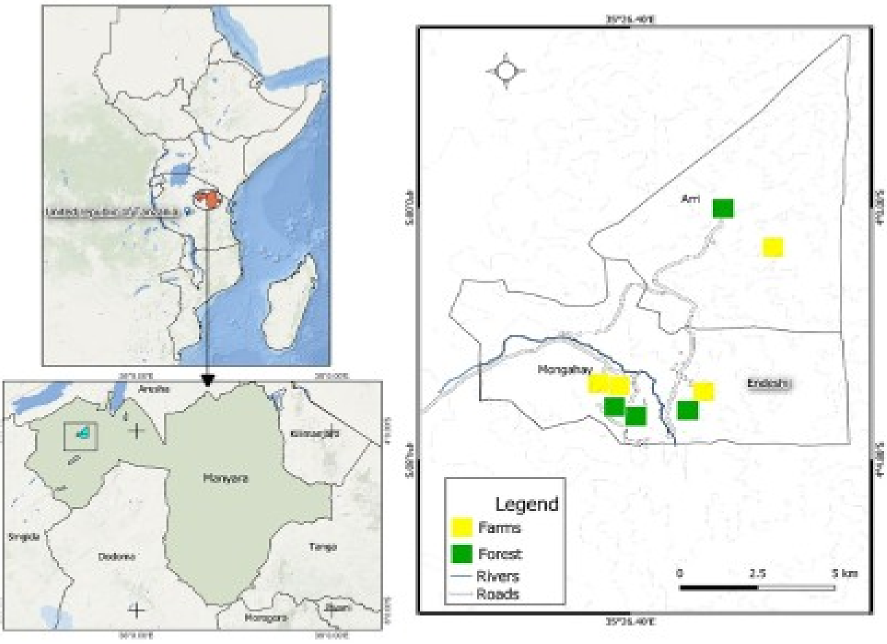


**Fig. 1** A map of Mbulu district in Tanzania showing location of the study site habitats in plague persistent and non-persistent localities

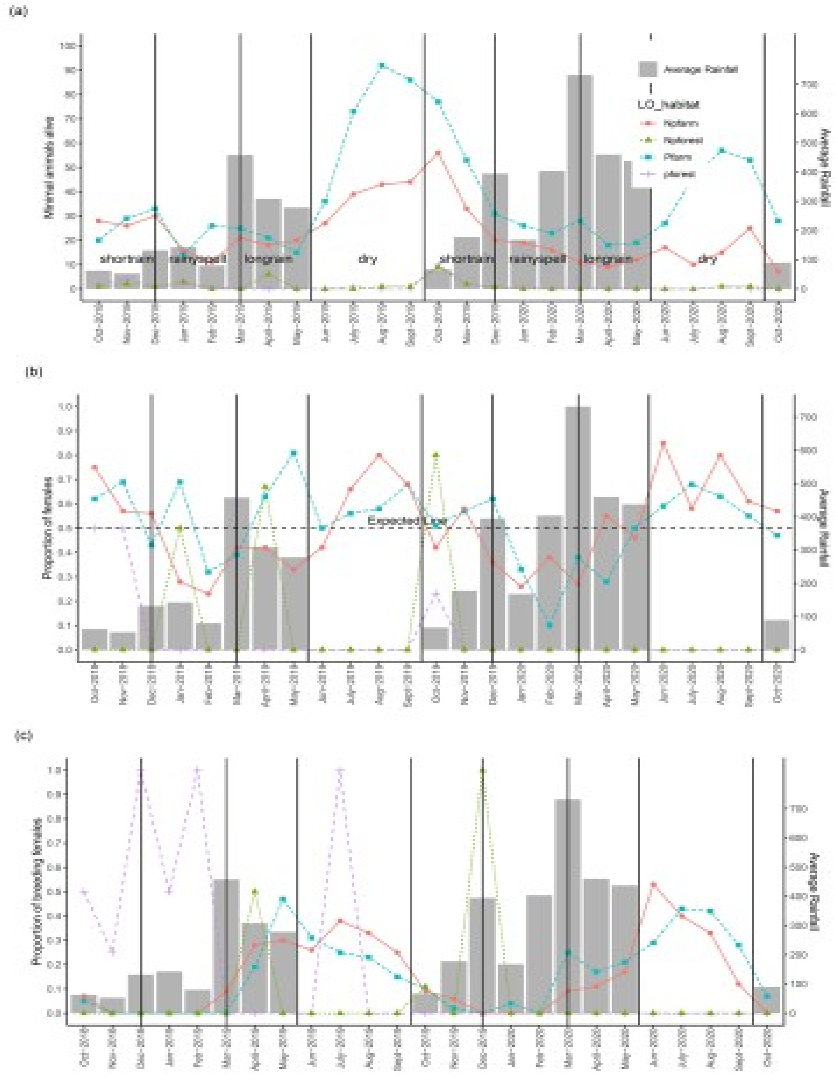
series of mostly dry days with shallow rains. Economically, the area is engaged in livestock keeping, including cattle, goats, and pigs, as well as crop farming, which involves maize, peas, and vegetables.

## Rodent trapping

Rodents were trapped with Sherman live-capture traps and followed the capture–mark–recapture (CMR) technique. Four grids each with 70 × 70 m were established in each locality, with two grids in agricultural land (mixed farms) and two grids in forest (natural forests), for a total of eight grids across all localities. Each grid had seven lines with seven trapping stations separated by ten meters apart, resulting in 49 trapping stations per grid. One Sherman trap (7.6 × 8.9 × 23 cm, H.B. Sherman Traps Inc) baited with peanut butter mixed with maize bran was set at every trapping station. Both the plague and non-plague localities had primary regenerating forests that were assumed to have similar forest structure, with a closed canopy of tall and medium-sized trees and were both managed by local villagers. Rainfall was measured and recorded using ordinary rain gauge. The rainfall data were recorded every day, and monthly mean values were calculated. Trapping was conducted every month for 25 consecutive months between October 2018 and November 2020.

## Data collection

Captured rodents were identified to species level using relevant keys (Happold 2013; Monadjem et al. 2015) followed by determination of sex, body weight, and reproductive status, i.e., the position of the testes, vagina, and nipples were noted as indicators of reproductive status. Females which were active (breeding females) were classified as virginal perforated (PSN), perforated and lactating (PLY), perforated small nipple with young ones (PSY) or perforated lactating not pregnant (PLN). Females


(a)
Minimal animals alive
Average Rainfall
LO_habitat
Nyfarm
Nyforest
Pfarm
pforest
shortrain
rainyspell
longrain
dry
Oct-2018
Nov-2018
Dec-2018
Jan-2019
Feb-2019
Mar-2019
April-2019
May-2019
Jun-2019
July-2019
Aug-2019
Sept-2019
Oct-2019
Nov-2019
Dec-2019
Jan-2020
Feb-2020
Mar-2020
April-2020
May-2020
Jun-2020
July-2020
Aug-2020
Sept-2020
Oct-2020
(b)
Proportion of females
Expected Line
(c)
Proportion of breeding females

◂**Fig. 2** Monthly abundance (MNA) variation for *Mastomys natalensis* (a) and proportion of females (both active and non-active) and breeding females (b and c) across different localities and seasons. The expected line represents the average sex ratio in the *Mastomys natalensis* population, indicating an equal number of males and females in the population

who were non-active had virginal closed (CSN). Active males had scrotal visible (SV) whereas non-active male testes were abdominal (AN) (Makundi et al. 2007a). Each newly captured animal was marked by toe clipping using unique number of codes generated from CMR software and released back at the trapping station previously captured. Toe clipping have shown to have no impact on behavioral and survival of the animal (Borremans et al. 2014). The clipped toes were then preserved in 70% alcohol and sent to Czech Republic's Institute of Vertebrate Biology, for species confirmation using molecular technique (mitochondrial cytochrome b gene).

### Data analysis

Data were analyzed focusing exclusively on *M. natalensis* and *L. makundii*, as they were the most abundant species captured in both localities. To assess the population abundance across different sampling periods and localities (Pfarm, Pforest, NPfarm, and NPforest), we used the minimum number of animals alive (MNA) since animals were not captured for three consecutive nights during the trapping sessions. The MNA was calculated based on the individuals' capture histories.

The sex ratio between localities and season was calculated by dividing the total number of females in the population by the total number of male and females. Sex ratio was calculated for females only, as males can be active throughout the season and encounter more than one female (Mulungu et al. 2013). To determine the proportion of breeding females across localities and sampling periods, we calculated the population abundance of breeding females for each trap session between the first and last capture. Subsequently, we divided the MNA of females by the MNA of both males and females. Furthermore, we used "ggplot" to generate line plots and rainfall bar plots, illustrating the population dynamics of the species over sampling period.

To assess the effect of each variable (locality and season) on population abundance and breeding females, we employed a generalized linear model with negative binomial error distribution for abundance after detecting over dispersion using the DHARMa package and a binomial distribution for the proportion of breeding females. We conducted post hoc tests to examine the relationships between abundance and the explanatory variables, as well as between proportion and the explanatory variables. To estimate the marginal means of abundance and the proportion of breeding females for each level of localities and seasons, and to compute pairwise comparisons between the levels, we used emmeans and the contrast functions.

Furthermore, we used the "ggplot2" package to visualize the relative magnitudes of effects for each predictor variable (seasons and localities) and identify which predictor variables had statistically significant effects on the response variables (abundance and proportion of breeding females). All analyses were performed using R statistical software version 4.0.2.

## Results

### Population dynamics of *M. natalensis*

The population abundance varied between seasons and localities. In the farms, the abundance gradually increased during the long rain season, starting in March. It reached its peak from July to September during the dry season and then gradually decreased from October to December during the short rain season. Similarly, in the forest habitats of both localities, the highest abundance was observed in April, corresponding to the long rain season, and another peak was observed in October, marking the start of the short rain season (Fig. 2a).

Furthermore, higher proportion of females were observed in the farm habitats of both localities with highest peaks during dry seasons in July, August, and September (Fig. 2b). The proportion of breeding females showed gradual increase starting March, peaking in June and July during dry season, and then gradually decreasing during the short rain season from October to December. In addition, the proportion of breeding females in the forest habitats showed variations between localities. In plague persistent forests, higher peaks of breeding females were observed in December, February, and July. Conversely, in non-plague persistent forests, higher proportions of breeding females were observed in December and in April. However, this pattern was observed only in the first year of the sampling period (Fig. 2c).

In the localities, the abundance was found to be significantly higher in the farm habitats than in forest habitats ($p < 0.001$). Furthermore, within the farms, the abundance in plague persistent areas was significantly higher compared to non-plague persistent areas (mean $= 0.49 \pm 0.14$SE, $p < 0.01$). Conversely, the abundance in forests in non-plague persistent locality was slightly higher than in the plague-persistent area, but the difference was not statistically significant (mean $= 0.17 \pm 0.31$SE, $p = 0.57$). Also, our model results showed a significant increase in abundance during the dry season compared to the rainfall spell period (mean $= 0.59 \pm 0.20$SE, $p = 0.003$) and the long rain season (mean = 0.70 ± 0.18SE, $p = 0.0001$). Contrarily, there were significant decreases in abundance during the dry season compared to short rain season (mean = − 0.75 ± 0.20SE, $p < 0.001$) and the long rain season compared to short rain season (mean = −0.86 ± 0.18SE, $p < 0.001$) (Fig. 3a).

The proportion of breeding females differed significantly between plague-persistent farms and plague-persistent forests (mean = − 1.87 ± 0.54 SE, $p = 0.001$) and between non-plague persistent farms and plague-persistent forests (mean = − 1.79 ± 0.54 SE, $p = 0.001$). However, no significant decrease in the proportion of breeding females was observed between non-plague persistent farms and non-persistent forests (mean = −0.59 ± 0.55SE, $p = 0.28$), as well as between non-persistent forests and persistent farms (mean = −0.68 ± 0.54SE, $p = 0.22$). In addition, the proportion of breeding females increased in non-plague persistent compared to plague persistent locality farms (mean = 0.08 ± 0.15SE, $p = 0.57$). The proportion of the breeding females in forests in non-plague persistent locality decreased when compared to that of forests in plague persistent areas (mean = − 1.19 ± 0.74SE, $p = 0.11$). However, these variations were not statistically significant. Furthermore, the proportion of breeding females showed significant seasonal differences. Dry season was associated with an increase in breeding females compared to the rainfall spell (mean = 3.14 ± 0.59SE, $p < 0.001$), the long rain season (mean = 0.48 ± 0.19SE, $p = 0.01$) and the short rain season (mean = 2.19 ± 0.24SE, $p < 0.001$). Moreover, there was a significant increase in the proportion of the breeding females during long rain season compared to the short rain season (mean = 1.71 ± 0.27SE, $p < 0.001$) (Fig. 3b).

### Population dynamics of *Lophuromys makundii*

In forests in plague-persistent and non-plague persistent localities, the population abundance showed a gradual increase in March during the long rain season, reaching its peak in June to July during the dry season and slowly decreased from October to December during the short rain season. Also, throughout the entire sampling period, the abundance in forests in the plague-persistent locality remained consistently higher than in the non-plague persistent area (Fig. 4a). Additionally, in the non-plague persistent locality, higher proportions of females were observed during specific months in the first year, in January, April, and September. However, in the second year, only May showed a higher proportion of females (Fig. 4b). In contrast, the plague-persistent locality showed a different pattern, with higher proportions of females observed in October, December, April, and August during the first year. Similarly, in the second year, December, January, and May (Fig. 4b). Furthermore, proportion of breeding females showed variations between the two forest localities. In the non-plague persistent locality, the proportion of breeding females peaked in May and August. In contrast, the plague-persistent locality showed a gradual increase in breeding females in the forest starting June and reaching a peak in November, and then rapidly declined in December. These trends were only noticed within the first year of the sampling period, no breeding females were observed during the entire second year (Fig. 4c).

Our model results revealed a significant variation in the abundance of *L. makundii* across different localities.

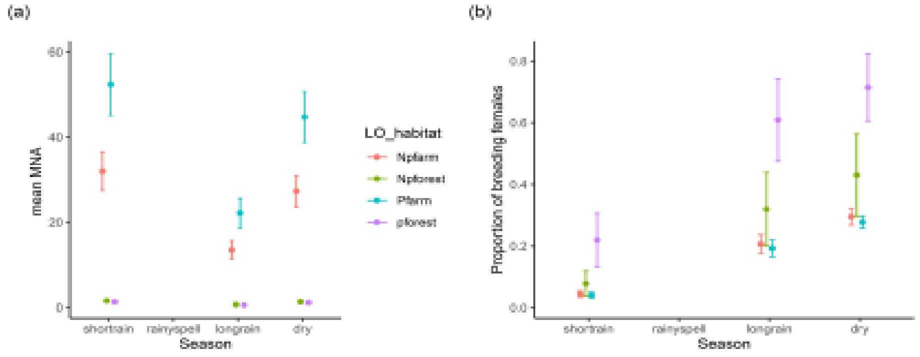


**Fig. 3** Predicted mean of abundance (MNA) (a) and proportion of breeding females (b) for *M. natalensis* by localities and seasons. The error bars represent the 95% confidence interval for the estimated mean effect

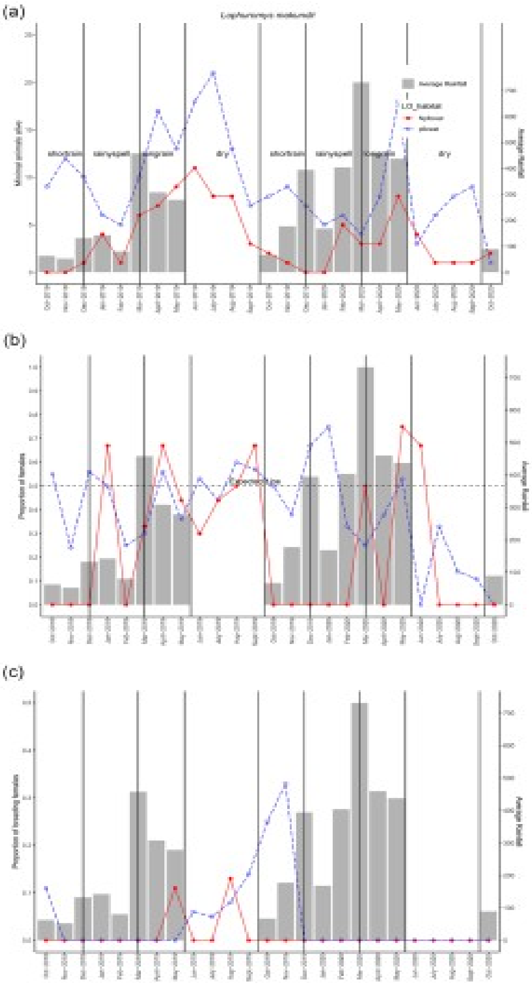


**Fig. 4** Monthly abundance (MNA) variation for *Lophuromys makundii* (a) and proportion of females (both active and non-active) and breeding females (b and c) across different localities and seasons. The expected line represents the average sex ratio in the *L. makundii* population, indicating an equal number of males and females in the population

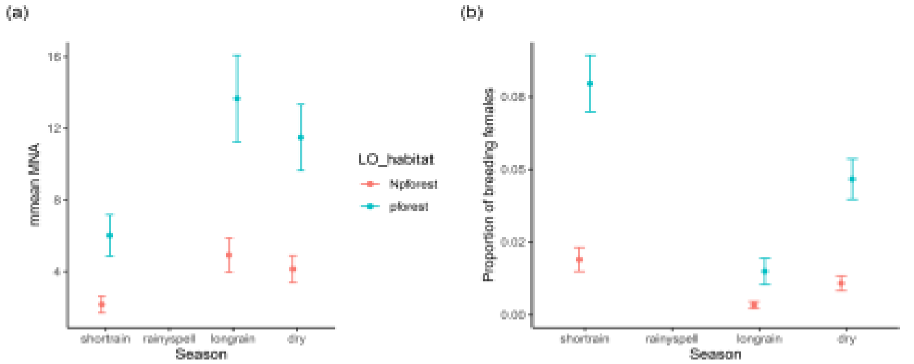


**Fig. 5** Predicted mean of abundance (MNA) (a) and proportion of breeding females (b) for *L. makundii* by localities and seasons. The error bars represent the 95% confidence interval for the estimated mean effect

The abundance was significantly lower in forests in the non-plague persistent locality (mean = − 1.02 ± 0.18SE, $p < 0.001$) compared to the plague-persistent area. Additionally, we observed seasonal variations in the abundance. During the dry season, there was a significant increase in the abundance compared to the rainfall spell period (mean = 0.65 ± 0.28SE, $p = 0.02$) and the short rain season (mean = 0.65 ± 0.23SE, $p = 0.01$). Moreover, the abundance showed a significant increase during the long rain season compared to the short rain season (mean = 0.82 ± 0.24SE, $p = 0.001$). Contrarily, the abundance decreased significantly during the rainfall spell period compared to the long rain season (mean = −0.82 ± 0.29SE, $p = 0.01$) (Fig. 5a).

Furthermore, the proportion of the breeding females in forests in the non-plague persistent locality increased significantly compared to the plague-persistent area (mean = − 1.5 ± 0.23SE, $p < 0.001$). Additionally, the proportion of breeding females increased significantly during short rain season compared to the dry season (mean = 0.57 ± 0.19SE, $p = 0.004$) and the long rain season (mean = 1.74 ± 0.33SE, $p < 0.001$). Moreover, the proportion of breeding females increased significantly during the dry season compared to the long rain season (mean = 1.17 ± 0.34SE, $p = 0.001$) (Fig. 5b).

## Discussion

The findings of this study shed light on how the population abundance and breeding patterns of *M. natalensis* and *L. makundii* respond to variations in seasons and in respect to the two localities within a plague epidemic focus. Our study examined the patterns of fluctuations in the abundance and breeding within the specific preferred habitats (agricultural land and forest) for the two species.

The abundance of *M. natalensis* showed distinct pattern in different habitats and seasons. In the farm localities, the abundance increased during the dry season and declined towards the end of the short rain season. This was a result of recruitment of new individuals into the population due to breeding activity during the long rain season. Similar findings have been reported in previous studies (Leirs et al. 1996; Mulungu et al. 2013). Contrarily, in the forest habitats of both localities, the abundance peaked during the long rain season in April and the short rain season in October. This behavior may have been contributed by the higher proportion of females observed crossing the expected line during these months as well as the food availability. This finding aligns with other studies that have reported similar behavior (Coetzee 1975; Leirs et al. 1990; Madsen and Shine 1999; Stuart et al. 2012). Overall, the abundance of *M.natalensis* was higher in agricultural lands compared to forest habitats for both localities which was consistent to the species preferences for agricultural farms land and fallow lands (Massawe et al. 2005; Mlyashimbi et al. 2019; Rwebuga et al. 2023). Furthermore, farms in plague-persistent areas were associated with increase in the abundance compared to non-plague persistent localities. Despite the similarity in mixed farming practices between the two localities, the higher abundance observed in plague-persistent farms locality could probably be associated with development of immunity toward bacterial infection in the persistent locality. Previous studies have reported that, when the susceptible host species are exposed to *Y. pestis* bacteria more frequently, this increases the likelihood of resistance and survival (Andrianaivoarimanana et al. 2018; Mitchell et al. 2022).

In terms of breeding females, higher proportions were observed in farms in both localities during dry season, gradually decreasing towards the short rain season. This could be influenced by breeding activity during the long rain season and food availability, supporting the specie's reproduction and survival. This is consistence to other previous studies by Massawe et al. (2012) and Mulungu et al. (2013). Furthermore, there was a variation in the proportion of breeding females between localities and seasons in the forests. In forests in the plague persistent locality, peaks in breeding females were observed during the short rain season, rainfall spell period, and dry season while in the non-persistent forests, higher breeding females were observed during short rain season and the long rain season. These variations may be influenced by the natural resources surrounding the forests.

The abundance of *L. makundii* in forests varied between localities and seasons with a significant increase during the long rain season and a decrease towards the end of the dry season. This increase could be linked to the duration of rainfall, allowing food availability and improved survival, consistent with previous study conducted in western Usambara Mountains (Makundi et al. 2007a). Also, the majority of these species prefer to feed on soil invertebrates, (Clausnitzer et al. 2003; Makundi et al. 2007a) which are particularly abundant during long rain season. Moreover, a higher proportion of breeding females was observed in the persistent forest compared to the non-persistent forest, possibly due to the high abundance and development of immunity. However, previous studies have shown that the time period during which young rodent enters the population, results in a decrease of human infections (Keeling and Gilligan 2000). The observed trends in rodent population dynamics have implications for disease persistence and transmission risks. Further study is necessary to understand the relationship between the state of seroprevalence and the population dynamics of the rodent species across localities. In addition, variations in the proportion of breeding females for both species were observed between the forest localities and seasons, but these differences were only noticed in the first year of the sampling period. No such variations were observed in the second year, possibly due to higher rainfall during that period, which affected the decline in breeding activity in the populations. However, Mwalimu et al. (2022) reported two cases of human plague in 2019 from a ward close to Nou forest reserve, which extends from persistent localities of Arri and Endeshi. Considering the sharp decline in breeding activity in 2019 and the absence of breeding in 2020, it may highlight the possibility that the foci still harbor the epizootic cycle.

## Conclusion

Our study provides information on the annual trends of rodent species in the foci, showing the high population density of both species in plague persistent localities with variations in their density and breeding pattern between habitats and seasons. This knowledge can be used to guide surveillance efforts and disease management strategies.

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**Author contribution** STK designed and conducted field data collection and data analysis, and wrote original draft manuscript. AAR reviewed the original drafts and supervised the research. RHM, AM, and CS reviewed the manuscripts. RHM and AM supervised the research. All authors read and approved the final version of the manuscript for submission.

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**Data availability** All data used in this analysis can be obtained from the corresponding author upon request.

## Declarations

**Ethical approval** Ethical clearance was obtained from Sokoine University of Agriculture Ref. no DPRTC/R/126/182/38, Manyara region Ref. no FA.262/347/01/H/247, Mbulu district Ref. no AB.323/381/01/‘B’/9. Animal handling followed the guidelines of the American Society of Mammalogists (ASM) for the use of wild mammals in research and education (Sikes & Animal Care and Use Committee of the American Society of Mammalogists, 2016).

**Conflict of interest** The authors declare no competing interests.

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# CHAPER FOUR

# PAPER III

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## Flea infestation of rodent and their community structure in frequent and non-frequent plague outbreak areas in Mbulu district, northern Tanzania

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ABSTRACT

Understanding rodent-ectoparasite interactions and the factors driving them is important in understanding the epidemiology of diseases involving an arthropod vector. Fleas are the primary vector for *Yersinia pestis*, the bacteria that causes plague and monitoring of flea population is essential for planning the potential mitigation measures to prevent the disease outbreak. In this study, we investigated flea abundance, community structure and the potential factors driving flea infestation in areas with frequent (persistent) and non-frequent plague (non-persistent) outbreaks. We collected fleas from captured rodents in two villages with both forest and farm habitats. We found 352 fleas belonging to 5 species with *Dinopsyllus lypusus* the most abundant overall (57.10%) and *Ctenophthalmus* spp. the lowest (1.70%). There were no significant differences of flea abundance between study localities, habitats and seasons ($p > 0.05$) but, flea infestation was significantly positively associated with the persistent locality and with the short rain season ($p < 0.05$). Further, flea abundance increased significantly with rodent body weight ($p < 0.05$). Furthermore, we found fleas broadly structured into two communities varying between the dry, long rain and short rain seasons. These findings have important implications for public health, as they may be used to assess and control the risks of plague transmission and other flea borne diseases in the foci.

### 1. Introduction

Fleas are bloodsucking insects with significant implications for human and animal health worldwide (Bitam et al., 2010). Fleas infest a wide range of hosts including wild and domestic animals, birds and human (Durden and Hinkle, 2019; Zając et al., 2020; Zurita et al., 2019). Infestation is influenced by environmental and human behavior modifications. For instance, when farmers share their dwellings with livestock or have corrals located in close proximity to their homes, it exposes domestic animals and humans to infestation, leading to the transmission of flea-borne diseases. Further, activities such as urbanization, deforestation, and encroachments into natural habitats, increase interactions between human and flea-infested environments that may also increase the risk of exposure to flea-borne pathogens (Gage et al., 2008; Bitam et al., 2010). Fleas are well known vectors of several illness including murine typhus caused by Rickettsia typhi, flea-borne spotted fever caused by *Rickettsia felis*, cat scratch disease caused by *Bartonella henselae*, and bubonic plague caused by *Yersinia pestis* (Krasnov, 2008; Durden and Hinkle 2019; Sherman 2007). Furthermore, some fleas such as the human fleas, act as vector for tape worms (Kandi et al., 2019; Ramana et al., 2011) and pose significant public health concerns. In regions with sporadic flea-transmitted disease outbreaks, such as plague, the absence of up-to-date information on flea dynamics and host infestation intensifies these concerns. Access to ssuch data could inform the development of strategies to counter potential outbreaks through, for example, targeting on reducing the population of fleas and rodents.

Several factors are known to influence flea richness and abundance including; host diversity (Krasnov et al., 2002; Young et al., 2015), host body condition (Bitam et al., 2010; Krasnov, 2008), host density (Krasnov et al., 2002; Stanko et al., 2002) and climatic conditions (Krasnov et al., 2004, 2005). However, it is not clear how such factors are directly linked to plague persistence especially in regions with

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history of disease outbreaks. Thus, understanding flea density, infestation and community structure in the plague foci may allow us to easily predict transmission risks of flea borne diseases among co-existing sympatric hosts. Plague is a zoonotic disease that is largely spread by fleas from rodents to humans (Gage and Kosoy, 2005). The disease continues to be a public health concern, with over 90% of all reported human cases worldwide originating from Sub-Saharan Africa and the Madagascar region (Bertherat and Bertherat, 2019; Vallès et al., 2020). In Tanzania, plague has been reported in several districts including Lushoto, Karatu and Mbulu and remains a significant potential health risk in case of outbreak. Studying host-parasite interactions therefore may help us to understand the risk of both persistence and outbreak of plague. The transmission of the bacteria causing plague (*Yersinia pestis*), is influenced by various factors, including flea density in the environment (Krasnov et al., 2006a; Pham et al., 2009; Tripp et al., 2009). Plague tends to persist in a particular locale or region when multiple fleas capable of transmitting *Y. pestis* infest hosts susceptible to plague infection (Eisen and Gage, 2009), thus making the disease more or less predictable based on known pre-disposing causes. Additionally, researchers have developed statistical models and used ecological data to predict the occurrence and distribution of plague in various regions. For instance, Eisen et al., (2007) used a GIS-based model to predict the habitat suitability for *Yersinia pestis*, in New Mexico, finding that 30.8% of the state as suitable plague habitat, Similarly, Neerinckx et al., 2008 used ecological niche modelling (ENM) to predict the potential distribution of plague occurrences across sub-Saharan Africa based on environmental variables and occurrence data. They identified elevation, potential evapotranspiration, mean diurnal temperature range, annual rainfall, and Normalized Difference Vegetation Index contributing to the plague occurrences in Sub-Saharan Africa. Furthermore, Poje et al., 2020 studying flea populations in black-tailed dog burrow in North America, found that the likelihood of prairie dog burrow being infested with fleas increased with high temperatures, while the prevalence of infested burrow declined with increased winter precipitation. This, in turn, impacted the dynamics of plague in prairie dog colonies.

Several studies have reported disease persistence and transmission conditions in Mbulu districts, Tanzania (Makundi et al., 2008; Ziwa et al., 2013). High flea diversity and rodent hosts richness, with a multiple host-flea interaction in different habitats are variables that contribute to plague persistence in this focus (Makundi et al., 2015). A more recent study has shown plague bacteria continues to circulate among susceptible rodents in Mbulu district (Haikukutu et al., 2022), suggesting potential risks of plague outbreak. These studies suggest that regular monitoring and updating data on the flea-rodent interactions and the likely factors driving potential outbreaks and disease persistence are important to control the disease in these rural communities. This can be achieved through public awareness campaigns and educational projects that inform and educate residents about lifestyle practices that encourage flea-rodent-human interaction. Additionally, community engagement is crucial, with health officers visiting local communities to identify possible risks and provide valuable guidance as well as developing strategies that target both flea vector and rodent hosts.

In this study, we aimed to provide current information on the flea infestation of rodents, their community structure and how infestation varied between plague persistent and non-persistent foci in Mbulu district, northern Tanzania. Specifically, we (i) assessed rodent flea abundance in different habitats, seasonality, and localities contrasting in plague outbreaks, (ii) examined which factors influence prevalence of flea infestation, (iii) assessed the effect of habitats, seasons, temperature, humidity and rodent species traits (sex, sex condition, species ID, weight) on overall flea abundance and, (iv) assessed how flea community structures between localities in different habitats and its potential hosts. We predicted that flea load would be greater in plague persistent than non-persistent localities and we predicted that flea abundance and infestation would be positively associated with seasonality and plague persistent locality due to available hosts and suitable habitats and environmental conditions that would provide flea population growth. Finally, we predicted that flea species would be structured according to similar resources such as blood meals from host animals, microclimate conditions, and habitats use, and that some flea species should show host preferences while others exhibit host sharing pattern between multiple hosts, providing conducive environment for the disease enzootic circulation.

## 2. Materials and methods

### 2.1. Study area

This study was conducted in two villages, Mongahay (04° 03′ S, 35° 26′E) and Endesh-Arri (04° 03′ S, 35° 27′E) located in Mbulu District, Manyara Region in Northern Tanzania from Jan 2019 to Dec 2019 (Fig. 1). The villages were chosen based on the plague outbreak history and presence of plague pathogen in the rodent population (Makundi et al., 2008; Ziwa et al., 2013; Mwalimu et al., 2022). Villages with and without human plague cases were purposefully selected in consultation with village leaders. Villages with a history of bubonic plague cases were identified as 'plague persistent' (Endeshi village), while those without a history of bubonic plague were identified as 'non-persistent' (Mongahay village). Both villages engaged in crop farming and livestock keeping as their primary economic activities.

The district where the study villages are lies between 1000 and 2400m above sea level and is characterized by bimodal rainfall pattern, with a long rainy season between March and May, and a short rainy season between November and January (Nyembo et al., 2021). The short rain season is characterized by sporadic and light rainfall, which is less predictable. During the short rain season the mean temperature was on average 16.84 °C ($SE = 0.13$). On the other hand, the long rain season is characterized by cloudy skies and heavy rainfall, with mean temperature of 14.79 °C ($SE = 0.12$).

### 2.2. Rodent trapping

Rodents were live trapped using Sherman traps (LFA 7.6 x 8.9 × 23 cm, H.B. Sherman Trap, Inc., Tallahassee, USA) baited with peanut butter mixed with maize flour. Five transect lines with 10 trapping stations set 10 m apart were established in the natural forest (natural forest) and farmland (mixed farming) habitats in each village (Kessy et al., 2023). Traps were left overnight and inspected each morning for three days. Trapping was conducted every month for 15 months between Jan 2019 to Dec 2019.

Captured animals were anaesthetized with diethyl ether for immobilization (Palomino et al., 2020). Morphological measurements (weight, head body length, tail length and ear length) and other characteristics of each captured animal (sex and reproductive status) were recorded. Sex condition were noted as indicators of reproductive status of the host species i.e. the position of the testes, vagina and nipples. Females were classified as virginal perforated (PSN), perforated and lactating (PLY), virginal closed (CSN), perforated small nipple with young ones (PSY) and perforated lactating not pregnant (PLN). Males were classified as scrotal visible (SV) as active males and testes were abdominal (AN) as non-active male (Makundi et al., 2007). Rodents were identified to species level using Happold (2013) and confirmed by sequencing the mitochondrial cytochrome *b* gene at the Institute of Vertebrate Biology, Czech Republic.

### 2.3. Flea collection

Rodents were removed from the holding bag and carefully brushed in a pan to remove fleas. Each bag was thoroughly checked to remove dislodged fleas and the tray was examined carefully with a hand lens to remove all ectoparasites using a moistened paint brush.

Fleas were grouped based on locality, habitat, month, and host

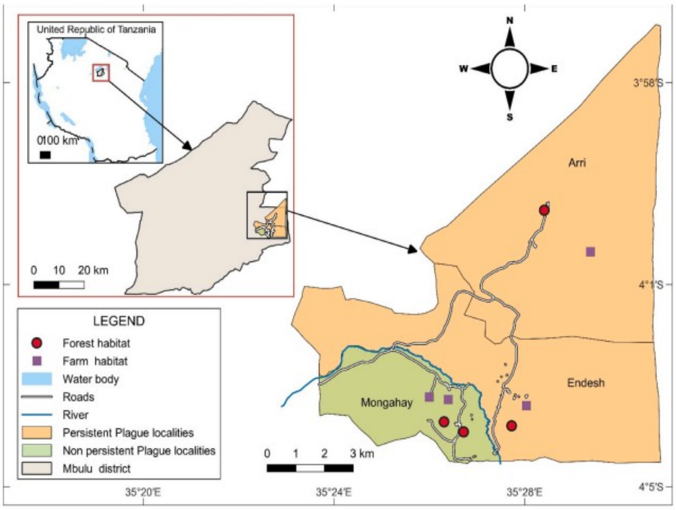

**Fig. 1.** A map of Mbulu district indicating the two study localities, Endeshi-Arri (Persistent locality and Mongahay (non-persistent locality), along with the two study habitats (Farmland and forests) in each locality.

species and were counted and preserved in 70% ethanol for future identification. The fleas were then processed by adopting a modified version of the method described in (Philip Samuel et al., 2021). Briefly, each group was exposed to NAOH 10%, dehydrated in various concentrations of ethanol (50%, 70%, 95%, absolute), cleared in clove oil, temporarily mounted using glycerin on a microscopic slide, and examined under a light microscope using a 10x objective.

To understand how local climatic parameters influence fleas in the area, rain data were measured and recorded using an ordinary rain gauge installed outside Mongahay village office between Jan 2019 to Dec 2019. Data were recorded every day, and monthly mean values were calculated. We also collected atmospheric temperature, and relative humidity data using data loggers (Thermochron iButtons ®), with two data loggers placed under trees in each locality. We considered trees that had dense canopy so that they could give enough shades throughout the day. The iButton data were downloaded once a month. The monthly mean values of temperature (∘C), and humidity (%) were calculated.

### 2.4. Data analysis

To establish flea abundance, we grouped flea data collected from each rodent host species and across the sampled sites and tested for normality using Shapiro test ($P < 0.05$). Flea abundance is used here to refer to the total number of fleas collected for each rodent host and sampled sites during the sampling period regardless of the species identity. To assess how flea abundance varied between localities, habitat types and season, we used the Mann-Whitney-Wilcoxon test to explored significant differences of flea load between habitats and localities. Similarly, the Kruskal-Wallis test was used to assess differences in flea abundance across flea species and seasons as well as differences of each flea species across rodent species and habitats in each locality (Npfarm = farm in non-plague persistent locality, Npforest = forest in non-persistent locality, Pfarm = farm in persistent locality and Pforest = forest in persistent locality).

Further, to assess how temperature, humidity, rainfall, and rodent species traits (sex, sex condition, rodent species, weight, head body length) influenced flea abundance, we built a negative binomial generalized linear mixed model (GLMM) implemented in the 'lme4' package. Prior to modelling, we examined the data variables for potential multicollinearity among temperature, rainfall, the weight, head and body length variables. We subsequently dropped rainfall from the model and retained temperature as these were highly correlated ($r = 0.51$) and because temperature is known to influence flea growth and development (Cavanaugh and Marshall 1972; Kreppel et al., 2016; Ming ming et al., 2013). The first model included sex, sex condition, head and body length, temperature, weight and humidity as fixed factor and rodent species as random factor. The relative influence of each variable in the model was evaluated by deleting non-significant model term in a backward step-wise process, assessing model variance at each step of the modelling. The drop1 function was used to delete non-significant term along each modelling steps and model significance assessed using the Wald test (Bolker et al., 2009). The best model fitting the data was chosen using the Akaike Information Criterion (AIC).

Furthermore, the binomial generalized linear model (GLM) implemented in the MASS package was used to examine the probability of flea infestation as a function of localities, habitats, seasons and rodent species. Flea infestation-referred as presence or absence was treated as a dependent variable in the model. To understand the relative influence of each variable in the model similar procedure as performed above was followed. Further, the relative risk ratio (RR) of each independent variable was computed from exponentials of coefficients generated from the best models. To understand how these factors from the best model were able to predict the flea load and prevalence of flea infestation, we built prediction models using the 'predict' function with the "ggplot2″ package. All modelling analyses were performed in R program, version 4.3.1.

Finally, to assesses species interaction and how flea community structures between localities, habitats and seasons we used cluster analysis based on a Bray-Curtis similarity matrix of grouped variables with the program PRIMER v6. To obtain this, abundance matrix data were first square root transformed to down weight high abundance data, normalizing them and creating a resemblance matrix. Further, we visualized whether flea species clustered based on locality, habitat, and season using a dendrogram plot.

## 3. Results

### 3.1. Abundance of fleas in the study area

A total of 352 fleas belonging to 5 species were collected, with *Dinopsyllus lypusus* being the most abundant species, comprising 57.10% of the total (n = 201), followed by *Xenopsylla brasiliensis* at 29.26% (n = 103), *Nosopsyllus* spp. at 8.52% (n = 30), *Xenopsylla cheopis* at 3.41% (n = 12) and *Ctenophthalmus* spp. at 1.70% (n = 6).

A total of 420 individuals belonging to 12 species within family Muridae were captured. Among all species, *Mastomys natalensis* had the highest number of captures compared to other species in different habitats and localities. Additionally, the short rainy season had higher number of rodent hosts compared to other seasons. The total number of rodent hosts for each species across habitats, localities, and seasons is presented in Table 1.

Flea abundance by flea species across rodent hosts and habitats revealed that, cultivated land, flea abundance was dominated by *D. lypusus*, accounting for 48.26% (n = 83) of the total flea population, followed by *X. brasiliensis* at 36.63% (n = 63), *Nosopsyllus* spp. at 9.30% (n = 16), and *X. cheopis* at 5.82% (n = 10). Among the rodent species, *Mastomys natalensis* had the highest flea abundance at 66.28% (n = 114), followed by *Aethomys kaiseri* at 24.42% (n = 42). In the forest habitat, *D. lypusus* was also the most abundant flea species, accounting for 65.56% (n = 118) of the total flea population, followed by *X. brasiliensis* at 33.89% (n = 40), *Nosopsyllus* spp. at 11.86% (n = 14), *Ctenophthalmus* spp. at 3.33% (n = 6), and *X. cheopis* at 1.69% (n = 2).

The plague persistent locality had the highest flea abundance 71.88% (n = 253) compared to non-persistent locality 28.13% (n = 99). On the habitat types, the forest had the highest flea abundance 51.14% (n = 180) compared to cultivated areas 48.86% (n = 172). Also, flea abundance was highest in the short rain season 59.94% (n = 211) than the long rain season and dry season (22.73%, n = 80 and 17.33%, n = 61; respectively). Furthermore, there were significant differences in flea abundance between flea species ($\chi^2 = 11.69$, df = 4, p = 0.02). There were no significant differences in flea abundance between localities (W = 1744, p = 0.68), habitats (W = 2157, p = 0.83) and seasons ($\chi^2 = 5.04$, df = 2, p = 0.08) (Fig. 2a–c).

The rodent species with the highest flea abundance were *Mastomys natalensis* at 32.22% (n = 58) and *Praomys delectorum* at 30.56% (n = 55) (Fig. 3). When assessing how each flea species varied between rodent species and habitats in each locality; there was a significant difference in *X. Brasiliensis* abundance between rodent species ($\chi^2 = 25.55$, df = 11, p = 0.01). A Significant higher abundance of *X. Brasiliensis* was observed on *M. natalensis* compared to *Mus* cf. *gratus* (p = 0.03), *Grammomys* cf. *macmillan* (p = 0.01) and *Lophuromys makundii* (p = 0.02). However, there were no significant difference in *X. brasilliensis* abundance between habitats of each locality ($\chi^2 = 1.03$, df = 3, p = 0.79). Similarly, the abundance of *D. lypusus* species varied significantly between rodent species ($\chi^2 = 26.16$, df = 11, p = 0.01). *Mastomys natalensis* had significantly higher abundance of *D. lypusus* compared to *Mus minutoides* (p = 0.03), *Mus gratus* (p = 0.02), *Lophuromys makundii* (p = 0.01), *Graphiurus* cf. *raptor* (p = 0.03), and *Lemniscomys striatus* (p = 0.01). No significant differences were found in *D. lypusus* abundance between habitats of each locality ($\chi^2 = 3.19$, df = 3, p = 0.36). Furthermore, the abundance of *X. cheopis* varies significantly between rodent species ($\chi^2 = 20.26$, df = 11, p = 0.04). *Mastomys natalensis* had significantly higher abundance of *X. cheopis* compared to *Mus minutoides* (p = 0.04), *Lophuromys makundii* (p = 0.02), *Graphiurus* cf. *raptor* (p = 0.02), *Lemniscomys striatus* (p = 0.02), *Grammomys* cf. *macmillan* (p = 0.01) and *Arvicanthis* sp. *"Masai Mara"*. No significant differences in *X. cheopis* were observed between habitats in the locality ($\chi^2 = 0.87$, df = 3, p = 0.83). Moreover, there was a significant difference in *Nosopsyllus* spp abundance between rodent species ($\chi^2 = 32.31$, df = 11, $p < 0.05$), with *M. natalensis* having higher abundance compared to all other rodent species ($p < 0.05$). However, there were no significant difference in *Nosopsyllus* spp abundance between habitats in the locality. Additionally, the abundance of *Ctenophthalmus* spp did not vary significantly between rodent species ($\chi^2 = 17.75$, df = 11, p = 0.08) and between habitats in the localities ($\chi^2 = 3.83$, df = 3, p = 0.28).

### 3.2. Factors influencing flea abundance

The model results indicated rodent weight was significantly and positively correlated with flea abundance (mean = 0.02 ± 0.004SE, $p < 0.05$) Fig. 4a). Furthermore, male rodents had higher flea abundance than females (mean = 0.27 ± 0.158SE, p = 0.09; Fig. 4b).

### 3.3. Effect of locality, season, habitat and rodent species on probability of flea infestation

The highest probability of flea infestation was mostly associated with the plague persistent locality. Similarly, there was a significant effect of the short rain season on the probability of higher flea infestation. (Table 2, Fig. 5).

### 3.4. Flea community structure in the plague foci

Cluster analysis based on the flea abundance data revealed two distinct flea community structures based on the habitats. The dendrogram plot (Fig. 6) showed that flea species were clustered into two main groups, group A comprising of four species (*Nosopsylla* spp., *Xenopsylla*

**Table 1**
Number of rodent species captured across localities, habitats (Pfarm = farm in plague persistent locality, Pforest = forest in plague persistent locality, NPfarm = farm in non-plague persistent locality and NPforest = forest in non-plague forest) and seasons.

| Rodent species | Localities | | | | | | Seasons | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pfarm (n) | Pforest (n) | Plague locality (n) | NPFarm (n) | NPForest (n) | Non-plague locality (n) | Dry | Long rain | Short rain |
| *Aethomys kaiseri* | 17 | 0 | 17 | 6 | 0 | 6 | 12 | 4 | 7 |
| *Arvicanthis* sp. *"Masai Mara"* | 6 | 0 | 6 | 2 | 0 | 2 | 0 | 0 | 8 |
| *Grammomys* cf. *macmillani* | 4 | 16 | 20 | 0 | 3 | 3 | 7 | 4 | 12 |
| *Graphiurus* cf. *raptor* | 0 | 5 | 5 | 0 | 4 | 4 | 0 | 0 | 9 |
| *Lemniscomys striatus* | 0 | 14 | 14 | 0 | 1 | 1 | 1 | 1 | 13 |
| *Lemniscomys zebra* | 2 | 0 | 2 | 3 | 0 | 3 | 1 | 0 | 4 |
| *Lophuromys makundii* | 0 | 32 | 32 | 0 | 0 | 0 | 9 | 12 | 11 |
| *Mastomys natalensis* | 113 | 9 | 122 | 54 | 31 | 85 | 49 | 36 | 122 |
| *Mus* cf. *gratus* | 0 | 2 | 2 | 0 | 1 | 1 | 0 | 2 | 1 |
| *Mus minutoides* | 8 | 0 | 8 | 1 | 0 | 1 | 6 | 2 | 1 |
| *Praomys delectorum* | 0 | 81 | 81 | 0 | 4 | 4 | 29 | 13 | 43 |
| *Rattus ratus* | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |

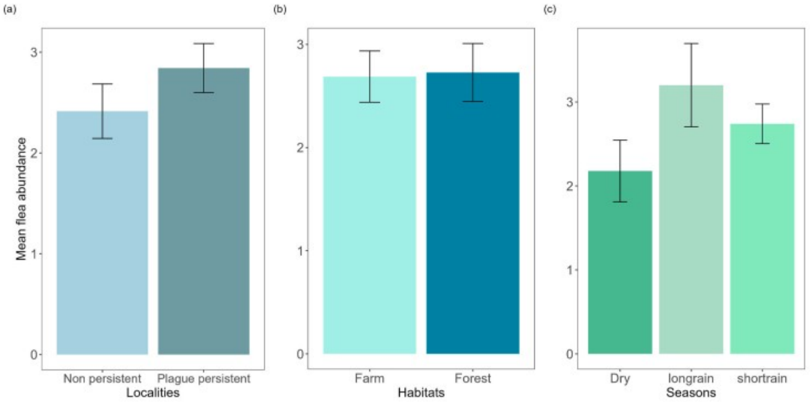

**Fig. 2.** Flea abundance in the (a) localities, (b) habitats and (c) seasons. Error bars represent the standard error. There were no statistically significant differences that were observed.

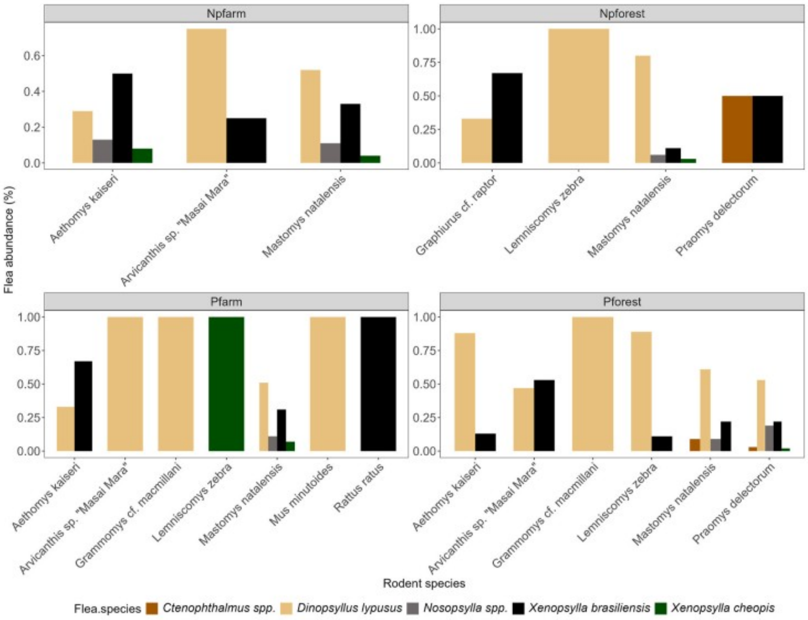

**Fig. 3.** Flea abundance for different flea species across habitat types in each locality and rodent species.

*cheopis*, *Dinopsyllus lypusus*, and *Xenopsylla brasiliensis*) and group B consisting of only one species (*Ctenophathalmus* spp). Group A had a finer-scale separation of the two subgroups, with *Nosopsylla* spp. and *Xenopsylla cheopis* clustering together and *Dinopsyllus lypusus* and *Xenopsylla brasiliensis* forming a separate cluster. Furthermore, flea communities were structured based on habitat, with some flea species

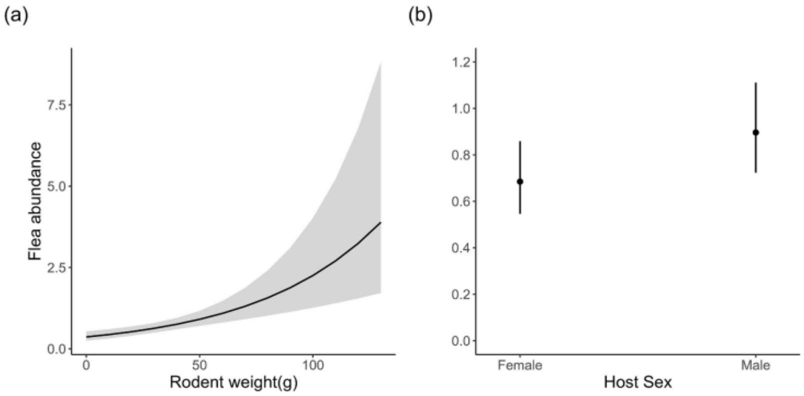

**Fig. 4.** Plots showing predicted effect of rodent traits on flea abundance, based on final best fitting generalized linear mixed model with a negative-binomial function. The plots (a) indicates that rodent weight increased with flea abundance. The gray shade in the plots represents the strength and direction of the correlation, with the width of the shade indicating the 95% confidence interval (CI) around the estimated effect. Furthermore, plot (b) indicates that male rodents are more likely to have higher flea abundance compared to female rodents, but this association was not statistically significant. The bars are 95% confidence intervals of the effects.

**Table 2**
Effect size with standard errors (±SE) and relative risk ratio (RR) of localities and seasons on the probability of flea infestation, from the final best fitting Generalized Linear Model (GLM) model.

| Predictors | Estimate (SE) | RR | RR 95% CI | z-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | −2.09 (0.49) | 0.12 | 0.05–0.32 | −4.23 | **<0.001** |
| **Locality** | | | | | |
| Plague persistent | 1.01 (0.45) | 2.75 | 1.15–6.58 | 2.27 | **0.02** |
| **Season** | | | | | |
| Long rain | −0.16 (0.57) | 0.84 | 0.27–2.63 | −0.29 | 0.77 |
| Short rain | 0.98 (0.51) | 2.69 | 0.98–7.36 | 1.93 | **0.05** |

Non-persistent locality and dry season were defined as reference.

associated with both forest and cultivated land, while others were associated with only forest habitats.

## 4. Discussion

This study aimed to understand the pattern of flea abundance between localities, habitat type, and season. Flea abundance was found to be similar between localities and seasons. However, the study found that flea infestation was mostly associated with the plague persistent locality and the short rain season. Furthermore, flea abundance was found to have a significant positive correlation with rodent weight. In addition, flea community was structured into two distinct groups.

We did not find significant difference in flea abundance between the localities, despite the hypothesis that the plague persistent locality would have higher flea abundance. This observation seems to contradict the hypothesis that high flea abundance in persistent localities increases the risk of bubonic plague. However, it is important to note that the study found that the probability of flea infestation was significantly higher in the plague persistent locality, indicating that the risk of plague pathogen spreading may still be elevated in this locality. One possible explanation for the lack of significant difference in flea abundance between the localities could be differences in the flea species assemblage and level of infestation among different hosts. Even if the flea abundance is similar, the composition of flea species and the levels of infestation on individual host species could still be important determinant of disease persistence, consistent with the available literature (Eisen et al., 2012). Moreover, we did not find any significant differences of flea abundance between seasons, but we observed that rodents were more frequently infested with fleas during the short rain season. This observation may be attributed to the warmer and more humid conditions during the short rain season, which create a favourable environment for flea development and survival, leading to increased infestation in rodents. These findings align with previous studies which have shown that warmer and humid condition promote flea development and survival, leading to higher flea abundance (Krasnov et al., 2001; Kreppel et al., 2016; Sharif 1949; Mboera et al., 2011; Ngeleja et al., 2017), Importantly, such condition has also been associated with an elevated incidence of human plague in some of the plague foci. For example, Debien et al. (2010) reported that precipitation resulted in higher flea abundance and an increased incidence of plague in Lushoto, Tanzania.

Further, we found a positive association between rodent weight and flea abundance. We also found a positive association between male rodents and flea abundance, which is often attributed to their larger body size (Moore and Wilson, 2002), but this relationship was not significant in our study area. Mostly, male rodents tend to have higher flea abundance due to their larger body size, ample blood supply, weaker immunity and less grooming ability (Eads and Hoogland, 2016; Kiffner et al., 2013). In addition, larger rodents tend to have higher activity levels, which could increase their exposure to fleas in the environment (Krasnov et al., 2006b). However, different species can vary from these patterns, and more studies are necessary to better understand relationship between rodent weight and flea-borne diseases enabling more insights into their specific host-flea relationships.

Furthermore, we found two communities of fleas in the foci, suggesting the flea community structures were influenced by the seasons, habitats types and hosts present in these habitats. These results are consistent with studies elsewhere which have shown strong flea-habitats (Brinkerhoff, 2008), host-habitat relationships (Krasnov et al., 2006) and environmental factors (Chotelersak et al., 2015). In the present study, the first flea community included *Dinopsyllus lypusus*, *X. brasilliensi, X.cheopis* and *Nosopsyllus* spp. which were found in both

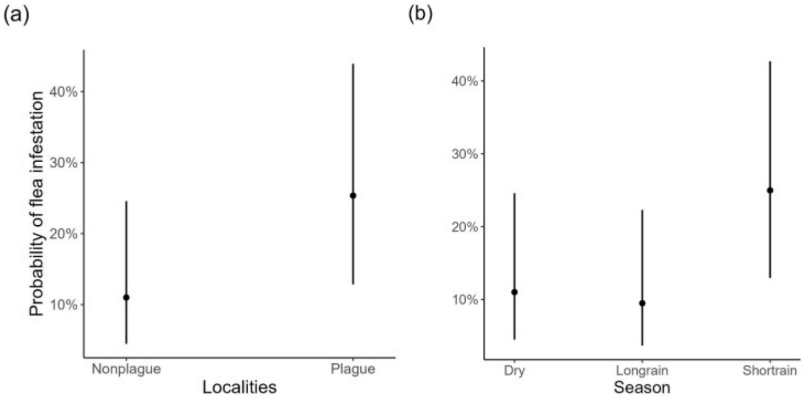

**Fig. 5.** Plot showing the predicted effect of locality and season on the probability of flea infestation, based on the final best-fitting generalized linear model with a binomial function. The analysis aimed to identify the factors that strongly influence flea infestation. The strongest predictors of flea infestation were plague persistent localities and short rain seasons. The probability of infestation on these predictors was found to be statistically significant ($p < 0.05$), suggesting a higher likelihood of flea infestation in this locality and season. The bars are 95% confidence interval of the effects.

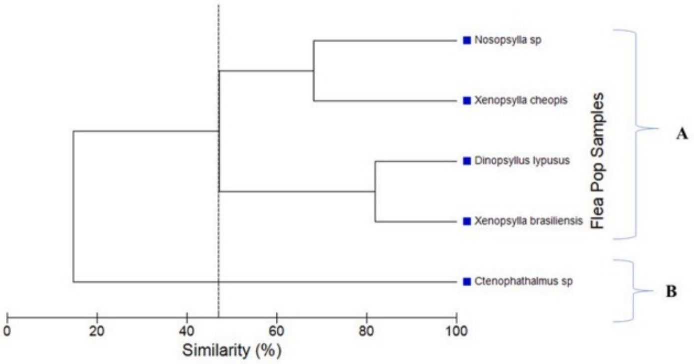

**Fig. 6.** Dendrogram of flea species showing two main groups (AB) of flea community based on farm and forest habitats.

farm and forest habitats. The species prefer rodents as primary hosts. For example, *X.cheopis, Dinopsyllus lypusus*, and *X. brasilliensis* prefer *R. ratus* as a primary host (Msangi, 2019), but they can also infest other rodent species (Palazzo, 2011; Trivedi, 2003). However, a fine scale separation observed in this group may be connected to the habitat types, seasons and/or other factors in the foci, further studies would be needed to confirm this hypothesis. *Dinopsyllus lypusus*, and *X. brasilliensis*, have been reported as potential vector of plague among sylvatic rodents (Ziwa et al., 2013) and the species were found on commensal rodent such as *Ratus rattus* and *M. natalesnsis*, and on wild rodents such as *P. delectorum* and *L. makundii* (Fig. 2a), indicating a flea-host-habitat association. In addition, other studies have revealed that Xenopsylla species is primarily an efficient vector of plague to humans (Zhang et al., 2015; Hinnebusch et al., 2017). Moreover, *M. natalensis* is a social species that nests in burrows and occasionally associates with other wild rodent species (Coetzee 1975); given that *Y.pestis* is still circulating in this species in the foci (Haikukutu et al., 2022), the diverse flea infestation on *M.natalensis* may be contributing to plague persistence in the foci and possibly influencing spreading of *Y.pestis* between other rodent species and/or other hosts sharing these habitats. In the second flea community, *Ctenophthalmus* spp. were found to be the only species. The species was only present in forest habitats, suggesting a strong association with areas characterized by vegetation, such as grassy and wooded environments. Additionally, their presence in rodent burrows and nests reinforces their connection to habitats where these particular hosts were commonly located. The species was found on host *M. natalensis* and *P. delectorum* which was consistent with previous findings conducted in the same study area (Haule et al., 2013). The state of the forest habitat supporting more diverse flea species compared to the farms, and presence of some of flea species infesting multiple rodent hosts that includes the susceptible species may encourage the potential of epizootic cycle of disease transmissions between rodent species. Alarmingly, these flea species have the ability to harbor other zoonotic pathogens such as *Bartonella* and *Richettsia typhi* (Leulmi et al., 2014; Occhibove et al., 2022) highlighting the need for more studies on flea borne pathogen pattern and their role as pathogen vector in the foci.

## 5. Conclusion

Our findings provide insight into the complex interactions between flea communities, rodent host species and environmental factors in the plague foci. The observed flea vector associating with sylvatic host, its ability to harbor other zoonotic pathogens influences the relevance of extending our study to a broader disease transmission dynamic within the foci. Our data about these ecosystems, provide opportunities for potential strategies to targeted public health interventions that can lower risks of bubonic plague and other flea borne diseases in these rural communities.

## Ethical approval

Ethical clearance was obtained from Sokoine University of Agriculture Ref. no DPRTC/R/126/182/38, Manyara region Ref. no FA.262/347/01/H/247, Mbulu district Ref. no AB.323/381/01/'B'/9. Animal handling followed the guidelines of the American Society of Mammologists (ASM) for the use of wild mammals in research and education (Sikes & Animal Care and Use Committee of the American Society of Mammologists, 2016).

## Author contributions

STK designed, conducted field data collection, data analysis and wrote original draft manuscript. AAR analyzed the data and reviewed the original drafts. RHM and AM reviewed the manuscripts. AAR, RHM & AM supervised the research. All authors read and approved the final version of the manuscript for submission.

## Data availability

All data used in this analysis can be obtained from the corresponding author upon request.

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## Declaration of competing interest

The authors declare that they have no conflict of interest.

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**TS1:** Rodent trap success (%) across localities, habitats (Pfarm =farm in plague persistent locality, Pforest =forest in plague persistent locality, NPfarm =farm in non-plague persistent locality and NPforest = forest in non-plague forest) and seasons.

| | **Localities** | | | | | | **Seasons** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Rodent species** | **Pfarm (%)** | **Pforest (%)** | **Plague locality (%)** | **NPFarm (%)** | **NPForest (%)** | **Nonplague locality n (%)** | **Dry n (%)** | **Long rain n (%)** | **Short rain n (%)** |
| *Aethomys kaiseri* | 0.75 | 0 | 0.38 | 0.27 | 0 | 0.13 | 0.8 | 0.44 | 0.58 |
| *Arvicanthis sp. "Masai Mara"* | 0.27 | 0 | 0.13 | 0.09 | 0 | 0.04 | 0 | 0 | 0.67 |
| *Grammomys cf. macmillani* | 0.18 | 0.71 | 0.44 | 0 | 0.13 | 0.07 | 0.47 | 0.44 | 1 |
| *Graphiurus cf. raptor* | 0 | 0.22 | 0.11 | 0 | 0.18 | 0.09 | 0 | 0 | 0.75 |
| *Lemniscomys striatus* | 0 | 0.62 | 0.31 | 0 | 0.04 | 0.02 | 0.07 | 0.11 | 1.08 |
| *Lemniscomys zebra* | 0.09 | 0 | 0.04 | 0.13 | 0 | 0.07 | 0.07 | 0 | 0.33 |
| *Lophuromys makundii* | 0 | 1.42 | 0.71 | 0 | 0 | 0 | 0.6 | 1.33 | 0.92 |
| *Mastomys natalensis* | 5.02 | 0.4 | 2.71 | 2.4 | 1.38 | 1.89 | 3.27 | 4 | 10.17 |
| *Mus cf. gratus* | 0 | 0.08 | 0.04 | 0 | 0.04 | 0.02 | 0 | 0.22 | 0.08 |
| *Mus minutoides* | 0.36 | 0 | 0.18 | 0.04 | 0 | 0.02 | 0.4 | 0.22 | 0.08 |
| *Praomys delectorum* | 0 | 3.6 | 1.8 | 0 | 0.18 | 0.09 | 1.93 | 1.44 | 3.58 |
| *Rattus ratus* | 0.04 | 0 | 0.02 | 0 | 0 | 0 | 0 | 0 | 0.08 |

# CHAPTER FIVE

## PAPER IV

## 5.0 Ecological and environmental correlates of flea abundances within human habitations in a plague focus, Mbulu district, Tanzania

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### Abstract

The persistence of plague is a major public health concern in some African countries. Although the role of flea vectors in its transmission is widely recognized, there is still limited understanding of the local-scale factors that contribute to outbreak recurrence, especially in plague-endemic areas characterized by a complex interplay of human lifestyle practices, behavior, environmental conditions, and host availability. This situation risks the continued persistence of the disease and its health impacts on humans. In this study, we used the removal method and light trap to collect data on flea abundance, to understand the variation within houses and across the sampling period, and the influence of human behaviors and practices on the flea abundance within houses in rural communities Mbulu District, Tanzania. We found 1134 fleas from seven species with *Ctenocephalides canis* 26.57% the most abundant, followed by *Pullex irritans* 26.02% and *Ctenocephalides felis* 17.02%. The abundance was significantly higher in dry than long rain season. Houses with a sleeping bed, mattress and loft had significantly higher flea abundance than houses with a bed and mat alone. Furthermore, households sharing with livestock under one roof had significantly more fleas than non-shared households suggesting strongly that human lifestyle, behaviors and hygiene could be playing a big role in exposing humans to flea bites and potentially increasing the risks of contracting plague and its persistence in these rural communities. Our findings call for improving community knowledge in disease epidemiology and hygiene and may help interventions to reduce disease exposure in these rural communities.

**Key words:** Flea abundance, household behavior, plague foci, plague persistence

### 5.1 Introduction

Fleas are parasitic insects that are known to infest a variety of mammals, including humans, dogs, and cats [1,2]. They are known to be highly mobile, with the ability to jump up to 150 times their own body length, allowing them to move quickly between hosts. Fleas can survive for long periods without a host and can lay eggs that remain viable until suitable conditions for development are met, allowing them to persist in the environment [3]. Further fleas serve as vectors of many pathogens that can be transmitted between animals and humans, establishing a cycle of disease transmission in both populations. Among these are the plague bacterium, *Yersinia pestis* [4], the bacteria that cause murine typhus, *Rickettsia typhi* [5] and bartonellosis which is caused by various species of bacteria in the genus Bartonella, including *Bartonella henselae*. Understanding the biology and behavior of fleas and how environmental conditions and human life style shape their population dynamics and disease transmission is germane for improving the strategies for reducing infection risks to both animals and humans.

Flea abundance is among the factors that influence plague disease transmission, and is influenced by a variety of factors including host availability, temperature, humidity, and habitat characteristics [6–8]. Other factors such as weather condition also regulate flea population [9].

When the temperature is very low, for example, flea development slows down, but when the temperature is high, flea growth and development increases, resulting in rapid population growth [10]. Further, immature fleas are more affected by very low humidity, particularly when the relative humidity (RH) falls below 50% even when the temperature is favorable [11,12]. However, flea population may respond to factors other than climatic changes, such as host density. For example, during the rainy season with warm temperatures, vegetation production increases, thereby increasing rodent densities, which favors flea populations due to available food and shelter [13]. Furthermore, previous studies have looked into the host preferences of different flea species [14,15] including a variety of rodent hosts which are the primary hosts of plague [16]. The human flea (*Pulex irritans*), for example, is known to prefer humans as hosts, but can also infest a range of other animals, including dogs, cats, pigs, and rodents [17] making it an agent of zoonoses. Similarly, the cat flea (*Ctenocephalides felis*) and the dog flea (*Ctenocephalides canis*), are more commonly found on their respective specific hosts, but also can infest other animals and even humans [9,18–20]. In this case, the complex interplay of host preference, environmental conditions, and habitat characteristics greatly impacts the dynamics and distribution of flea populations.

Previous studies that have looked into the relationship between household behavior and flea abundance suggest that households with poor sanitation and hygiene practices are more likely to have higher flea abundance due to increased food and shelter availability for fleas [21,22]. This is because, fleas may feed on organic debris on carpets, furniture or soil [23,24], and may use cluttered or poorly maintained areas as favorable habitats, such as poorly maintained walls or floor and domestic animal resting places [25–27]. Further, fleas may also be attracted to the warmth and moisture generated by certain household appliances, such as carpets, curtains, beddings as well as house designs such as thatched roof mud wall houses [28,29]. Overall, all these promote flea survival and infestation while also increasing the possibility of fleas, human and other host interactions, which may encourage flea borne diseases such as plague to persist in the foci. Previous studies in Lushoto district, a plague focus in Northern Tanzania, showed that sporadic outbreaks were linked to poor housing and environmental sanitation [30,31]. Despite this knowledge, little is known about the links between flea population dynamics and household behavior in the plague foci, Mbulu district, Tanzania [32] and it is still unclear whether a similar situation reported elsewhere in other plague foci also applies in the Mbulu focus.

In this study, we aimed to examine the fluctuations of flea abundance across the sampling period and how household sleeping behavior (such as mat and sleeping loft) influenced flea abundance within houses. Specifically, we assessed (i) how flea population change over the sampling period, (ii) how do season and flea species identity influence flea abundance and (iii) how household behavior and house structure affects flea abundance inside houses. We hypothesized that flea abundance within houses would vary during the course of the sampling period due to changes in seasonality. We also hypothesized that, dry season will have a higher flea abundance than other seasons and some flea species such as *Pulex irritans*, *Ctenocwphalides canis* and *Ctenocphalides felis* will be more abundant than others due to variety of host choices within houses. Further, we predict that houses with poor sanitation and hygiene practices such as keeping livestock within sleeping houses, will show higher probability of having more fleas due to increased availability of food and shelter for fleas. This information could be useful in designing the appropriate strategies to reduce flea predisposing causes, thereby reducing the likelihood of a plague outbreak in these rural communities.

## 5.2 Material and Methods

### 5.2.1 Study area

The study was conducted between January to December 2019 in Mbulu district located in Northern Tanzania, at 3° 57′ 097″S, 35° 18′ 39.60″E (Fig. 1). The district is located between 1000-2400 m above sea level, and has a semi-arid to sub-humid with biannual rainfall of <400 and >1200mm between March and May, and between October to December. The economic activities include livestock keeping and agriculture. Two villages Endesh and Mongahay, were chosen for the study due to the history of plague outbreaks in the last 12 years following a relatively long period of asymptomatic infection [31,33,34].

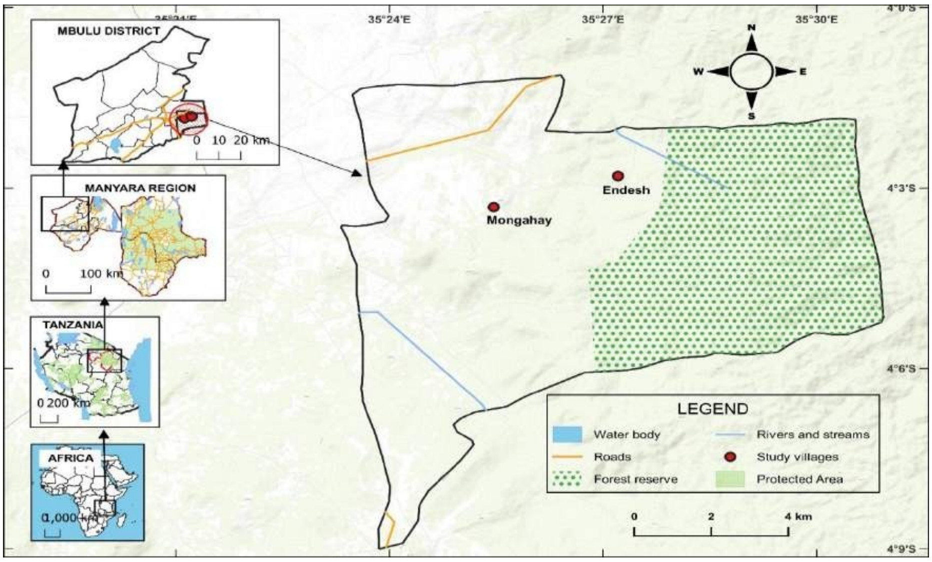


**Figure 1:** Map of Mbulu district in Tanzania showing the locations of the study sites Endeshi and Mongahay villages surveyed. Map was generated using QGIS software (version 3.8.3 Zanzibar). Based layer were sourced from Esri map; https://esri.maps.arcgis.com/home /item.html?id=273ffd9a4c05 4d47843ed9642ecb143 e , licensed under the Esri Master license agreement; https://goto.arcgis.com/ termsofuse/viewtermsofuse.

### 5.2.2 House selection

A randomly sampled group of 44 houses were selected for rodent trapping and flea collection. The majority of the houses were made of thatched and corrugated mud huts. Further, crops were stored inside the sleeping houses, while livestock were kept in corrals near the sleeping and/ or inside sleeping houses. For this study, we delineated houses sharing or not sharing with livestock for our study this factor is known to influence flea infestation in other plague foci elsewhere.

### 5.2.3 Flea collection

To collect fleas from rodents, animals were live trapped using sherman traps and locally made wooden box traps baited with peanut butter mixed with maize flour to collect fleas from rodents. Captured animals were anaesthetized with diethyl ether for immobilization [35]. Other morphological characteristics were recorded such as weight, sex and reproductive status and rodents were identified to species level using relevant keys [36,37] and confirmed by sequencing the mitochodrial cytochrome b gene at Czech Republic, Institute of Vertebrate Biology, and has been reported elsewhere [32]. Three traps were left overnight and inspected each morning for three consecutive days between January 2019 to December 2019.

To collect fleas from the surroundings inside the houses, a light trap (Ltrap) was also installed in the house. The Ltrap consisted of a flash light and a metal tray quarter filed with water. Vaseline jelly was smeared to inner edge of the tray to prevent fleas from escaping. The flash light was directed to the center of the tray to attract fleas. However, in the morning when the traps were removed, the flashlight was switched off. Fleas captured overnight were removed from the tray in the morning.

Further, fleas were sorted according to villages, month, and whether they were collected from the rodents or house floor, counted and preserved in 70% ethanol for future identification. The fleas were identified to genus and/or species level, following the methods described in [38,39]. The fleas were then processed by adopting a modified version of the method described in [40]. Briefly, each pool from the sorted groups was exposed to NAOH 10%, dehydrated in various concentrations of ethanol (50%, 70%, 95%, absolute), cleared in clove oil, temporarily mounted using glycerin on a microscopic slide, and examined under a light microscope using a 10x objective. The processes were applied to improve flea visibility, preserve flea morphology for proper analysis and enable examination under a light microscope.

### 5.2.4 Characterization of the houses and household practices

House characteristics and household practices were recorded in the residences where fleas trapping was conducted. Before recording, we obtained verbal consent from the owner of the house through a clear and transparent explanation of the observation process. A mutual agreement was reached regarding the recording of these characteristics for the purpose of understanding how these factors could influence flea abundance and dynamics in the rural settings [41,42]. After obtaining the consent, we proceeded and characterized the human household structure based on house type (brick with thatched roof, brick with corrugated iron sheet roof, mud with thatched roof, mud with corrugated iron sheet roof), floor type (mud, cement), and wall type (mud, plastered with bricks), as potential correlates of flea abundance (Fig. 2). Also, household practices or human behaviors, including crop storage (inside or outside the sleeping house), cattle keeping (inside or outside the sleeping house), and sleeping practices (bed with mattress, bed with some mattresses and some mats, bed with mats and a loft room) were also recorded (Fig. 2).

**Fig 2. (a)** Visual presentation of local house types, indoor and outdoor view and (b) illustration of house designs outside and inside houses in the study community

### 5.3 Data Analysis

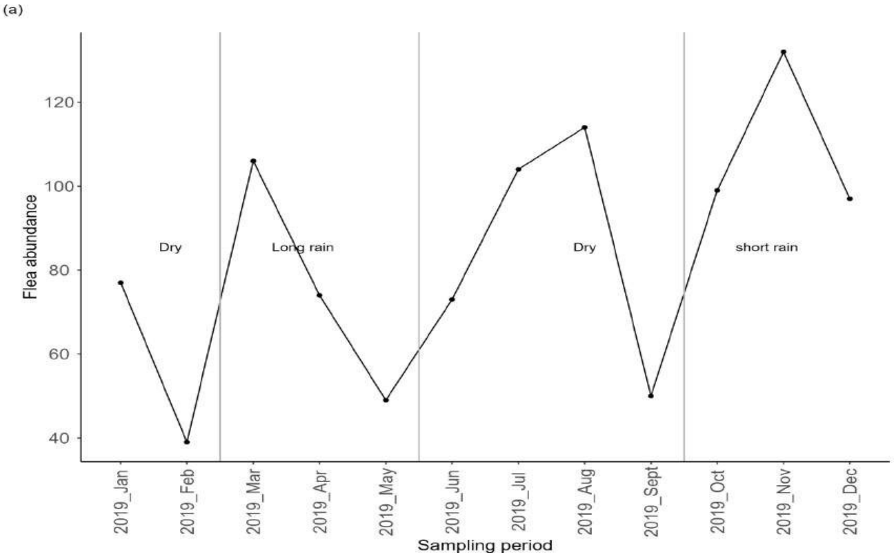
Flea abundance was referred as the total number of fleas collected from the rodents and Ltrap during the sampling period regardless of the species identity as they constituted the inside flea pool potential threat to humans. To assess how the flea abundance fluctuated throughout the sampling period and to understand pattern of flea distribution within each season, we conducted descriptive analysis using line plots and stacked bar plots using the 'ggplot' function. To assess the influence of seasonality and flea species on flea abundance, a generalized linear model (GLM) with negative binomial error distribution was used after confirming overdispersion from using relevant function in the DHARMa package. Since all variables in the initial model were found to be statistically significant, indicating potential relationships with the response variable, we reported this result without removing any variables from the model. To assess the model's goodness of fit, we generated a four-panel plot that included a residuals histogram, a Q-Q plot of standardized deviance residuals, a plot of fitted values versus residuals, and a plot of Cook's distance. These diagnostic plots allowed us to assess the assumptions of the model, identify influential observations, and ensure the model's validity. To estimate the marginal means of flea abundance for each level of the seasons and flea species variables and compute pairwise comparisons between the levels, we used emmeans and the contrast functions.

Further, to examine the effect of house characteristics and house behavior on flea abundance, we build generalized liner model (GLM) with a negative binomial error distribution after confirming overdispersion. We first build a global model that includes six variables, house type, floor type, wall type, crop storage, cattle keeping and sleeping behavior as predictors. Flea abundance was the response variables. A chi-square test was used to examine model significance whereby, each model covariate was assessed using the drop1 function in which a non-significant variable was removed until a final model was reached. To assess the goodness of fit for this model, we used the same analysis approach as in earlier steps. Finally, a "ggplot2" package was used to visualize the relative magnitudes of effect for each predictor variable, as well as to identify which predictor variables have had statistically significant effects on the response variable.

## 5.4 Results

### 5.4.1 Flea abundance across the sampling period

We collected 1134 fleas from seven species, with the most abundant species *Ctenocephalides canis* comprising 26.57% of the total (275). Following in order of decreasing abundance were *Pullex irritans* (26.02%, n = 269), *Ctenocephalides felis* (17.02%, n = 176), *Echidnophage gallinacean* (14.22%, n = 147), *Xenopsylla brasilliensis* (12.19%, n = 126), *Dinopsyllus lypusus* (3.68%, n = 38), and *Xenopsylla cheopis* (0.29%, n = 3). Flea abundance varied during the sampling period, decreased at the end of each season and increased at the beginning of each new season (Fig 3). Further, the abundance of each flea species varied across the different seasons. Dry seasons had the highest abundance, accounting for 44.87% of the total (n = 464), with *C. canis* and *P. irritans* the most abundant species (26.51%, n = 128) and (23.71%, n = 110) respectively. The short rain season had the second-highest abundance (n = 340) accounting for 32.88% of the total, with most species increasing in abundance. *Ctenocephalides. canis* was the most abundant species in this season accounting for 33.24% of the total (n = 113), followed by *P. irritans* (18.82%, n = 64), *E. gallinacean* (17.65%, n = 60), and *C. felis* (17.65%, n = 60). The long rain season had the lowest abundance (22.24%, n = 230), with *P. irritans* and *C. felis* the most abundant species comprising of 33.24% (n = 95) and 33.91% (n = 78) respectively (Fig. 3).



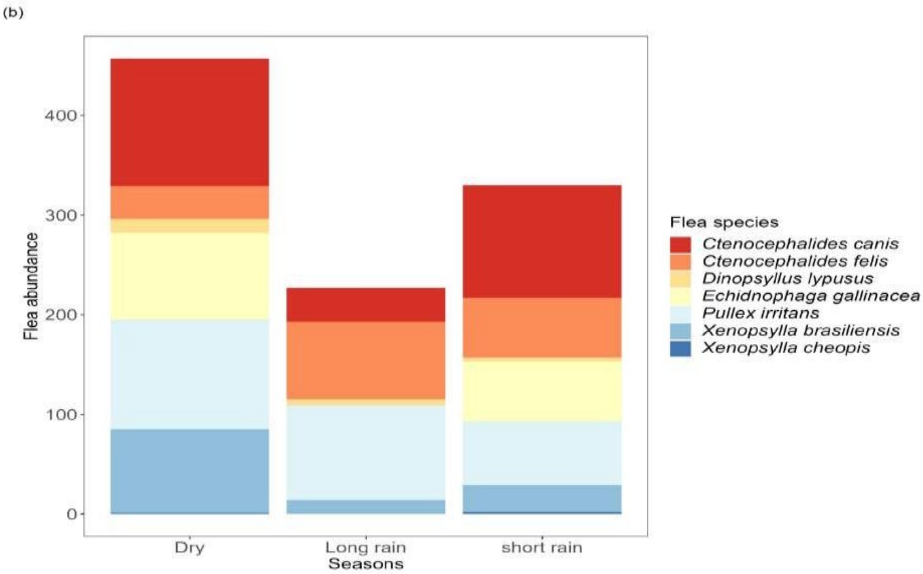


**Fig 3. (a)** Flea abundance across the sampling period January to December 2019 and (b) flea abundance by species across seasons

### 5.4.2 Influence of season and flea species on flea abundance

Our model results showed dry season had a significant increase in flea abundance than the long rain season (mean = 0.93±0.33SE, p = 0.004). Also, flea abundance decreased during the long rain season compared to the short rain season (mean = -0.61±0.33SE, p = 0.06) whereas in dry season the flea abundance increased compared to the short rain season (mean = 0.32±0.31SE, p = 0.32). However, both variations were not statistically significant. In addition, the flea species *C. canis* significantly increased than *D. lypusus* (mean = 1.89±0.48SE, p < 0.05) and *X. cheopis* (mean = 4.48±0.74SE, p < 0.05). Also, the abundance of *Ctenocephalides feli*s significantly increased compared to *D. lypusus (*mean = 1.73±0.48SE, p < 0.05) and *X. cheopis* (mean = 4.32±0.74SE, p < 0.05)*. Dinopysllus.lypusus* had a significantly higher abundance (mean = 2.59±0.76SE, p = 0.001) compared to *X. cheopis*. However, the abundance of *D. lypusus* decreased significantly compared to *P. irritans* (mean = -2.05±0.48SE, p = 0.05), *E. gallinacea* (mean = -1.13±0.48SE, p = 0.02), and *X. brasilliensis* (mean = -1.05±0.48SE, p = 0.03). Further, *Pulex.irritans* had a significant increase in flea abundance compared to *X. brasilliensis* (mean = 1.003±0.46SE, p = 0.03) and *X. cheopis* (mean = 4.64±0.74SE, p = 0.05) (Fig. 4).

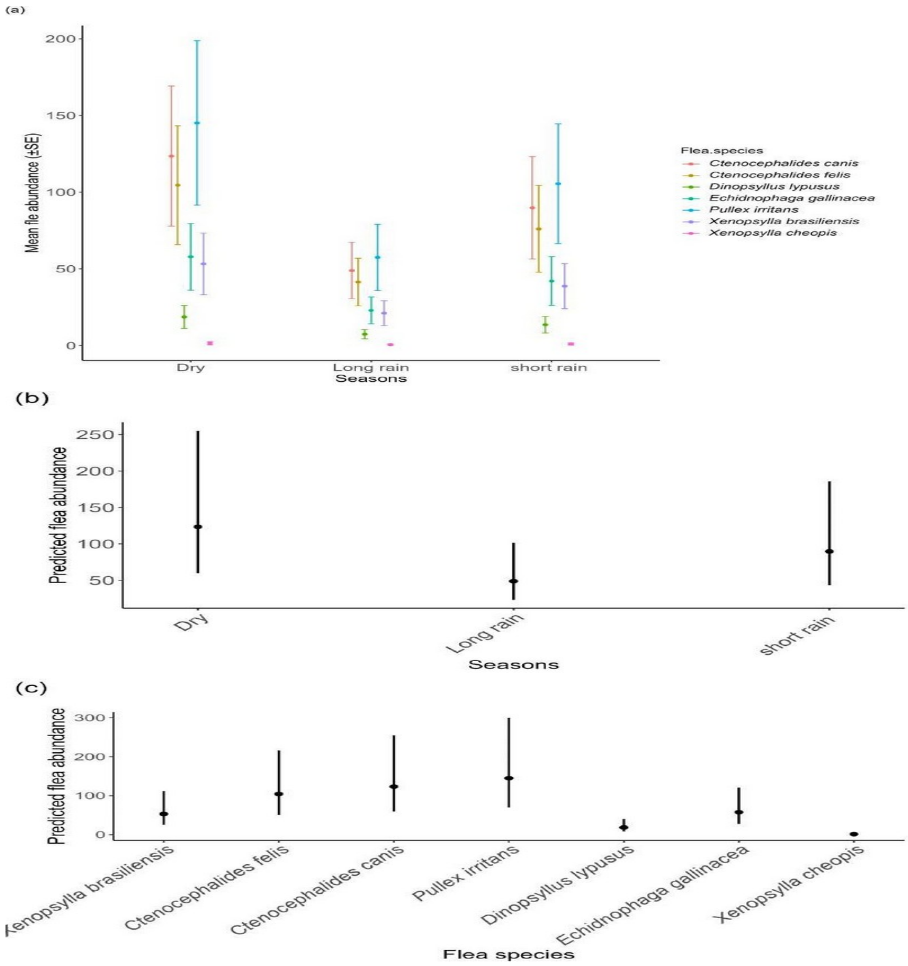


**Fig 4. (a)** Seasonal differences in flea abundance by species, (b) Seasonal probability of flea abundance in dry, long rain, and short rain seasons. The higher probability of flea abundance was most likely associated with a dry season followed by a short rain season, and (c) the increased flea abundance by species was mostly likely influenced by *Pulex irritans*, *Ctenocephalides canis*, and *Ctenocephalides felis*. The error bars represent the 95% confidence intervals around the predicted means.

### 5.4.3 Effect of house characteristics on flea abundance

The model results indicated that houses with no livestock and those maintained livestock outside sleeping houses had significantly lower flea abundance than houses with livestock kept within sleeping house. Also, houses with sleeping behavior; bed, mattress and sleeping loft had much more fleas than houses with only bed and mat. (Table 1, Fig 5).

**Table 1:** Estimated effect size with standard errors (±SE) from the final best fitting GLM model

| **Predictors** | **Estimate±SE** | **z-value** | **p-value** |
|---|---|---|---|
| (Intercept) | 3.07±0.18 | 17.02 | **<0.001** |
| **Cattle keeping** | | | |
| No cattle | -0.94±0.38 | -2.49 | **0.01** |
| Outside sleeping house | -0.44±0.18 | -2.41 | **0.02** |
| **Sleeping behavior** | | | |
| Bed, mattress | -0.18±0.25 | -0.74 | 0.46 |
| Bed, mattress and mat | 0.08±0.19 | 0.44 | 0.66 |
| Bed, mattress and sleeping loft | 0.94±0.38 | 2.49 | **0.01** |

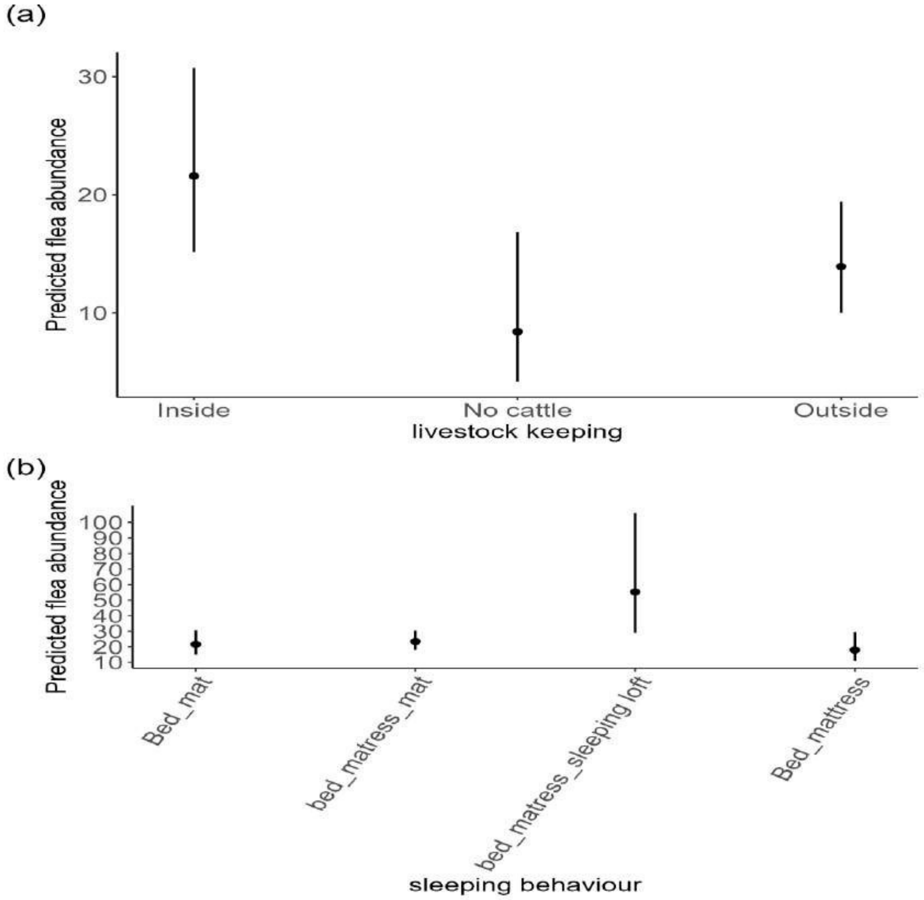


**Fig 5.** (a) Prediction plots showing the effects of house behavior on flea abundance. Houses with livestock inside their sleeping houses were more likely to have more fleas than houses with no cattle or those with livestock outside, and (b) houses with a combination of bed, mattress, and sleeping loft showed a higher probability of having more fleas than houses with only bed and mat.

### 5.5 Discussion

The purpose of this study was to understand flea populations over the sampling time, how season and flea species influences flea abundance, and the impact of household behavior on flea abundance inside houses. We found flea abundance fluctuating throughout the sampling period, with decreases at the end of each season and rises at the beginning of each new season. Also, dry season was found to be most likely connected with the increased flea species *P. irritans*, *C. canis* and *C. felis*, followed by the short rain season. Further, houses with sleeping behavior bed, mattress and sleeping loft had much higher flea abundance than houses without livestock and those kept outside sleeping houses.

Changes in flea abundance with decrease at the end of each season and increase at the beginning of each season may be contributed by the changes in weather conditions as has been described previously [43]. Higher temperatures and humidity may generate favorable conditions for flea development and survival, resulting in increased flea abundance [44]. Lower temperatures and humidity, can have a negative impact on flea development and survival, resulting in a decrease in flea population [45]. Although we did not record the exact interior temperature and humidity, changes in outer weather conditions may have altered the indoor environment, thereby changing the developmental stages of fleas present indoors. Further, temperature and humidity have a significant impact not only on flea abundance, but also on their behavior and disease transmission. Low temperatures below 10°C have been linked to increased flea vector mortality in connection to *Y. pestis* infection. Higher temperature above 20°C, on the other hand, enhance biofilm blockage of flea proventriculus, hence increasing flea biting activities. Also, humidity at a moderate level of 50% is essential for larval development. Excessive humidity level above 80% have been shown to increase flea bite rates, while humidity level above 95% increase the risk of larval and eggs being destroyed [12]. These findings suggest that, observed changes in flea abundance, which may be influenced by weather conditions, may be contributing disease persistence in the foci. Also, variation in flea abundance by species across seasons was higher during warm periods of the year. From June to august (Dry season) and October to December (season with warm and moist conditions). Previous studies have shown that warmer and humid condition promote flea development and survival, resulting in increased flea abundance [46–48]. Importantly, *Ctenocephalides canis, Pulex irritans* and *Ctenocephalides felis* were more most abundant during this period. This may be influenced not only by these climatic conditions but also by other factors within the indoor environment, such as the presence of preferred hosts, as host availability aids in their development and survival.

Moreover, we found increase in flea abundance in houses where livestock were kept indoors. This finding is similar to other studies [42,49], which found livestock within houses offer adequate food and shelter for fleas. Also, due to grazing activities conducted in the study area, dogs and cattle may play a significant role in the spread of fleas as bridging hosts, increasing the risk of spreading flea pathogens from the wild to domestic areas. Furthermore, sleeping behavior (bed, mattress and sleeping loft) contributed in the increase of flea abundance. Most houses, on the other hand, lacked enough beds to accommodate the number of people, with the rest sleeping on floor mats, thereby exposing to flea bite[22,30,50]. Further, sleeping on mats more likely increases the propensity for flea infestation than other types of beddings [22]. In this study area, the loft was used to store crops, which may have influenced persistence of rodent host such as *M. natalensis* and *R. rattus* [32] both of which are susceptible hosts [31,34,51]. In addition, the majority of the houses in the study area were close to the farm and had livestock kept inside or outside cattle pens, which were still close to the sleeping houses. These sleeping houses had mud floors and walls, as well as poor indoor and outdoor hygiene (Fig. 3), indicating that poor house conditions and sanitations may have contributed to flea abundance and persistence in the study area.

Finally, our findings highlight the importance of considering both environmental factors and household behaviors into domestic flea control strategies. They also contribute to the understanding of flea populations and their relationship with household behaviors, as well as emphasizing the importance of providing community knowledge about the importance of flea control and implementing integrated pest management strategies that can contribute to long- term reduction in flea abundance.

### Acknowledgements

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### Data availability

The data underlying the findings of this study and R codes used in the analysis can be accessed from a repository using https://doi.org/10.5061/dryad.cz8w9gjbr

## Contribution

Conceptualization, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing: **Stella T. Kessy** Supervision, Writing – Review & Editing: **Alfan A. Rija**

## Ethical Approval

Ethical clearance was obtained from Sokoine University of Agriculture Ref. no DPRTC/R/126/182/38, Manyara region Ref. no FA.262/347/01/H/247, Mbulu district Ref. no AB.323/381/01/`B`/9. Animal handling followed the guidelines of the American Society of Mammalogists (ASM) for the use of mammals in research and education (Sikes and Animal Care and Use Committee of the American Society of Mammalogists, 2016).

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# CHAPTER SIX

## PAPER V

## 6.0 Knowledge and practices related to plague persistence in plague endemic foci, Mbulu District, Tanzania

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### Abstract

**Introduction**

Plague continues to be a major public health concern in African countries. Several social practices and environmental conditions have been associated with the reoccurrence of bubonic plague, especially in places where the disease is prevalent. Therefore, it remains important to understand people knowledge, behavior and practices related to disease risks in order to identify factors that may hinder prevention and control strategies in the foci.

**Methods and results**

A study survey of 100 households was conducted in Mbulu district to assess plague knowledge, factors that influence flea bite and measures used for rodent and flea control. Majority of participants (86%) were familiar with the plague disease and about (50%) mentioned swelling lymph nodes as a common symptom. Most of the participants (62%) claimed to observe human plague cases during the long rain season. The majority of participants (97%) reported to experience flea bite in their domestic settings, with most stating that they experienced more flea bites during the dry season. Houses with livestock had a greater likelihood of flea bite (OR = 2.7; 95% CI: 0.36-18.80, p = 0.267) compared to houses with no livestock. Furthermore, residents reported using both local and chemical methods to control rodents and flea inside houses. Most respondents preferred using local methods in flea control. Respondents stated that the efficacy of flea control methods being applied ranged from few days to several months. There was limited knowledge of the residual effects of the agricultural chemicals being used to control fleas among the surveyed community.

### Conclusion

Our study highlights the importance of raising awareness and adopting effective control methods for controlling fleas and lower the risk of plague transmission and other flea borne diseases in the local communities. Sensitization of the local community on the use of appropriate chemicals for flea control is urgent to avoid any potential long-term impacts of the residual effects on the health of the local communities.

**Key words:** agricultural chemicals; cultural practices, flea bites; local knowledge, plague disease; human health.

### Author summary

Plague is a significant health challenge in Africa due to environmental conditions, lifestyle, and behaviors of the people living mostly in persistent areas. Understanding people’s knowledge on the environmental conditions and lifestyle practices related to plague transmissions is essential to preventing the disease spread.

We assessed community understanding on plague, factors influencing flea bites, and methods used to control rodents and fleas in the plague foci to inform the development of the potential mitigation strategies for the disease. Majority of respondents were familiar with the disease; however, few were aware of its symptoms. They reported that human cases occurred during the rainy season, with frequent flea bites experienced during the dry season. Also, households keeping livestock inside sleeping houses had a higher likelihood of flea bites compared to those without livestock. Furthermore, residents reported using both local and chemical methods to control rodents and fleas with the majority preferring local methods for flea control. Respondents stated the efficacy of the flea control methods used ranged from days to months, although there was limited understanding of the long-term health effects of the chemical residuals used among the surveyed community. The study findings emphasize the importance of understanding community awareness and preventive measures in controlling the spread of plague and call for greater community awareness raising on using appropriate flea control methods. Understanding local practices and preventive methods aids the development of interventions that are safer and more acceptable to the community, ultimately leading to more effective control strategies in these rural settings.

## 6.1 Introduction

Plague persistence threatens the health and economy of affected communities in low- and middle-income countries [1-3]. Since the early 2000s, there have been many reports of human plague cases associated with fleas and rodents in three major continents; Asia, America, and Africa [4]. Countries like Madagascar and the Democratic Republic of Congo in Africa, and Peru in South America represent the highest incidence of plague [5,6]. However, the occurrence of plague is not limited just to these countries. Human plague cases for instance, have been reported sporadically in some countries, such as Tanzania, particularly in the regions of Tanga, Arusha, and Manyara [7-10].

Although the frequency of plague outbreaks in the country has decreased compared to the past, the conditions still provide a conducive environment for future occurrences [10]. Moreover, an increasing association between persistence of plague and social and environmental factors has been observed not only in Tanzania [7,10], but also in other countries such as Peru, Zambia and Uganda [11-13]. This highlights the need for understanding how human practices and behaviour in their surroundings influence the continued existence of plague. This would facilitate the identification of practices and of any other social factors that may hinder the implementation of intervention approaches aimed at preventing and controlling both the host reservoirs and vectors of the plague in the foci.

Plague is an infectious disease transmitted by the flea vector and caused by the bacterium *Yersinia pestis* [14]. Plague can be transmitted to humans through two routes: indirectly via fleabites or directly by contact with infected droplets and tissues, either from rodent hosts or other hosts [14,15]. Further, the dynamics of flea vectors and rodent hosts are mostly affected by rainfall, humidity and temperature [16-18]. These conditions increase risks of plague outbreaks especially in areas with a large population of rodent and flea species that are susceptible to the disease [19,20]. In Tanzania, for instance, rodent species such as *Mastomys natalensis* and *Rattus rattus,* and flea species including *Xenopsylla cheopis*, *Xenopsylla brasilliensis* and *Dinopsylla spp* have been associated with ongoing presence and spread of the diseases [21-23]. Additionally, some flea species including *Pulex irritans* inhabit peri-domestic areas and are known to spread plague, especially in residential areas [24-26] where both social and environmental conditions are closely linked to plague incidences [27-30]. Studies conducted in Lushoto District, Tanzania, for instance, have shown several variables related to social, cultural, economic, ecological and climate to contribute to the persistence and periodic outbreak of the disease in the area [7, 31-33]. These studies altogether strongly suggest heightened health risks to human under plague persistent conditions.

Proper flea control is important in reducing the increasing likelihood of plague outbreaks. Usually, it is important to prioritize flea control before rodent control to minimize the spread risks of infected fleas and plague bacteria [34]. Insecticide dusting has been widely used and recommended as an effective control method for rodent fleas during epidemics [35]. However, there are concerns regarding the potential health risks and environmental side effects due to its toxicity [36]. To do away with this potential health risks, alternative methods are in use such as bait station that uses a host as a target to deliver insecticide to the ectoparasites. The approach is considered as cost-effective and specifically targets ectoparasites on the host [37,38], although additional experimental studies to enhance its effectiveness are required[39]. Additional challenge encountered in flea control is the improper use of chemical insecticides that leads to flea resistance [40-42] and increased exposure of humans to the side-effects of these chemicals. These studies altogether, highlight the importance of understanding the social-cultural dynamic factors of flea and rodent control measures towards preventing plague in affected communities.

Plague dynamics and control in affected regions in Tanzania is relatively well studied. In the early stages of plague outbreaks in Lushoto Tanzania [7,22,43], several strategies were used to minimize the spread of the disease. These included eliminating fleas and rodents, giving chemotherapy to patients, providing chemoprophylaxis to anyone in contact with infected individuals, and isolating the affected localities. However, these measures did not provide expected results in response to sporadic outbreaks. Additional strategies such as environmental sanitation, house improvements, and health education for village leaders and communities in the affected areas were introduced and implemented to eliminate rodents and fleas in their surrounds leading to decreased plague incidents in the affected communities [7,8]. Further, in Mbulu District, Tanzania, occasional cases of human plague were reported [23,44] and indicated continued spread from the primary locality. Studies conducted in these subsequently affected areas found the rodent host and flea vector, and the circulation of *Y. pestis* in the rodent host population was closely linked [10,45]. Despite this valuable knowledge, there is yet, limited information on the methods used by residents to control flea and rodent population and peoples' awareness about plague disease and related risks of flea bite in their surroundings remain unclear, potentially contributing to the persistence of plague in these areas. This information, when available could be used to devise the potential strategies and to prioritize the mitigation measures to curb the diseases and associated risks to humans, thereby serving lives.

In this study, we aimed to assess peoples' knowledge and awareness on plague disease, the risk factors related to flea bite and control measures related to the spread of plague disease in the foci. We assessed the following research questions; (i) To what extent are people in the plague foci familiar with the plague disease? (ii) What household practices and/ or other factors mentioned by the respondents influence flea bites? and (iii) What local methods are mostly used for both rodent and flea control in the study area. We hypothesized that residents in the plague foci will have different levels of familiarity with the plague disease making some people more vulnerable to plague infection. We also hypothesized that majority of the households will be experiencing flea bites influenced mostly by household practices such as keeping livestock in same residence with human.

## 6.2 Material and methods

### 6.2.1 Study area

The study was conducted in Mbulu district located in northern Tanzania at coordinates 03◦ 57′ 097″S, 35◦ 18′ 39.60″E, with altitudes ranging from 1000 to 2400m above sea level. The study specifically focused on two villages, Arri-Endesh and Mongahay (Fig 1). The selection of this area was based on the persistence of plague outbreaks over the last 12 years, following a relatively long period of asymptomatic infection [8,10,23]. The district has a population of 238 272 with an average household size of 5.5 [44].

The climate in the study area is semi-arid to sub-humid, characterized by biannual rainfall ranging from less than 400mm to more than 1200mm. Relative humidity ranges from 55% to 75% and mean annual temperature ranges from 15 to 24°C [46] and two rainy seasons, with a long rain season from March to May and short rain season from October to December.

The residents in the area practice mixed farming cultivating short-cycle crops such as beans in December and harvested in March. The long-cycle crops such as maize and peas are planted in December and harvested in July or August. Intercropping is a common practice, with maize, beans, and peas grown together, while onions, garlic, Irish potatoes, and sweet potatoes are often grown singly. Further, livestock grazing practice varies across the two seasons: with the livestock taken outside for grazing mostly in the fallow lands, during the rainy season while animals are fed hay and stalks harvested from the farms indoors during the dry season.

Across the studied communities, majority of the human dwellings were thatched and corrugated roof with mud walls and mud floor. Most of these houses were also located near farmlands (Fig 2). Crop harvests were stored inside the sleeping houses, while livestock were kept in corrals near the sleeping and/ or inside sleeping houses. Further, sleeping practices varied, with some houses having beds with mattress or mat, while some had only one bed with mattress and several mats to sleep on the floor. Also, some households had all or one the aforementioned features and a loft chamber on the roof with a sleeping mat (Fig 2). These characteristics were recorded since they are known to influence flea infestation and survival[7,47]and so might increase the risks of flea bite inside houses and potential transmission of plague.

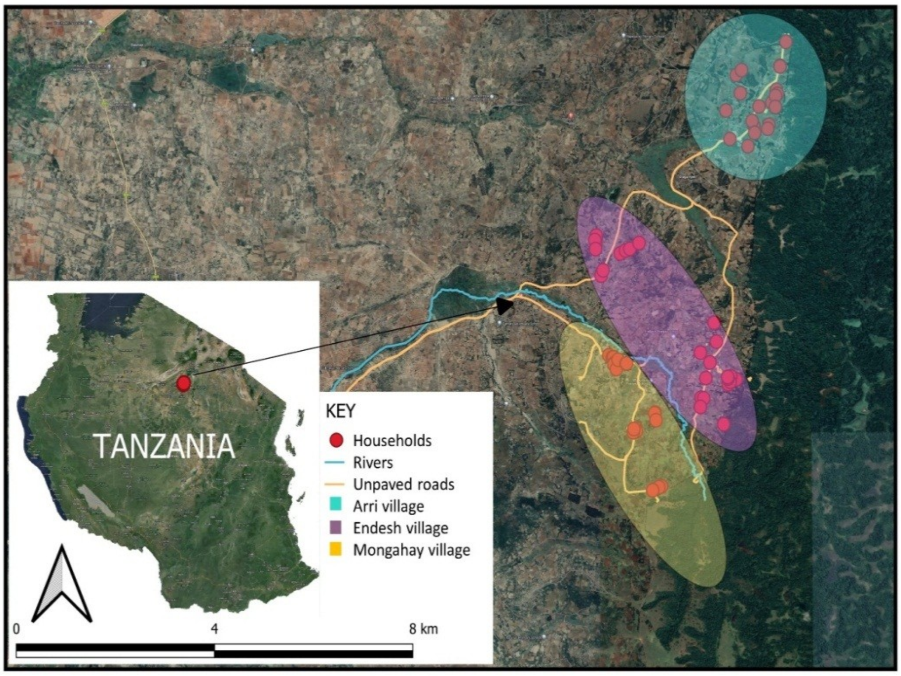


**Fig 1.** Map of Mbulu district in Tanzania showing locations of the study sites: Arri-Endeshi and Mongahay village communities surveyed. The map was generated using QGIS software (version 3.8.3 Zanzibar). Based layer sourced from Esri maps: https://esri.maps.arcgis.com/home/item.html?id=273ffd9a4c054d47843ed9642ecb143e, licensed under the Esri Master License Agreement; https://goto.arcgis.com/termsofuse/viewtermsofuse. Administrative boundaries and base layer image obtained from Administrative boundaries and the base layer image were downloaded from Open Street Map (google hybrid) https://www.nbs.go.tz /nbs/takwimu/census2012/Districts_Shapefiles_2019.zip.

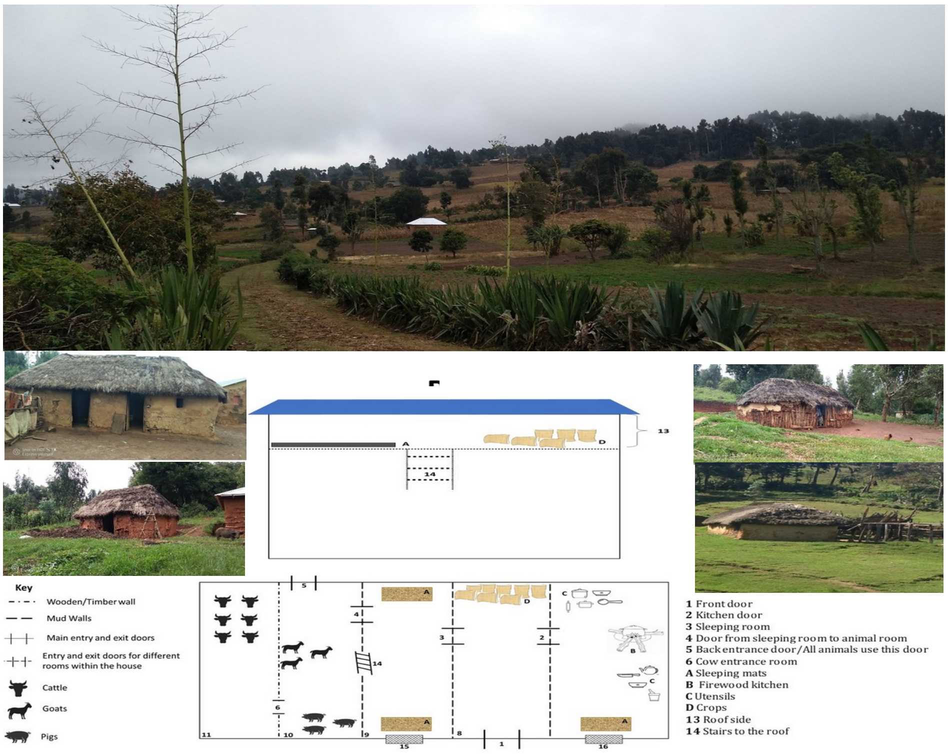


**Fig 2.** Illustration of the house design inside and outside surroundings in the study community

### 6.2.2 Sampling design

To select houses for questionnaire, we used a list of households from the villages log books accessed from the village government office. The name of each resident was recorded in an excel spread sheet and given a unique number. Further, the RANDBETWEEN function in excel was used to randomly select houses for this survey. Mongahay village had 261 houses, 46 of which were chosen for the questionnaire survey and 64 households taken Arri-endesh (N=531 houses). During data collection, not all household owners were present even after multiple visits perhaps were away for the farming activities so, we selected additional houses to fill the gap enabling us to survey 100 households in total. All interviews were conducted with either the head of the house or any adult household member chosen to represent the head of the house for the interview.

The questionnaire included both open and closed ended questions and aimed to understand information on the plague, the existence of fleas and rodent in their household environments, flea bites, social factors associated with the plague disease as well as the methods employed by residents to control both rodent and flea. Before beginning the interview, we briefly explained the purpose of the research to the participant and thereafter a written consent was collected from each participant. We did not ask respondents of their names during interview to ensure confidence in them and confidentiality of the data. All interviews were conducted in Kiswahili, with notes taken to ensure accuracy and reliability of the gathered data. Field data collection for this particular study was completed for 3 months.

### 6.3 Data analysis

Before analysing the data, the responses from the survey were entered into a spread sheet and reviewed for errors with some coded to support some of the analysis. For instance, a question about experiencing flea bites was coded as 1 for “Yes replies” and 0 for “No replies” to create binomial response variable data. Also, questions with multiple responses were sorted based on their similarities and presented as percentage. To assess the extent of respondents' knowledge about plague disease in the foci, their experiences with flea bites throughout different seasons, and the methods they use to control rodents and fleas, we did a descriptive analysis. Furthermore, to assess factors that influence the probability of flea bites in the residential areas, we build a generalized linear model (GLM) implemented in the MASS package. The first model included four factors; the presence of livestock, animal corral, flea seasonality and house type as predictor variables. The response variables were the "the “yes" and "no" indicating occurrence or absence of flea bites. The relative influence of each variable in the model was evaluated by using drop 1 function, deleting non-significant model term in a backward step-wise process until the final model was reached. At each model step, the significant effect of each variable was assessed independently using the Wald test [48]. The best model fitting the data was chosen using the Akaike Information Criterion (AIC). Furthermore, a tab model function from the sjPlot package was used to generate a table summarizing the results of the best model and provide information including odds ratios, confidence intervals, p-values. A p-value less than 0.05 was considered significant. The data analysis was conducted in R version 4.3.1.

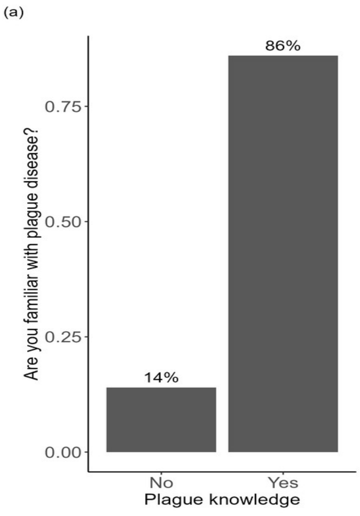
## 6.4 Results

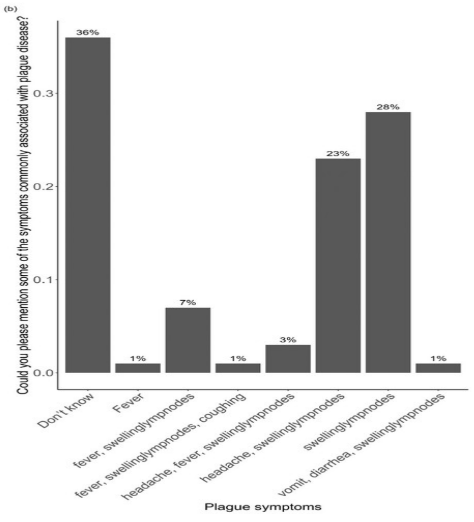
### 6.4.1 Demographic information

A total of 100 residents were interviewed, of which 63% were male and 37% were female. The respondent age ranged from 19 to 91 years old. Irrespective of gender, the majority of the interviewees were between the ages of 19-60 years old (86%). Approximately 68% of the houses had a household size of six or more people, with the average household size of 7 members per household. In the study area, the majority of the respondents (85%) had primary education. Also, the majority (93%) were engaged in farming and livestock keeping, while others (7%) pursued different occupations, such as business and government employment and few were secondary school students.

### 6.4.2 Knowledge and awareness of the plague disease among studied local community

The majority of respondents (86%) were aware of the plague disease (Fig 3a). However, when asked about specific symptoms of the disease, 36% were unable to provide a response. Interestingly, about 51% respondents mentioned swelling lymph nodes as a common symptom (Fig 3b). But when asked if they or any member of their household had ever been affected by plague, the majority 82% responded with NO and only 18% reported to either have experienced personal illness or the occurrence of plague within their family members (Fig 3c). Additionally, when asked about where they would initially seek assistance or medical help in the event of experiencing plague, the majority of respondents (82%) indicated a preference for hospital. However, a few respondents mentioned alternative sources of help, such as their father (1%), health officers (1%), or village leaders (6%). Moreover, the participants' understanding of the plague's seasonality was evident, with a majority (62%) associating human plague cases with the long rain season. In contrast, only few (2%) linked the disease to the short rainy season (Fig 3d).





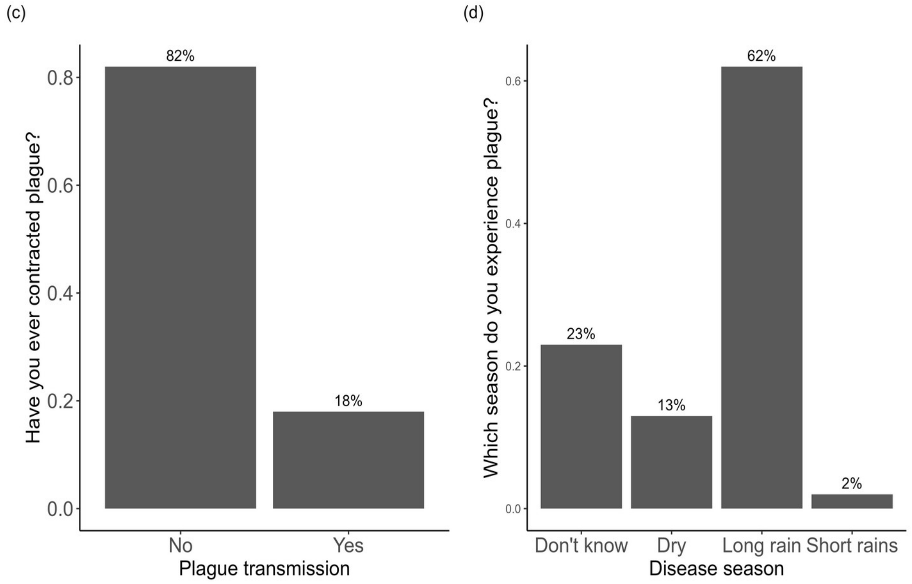


**Fig 3.** The frequency of responses in relation to different aspects of plague knowledge and experiences, (a) plague knowledge, (b) plague symptoms, (c) plague transmissions and (d) human plague cases seasonality

### 6.4.3 Factors that influence flea bite

Most participants (97%) reported experiencing flea bites in their domestic environments. When asked about the specific time of the season when they mostly get these flea bites, majority (84%) indicated the dry season. Also, our GLM results showed that residents who keep livestock have 2.77 times higher odds of experiencing flea bites than those who do not keep livestock. However, this association was not statistically significant (Table 1; Fig 4).

**Table 1.** Odds ratio and corresponding confidence intervals and p-value from the final best-fitting Generalized Linear Model (GLM)

| | | | **Flea bite** | | | |
|---|---|---|---|---|---|---|
| *Predictors* | *(No respondense)* | *Estimate (SE)* | *Odds Ratios* | *95%CI* | *Z-value* | *p-value* |
| (Intercept) | | 1.87(0.76) | 6.5 | 1.80 – 41.57 | 2.46 | **0.014** |
| keeplivestock [Yes] | 76 | 1.02(0.92) | 2.77 | 0.36 – 15.80 | 1.11 | 0.267 |
| keeplivestock [No] | 24 | | | | | |

**Fig 4.** Effect predicted by generalized linear model assessing the effect of livestock keeping on probability of flea bite. The analysis revealed that, respondent who reported keeping livestock had a higher probability of experiencing flea bite compared to those who did not keep livestock.

### 6.4.4 Methods employed by the residents for controlling rodents and fleas

The residence in the community used a combination of chemical and natural methods to control rodents and fleas in their homes. These methods were used either separately or blended or blended natural and chemical approaches (Fig 5a). On rodent control, the application methods were relatively similar. About 38% of the response reported to be using only chemical methods, 31% relied on natural methods, and 30% of the respondents reported to employ both chemical and natural methods. The chemical methods used for rodent control specifically involved the use of rodenticide, while the local methods included the presence of cat and baited traps.

In flea control, the majority of the respondents (54%) relied on local methods (such as sprinkling water on the floor and spreading pyrethrum flowers inside house), followed by 26% who preferred chemical methods (26%). A small proportion of the respondents (17%) reported using a combination of both local and chemical methods. The chemical methods used for flea control involved the use of indoor sprays, such as Rungu, (which contain carbamate and pyrethroid insecticide like propoxur and Tetramethrin respectively), and other insecticides such as Dursban, Diazinon and Servin powder (dudu dust). Further, some respondents even mentioned combining certain chemicals, such as Dursban and Diazioan. Additionally, the local-based methods used for flea control consisted of marigold plants, locally known as “Majani ya bangi- translated as resembling marijuana leaves”, pyrethrum flowers and sprinkle water on the house floor, either alone or mixed with powder soap. Furthermore, upon asked on the effectiveness of these local methods in flea control, the respondents said the impacts varied from few days to several months (Fig. 5b).

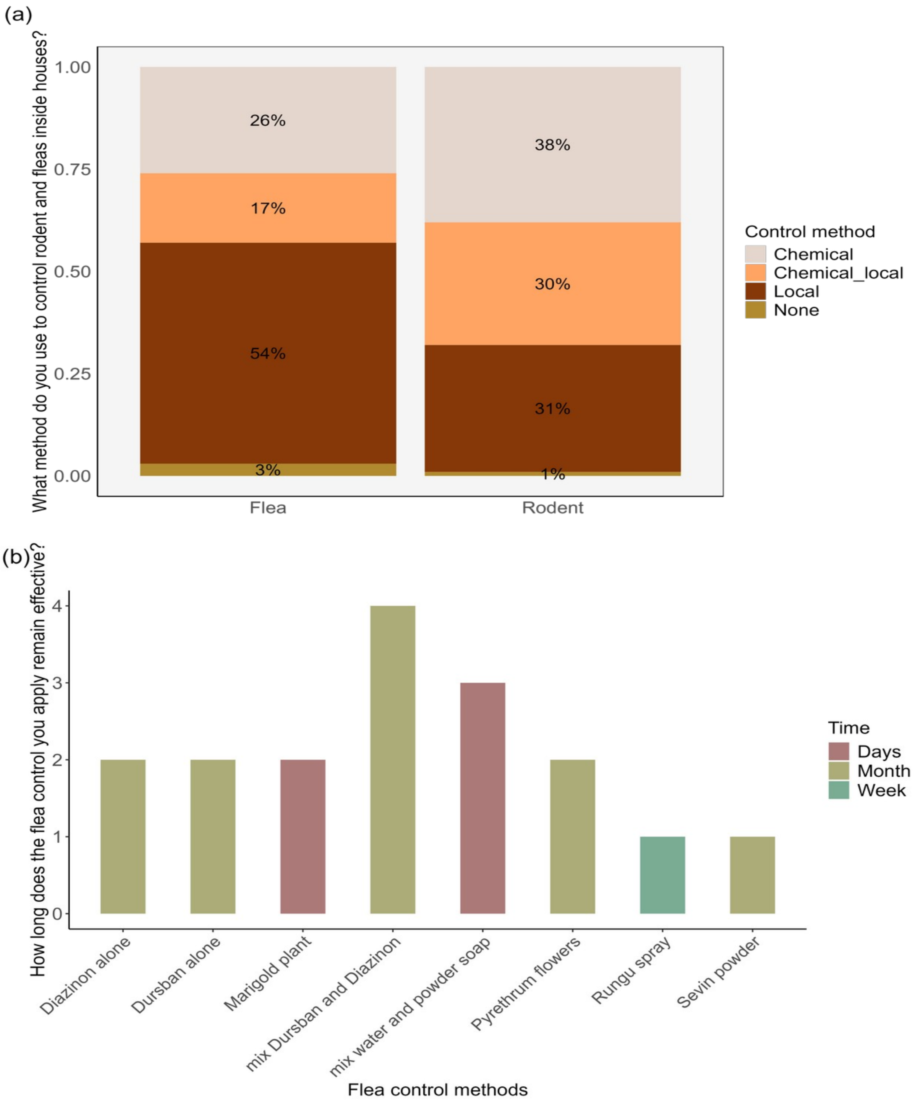


**Fig 5. (a)** Percentage of respondents using rodent and flea control methods and (b) Duration of effectiveness of flea control methods as claimed by respondents after application.

## 6.5 Discussion

The purpose of this study was to examine the general knowledge and awareness of the plague disease within the human population in the foci, explore factors that influence flea bite in domestic settings, and assess the commonly used methods employed by residents for rodent and flea control. We found majority of participants were aware with the plague disease and most of them claimed to observe human plague cases during the long rain season. Also, most of the participants reported to experience flea bite in their domestic settings, especially during the dry season. Presence of livestock in domestic areas was found to increase the probability of being bitten by fleas. Furthermore, residents reported to use both local and chemical methods to control rodents and flea inside houses. In flea control, majority of respondents relied on the locally-available methods, followed by chemicals and few used a combination of both. Respondents claimed that the effectiveness of flea control methods varied from few days to several months.

The majority of participants reported their awareness and familiarity with the existence of the plague disease. However, there were some gaps in specific knowledge. Although over 50% correctly mentioned swelling of lymph nodes as a common symptom, almost one-third were unable to name any symptoms. This indicates a general awareness of plague exists, but details of clinical symptoms are lacking to some residents. Inability to identify symptoms may result in delays in seeking medical attention, which can lead to higher rates of disease transmissions and potential deaths. For instance, [49] reported that early detection and immediate treatments are crucial for controlling the spread of the disease. Further, the majority of participants knew of the association between rainy season and plague cases which may likely be associated with the decrease in rodent abundance in these areas observed during this season in our previous study [50]. This reduction in rodent abundance disrupts the natural reservoir dynamics, potentially increasing the likelihood of infected fleas seeking alternative hosts, such as humans or domestic animals. This increased contact between fleas and human in the absence of sufficient rodent population increases the risk of plague transmission to humans [51,52]. Almost all participants reported to have experienced flea bites in their homes, indicating greater flea-human interactions. In another study (Kessy et al-*in review*), we found seven flea species in human habitations which are potentially interacting with humans in this study area.  The presence of multiple flea species increases the likelihood of plague bacteria spreading to human population [1], hence understanding these interactions and the specific species and competence as vector is crucial for informing the effective plague control and prevention measures.

The majority of the respondents reported to experience these flea bite during dry season. This is possibly due to the favourable conditions in this season which promotes flea growth and hence increase the incidence of flea bites. Flea-host-seeking behaviour is influenced by environmental cues like temperature, humidity, and host odours, all of which can be affected by climatic changes. Warmer temperatures and humidity level above 70% during the dry season, for instance, promote flea development and population growth [53,54]. Further, presence of livestock was among factors that showed higher risk of flea bites. This is because the presence of livestock enhances flea development and survival because they provide a source of blood for adult fleas to feed, shelter and nesting sites and serve as vehicle for fleas spreading to the new hosts and environments when moving between different areas or come into contact with other hosts [55,56]. Importantly, our previous study found that rodent abundance was higher in agricultural and forest habitats within the study area [50]. Since most of the surveyed households were located closer to the farm and forest habitats, this created an ideal condition for rodents and fleas to disperse between the houses and wild places. Furthermore, the existing human practices such as storing crop indoors and cattle corals around human abodes could be providing favourable sites for rodents and flea breeding facilitating more contacts with humans, thereby directly influencing persistence and repeated human plague cases in the area. Additionally, the existing poor living style such as sleeping on mats on mud floor increased risks to flea bites and potential disease transmission as has been observed in Lushoto District, Tanzania- another plague focus [7]. Despite the existing health challenges, yet local people demonstrated efforts to minimizing the negative impacts of the disease. Both chemical and locally-based methods were in use for controlling rodents and fleas in their abodes. Rat poisoning was the main chemical choice while the majority used cats as local biological control. These findings indicate that the integrated pest management practices were common in the rural communities corroborating with previous studies on the existing use of integrated pest management in rural communities [57,58]. Interestingly, majority of the respondents relied only on plant-based approaches for flea control, either spreading leaves of certain plant species on the floor and/or under the bed to repel or kill fleas indoor. Common local methods included using marigold leaves (*Tagetes erecta*), pyrethrum flowers (*Chrysanthemum cinerariifolium*) and water sprinkling to deter or minimize risks of flea bites, similar to reports from previous studies on using botanical repellents for the insect pests [59-61]. Further, the existing use of agricultural insecticide for controlling fleas has potential danger due to lack of proper protocols, guidelines or consideration of the potential health risks due to exposure to these chemicals and their residual effects in residential settings. The majority of the chemicals used were pesticides primarily applied to kill pests in the fields, with some also being sprayed inside houses. However, it is important to note that the pesticides used indoors were not specifically intended for indoor insect control, making their use unacceptable. Despite this, many respondents reported that these pesticides were helpful as they experienced a significant reduction in flea bite even after more than three months of application. This suggests that, these chemicals will remain in use for longer time in these rural communities if alternative interventions are not available. Further, unregulated chemical use may lead to flea resistant [40-42] thereby further accelerating disease persistence in the foci.

Finally, our findings have several implications on the human health in these and similar rural communities in the tropics where plague disease is still persistent. One, to improve the health of the local communities, continued efforts to control flea and reduce risks of plague transmissions are needed. This can be achieved through increasing local awareness about the clinical symptoms of plague across the village communities, instituting cleanness policy within the village communities and deterring the cultural practices that may increase the risk of flea bite in their surroundings through community workshops and educational campaigns. Second, our results suggest poor living condition of most rural communities increase their exposure to flea bites and potential disease infection, the local government should strive to engage the local communities in economic production ventures to help lift them out of poverty to afford a decent life. Improved living standards due to elevated incomes will greatly reduce the social and cultural factors exposing them to the bites and disease thereby eradicating plague in these rural communities. Third, local transmission cycle of the disease requires further study to inform the potential improvement of the currently used local control methods. Such studies should also look into the efficacy of the plant species and the appropriate use of proper insecticides used to control flea population. Use of these integrated approaches may greatly diminish the disease persistence in these rural communities.

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**Author contributions**

Conceptualization, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing: **Stella T. Kessy**

Supervision, Writing – Review & Editing: **Alfan A. Rija**

**Data availability statement**

All data analyzed during this study, R codes used and figures produced are included in this published article under a public repository link https://datadryad.org/stash/share/iDOvCqdJs68ODhDL3g_t4GiAxwd2P3TLRXTFhb7tlw4

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# CHAPTER SEVEN

## 7.0 GENERAL DISCUSSION

### 7.1 General Discussion

The purpose of this study was to examine the ecological and socio factors that contribute to the ongoing persistence of plague in Mbulu district, Tanzania. We examined rodent abundance, diversity and community structure as well as the population dynamics of two rodent species *M. natalensis* and *L. makundii*. We also assessed the pattern of flea infestation between localities and habitats and explored the local community knowledge and practices about plague disease, as well as the methods used to control rodent and flea.

The study found that plague persistent locality had higher rodent abundance, species richness and diversity than non-persistent locality. The abundance in the farm and forest habitats was higher than that of house premises, indicating that these particular habitats play a role in shaping the rodent community structure. This finding aligns with previous study, Bonvicino *et al.* (2015), which found that countries with high rodent species richness in plague foci are more likely to maintain plague in the wild. Furthermore, higher rodent abundance was found during short rain season, which may have been due to breeding and reproduction activities that normally occur during long rain seasons. A recent study conducted in the same study area found that majority of rodents tested positive for *Y. petsis* during this short rain season and the long rain season (Haikukutu *et al.,* 2022). This observation is consistent with the findings of Davis *et al.* (2005) that reviewed studies on rodent reservoirs for plague and other zoonoses and found that there is frequently a positive correlation between host abundance and host prevalence at a seasonal time frame.

In addition, the study revealed three distinct communities of rodents based on habitat type. Specifically, rodent community structure varied significantly between forest, house, and farm habitats indicating potential differences in resource utilization patterns, such as food and habitats. These variations likely contribute to the observed differences in community structure.

Furthermore, the study revealed distinct pattern of abundance and proportion of breeding females of the two species, *M. natalensis* and *L. makundii* across various habitats, seasons and localities. The abundance of both species was significantly higher in plague persistent locality compared to non-persistent localities. Previous studies by Samia *et al.* (2011) and Sun *et al*. (2019) has shown the abundance and distribution of rodent hosts is directly linked to plague persistence which also corroborates with our study findings. This may suggest that the disease is being maintained by the persistent locality in our study area. Also, the proportion of breeding females in both species varied between habitats and seasons. This is similar to other study Pinot et al 2014, which indicated that such variation in breeding paatern may be influenced by factors such as food availability.

On top of that, the study found that flea abundance did not differ between localities, habitats, and seasons, but the probability of flea infestation was significantly higher in the plague persistent locality and during the short rain season. Despite the fact that, there are no significant differences in flea abundance between localities, the composition of flea species and the levels of infestation on individual host species may still be important driver of disease persistence. Several studies have found that flea species composition and their infestation levels on different hosts species are related to plague persistence (Gage et al 2005; Stenseth *et al.,* 2006). Additionally, the study observed that rodents were more frequently infested with fleas during the short rain season, which may be linked to warmer and more humid conditions that favor flea development and survival. Previous study in the same area observed higher rodent abundance (Kessy *et al.,*2023) and higher rodents with positive *Y. pestis* (Haikukutu *et al.,* 2022) during short rain season, indicating that during this season there is a higher probability of plague bacteria circulating between the rodent host and the flea vector influencing a risk of bacteria spreading to other hosts.

Moreover, the study found that flea abundance inside houses fluctuated at the end and beginning of each season during the sampling period which might have been impacted by the seasonal variations. Although, we did not monitor temperature and humidity changes indoor, still, variations outside may have had an effect indoor. Several studies have reported how seasonalchanges influence flea growth and survival (Kreppel *et al.,* 2016; Samuel *et al.,* 2022). According to Alderson *et al.,* (2020) these changes affect not only the flea abundance but also flea behavior and transmission rates. Some of the effects mentioned includes, increase in flea vector mortality due to *Y. pestis* infection and enhancing biofilm blockage hence increasing flea biting activities. Also, the study found that dry season had higher flea abundance than the long rain season and short rain season and some flea species such as *Pullex irritans*, *Ctenocephalides canis*, and *Ctenocephalides felis* were more abundant than other species. This may have been contributed by the presence of variety hosts within houses and warmer condition. Further, houses with poor sanitation and hygiene practices, such as keeping livestock within sleeping houses, showed higher probability of having more fleas. The findings are consistent with previous studies Andrianaivoarimanana *et al.* (2019) and Duplantier *et al*. (2005) which also reported that the presence of food and shelter in such environments contributes to increased flea infetations.

Furthermore, studies on knowledge and practices related to plague persistence in the plague foci found that, participants had a high level of awareness and understanding about plague disease, with the majority linking human plague cases with the long rainy season. This is consistent with previous study that have found a link between rainfall and plague cases (Cavanaugh *et al.,* 1972; Parmenter *et al.,* 1999). Also, almost all participants reported their experiences with flea bites, with majority reporting a higher frequency of bites during the dry season. Furthermore, several natural and chemical control strategies for rodent and flea were reported to be employed in the house premises. Our findings are simlar with prior studies that have reported integrated pest management practices are common in rural communities (Roomaney *et al.,* 2012; Saez *et al.,* 2018). Notably, the majority of participants reported using natural flea control methods, with quarter of the respondents reporting using insecticides, sometimes in combination. The use of multiple insecticide classes have been shown to promote flea resistant in Madagascar (Boyer *et al.,* 2014; Miarinjara and Boyer, 2016) and such risks of flea resistance have been reported in the study area (Rugalema and Mnyone, 2020), which may hinder flea vector control strategies in the foci. Further, participants reported durations of efficacy for the used flea control methods, ranging from short-term effects lasting days to longer-term effects lasting months. This variation in efficacy highlights the importance of continuing to investigate, improve, and assess these various control strategies in order to target not only flea vector pathogen of plague but also other multiple flea-borne pathogen present in the foci.

# CHAPTER EIGHT

## 8.0 GENERAL CONLUSION AND RECOMMENDATIONS

### 8.1 General Conclusion

The study provides information on the abundance, diversity, and community structure of rodents in bubonic plague endemic areas. It emphasizes the need of understanding local-scale population dynamics in order to develop effective disease control strategies. It also contributes to our understanding of rodent population dynamics and their implications for plague persistence as well as highlighting the importance of localized ecological factors, such as habitat types and seasonal variations, in shaping potential disease reservoirs. Furthermore, the study highlights the complex interactions between flea communities, rodent hosts, and environmental factors in plague foci. It emphasizes the importance of studying flea infestations to predict transmission risks of flea-borne diseases and emphasizes the need to incorporate local climatic conditions and rodent species traits into flea control and prevention strategies. Moreover, the study highlights the importance of considering environmental factors and household behaviors and practices into account when developing domestic flea control methods. It also emphasizes the need of raising awareness and designing effective control methods for controlling fleas and lower the risk of plague transmission in the local communities.

### 8.2 Recommendations

The study findings have led to the formation of the following recommendations;

i. Further study is needed to examine the association between seropositive rates for plague bacteria in rodent and flea species across different localities, while also considering potential variations influenced by seasonal factors.
ii. Additional studies are also needed to explore specific rodent host-flea interactions and their role as pathogen vector in the foci.

iii. Efforts should be made to further explore and advance the use of natural methods for flea control, with a particular focus on the designing and developing sustainable and eco-friendly approaches for long-term prevention of flea infestations in our local communities.

iv. Continued efforts are necessary to raise awareness in the affected areas and to remind people about proper practices that may effectively decrease and/ or prevent rodents and flea infestation in their domestic settings. This can minimize the occurrence of multiple interactions between rodent, flea, and humans.

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# APPENDICES

## Appendix 1: Questionnaire used in the study

My name is Stella Kessy. I am a PhD student from Sokoine University of Agriculture. I am conducting a research study to gather information on people's knowledge and attitudes regarding the plague disease, as well as the methods employed to control rodent and flea ectoparasites among community residents. The questionnaire consists of 16 questions, which will take less than 15 minutes to complete. Additionally, during the interview, the interviewer will make observations of the house surroundings and note down relevant information. Please be assured that all responses will be treated as confidential

Contact person; Stella Kessy
Tell; 0782192143
Email; kessystella78@gmail.com

### I. Identifications

1) Household ID
2) Village
3) Ward
4) District
5) Interview date
6) Name of interviewer

### II. Demographic Information

| | Sex | Marital status | Age(yrs) | Education level | Main occupation | Household size |
|---|---|---|---|---|---|---|
| Respondent | | | | | | |

**Knowledge and Attitudes Regarding the Plague Disease in the Community**

1. Are you familiar with the disease called plague?
2. Are you aware of the symptoms associated with the plague?

A) Yes; If yes mention some of the symptoms commonly associated with the plague
B) No

3. Have you or any member of your household experienced or been affected by the plague?

A) Yes
B) No

4. In which season or time of the year do human plague outbreaks primarily occur?

A) Dry season
B) Long rain season
C) Short rain season

5. If you or a family member were to experience plague, where would you initially seek assistance or medical help?
6. Do you engage in hunting small mammals?

A) Yes; If yes; which wild animals do you typically hunt?
B) No

7. Do you include bush meat as part of your regular diet?

A) Yes
B) No

8. Do you commonly encounter rodents inside houses?

A) Yes
B) No

9. Do you frequently experience flea bites inside your house?
A) Yes
B) No

10. During which time of the year do you tend to experience a higher occurrence of fleas indoors?
A) Dry season
B) Long rain season
C) Short rain season

11. During which time of the year do you mostly observe a higher presence of rodents inside houses?
A) Dry season
B) Long rain season
C) Short rain season

12. What methods do you use to control fleas within residential premises?( provide a list of the specific control methods used to manage fleas inside your house)

13. If you have rodents in your residential premises, What methods do you employ to control them? (provide a list of the specific control methods used to manage rodents inside your house)

14. Do you keep cattle in your home?
A) Yes; if yes, provide a list of the livestock that are currently present in your home
B) No
15. Are your animals housed inside the sleeping quarters or do they sleep outside?
A) Inside
B) Outside

16. Are your crops stored inside the sleeping houses or outside?
C) Inside
D) Outside

## III. Observations

1. House type
2. Wall and floor type
3. Types of bedding
4. Absence or presence of livestock

## Appendix 2: Dodoso

Jina langu ni Stella Kessy, ni mwanafunzi wa shahada ya uzamili (PhD) kutoka Chuo Kikuu cha Kilimo Sokoine. Ninafanya utafiti wa kukusanya taarifa za uelewa na mtazamo wa watu kuhusu ugonjwa wa tauni, pamoja na njia zinazotumiwa kudhibiti panya na viroboto katika mazingira mnayoishii. Maswali 16 yameandaliwa na itachukua chini ya dakika 15 kukamilisha kujibu dodoso. Aidha, baada wa mahojiano, msaili ataangalia mazingira ya nyumba na kukusanya taarifa muhimu. Tafadhali, amini kuwa majibu yote yatahifadhiwa kwa usiri.

Mtu wa mawasiliano; Stella Kessy
Namba ya simu; 0782192143
Barua pepe; kessystella78@gmail.com

### I. Utambulisho

1) Namba ya nyumba
2) Kijiji
3) Kata
4) Wilaya
5) Tarehe ya usaili
6) Jina la msailiwa

### II. Demografia

| | Jinsia | Umeoa/ Umeolewa | Umri | Elimu | Aina ya kazi | Idadi ya wanafamilia |
|---|---|---|---|---|---|---|
| Msailiwa | | | | | | |

### III. Ufahamu na mtizamo hukusu ugonjwa wa Tauni wa wanajamii

1. Unafahamu ugonjwa wa tauni?
2. Unajua dalili za ugonjwa?

A) Ndiyo; Kama ndiyo, zitaje
B) Hapana

3. Ulishawahi kukabiliana na ugonjwa wa tauni hata ukasababisha kifo kwa familia yako au mwanakaya?

A) Ndiyo
B) Hapana

4. Ni msimu gani wa mwaka ugonjwa huu hutokea?

A) Kiangazi
B) Masika
C) Vuli

5. Unachukua hatua gani kujikinga pale unapopata dalili za ugonjwa au mwanafamilia ndani ya nyumba akiupata?

6. Je unawinda wanyama wadogo?

A) Ndiyo; kama ndiyo taja wanyama unaowawinda
B) Hapana

7. Je unatumia wanyama wadogo kama chakula?

A) Ndiyo
B) Hapana

8. Je tabia ya kuwa na panya ndani ya nyumba?

A) Ndiyo
B) Hapana

9. Je kuna tabia ya kung’antwa na voroboto ndani ya nyumba?

A) Ndiyo
B) Hapana

10. Ni wakati gani katika mwaka panya wanatokea kwa wingi ndani ya nyumba?

A) Kiangazi
B) Masika
C) Vuli

11. Ni wakati gani katika mwaka viroboto wanatokea kwa wingi ndani ya nyumba?

A) Kiangazi
B) Masika
C) Vuli

12. Ni njia gani unatumia kudhibiti viroboto ndani ya nyumba yako? (Tafadhali toa orodha ya njia maalum za udhibiti unazotumia kudhibiti viroboto ndani ya nyumba yako)

13. Ikiwa una panya katika eneo lako la makazi yako, unatumia njia gani kuwadhibiti? (Tafadhali toa orodha ya njia maalum za udhibiti unazotumia kudhibiti panya ndani ya nyumba yako).

14. Je unamifugo kwenye kaya yako?

A) Ndiyo; kama ndiyo, taja aina ya mifugo iliyopo kwenye kaya yako
B) Hapana

15. Je, wanyama wako hulazwa ndani au nje ya sehemu ya kulala?

A) Ndani
B) Nje

16. Je, mazao yako huhifadhiwa ndani au nje ya nyumba za kulala?

A) Ndani
B) Nje

## IV. Kuangalia

1. Aina ya nyumba
2. Aina ya ukuta na sakafu
3. Aina za malazi
4. Kuwepo au kutokuwepo kwa mifugo

## Kuhusu Tasnifu Hii

Utafiti huu ulilenga kutathmini sababu zinazosababisha kuendelea kwa tauni katika Wilaya ya Mbulu, Tanzania. Ugonjwa huu huenezwa na panya, kusambazwa na mdudu kiroboto na kusababishwa na bakteria *Yersinia pestis*. Utafiti ulichunguza wingi na spishi za panya na viroboto, na muundo wa jamii katika maeneo tofauti (eneo lenye mlipuko wa tauni na lisilokuwa na historia ya tauni), makazi (mashambani na misitituni) na misimu tofauti. Pia utafiti huu uliangalia mabadiliko ya wingi wa viroboto ndani ya nyumba katika kipindi chote cha utafiti na kutathmini uelewa wa watu juu ya ugonjwa wa tauni na tabia hatarishi zinazohusiana na kuongezeka kwa kung'wata na viroboto. Maeneo yenye milipuko ya tauni na makazi ya shamba na misitu yalionyesha wingi mkubwa wa panya na viroboto katika msimu wa mvua fupi. Wingi wa viroboto ndani ya nyumba ulitegemea misimu, usafi, na uwepo wa wanyama. Wanajamii walionyesha kuwa na uelewa juu ya ugonjwa wa tauni na waliripoti kuumwa na viroboto mara kwa mara wakati wa kiangazi. Pia wanajamii walidhibiti viroboto kwa njia za asili na kemikali, hali iliyoonyesha utumiaji wa mara kwa mara wa kemikali kwa hawa viroboto unaweza kutengeneza usugu ambao utapunguza nguvu ya njia zinazotumiwa kuwazuia. Matokeo ya utafiti huu yanasisitiza umuhimu wa kuelewa namna makazi, misimu na tabia za binadamu zinavochangia kuongezeka kwa panya na viroboto kwenye maeneo yaliyoathiriwa na tauni. Pia, yanatoa habari muhimu itakayo tumika na watafiti pamoja na wanajamii kupanga na kubuni mikakati madhubuti ya kudhibiti panya na viroboto hasa maeneo yaliyo na milipuko ya ugonjwa kwa binadamu.