

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

Stella T. Kessy^{a,b,d,*}, RhodesH. Makundi^{b,c}, Apia W. Massawe^{b,c}, Alfian A. Rija^a

^a Department of Wildlife Management, Sokoine University of Agriculture, P.O. Box 3073, CHUO KIKUU, Morogoro, Tanzania

^b The African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE IRPM&BT), Tanzania

^c Institute of Pest Management, Sokoine University of Agriculture, P. O. Box 3110, Morogoro, Tanzania

^d School of Life Science and Bio-Engineering (LiSBE), Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha, Tanzania

ARTICLE INFO

Keywords:

Plague
Flea abundance
Flea community
Flea-rodent interactions

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 lypus* 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; Zajac 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 such 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

* Corresponding author. Department of Wildlife Management, Sokoine University of Agriculture, P.O. Box 3073, CHUO KIKUU, Morogoro, Tanzania.
E-mail addresses: kessystella78@gmail.com (S.T. Kessy), rmakundi@yahoo.com (RhodesH. Makundi), apiamas@yahoo.com (A.W. Massawe), al.rija10@gmail.com (A.A. Rija).

<https://doi.org/10.1016/j.ijppaw.2024.100921>

Received 5 December 2023; Received in revised form 1 March 2024; Accepted 2 March 2024

Available online 4 March 2024

2213-2244/© 2024 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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, Neerincx 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 Hapold (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

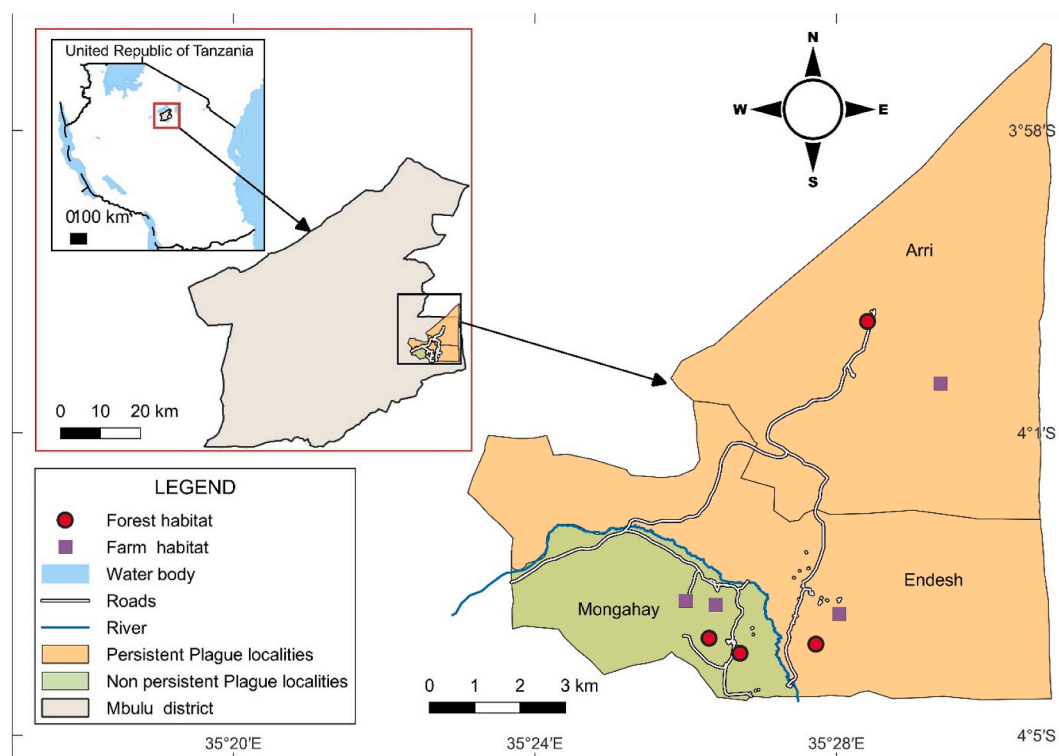


Fig. 1. A map of Mbulu district indicating the two study localities, Endesh-Arri (Persistent locality and Mongahay (non-persistent locality), along with the two study habitats (Farmland and forests) in each locality.

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 explore 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 assess 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 lypus* 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. lypus*, 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. lypus* 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. brasiliensis* abundance between habitats of each locality ($\chi^2 = 1.03$, df = 3, p = 0.79). Similarly, the abundance of *D. lypus* species varied significantly between rodent species ($\chi^2 = 26.16$, df = 11, p = 0.01). *Mastomys natalensis* had significantly higher abundance of *D. lypus* 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. lypus* 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
<i>Aethomys kaiseri</i>	17	0	17	6	0	6	12	4	7
<i>Arvicanthis</i> sp. “Masai Mara”	6	0	6	2	0	2	0	0	8
<i>Grammomys</i> cf. <i>macmillani</i>	4	16	20	0	3	3	7	4	12
<i>Graphiurus</i> cf. <i>raptor</i>	0	5	5	0	4	4	0	0	9
<i>Lemniscomys striatus</i>	0	14	14	0	1	1	1	1	13
<i>Lemniscomys zebra</i>	2	0	2	3	0	3	1	0	4
<i>Lophuromys makundii</i>	0	32	32	0	0	0	9	12	11
<i>Mastomys natalensis</i>	113	9	122	54	31	85	49	36	122
<i>Mus</i> cf. <i>gratus</i>	0	2	2	0	1	1	0	2	1
<i>Mus minutoides</i>	8	0	8	1	0	1	6	2	1
<i>Praomys delectorum</i>	0	81	81	0	4	4	29	13	43
<i>Rattus rattus</i>	1	0	1	0	0	0	0	0	1

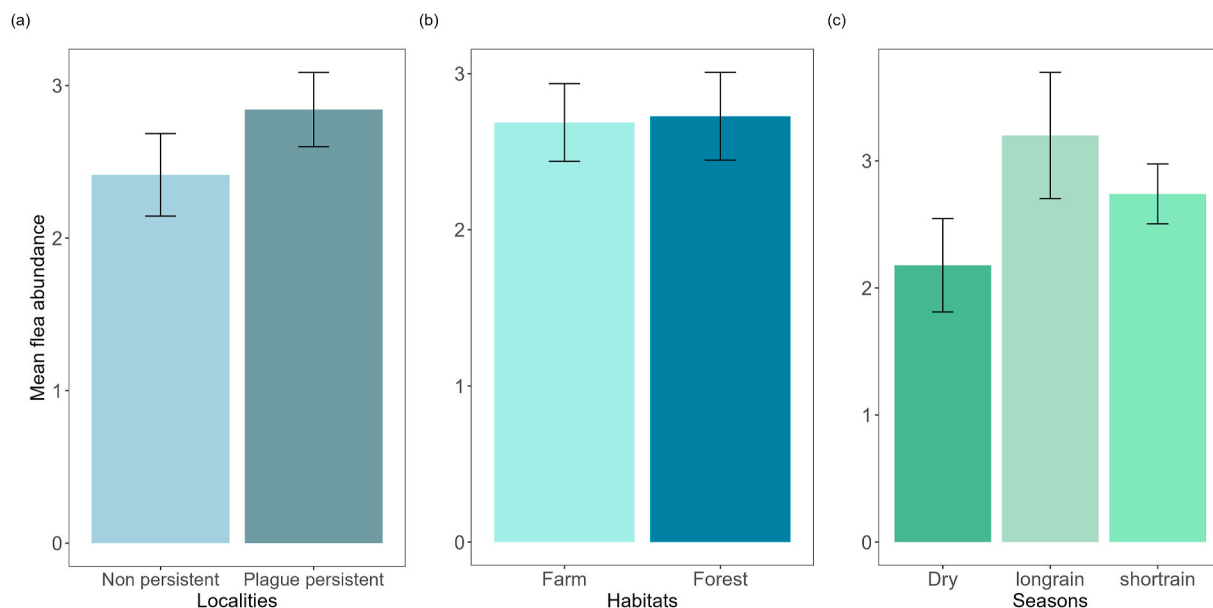


Fig. 2. Flea abundance in the (a) localities, (b) habitats and (c) seasons. Error bars represent the standard error. There were no statistically significant differences that were observed.

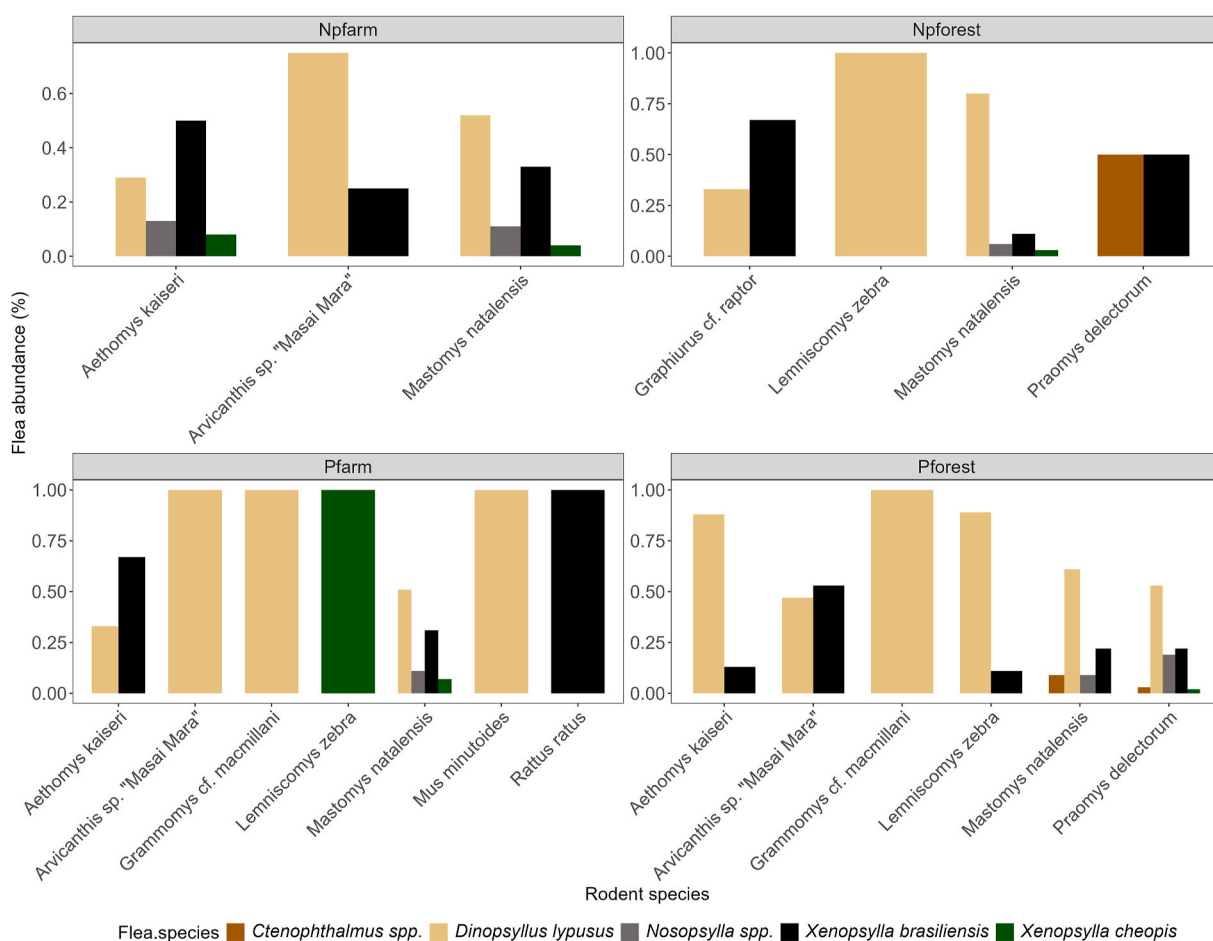


Fig. 3. Flea abundance for different flea species across habitat types in each locality and rodent species.

cheopis, *Dinopsyllus lypus*, and *Xenopsylla brasiliensis*) and group B consisting of only one species (*Ctenophthalmus* spp). Group A had a finer-scale separation of the two subgroups, with *Nosopsylla* spp. and

Xenopsylla cheopis clustering together and *Dinopsyllus lypus* and *Xenopsylla brasiliensis* forming a separate cluster. Furthermore, flea communities were structured based on habitat, with some flea species

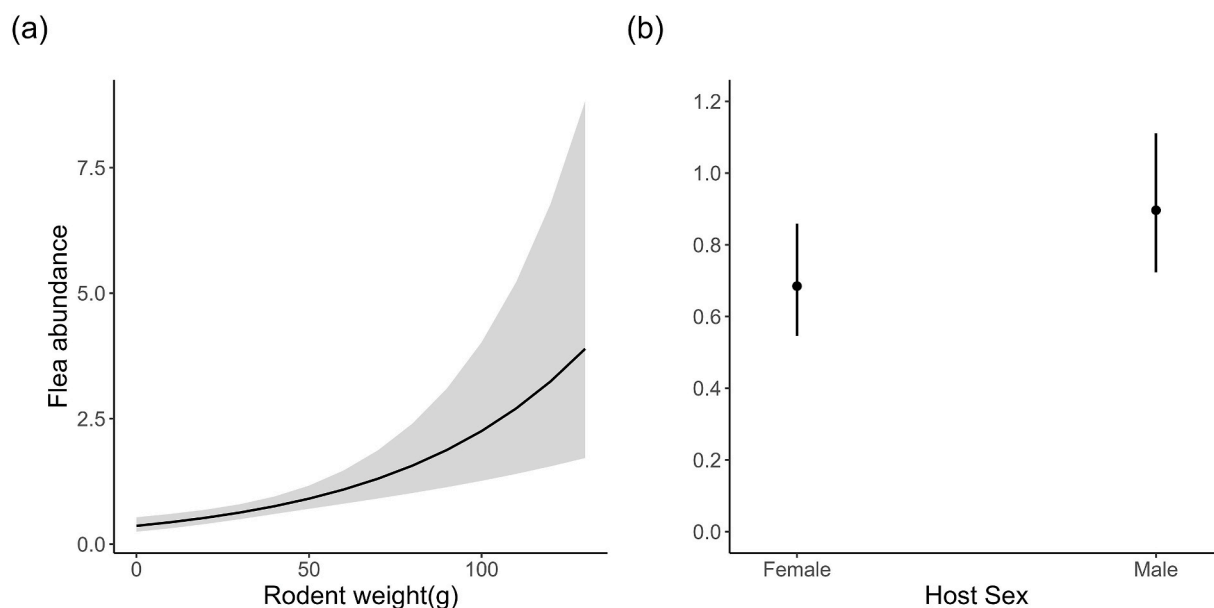


Fig. 4. Plots showing predicted effect of rodent traits on flea abundance, based on final best fitting generalized linear mixed model with a negative-binomial function. The plots (a) indicates that rodent weight increased with flea abundance. The gray shade in the plots represents the strength and direction 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 (\pm 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 lypus*, *X. brasiliensis*, *X. cheopis* and *Nosopsyllus* spp. which were found in both

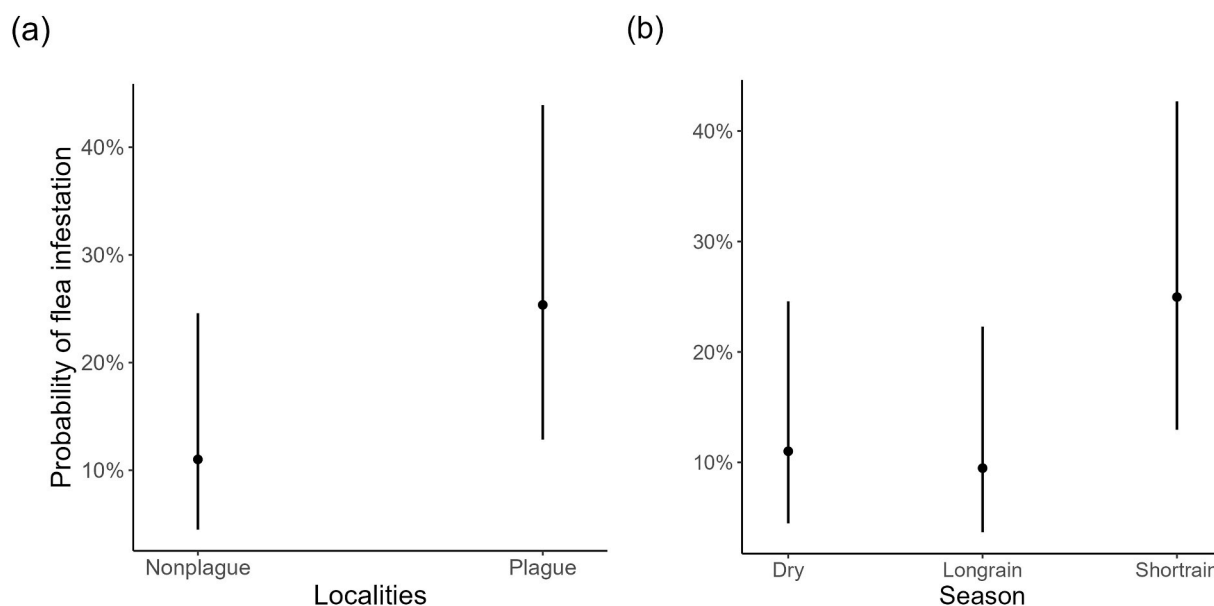


Fig. 5. Plot showing the predicted effect of locality and season on the probability of flea infestation, based on the final best-fitting generalized linear model with a binomial function. The analysis aimed to identify the factors that strongly influence flea infestation. The strongest predictors of 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.

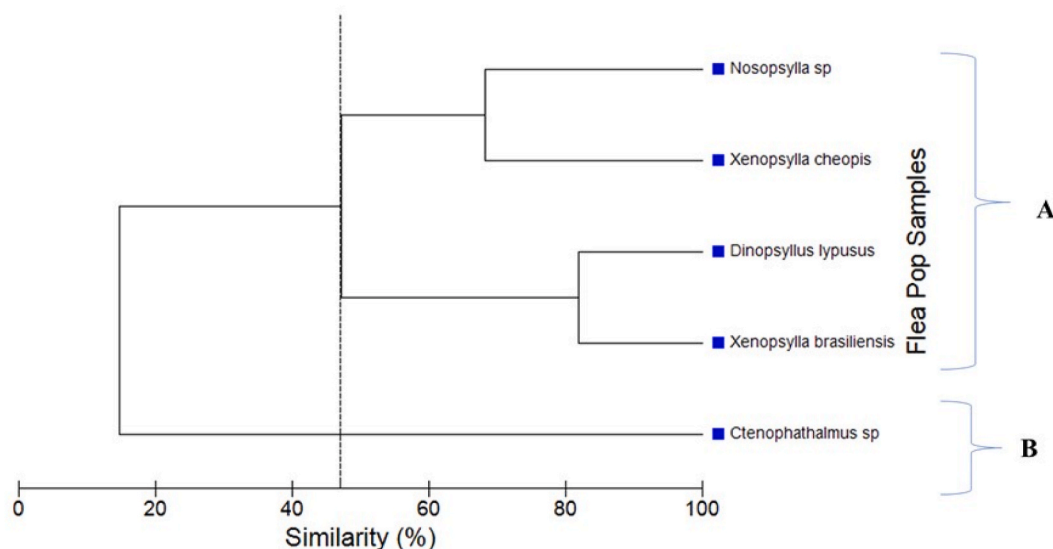


Fig. 6. Dendrogram of flea species showing two main groups (AB) of flea community based on farm and forest habitats.

farm and forest habitats. The species prefer rodents as primary hosts. For example, *X. cheopis*, *Dinopsyllus lypus*, and *X. brasiliensis* prefer *R. rattus* 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 lypus*, and *X. brasiliensis*, 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. natalensis*, 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. Alarming, these flea species have the ability to harbor other zoonotic pathogens such as *Bartonella* and *Rickettsia 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 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).

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.

Funding

The study was funded by the African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE IRPM&BTD) ACE II–Credit number 5799–TZ at Sokoine University of Agriculture, Morogoro, Tanzania.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

Many thanks to the community leaders and local people of Endesh and Mongahay villages in Mbulu district for allowing us to conduct this study. Thanks to the technical staffs for the assistance in field trapping and animal processing. We also extend our thanks to Professor Josef Bryja, Institute of Vertebrate Biology, Czech Republic for rodent species identification.

References

Bertherat, E., Bertherat, É., 2019. Plague around the world in 2019. *Wkly. Epidemiol. Rec.* 25, 289–292.

- Bitam, I., Dittmar, K., Parola, P., Whiting, M.F., Raoult, D., 2010. Fleas and flea-borne diseases. *Int. J. Infect. Dis.* 14, e667–e676.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24 (3), 127–135.
- Brinkerhoff, R.J., 2008. Habitat-associated differences in flea assemblages of striped skunks (*Mephitis mephitis*). *Comp. Parasitol.* 75, 127–131.
- Cavanaugh, D.C., Marshall, J.R.J.D., 1972. The influence of climate on the seasonal prevalence of plague in the Republic of Vietnam. *J. Wildl. Dis.* 8 (1), 85–94.
- Chotlersak, K., Apiwathnasorn, C., Sungvornyothin, S., Panasoponkul, C., Samung, Y., Ruangsittichai, J., 2015. Correlation of host specificity, environmental factors and oriental rat flea abundance. *Southeast Asian J. Trop. Med. Publ. Health* 46, 198.
- Coetzee, C.G., 1975. The biology, behaviour, and ecology of *Mastomys natalensis* in southern Africa. *Bull. World Health Organ.* 52 (4–6), 637.
- Debien, A., Neerincx, S., Kimaro, D., Gulnick, H., 2010. Influence of satellite-derived rainfall patterns on plague occurrence in northeast Tanzania. *Int. J. Health Geogr.* 9, 60.
- Durden, L.A., Hinkle, N.C., 2019. Fleas (siphonaptera). In: *Medical and Veterinary Entomology*. Elsevier, pp. 145–169.
- Eads, D.A., Hoogland, J.L., 2016. Factors that affect parasitism of black-tailed prairie dogs by fleas. *Ecosphere* 7, e01372.
- Eisen, R.J., Borchert, J.N., Mpanga, J.T., Atiku, L.A., MacMillan, K., Boegler, K.A., Monteneri, J.A., Monaghan, A., Gage, K.L., 2012. Flea diversity as an element for persistence of plague bacteria in an East African plague focus. *PLoS One* 7, e35598.
- Eisen, R.J., Gage, K.L., 2009. Adaptive strategies of *Yersinia pestis* to persist during inter-epizootic and epizootic periods. *Vet. Res.* 40, 1.
- Eisen, R.J., Reynolds, P.J., Ettestad, P., Brown, T., Ensore, R.E., Biggerstaff, B.J., Cheek, J., Bueno, R., Targhetta, J., Monteneri, J.A., Gage, K.L., 2007. Residence-linked human plague in New Mexico: a habitat-suitability model. *Am. J. Trop. Med. Hyg.* 77 (1), 121–125.
- Gage, K.L., Kosoy, M.Y., 2005. Natural history of plague: perspectives from. *Annu. Rev. Entomol.* 50, 505–528.
- Gage, K.L., Burkot, T.R., Eisen, R.J., Hayes, E.B., 2008. Climate and vector borne diseases. *Am. J. Prev. Med.* 35, 436–450.
- Haikukutu, L., Lyaku, J.R., Lyimo, C., Kasanga, C.J., Kandusi, S.E., Rahelinirina, S., Rasoamalala, F., Rajerison, M., Makundi, R., 2022. Plague in Tanzania: first report of sylvatic plague in Morogoro region, persistence in Mbulu focus, and ongoing quiescence in Lushoto and Iringa foci. *IJID Regions* 4, 105–110.
- Happold, D.C.D. (Ed.), 2013. *Mammals of Africa. Volume III: Rodents, hares and rabbits*. Bloomsbury Publishing, London, UK, p. 784.
- Haule, M., Lyamuya, E.E., Hang'ombe, B.M., Matee, M.I., 2013. Investigation of Fleas as Vectors in the Transmission of Plague during a Quiescent Period in North-Eastern, Tanzania.
- Hinnebusch, B.J., Bland, D.M., Bosio, C.F., Jarrett, C.O., 2017. Comparative ability of *Oropsylla montana* and *Xenopsylla cheopis* fleas to transmit *Yersinia pestis* by two different mechanisms. *PLoS Neglected Trop. Dis.* 11, e0005276 <https://doi.org/10.1371/journal.pntd.0005276>.
- Kandi, V., Koka, S.S., Bhoomigari, M.R., 2019. Hymenolepiasis in a pregnant woman: a case report of *Hymenolepis nana* infection. *Cureus* 11.
- Kessy, S.T., Makundi, R.H., Sabuni, C., Massawe, A.W., Rija, A.A., 2023. Rodent Abundance, Diversity and Community Structure in a Bubonic Plague Endemic Area, Northern Tanzania. *Mammalia*.
- Kiffner, C., Stanko, M., Morand, S., Khokhlova, I.S., Shenbrot, G.I., Laudisoit, A., Leirs, H., Hawlena, H., Krasnov, B.R., 2013. Sex-biased parasitism is not universal: evidence from rodent–flea associations from three biomes. *Oecologia* 173, 1009–1022.
- Krasnov, B., Khokhlova, I., Shenbrot, G., 2002. The effect of host density on ectoparasite distribution: an example of a rodent parasitized by fleas. *Ecology* 83, 164–175.
- Krasnov, B.R., 2008. *Functional and Evolutionary Ecology of Fleas: a model for ecological parasitology*. Cambridge University Press.
- Krasnov, B.R., Khokhlova, I.S., Fielden, L.J., Burdelova, N.V., 2001. Effect of air temperature and humidity on the survival of pre-imaginal stages of two flea species (Siphonaptera: pulicidae). *J. Med. Entomol.* 38, 629–637.
- Krasnov, B.R., Mouillot, D., Shenbrot, G.I., Khokhlova, I.S., Poulin, R., 2004. Geographical variation in host specificity of fleas (Siphonaptera) parasitic on small mammals: the influence of phylogeny and local environmental conditions. *Ecography* 27, 787–797.
- Krasnov, B.R., Shenbrot, G.I., Khokhlova, I.S., Poulin, R., 2005. Diversification of ectoparasite assemblages and climate: an example with fleas parasitic on small mammals. *Global Ecol. Biogeogr.* 14, 167–175.
- Krasnov, B.R., Shenbrot, G.I., Mouillot, D., Khokhlova, I.S., Poulin, R., 2006a. Ecological characteristics of flea species relate to their suitability as plague vectors. *Oecologia* 149, 474–481.
- Krasnov, B.R., Stanko, M., Khokhlova, I.S., Mošanský, L., Shenbrot, G.I., Hawlena, H., Morand, S., 2006b. Aggregation and species coexistence in fleas parasitic on small mammals. *Ecography* 29, 159–168.
- Krasnov, B.R., Stanko, M., Mikšlová, D., Morand, S., 2006. Habitat variation in species composition of flea assemblages on small mammals in central Europe. *Ecol. Res.* 21, 460–469. <https://doi.org/10.1007/s11284-005-0142-x>.
- Kreppel, K.S., Telfer, S., Rajerison, M., Morse, A., Baylis, M., 2016. Effect of temperature and relative humidity on the development times and survival of *Synopsyllus fonquerniei* and *Xenopsylla cheopis*, the flea vectors of plague in Madagascar. *Parasites Vectors* 9, 1–10.
- Leulmi, H., Socolovschi, C., Laudisoit, A., Houemenou, G., Davoust, B., Bitam, I., Raoult, D., Parola, P., 2014. Detection of *Rickettsia felis*, *Rickettsia typhi*, *Bartonella*

- species and *Yersinia pestis* in fleas (siphonaptera) from Africa. *PLoS Neglected Trop. Dis.* 8, e3152.
- Makundi, R.H., Massawe, A.W., Borremans, B., Laudisoit, A., Katakweba, A., 2015. We are connected: flea–host association networks in the plague outbreak focus in the Rift Valley, northern Tanzania. *Wildl. Res.* 42, 196–206.
- Makundi, R.H., Massawe, A.W., Mulungu, L.S., Katakweba, A., Mbise, T.J., Mgode, G., 2008. Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, northern Tanzania. 2007. *Mammalia* 72, 253–257. <https://doi.org/10.1515/MAMM.2008.038>.
- Makundi, R.H., Massawe, A.W., Mulungu, L.S., 2007. Breeding seasonality and population dynamics of three rodent species in the Magamba Forest Reserve, Western Usambara Mountains, north-east Tanzania. *Afr. J. Ecol.* 45, 17–21. <https://doi.org/10.1111/j.1365-2028.2006.00667>.
- Mboera, L.E., Mayala, B.K., Kweka, E.J., Mazigo, H.D., 2011. Impact of climate change on human health and health systems in Tanzania: a review. *Tanzan. J. Health Res.* 13 (5).
- Moore, S.L., Wilson, K., 2002. Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* 297, 2015–2018, 1979.
- Msangi, S., 2019. The surveillance of rodent populations in East Africa in relation to plague endemicity. *Tanzan. J. Sci.* 1, 8–20.
- Mwalimu, C.D., Mgode, G., Sabuni, C., Msigwa, F., Mghamba, J., Nyanga, A., Mohamed, A., Kwesi, E., Nagu, N., Kishimba, R., John, L., 2022. Preliminary investigation and intervention of the suspected plague outbreak in Madunga, Babati District-Tanzania. *Acta Trop.* 233, 106566 <https://doi.org/10.1016/j.actatropica.2022.106566>.
- Neerincx, S.B., Peterson, A.T., Gulinck, H., Deckers, J., Leirs, H., 2008. Geographic distribution and ecological niche of plague in sub-Saharan Africa. *Int. J. Health Geogr.* 7 (1), 1–12.
- Ngeleja, R.C., Luboobi, L.S., Nkansah-Gyekye, Y., 2017. The effect of seasonal weather variation on the dynamics of the plague disease. *Int. J. Math. Math. Sci.* 2017, 25.
- Nyembo, L.O., Larbi, I., Rwiza, M.J., 2021. Analysis of spatio-temporal climate variability of a shallow lake catchment in Tanzania. *Journal of Water and Climate Change* 12, 469–483.
- Occhibove, F., McKeown, N.J., Risley, C., Ironside, J.E., 2022. Eco-epidemiological screening of multi-host wild rodent communities in the UK reveals pathogen strains of zoonotic interest. *Int J Parasitol Parasites Wildl* 17, 278–287.
- Palazzo, S., 2011. *Nosopsyllus fasciatus* [WWW Document]. *Animal Diversity Web*. URL. https://animaldiversity.org/accounts/Nosopsyllus_fasciatus/, 5.16.23.
- Palomino, P.S., Montenegro, O.L., Mesa-González, E., 2020. Anesthesia of sigmodontine rodents under field conditions. *J. Vet. Med.* 69–83.
- Pham, H.V., Dang, D.T., Tran Minh, N.N., Nguyen, N.D., Nguyen, T.V., 2009. Correlates of environmental factors and human plague: an ecological study in Vietnam. *Int. J. Epidemiol.* 38, 1634–1641.
- Philip Samuel, P., Govindarajan, R., Krishnamoorthi, R., Venkatesh, A., 2021. A rapid protocol for clearing, staining, and mounting of Arthropoda: trombiculidae, Pediculidae and Pulicidae. *North West J Zool* 17.
- Poje, J.E., Locke, T.E., Samuel, M.D., 2020. Impacts of environmental conditions on fleas in black-tailed prairie dog burrows. *J. Vector Ecol.* 45 (2), 356–365.
- Ramana, K.V., Rao, S.D., Rao, R., Mohanty, S.K., Wilson, C.G., 2011. Human dipylidiasis: a case report of *Dipylidium caninum* infection from Karimnagar. *Online J. Health Allied Sci.* 10.
- Sharif, M., 1949. Effects of constant temperature and humidity on the development of the larvae and the pupae of the three Indian species of *Xenopsylla* (Insecta: siphonaptera). *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 233, 581–633.
- Sherman, D.M., 2007. *Tending Animals in the Global Village: a Guide to International Veterinary Medicine*. John Wiley & Sons.
- Stanko, M., Miklišová, D., Göüy de Bellocq, J., Morand, S., 2002. Mammal density and patterns of ectoparasite species richness and abundance. *Oecologia* 131, 289–295.
- Tripp, D.W., Gage, K.L., Monteneri, J.A., Antolin, M.F., 2009. Flea abundance on black-tailed prairie dogs (*Cynomys ludovicianus*) increases during plague epizootics. *Vector Borne Zoonotic Dis.* 9, 313–321.
- Trivedi, J., 2003. *Xenopsylla cheopis* [WWW Document]. *Animal Diversity Web*. URL. https://animaldiversity.org/accounts/Xenopsylla_cheopis/, 5.16.23.
- Vallès, X., Stenseth, N.C., Demeure, C., Horby, P., Mead, P.S., Cabanillas, O., Ratsitorahina, M., Rajerison, M., Andrianavaoimanana, V., Ramasindrazana, B., 2020. Human plague: an old scourge that needs new answers. *PLoS Neglected Trop. Dis.* 14, e0008251.
- Young, H.S., Dirzo, R., McCauley, D.J., Agwanda, B., Cattaneo, L., Dittmar, K., Eckerlin, R.P., Fleischer, R.C., Helgen, L.E., Hintz, A., 2015. Drivers of intensity and prevalence of flea parasitism on small mammals in East African savanna ecosystems. *J. Parasitol.* 101, 327–335.
- Zajac, Z., Kulisz, J., Woźniak, A., 2020. Flea communities on small rodents in eastern Poland. *Insects* 11, 894.
- Zhang, Y., Dai, X., Wang, Q., et al., 2015. Transmission efficiency of the plague pathogen (*Y. pestis*) by the flea, *Xenopsylla skrjabini*, to mice and great gerbils. *Parasites Vectors* 8, 256. <https://doi.org/10.1186/s13071-015-0852-z>.
- Ziwa, M.H., Matee, M.I., Hang'ombe, B.M., Lyamuya, E.F., Kilonzo, B.S., 2013a. Plague in Tanzania: an overview. *Tanzan. J. Health Res.* 15.
- Ziwa, M.H., Matee, M.I., Kilonzo, B.S., Hang'ombe, B.M., 2013b. Evidence of *Yersinia pestis* DNA in rodents in plague outbreak foci in Mbulu and Karatu Districts, northern Tanzania. *Tanzan. J. Health Res.* 15.
- Zurita, A., Callejón, R., García-sánchez, Á.M., Urdapilleta, M., Lareschi, M., Cutillas, C., 2019. Origin, evolution, phylogeny and taxonomy of *Pulex irritans*. *Med. Vet. Entomol.* 33, 296–311.