Associated factors contributing abundance of fleas on rodents in plague endemic area of Karatu district, northern Tanzania.

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July 10, 2023

Abstract

Fleas are small wingless hematophagous insect that are frequently infesting on rodents and other small mammals while acting as reservoirs and vectors of many rodent borne zoonotic diseases including plague infectious disease which is threat to the public health in many part of the world including Tanzania. 291 rodents from nine species were captured with Sherman traps in farm land, peridomestic areas, bush land and forest buffer zone across wet and dry season in plague and non-plague foci villages. Captured rodents were anaesthetized and 190 fleas comprising four species were collected and morphologically identified with available dichotomous key. Dinopsyllus lypusus were (46.32%), Ctenophthalmus spp (26.84%), Xenopsylla brasiliensis (16.32%) and Xenopsylla cheopis (10.53%). 38.42% of fleas were collected from Mastomy natalensis, 22.63% from Lemniscomys striatus and 18.42% from Rattus rattus. Highest flea infestation prevalence was found on R.rattus and was strongly associated with X.cheopis and X.brasiliensis. Specific flea index of X.cheopis on R.rattus was (01) in plague foci and (<0.5) in non-plague foci villages. Result of GLM final model indicated that flea abundance was significant influenced by rodent species ($p < .001$), season ($p = .031$), habitat type ($p = .02$), rodent weight ($p < .001$), rodent sex ($p < .001$) and plague locations ($p = .02$). There was significance difference in variation of flea abundance between rodent sexes ($W = 9158.5$, $p = .009$) and a weak positive correlation between rodent's weight and abundance of fleas ($R = 0.17$, $p < 0.05$). Despite that, specific flea index of X.cheopis on rats in both plague foci and non-plague foci villages were not indicating alarming condition that would require urgent control of fleas, still society should consistently adhere to rodent and fleas control methods in order to limit their interaction to the society especially in farm land and peridomestic areas where human activities are high.

CHAPTER THREE

MANUSCRIPT TWO

3.0 Associated factors contributing abundance of fleas on rodents in plague endemic area of Karatu district, northern Tanzania.

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3.1 Abstract

Fleas are small wingless hematophagous insect that are frequently infesting on rodents and other small mammals while acting as reservoirs and vectors of many rodent borne zoonotic diseases including plague infectious disease which is threat to the public health in many part of the world including Tanzania. 291 rodents from nine species were captured with Sherman traps in farm land, peridomestic areas, bush land and forest buffer zone across wet and dry season in plague and non-plague foci villages. Captured rodents were anaesthetized and 190 fleas comprising four species were collected and morphologically identified with available dichotomous key. *Dinopsyllus lypusus*were (46.32%)*, Ctenophthalmus spp* (26.84%), *Xenopsylla brasiliensis* (16.32%) and *Xenopsylla cheopis* (10.53%). 38.42% of fleas were collected from *Mastomy natalensis* , 22.63% from*Lemniscomys striatus* and 18.42% from *Rattus rattus* . Highest flea infestation prevalence was found on *R.rattus* and was strongly associated with *X.cheopis* and *X.brasiliensis* . Specific flea index of *X.cheopis* on *R.rattus* was (01) in plague foci and (<0.5) in non-plague foci villages. Result of GLM final model indicated that flea abundance was significant influenced by rodent species ($p < .001$), season ($p = .031$), habitat type ($p = .02$), rodent weight ($p < .001$), rodent sex ($p < .001$) and plague locations ($p = .02$). There was significance difference in variation of flea abundance between rodent sexes ($W = 9158.5$, $p = .009$) and a weak positive correlation between rodent's weight and abundance of fleas ($R = 0.17$, $p < 0.05$). Despite that, specific flea index of*X.cheopis* on rats in both plague foci and non-plague foci villages were not indicating alarming condition that would require urgent control of fleas, still society should consistently adhere to rodent and fleas control methods in order to limit their interaction to the society especially in farm land and peridomestic areas where human activities are high.

Key words: Ectoparasite, Flea, Rodent, Plague endemic area. Plague foci, Specific flea index

3.2 Introduction

Fleas (Siphonaptera) are hematophagous insects and obligatory ectoparasites of vertebrates that have colonized variety of habitats from wet tropical forests to semi-arid and desert areas (Zając *et al* ., 2020; Zhang *et al* ., 2022). They have an obligate parasitic life with a wide range of potential hosts, primarily small mammals and less often birds (Eads *et al* ., 2020). Fleas exhibit a holometabolous type of lifecycle and complete their life cycle in 14 days to 140 days, depending mainly on temperature and humidity (Krämer and Mencke, 2012; Ashwini *et al* ., 2017; Gram and Short, 2020).

Fleas contribute significantly in the circulation of pathogens hence receiving considerable attention mostly because they are vector of many zoonotic diseases (Eads *et al* ., 2020). They play role in spreading plague disease as well as other emerging pathogens that causes zoonoses such as bartonelloses, tularemia and rickettsioses (flea-borne spotted fever, Q fever and murine typhus), fleas also maintains and transmits pathogens of myxomatosis and trypanosomiasis and can act as intermediate hosts of some helminthes (Zając *et al* ., 2020; Cófreces *et al* ., 2021). However, the most known flea borne zoonotic disease is plague caused by bacteria known as *Yersinia pestis* ; a zoonotic bacteria renowned for killing millions of humans during the Black Death in Europe in 14 century (Ditchburn and Hodgkins, 2019; Barbieri *et al* ., 2021; Izdebski *et al* ., 2022). Plague is still threatening the public health in some parts of the world, especially in African countries (Ditchburn and Hodgkins, 2019). By doing surveillance studies on flea assemblage on rodent community in plague endemic areas we can understand factors that influence flea abundance and infestation potential on rodents and being aware of significant factors that may endanger the public health and therefore we can be in a good position to suggest effective intervention strategies to control their spread.

Plague persistence in plague endemic areas is characterized by coexistence of interactions among rodent communities and flea species, whereby some species of rodents and fleas are better at maintaining, amplifying and transmitting *Yersinia pestis* (Gage and Kosoy, 2006; Antolin *et al* ., 2010). Some of frequently reported fleas and rodents species involved in plague maintenance and transmission are*Xenopsylla cheopis, Xenopsylla brasiliensis* and*Dinopsyllus lypusus* and rodent species *Lophuromys spp* ,*Praomys delectorum* , *Graphiurus murinus* , *Lemniscomys striatus, Mastomys natalensis* , and *Rattus rattus* (Kilonzo*et al* ., 2006; Makundi *et al* ., 2008; Eisen and Gage, 2009). A study done by Ziwa et al. (2013) has reported the concurrence of host-vector interaction to be responsible for transmission of plague disease. Also Makundi et al. (2015) has reported the presence of multiple associations between domestic and peridomestic rodent species infested with fleas to be among major contributing factors for persistence and spread of plague in plague endemic areas. Moreover, the roles of hosts and fleas, for instance in plague maintenance or amplification, is mostly affected with change of space and time (Kosoy*et al* ., 2017).

The distributions and community structure of fleas are influenced by numerous biotic and abiotic factors, that include, host species diversity, sex, age, body size, immune status, host population abundance, habitat diversity and seasonal variation of temperature and precipitation (Lopez-Perez *et al* ., 2017, 2022). Host diversity is a relevant factor to consider since it involves variation in flea species richness (Fantozzi *et al* ., 2022), yet it is not a fixed rule, fleas can infest hosts phylogenetically close, switching between coexisting species within guilds (Cofreces *et al* ., 2021).

Noting this uncertainty plague management in plague foci is mostly efficient when encompassing rodents and their flea parasites. As described by Garcia-Longoria *et al.* (2019) host communities can influence parasitism. Community organization of parasites is considered to be determined mainly by their hosts because host present a habitat for parasites, providing them with place for living, feeding and mating (Fellin and Schulte, 2022). Thus, a host can be reflected as a biological shelter for parasites. Unlike endoparasites, ectoparasites are influenced not only by host characteristics, but also by characteristics of the host environment (Krasnov *et al* ., 1997). Therefore, a habitat of the ectoparasite should not be just a particular host, but a particular host in a particular habitat. If so, an important determinant of parasite community structure should be a complex host-habitat relationship. Quantifying variation of ectoparasites load among host species and comparing these variation with other ecological factors which are known to shape host communities is the good approach for understanding dual nature of host-parasite interaction (Merrill*et al* ., 2020; Veitch *et al*

., 2020).

Concisely, the aim of this study was to examine rodent's flea load and their associated factors in plague endemic area of Karatu district by i) quantifying and assessing the variation of flea's parasitological indices (specific flea index, total flea index and flea infestation prevalence on rodents), ii) assessing the association of abundance of species of fleas with rodent species, plague and non-plague foci villages, season (wet and dry season), habitat type and rodent sex, iii) investigating flea biased parasitism on rodent's weight and sex (male and female rodents) and lastly, iv) assessing the influence of host characteristics (rodent species, sex and weight), habitat type, season and plague locations on abundance of fleas. Result of this study will help to create awareness to the public health sectors and vector control programs by providing valuable information that will help in decision making process regarding flea control in plague endemic area of Karatu district, northern Tanzania.

3.3 Material and methodology

3.3.1 Study area

This study was conducted at Karatu district, Arusha region in northern part of Tnzania. The district is bordered by Monduli district to the east, Shinyanga region to the west, Ngorongoro district to the north, and Manyara region to the south and southeast. The study was conducted in four villages found in Karatu district which are Rhotia Kati 3deg19'16.4"S 35deg44'22.3"E, Kambi ya Simba 3deg17'05.28"S 35°48'30.84"E, Kitete 3°13'58.5"S 35°50'30.8"E and Marera 3°18'32.2"S 35°43'26.6"E. Rhotia Kati and Kambi ya Simba are plague foci villages while Marera and Kitete are non-plgue foci villages (Kilonzo *et al* ., 2006)**(Figure 1 below)** . Economic activities of residents in the study area involve crop cultivation, livestock keeping and small retail shops.

Figure 1 : Showing map of Tanzania highlighting Rhotia and Mbulumbulu wards where four villages were selected for rodent sampling in this study.

3.3.2 Selection of study area

In this study four villages were selected depending on the information of whether been involved or not involved in the past human plague outbreak (Kilonzo *et al* ., 2006). Rodents were sampled from four habitats that include, farm land, bush land, peridomestic area and forest buffer zone that were selected purposefully from each village. Selection criteria of habitats was relying on the following factors; a habitat characterized with bushes comprising shrubs and little dwarf scattered trees away from settlements were considered as bush land habitat, a land cultivated with cereal and other agricultural crops were considered as farm land habitat, a forest buffer zone habitat was declared by the conservation authority where they provided permission and guidance to trap in the area. Forest buffer zone was defined as a part of protected land adjoining conservation area and community area with no human activities allowed in that area. Peridomestic area was considered to be an area of land surrounding human settlement within a range of one hundred meter. Bush land, farm land and forest buffer zone were all considered as sylvatic areas and were selected at least 300 to 500 m away from the human settlements.

3.3.3 Study design and sample size

This study was repeated cross sectional design involving collection of rodent sample across season (wet and dry season). Preliminary survey was conducted in a study area one week before rodent sampling begun to request permission and inform authority about this study. Sherman live animal traps were used to capture rodents in farm land, bush land, peridomestic areas and forest buffer zone.

Rodent sample size was estimated with formula described by Naing*et al* . (2006)

$$n = \frac{Z^2 \ \mathrm{P} \ (1-P)}{d^2}$$

Whereby n= sample size; Z= test statistics at 95% confidence level;P = expected prevalence of *Yersinia pestis* in rodents; (1-p) = probability of having no infected rodent; d = desired level of precision. Values in the formula are: Z=1.96, d = 5% and P = 10%. By inserting the values into the formula(

1.96^2 *amp*; $\times 0.1(1-0.1)\frac{}{0.05^2}$ = 138.29). Therefore, sample size for this study was estimated to be 140 rodents in each season (wet and dry) giving a total sample size of 280 rodents.

3.3.4 Rodent trapping and processing

A total of 120 Sherman traps baited with peanut butter and mixed with maize flour were used to capture rodents in plague and non-plague foci villages in farm land, bush land, peridomestic and forest buffer zone habitats. Selection of trapping sites was made successfully with the help of the residents and involved observation of rodent's signs such as rodent's pathway, droppings, movements sign or observation of gnawed seeds, and depending on the information from the residents about presence of rodents in their surroundings. Information about this study was clearly provided to the owner of trapping site for requesting permission before trapping. 35 traps were set at a distance of 10 m apart in seven transect lines containing five trapping station in all habitats except in peridomestic areas were 35 Sherman traps were set at 2 to 5 m apart by targeting areas with rodent burrows or other rodent signs around human houses and livestock shelters. Traps were usually sets on the field in the evening at 5:00 pm and left overnight for at least three consecutive days while inspecting the traps for captured rodents every day during morning and afternoon.

Captured rodents were carefully removed from the trap using animal handling bag and were anaesthetized using diethyl ether in a bottle contained cotton wool. Blood sample were collected from anaesthetized rodents for making blood smear for observation of *Yersinia pestis*coccobacilli under light microscope; organ such as lugs and spleen were collected from the euthanized rodent for confirmation of (*pla*) gene of *Y.pestis* DNA under qPCR (results in another manuscript). Fleas were collected from a euthanized rodent before dissection by brushing their body thoroughly over a plastic basin (Makundi *et al* ., 2008). Rodents were brushed from the head to the base of the tail using small shoeshine brush until all fleas stop to fall in a basin. Animal handling bag and anaesthetizing bottle were checked for presence of fleas and were all collected with a fine forceps. Collected fleas were counted, recorded and preserved in a well labeled eppendorf tubes containing 70% ethanol and stored at room temperature until morphological identification using dichotomous key in the laboratory.

3.3.5 Rodent identification

Rodents were identified to genus and specie level using an established taxonomic nomenclature of rodents identification (Happold, 2013). Identification involved observation of rodent's fur color and morphometric measurements that were taken in millimeters using digital vernier caliper to measure length of hind foot (*pes*), ear length, tail length and head to body length and also the rodent's weight were measure in gram by using digital weighing balance.

3.3.6 Fleas processing and identification

Fleas were processed in the laboratory following standard procedure in (Hastriter and Whiting, 2003) cited in (Berrizbeitia *et al* ., 2017), involving passing fleas through series of reagents to make their feature clear enough to be identified with dichotomous key. After processing, fleas were examined under digital stereo microscope OPTA-TECH® and identified to genus and specie level following conventional dichotomous key as described in (Harimalala*et al* ., 2021; Friggens *et al* ., 2020). Fleas were then mounted on a microscope glass slides in accordance with conventional procedures for fleas processing before identification.

Flea processing/clearing were involving puncturing of fleas at the region between abdominal sterna II and III using thin-fine needle after placing them on a wax-block. Punctured fleas were soaked in potassium hydroxide (10%) for 24 h to decolorize their dark color and make their features visible for identification. After this process fleas were immersed in distilled water for 30 min to wash excess potassium hydroxide and stop decolonization process. After 30 min fleas were removed and gently compressed on their abdomen to

expel marinated soft tissues throughout the punctured hole followed with dehydration process in a series of ethanol solutions (70%, 80%, 95% and absolute) for 30 min in each step.

For clarification of flea's exoskeleton, fleas were immersed in methyl salicylate for 15–20 min and then transferred to xylene for a minimum of 1 h. After then, they were mounted on a microscope glass slide in a Canada balsam (Campbell *et al* ., 2018) which finalized the process and fleas were ready for identification. Fleas voucher specimen of each species identified were reserved at college of veterinary medicine and biomedical sciences at Sokoine University of Agriculture (SUA), Morogoro Tanzania.

3.3.7 Data analysis

Flea data were recorded in Microsoft Excel 2016 and prepared in a readable data set format, data set were imported in R programming language (R Core Team version 4.2.2 of 2022) from which all statistical analyses were conducted with a significance level (α) of 0.05. Data were tested for normality before analyzed and were found not normally distributed (Shapiro-Wilk test p <.05) leading us to use non-parametric test for inferential statistics. Rodent's flea load on examined rodents were evaluated by assessing total flea indices and specific flea indices (Zimba *et al* ., 2012). Percentage prevalence of rodents infested with fleas (proportion of examined rodents positive for fleas) were computed with 95 % confidence interval for proportion and multiplied by 100%.

Chi-square χ^2 test of association was applied to examine the relationship of abundance of species of fleas with rodent species, plague and non-plague foci villages, season, habitat type and rodent sex. The nature of association was further analyzed to find the contribution of level of factors to the Chi-square result using absolute standardized residuals and the relationship were presented in balloon chart (Kassambara, 2022). Flea biased parasitism on rodent's sex and weight was analyzed by assessing the variation of flea abundance between sexes using Wilcoxon rank sum test and through evaluation of correlation between flea abundance and rodent's weigh using Spearman rank correlation test (rho) respectively; these factors were further analyzed in generalized linear model (GLM) to assess their influence on flea abundance. The GLM was used to establish the model variables that described the influence of predictors of abundance of flea. Model was selected based on AIC in which model with possible lowest AIC was selected with help of step-AIC function in MASS package of R programming language (Venables and Ripley, 2002). Predictor variables were both numerical and categorical variables that include, host characteristics (rodent species, sex and weight), habitats, season and plague locations.

3.4 Results

3.4.1 Flea collected from rodents

Four species of fleas (n= 190) were collected from six species of rodents (n=73/291) from a total of nine rodent species that were captured with Sherman animal live traps in plague and non-plague foci villages across wet season (January-February) and dry season (July) of 2022. We found that total flea index in wet season was 0.53 (SD=1.54), dry season was 0.78 (SD= 1.66), plague foci was 0.71 (SD= 1.81) and non-plague foci was 0.55 (SD= 1.13). Flea species that were collected in this study were *Dinopsyllus lypusus* (46.32 %)*, Ctenophthalmus spp* (26.84 %), *Xenopsylla brasiliensis* (16.32 %) and *Xenopsylla cheopis* (10.53 %). Collected fleas were evaluated to estimate total flea indices (TFI) and prevalence of flea infestation among rodent species **(table 1)** . Apparently, there was no flea collected from three species of rodents *Grammomys spp, Praomys delectorum* and *Otomys spp* .

3.4.2 Variation of flea infestation prevalence on rodent community

We found that estimated prevalence of all rodents infested with fleas was 25 % (CI= 20 % - 30 %) and was statistically significant lower than 50 % of infestation prevalence p < .001. Variations of prevalence of rodents infested with fleas were estimated across different habitats, plague locations and season. It was found that farm land and peridomestic area had high prevalence of fleas 30 % (CI= 21% -40%) and 26 % (CI= 18% -35%) respectively compared to forest buffer zone and bush land 24% (CI= 14% -38%) and 17% (CI= 09% -29%) respectively. Plague foci villages had high fleas prevalence 26% (CI= 20% -33%) compared

to non-plague foci villages 23% (CI= 16% -32%). Dry season had high fleas prevalence 30% (CI= 23% -38%) compared to wet season 20% (CI= 15% -27%).

3.4.3 (i) Specific flea indices (SFI) on rodents in different habitats

Fleas collected from all rodent species were involved in the estimation of specific animal flea indices across different habitats **(table 2)** . Generally *D. lypusus* infested five species of rodents covering (42.47%) of all infested rodents (n=73/291);*Ctenophthalmus spp* infested four species of rodents (32.88%),*X. brasiliensis* infested three species of rodents (12.33%) and*X. cheopis* infested two species of rodents (12.33%).

3.4.3 (ii) Variation of specific flea indices (SFI) on rodents in plague and non-plague foci

Rodents in non-plague foci villages were mostly infested with*Ctenophthalmus spp* and *D. lypusus* **fig (2A and 2B)**below from which *Ctenophthalmus spp* was mostly infested two rodent species i.e. *L.striatus* and *M.natalensis* while*D.lypusus* was mostly infested *G.murinus* compared to other captured rodents species. In plague foci villages most captured rodents were infested with *X.cheopis* and *X.brasiliensis***fig (2C and 2D)** . *X.brasiliensis* and *X.cheopis*were mostly infested *R.rattus* than other rodent species.

Fig 2 . Bar charts showing comparison of specific animal flea index (SFI) on rodent species collected in plague and non- plague foci villages in plague endemic area of Karatu district northern Tanzania. The above numbers on bar chats are percentage trap success of each individual rodent.

3.4.4 Association of abundance of flea species with other factors

We found that abundance of flea species was statistically significant associated with type of habitat ($\chi 2$ (9) = 41.701,$p< .001$), season ($\chi 2$ (3) = 17.839,$p< .001$), rodent species ($\chi 2$ (15) = 134.35,$p< .001$) and rodent sex ($\chi 2$ (3) = 10.543,$p= .014$), but was not statistically significant associated with plague locations (plague and non-plague foci) ($\chi 2$ (3) = 2.4006,$p= 0.49$). Balloon chart was plotted to describe this relationship by using Pearson residuals (standardized residuals) of all level of each factor. The high value of Pearson residual was described with the big and bright red balloon in a gradient of size and color from dark to pale red suggesting particular level of factor had strong association with abundance of particular flea specie and a gradient of small balloons of white to bright blue suggesting weak or insignificant association of abundance of particular flea specie **(fig 3)** .

Fig3 . Balloon chart showing association of abundance of fleas species with other factors; the abbreviations Cteno=*Ctenophthalmus spp* , Dino= *Dinopsyllus lypusus* . X.br=*Xenopsylla brasiliensis* and X.cheo= *Xenopsylla cheopis* .

3.4.5 Flea biased parasitism on rodent's sex and weight

We found statistical significant difference in flea abundance between male and female rodents (Wilcoxon rank sum test W = 9158.5, $p = .009$) with small magnitude of effects size (r= .153) (Rules: funder 2019). The median weight of these two sexes was 40.1g (IQR= 25.3g) and 39.4g (IQR= 23g) for male and female rodents respectively and were not statistically significant different (W = 10140, $p = .54$). Correspondingly, we found statistical significant correlation between flea abundance and rodent's weight with Spearman's rank correlation coefficients ($R = .17$, $S = 34176$ and $p < .05$) indicating weak positive correlation **(fig. 4 A)** .

3.4.6 Factors influencing flea abundance

A best final fitted model obtained from the GLM (Poisson family) was statistically significant to explain the relationship of predictor variables to the abundance of flea χ^2 (15) = 106.63, $p< .001$, model coefficients in **(table 3)** , predictors chart in **fig. 4 (4B-4F)** showing the probability of fleas in different factors.

Figure 4 . (A) Scatter plot for Spearman's rank correlation test of rodent's weight and flea abundance. (B) is the GLM prediction curve of influence of rodent's weight on abundance of fleas. (C-F) are the GLM categorical predictor variables with their influence to abundance of fleas.

3.5 Discussion

3.5.1 Flea species collected from rodents

Four species of fleas *X.brasiliensis, X.cheopis, D.lypusus* and*Ctenophthalmus spp* that were collected from rodent community in both plague and non-plague foci villages are all considered as potential or likely to be potential for harboring and transmitting plague infection from rodent to rodent or rodent to human being during the past human plague outbreak in Karatu plague endemic area and elsewhere, however their efficiency in plague transmission is different (Kilonzo*et al* ., 2006; Eisen *et al* ., 2007; Makundi *et al* ., 2008). The most efficient flea vector of plague infectious disease is*X.cheopis* that serves in circulating plague bacilli in both enzootic and epizootic plague periods (Eisen and Gage, 2009). The high vectoring potential of *X.cheopis* is depending on its exclusive characteristic of proventricular spines that provides attachment area for colonization of *Y.pestis* leading into formation of proventricular blockage after taking blood meal from an infected host, a situation which make *X.cheopis* to increase its daily biting rate for sucking blood meal with multiple regurgitation on the biting site on an attempt to unblock its proventriculus; therefore facilitating the increase of transmission of infection compared to other fleas (Gage and Kosoy, 2005; Korzun and Nikitin, 1997).

Similarly, *D.lypusus* and *Ctenophthalmus spp* are conceived as enzootic plague vectors and they also facilitate rapid transmission of the diseases during plague outbreak (Eisen and Gage, 2009; Ziwa*et al* ., 2013; Enscore *et al* ., 2020). *D.lypusus* and*X.brasiliensis* were conveyed as competent vectors for transmission of plague disease during 2007 plague outbreak in Mbulu and (1996/7) in Karatu plague endemic area (Kilonzo *et al* ., 2006; Makundi *et al* ., 2008). Furthermore, *D.lypusus* were reported in East Africa as a plague vector and an important enzootic flea in the maintenance of *Y.pestis* (Devignat, 1949; Arap*et al* ., 1977; Kilonzo 1992). This suggested that these flea species *X.brasiliensis, X.cheopis* and *D.lypusus* are important fleas in the transmission and maintenance of plague bacilli in plague endemic foci of Karatu district.

3.5.2 Specific flea index (SFI)

Since oriental rodent fleas (*X.cheopis*) received high public health concern regarding their efficiency in amplification and transmission of *Y. pestis* from rodent to rodent and from rodent to human (Eisen and Gage, 2009; Maestas and Britten, 2017), we have evaluated their specific flea index as one of the potential factor that may cause risk for epizootic plague among rodents and eventually to the society (Gage, 1999; Eisen *et al* ., 2012, 2020). It has been reported that a specific flea index >1 for *X.cheopis*on rats represents a potentially dangerous situation with respect of increased plague risk to human in the event of an outbreak of plague (Gage, 1999). Similarly an outbreak of human plague is more likely to happen if the specific flea index is >5 (Singchai *et al* , 2003).

Based on result obtained in this study, we found that specific flea index of *X.cheopis* was equal to one (1.0 SFI) in plague foci villages and less than 0.5 (<0.5 SFI) in non-plague foci villages. Despite that, these indices do not really call for urgent control of fleas in these areas, it provide the basic information to understand how plague foci villages require more attention to prevent situation from exceeding the risk level. The high specific flea index in plague foci villages is probably due to high abundance of host of oriental rodent fleas i.e. *Rattus rattus* and less frequently*Mastomys natalensis* as compared to non-plague foci villages.

3.5.3 Associated factors influencing flea abundance on rodents

Basically we found that flea abundance on rodent community were influenced by variation of season of the year (wet and dry season), plague and non-plague foci villages, variation of habitat types and host characteristics. Among all these factors, variation of season of the year has the major impact on flea abundance as it determine distribution of food and breeding cycle of their rodent host (Leirs *et al* ., 1996). Change in temperature and humidity of the year in response to changing season (wet and dry season) is significantly controlling the growth and survival of immature fleas (Ziwa *et al* ., 2013). Different studies have reported the influence of seasonal change on abundance of rodents and fleas to have the impact on maintenance and transmission of plague disease (Njunwa *et al* ., 1989; Makundi*et al* ., 1994; Eads *et al* ., 2016; Shuai *et al* ., 2022).

Variation of wet and dry season is generally affecting growth, reproduction and survival of immature stage

of fleas (Krasnov *et al* ., 2001; Ngeleja *et al* ., 2017). Low abundance of fleas during wet season could be explained by the fact that, the survival of immature fleas in rodent burrows is affected by soil moisture which is controlled by precipitation outside the burrow. Excessive wet conditions in rodent burrows contained with organic matter at a relative humidity >95% can promote growth of vicious fungi that reduces larval and egg survival (Eisen, 2009; Ben Ari *et al.* , 2011). The increase in flea abundance during dry season was probably due to the increase in the abundance of their rodent host as well as the supportive weather with moderate warm and moist condition during early dry season. Reportedly, rodent's flea abundance is mainly affected by ambient temperatures, precipitation, and relative humidity, where as warm-moist weather is providing the explanation for higher flea indices (Ben Ari*et al.,* 2011; Wale *et al* ., 2023).

Plague foci villages was significantly increasing abundance of fleas compared to non-plague foci villages. The increase of flea abundance could be encouraged by high proportion of abundance of rodents in plague foci that presented many potential biological habitats and source of blood meal for fleas. The increase in abundance of rodents influences the increase in abundance of fleas and subsequently enhances the distribution and composition of flea communities (Krasnov *et al* ., 2002; Eads *et al* ., 2016).

Habitat type was another factor that influenced abundance of fleas in our study area. Different habitats have different characteristics that are important for determining distribution and abundance of rodent host of fleas. As reported by Brinkerhoff, (2008) and Laudisoit et al. (2009), the change of habitats is affecting the composition of rodent species and their fleas, making it an important factor in ecological surveillance of abundance of flea species on rodents. In this study we found that abundance of fleas was significantly increasing in farm lands as compared to other habitats. Farm land habitat was observed to encourage rodent colonization as it promote availability of food such as maize and wheat seeds left after harvest during dry season. Habitually rodents prefer to reside in areas with adequate foods where they can make burrows and nest to protect them and their young from predation. Habitats with high food availability and less disturbance and control facilitate the increase in abundance of rodents and their fleas.

Moreover, we found that different rodent species have different influence on flea abundance. *Rattus rattus* was significantly influencing abundance of fleas as compared to other rodents. The characteristics of individual rodent host have been reported to affect responsible mechanism for flea acquisition (Kiffner *et al* ., 2013). Behavior and tendency of *R.rattus* to live in human habitats facilitates the interactions and sharing of fleas among them due to existence of small range of this habitat. Movements of*R.rattus* from one house to another and from nearby farms and livestock shelters is also predisposing this specie to encounter many fleas which are subsequently shared to other *R.rattus* . In addition, human habitats provide suitable environment with availability of food and warm condition supporting growth and reproduction of both rodent and fleas especially when the control measures are abandoned leading to increase flea index among rodents in this habitat. Tactics employed by fleas and many other ectoparasites to colonies microhabitats of the host species (such as rodent's burrows) or wait until a suitable host is present helps parasites to infest host easily especially a new born or when new host has visited the burrow (Bitam *et al* ., 2010).

3.5.4 Flea biased parasitism on rodent's sex and weight

We observed that sex biased fleas infestation was frequently recorded on male rodents than females; male rodents were significantly increasing flea infestation load. A study conducted by Moore and Wilson (2002) has shown that arthropods (especially sticking ectoparasites) obviously exhibit male-biased parasitism because most of them wait for the host to visit their area rather than them searching for the host. An animal that explore many habitats and visit many ecological niches has high probability to encounter many parasites frequently compared to less explorative animal with small movements within its ecological niche. Our result is arguably with different studies (Gaines and McClenaghan, 1980; Bitam *et al* ., 2010 and Buchholz and Dick, 2017) which also observed a similar relationship with males having high flea infestation than female because of their large home range and wide dispersal area as compared to female rodents. The little difference in weight between male and female rodents obtained in this study could or could not influence flea sex biased parasitism on rodents. Sex biased parasitism should not be taken as a general rule since in other study elsewhere it was found that in some host-parasite relationship both male and female rodent hosts were found

equally infested with fleas (Wirsing *et al* ., 2007; Kiffner *et al* ., 2011)

Similarly, weight of rodents in the general community was significantly influencing the increase in abundance of fleas. Under natural phenomenon weight of rodents is equivalent to the size and age of respective rodent specie. The change of these parameters affects the physiological and behavioral status of the individual rodent in particular ecological habitat whereby the increase of these parameters could facilitate rodents to become more explorative with large home range and dispersion in searching for food, mates or better shelter and in the meanwhile they keep increasing the frequency of encountering many fleas. Correspondingly, large rodents are easy target for ectoparasites compared to small one, they can also tolerate many fleas, while the youngest one would perform anti-parasitic grooming frequently (Wilson*et al* , 2002; Hawlena *et al* ., 2008).

3.6 Conclusion and recommendation

This study was intended to contribute knowledge about rodent's flea in plague and non-plague foci villages of plague endemic area of Karatu district, northern Tanzania. In this study we found that plague foci villages was leading with high abundance of rodents and fleas with reservoir potential for plague disease compared to non-plague foci villages. Also the result of specific flea index for *X.cheopis* on rats in both plague and non-plague foci villages did not exceed the risky level i.e. (>1 potentially dangerous situation and >5 urgent situation as an outbreak of human plague is more likely to happen), from which the society is advised to keep implementing rodent and flea control methods in their surroundings that help in reducing the interaction of these plague agents with human society especially in farm lands and peridomestic areas. Furthermore this study recommends that, for effective prevention of emergence of plague disease and other flea borne zoonotic diseases, it is advisable to make regular surveillance of abundance of rodents and fleas in potential plague endemic areas while monitoring strategies placed for plague prevention and their implementation in the society in an attempt of improving them or replacing them with a modern one.

Acknowledgements

We are extremely grateful to all the people whose extensive efforts made this study successful. We would like to express our sincere gratitude to Africa Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology and Development (ACE IRPM&BTD) for funding this research work. We also acknowledge Mr Omary Milango a field assistant and rodent technician of Institute of Pest Management at Sokoine University of Agriculture, Morogoro Tanzania who assisted in rodent data collection and Miss Miriam Jophrey Bukuku and Mr Mwita Wambura of department of microbiology, parasitology and biotechnology at SUA who assisted in flea processing and identification.

Conflict of interest statement

No one declared conflict of interest in this study.

Ethical approval

This study was approved by the Institutional Review Board of Sokoine University of Agriculture, Tanzania (Ref. No. SUA/DPRTC/R/186/15).

Data availability statement

Authors agree to deposit the data associated with this study in a Mendeley data repository and make it publicly available once the manuscript is accepted for publication under the Journal of Ecology and Evolution. Data reference link: Jakoniko, J., Massawe, A., Mwega, E., Kessy, S., (2023), "Contributing factors for abundance of fleas on rodents in plague endemic area. Karatu District, Northern Tanzania", Mendeley Data, v1http://dx.doi.org/10.17632/mydn68pyzz.

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Table1 . Fleas collected from rodents, showing TFI and percentage prevalence of infestation

Rodent species	**No. of rodents examined (n %)**	**No. of rodents infested with fleas (prevalence)**
Arvicanthis niloticus	70 (24.05)	12 (0.17)

Rodent species	**No. of rodents examined (n %)**	**No. of rodents infested with fleas (prevalence)**
Grammomys spp	12 (4.12)	0
Graphiurus murinus	2 (0.69)	1 (0.5)
Lemniscomys striatus	43 (14.78)	13 (0.3)
Lophuromys flavopunctatus	13 (4.47)	4 (0.31)
Mastomys natalensis	122 (41.92)	31 (0.25)
Otomys spp	3 (1.03)	0
Praomys delectorum	1 (0.34)	0
Rattus rattus	25 (8.60)	12 (0.48)
Total	**291**	**73 (0.25)**

Table2 . Rodent specific flea indices (SFI) across different habitats

Habitats	**Rodent species**	No. rodents examined	No. rodents infested (prevalence)	**No. fleas collected and (SFI)**	**No. fleas collected and (SFI)**	**No. fleas collected and (SFI)**	**No. fleas collected and (SFI)**
				X. brasiliensis	*X. cheopis*	*D. lypusus*	*Ctenophthalmu spp*
Bush land	*A. niloticus*	27	4 (0.15)	0	0	6 (0.22)	3 (0.11)
	Grammomys spp	5	0	0	0	0	0
	Lemniscomys striatus	7	0	0	0	0	0
	Mastomys natalensis	12	5 (0.42)	0	0	4 (0.33)	1 (0.08)
	Otomys spp	2	0	0	0	0	0
Farm land	*A. niloticus*	10	2 (0.2)	0	0	4 (0.4)	0
	Grammomys spp	1	0	0	0	0	0
	Lemniscomys striatus	29	10 (0.34)	4 (0.14)	0	19 (0.66)	12 (0.41)
	L. flavopunctatus	2	1 (0.5)	0	0	2 (1)	0
	M. natalensis	45	13 (0.29)	3 (0.07)	3 (0.07)	16 (0.36)	15 (0.33)
Forest buffer zone	*Grammomys spp*	5	0	0	0	0	0
	G. murinus	2	1 (0.5)	0	0	3 (1.5)	0
	L. flavopunctatus	11	3 (0.27)	0	0	6 (0.55)	1 (0.09)
	M. natalensis	27	7 (0.26)	6 (0.22)	0	8 (0.3)	3 (0.11)
	P. delectorum	1	0	0	0	0	0
Peridomestic	*A. niloticus*	33	6 0.18)	0	0	5 (0.15)	9 (0.27)
	Grammomys spp	1	0	0	0	0	0

Habitats	**Rodent species**	No. rodents examined	No. rodents infested (prevalence)	**No. fleas collected and (SFI)**	**No. fleas collected and (SFI)**	**No. fleas collected and (SFI)**	**No. fleas collected and (SFI)**
	L. striatus	7	3 (0.43)	0	0	7 (1)	1 (0.14)
	M. natalensis	38	6 (0.16)	0	0	8 (0.21)	6 (0.16)
	Otomys spp	1	0	0	0	0	0
	Rattus rattus	25	12 (0.48)	18 (0.72)	17 (0.68)	0	0
	Total	**291**	**73 (0.25)**	**31**	**20**	**88**	**51**

Table 3 . Summary of the best fitting coefficients of GLM (Poisson family) describing the influence of predictor variables (parameters) on abundance of fleas.

Parameters	**Estimate**	**Std. error**	**Z value**	***p*-value**
Intercept	-2.48	0.41	-6.05	$< .001$
Rodent species	-14.96	573.36	-0.03	0.98
Grammomys spp				
G.murinus	1.40	0.72	1.96	0.05
L. striatus	0.56	0.29	1.91	0.06
L. flavopunctatus	0.47	0.50	0.95	0.34
M. natalensis	0.24	0.25	0.93	0.35
Otomys spp	-14.91	1206.53	-0.01	0.99
P. delectorum	-15.46	2103.36	-0.01	0.99
R. rattus	1.34	0.29	4.66	$< .001$
Season Wet season	-0.41	0.19	-2.15	0.03
Habitats Farm land	0.86	0.31	2.79	0.005
Forest habitat	0.58	0.38	1.50	0.13
Peridomestic	0.39	0.32	1.21	0.23
Rodent Weight	0.017	0.004	4.20	$< .001$
Rodent sex Male	0.53	0.15	3.46	$< .001$
Plague location Plague foci villages	0.40	0.18	2.20	0.03