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# Trypanosomiasis in Rodents from Selected Plague Endemic Foci of Tanzania

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***Author's contribution***

*The sole author designed, analysed, interpreted and prepared the manuscript.*

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## ABSTRACT

Trypanosomiasis is among the zoonotic diseases that cause threat to public health, rodents are known to be reservoir of various Trypanosomes which are zoonotic. The cross-sectional studies were conducted in selected areas of plague endemic foci in Tanzania, aiming at establishing the prevalence of *Trypanosoma* sp. infection in rodent species. A total of 105 rodents comprising nine species were captured in different habitats during the study period conducted between March and May 2022. Thin and thick smears were used to detect the *Trypanosoma* sp. infection in rodent species. The prevalence recorded was 4.8%(5/105), with individual prevalence of *Mastomys natalensis* 1.9%(2/105), *Rattus rattus*1.9%(2/105) and *Lophuromys kilonzoi* 1.0%(1/105). Prevalence of *Trypanosoma* sp. infection was not differed significantly between host species (*P*>0.05), host sex (*P*>0.05), habitat type (*P*>0.05) and wards (*P*>0.05). The present study has confirmed the presence of *Trypanosoma* sp. infection in rodents in plague endemic foci of Tanzania, hence raising the public health concern due to their zoonotic potential.

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## 1. INTRODUCTION

Rodents are small mammals that belongs to the order Rodentia, the largest order of mammals comprising more than 32 families, 481 genera and 2277 species [1]. Rodent species are widely distributed occurring almost in all continents and vastly diversified [2,3]. Rodents are highly adapted to various habitats and environments [4]. Regardless of showing greater diversity in their behaviors, morphology, ecology and physiology, all rodents species have the common dentition feature. Indeed, they are characterized by the single pair of continuously ever-grown incisors, that has to be maintained in a short lengthy by a gnawing process [5,6]. Rodent species are known to harbor many different microorganisms that are pathogenic to animals and of public health concern, various Bacteria, Viruses, Rickettsia, Fungi, Arthropods, Helminths and Protozoans have been reported [7,8].

Trypanosomiasis is a disease caused by protozoans of the genus *Trypanosoma* which can infect both human beings and animals [9]. These parasites are transmitted by arthropods vectors such as Tsetse flies, Tabanus, Mites, Reduviid bugs and Fleas both mechanically and biologically [9,10]. *Trypanosoma lewisi, Trypanosoma evansi* and *Trypanosoma lewisi* like, have been reported to infect different types of rodent species in Europe, Asia, Australia, America and Africa [11-14].Rodents usually gets infected by *Trypanosom*a spp. by ingesting the infected fleas or through ingestion of the feces of infected fleas [15].Several fleas have been implicated to transmit *Trypanosoma* spp. in various countries and this species includes the members under genera *Xenopsylla*, *Ctenopthalmus*, *Nosopsyllus*, *Dinopsylla* to mention a few, which are also very prevalent in the plague endemic foci of Tanzania [15-17,10].

Despite of being infecting rodent species, these *Trypanosoma* species are zoonotic and may cause Atypical Human Trypanosomiasis, indeed, several cases have been reported with few cases resulting into mortalities [18-22].Due to close interaction between the rodents and Humans there is a high possibility of Humans to get infected with pathogenic agents from the rodents such as *Trypanosoma* spp. The closeness interaction between the rodents and humans especially in the rural areas could facilitate the possible transmission of zoonotic agents [23].In addition, human activities such as agricultural activities increase the abundance of the rodents, in turns the risks of zoonotic infections are also increasing.

In Tanzania, to the best of our knowledge there are few studies that have been done to ascertain the magnitude of rodent Trypanosomiasis [24-26,6].Furthermore, most of these studies have been conducted in one region only which is Morogoro region neglecting other regions. Therefore, the proposed study was sought to determine the prevalence of *Trypanosoma* species infection in rodents from selected area of plague endemic foci of Tanzania, with a view of providing a baseline information on possibility of zoonotic infection in rodent species by *Trypanosoma* species in the area and hence the need of advocating the plausible control and/or prevention measures to the stakeholders.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted in two districts within Tanga and Morogoro Regions namely Lushoto and Mbulu districts respectively which are plague endemic foci. Lushoto district location lies between 4°32'S and 38°37'E, with majority of the part lying within Western Usambara Mountains that forms a part of Eastern Arc Mountains. Mbulu district lies between 3°51'S and 35°32'E. These districts experienced plague outbreak in last thirteen years [27].Within each districts two wards were selected and within each ward two villages were selected. In Lushoto district, Shume (Mavumo and Gologolo villages) and Manolo (Madala and Manolo villages) wards were selected and sampled. In Mbulu district, Nahasey (Haysali and Nahasey villages) and Yaeda Ampa (Hayaseng and Arri villages) wards were selected and sampled.

### 2.2 Study Design

A cross-sectional study design was employed.

### 2.3 Rodents Trapping, Identification and Blood Collection

Rodents were captured in different habitats by Sherman traps which was baited by a mixture of maize flour and peanut butter, from March to May 2022. The habitats selected for sampling were as follows; fallow land, crop field, bushes,

plantation forests, indoor and near natural forest areas. Sixty traps were placed on the habitat overnight while five traps were placed indoor per house, the number of days for trapping in each village was three days which is equal to six days of trapping per ward. Five households were selected randomly from the list provided by local administration for indoor rodents sampling, verbal consent was sought prior to trap setting. The traps were set during the evening (1700hrs-1800hrs) and inspected the following day in the morning (0600-0700hrs). After carefully inspection the trapped rodents were carried to the nearby area where the rodents were taken out from the traps into clothing bag, thereafter, each individual was anaesthetized by soaking the clothing bag contain the rodent into a jar that contain 98% diethyl ether one by one [6,28]. Thereafter, the individuals was identified to genus or specie level by the aid of keys described by Kingdon, [3] and the blood was collected via cardiac puncture by using 2 milliliters syringes, then the blood was placed on the well labelled 5 milliliters Sodium Ethylene Diamine Tetraacetate (EDTA) vacutainer tube for storage and transportation to Parasitology laboratory at the Department of Veterinary microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture. Various parameters were recorded from the rodents to aid their identification including tail length, head body length, pes length, weight, ear length, sex and breeding status.

### 2.4 Smear Preparation and Identification of *Trypanosoma* sp.

From the stored EDTA vacutainers containing blood sample in the refrigerator, the thin and thick smears was prepared. Thin blood smear was prepared by placing a small drop of blood on a near short end of the clean slide. By using the spreader slide holding it at an angle of 35 degree, the slide was placed in front of the drop of blood then slowly it was pulled back until it was touching the drop of blood, then the drop of blood was allowed to spread along the juncture of the two slides. Thereafter, the spreader slide was smoothly and rapidly pushed forward to produce a smear. The slide was allowed to air dry and then stained with 10% Giemsa stain for 30 minutes. The same procedure was done in preparing the thick smear except that, after air dry the smear was fixed in methyl alcohol for 10 minutes, then stained with 10% Giemsa stain for 30 minutes. The excess stain in both smears was removed by tap water, all the procedures were performed according to Hendrix and Robinson, [29]. The smears was then observed under compound microscope by using 100X magnification with the aid of oil immersion.

### 2.5 Data Analysis

The raw data was double entered and cleaned on Microsoft excel 2019. Descriptive data were summarized in tables and figures. The prevalence was calculated according to Bush et al. [30]. The prevalence of Trypanosomiasis was compared between host species, host sex, habitat type and wards by Chi square test. All the statistical analysis was computed on Statistical Package for the Social Sciences (SPSS) version 20 IBM. In all analysis the *P*-value was set at 0.05 as significant.

## 3. RESULTS

### 3.1 Rodents Captured

A total of 105 rodent individuals were captured during the study period, with 9 species encountered including *Arvicanthis nairobe, Rattus rattus, Grammomys* sp*., Mus* sp*., Acomys* sp*., Lophuromys kilonzoi, Praomys delectorum, Lophuromys flavopunctatus* and *Mastomys natalensis* (Table 1). Their abundance is also shown in Table 1. The distribution of different rodent species in various habitats is shown in Table 2. The percentage of females and males captured is shown in Fig. 1. *R. rattus* and *M. natalensis* dominated the capture with more than 70% of the total capture. The number of rodent individuals captured within different wards are shown in Table 3.

### 3.2 Prevalence of Trypanosomiasis

Prevalence of Trypanosomiasis was found to be 4.8%, 95% CI (1.6, 10.8), with two individuals from *R. rattus* (1.9%) and *M. natalensis* (1.9%) infected while one *L. kilonzoi* (1.0%) was infected. The rest of the species was found to be not infected by *Trypanosoma* sp. The Chi square test revealed that the *Trypanosoma* sp. infection prevalence was not statistically different when compared to host species ($X^2$=7.198, df=8, P=0.515), host sex ($X^2$=0.008, df=1, P=0.930), habitat type ($X^2$=2.965, df=5, P=0.705) and wards ($X^2$=3.837, df=3, P=0.280) as shown in Tables 4 and 5.

**Table 1. Number of captured individual rodents during the whole study and their abundance**

| Rodents species | Number of captured individuals | Relative abundance (%) |
|---|---|---|
| *Mastomys natalensis* | 62 | 56.0 |
| *Rattus rattus* | 16 | 15.2 |
| *Luphorumys flavopunctatus* | 6 | 5.7 |
| *Luphorumys kilonzoi* | 4 | 3.8 |
| *Acomys* sp. | 1 | 1.0 |
| *Grammomys* sp. | 3 | 2.9 |
| *Praomys delectorum* | 5 | 4.8 |
| *Mus* sp. | 2 | 1.9 |
| *Arvicanthis nairobe* | 6 | 5.7 |
| **Total** | 105 | 100 |

**Table 2. Distribution of different species of rodents according to various habitats on selected plague endemic areas**

| Rodent species | Habitats | | | | | |
|---|---|---|---|---|---|---|
| | Fallow land | Crop field | Indoor | Bush | Plantation forest | Near natural forest |
| *Mastomys natalensis* | × | × | × | × | × | |
| *Rattus rattus* | | | × | | | |
| *Luphorumys flavopunctatus* | × | × | | | | × |
| *Luphorumys kilonzoi* | | × | | | × | |
| *Acomys* sp. | | × | | | | |
| *Grammomys* sp. | | | | × | × | |
| *Praomys delectorum* | | × | | × | × | |
| *Mus* sp. | | × | × | | | |
| *Arvicanthis nairobe* | × | × | | | | |

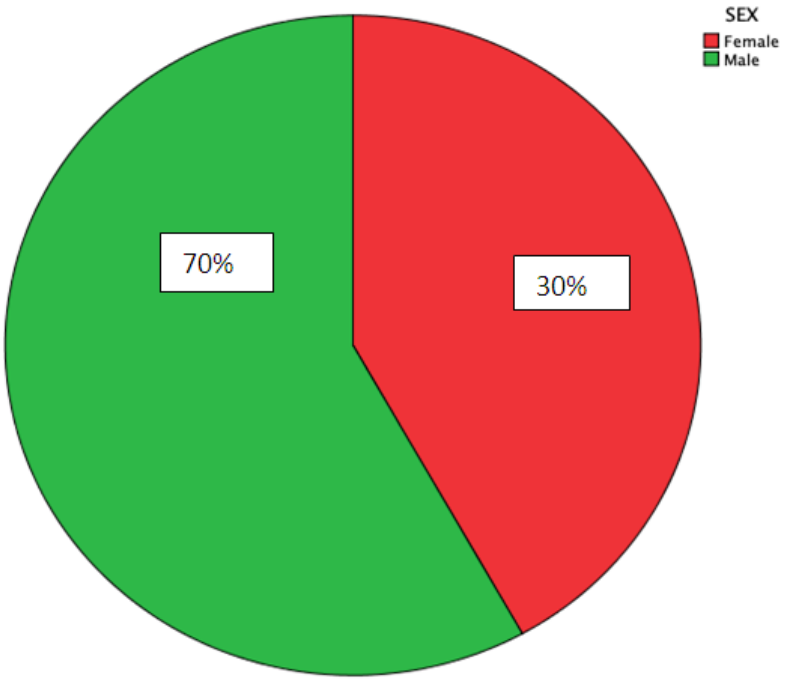


**Fig. 1. Percentage of captured rodent species according to sex**

**Table 3. Number of captured individual rodents during the study with respective to their sampling wards**

| Rodent species | Wards | | | | |
|---|---|---|---|---|---|
| | **Manolo** | **Nahasey** | **Shume** | **YaedaAmpa** | **Total** |
| *Mastomys natalensis* | 14 | 15 | 21 | 12 | 62 |
| *Rattus rattus* | 0 | 4 | 7 | 5 | 16 |
| *Luphorumys flavopunctatus* | 0 | 2 | 0 | 4 | 6 |
| *Luphorumys kilonzoi* | 1 | 0 | 3 | 0 | 4 |
| *Acomys* sp. | 0 | 1 | 0 | 0 | 1 |
| *Grammomys* sp. | 1 | 0 | 2 | 0 | 3 |
| *Praomys delectorum* | 0 | 0 | 5 | 0 | 5 |
| *Mus* sp. | 0 | 0 | 1 | 1 | 2 |
| *Arvicanthis nairobe* | 4 | 2 | 0 | 0 | 6 |
| **Total** | **20** | **24** | **39** | **22** | **105** |

**Table 4. Prevalence of *Trypanosoma* sp. in rodents regarding to host species and host sex**

| Variable | Sample size | Infected no.(%) | Chi-square | P-value |
|---|---|---|---|---|
| **Host species** | | | | |
| *Mastomys natalensis* | 62 | 2(1.9) | | |
| *Rattus rattus* | 16 | 2(1.9) | | |
| *Luphorumys flavopunctatus* | 6 | 0(0.0) | | |
| *Luphorumys kilonzoi* | 4 | 1(1.0) | | |
| *Acomys* sp. | 1 | 0(0.0) | 7.198 | 0.515 |
| *Grammomys* sp. | 3 | 0(0.0) | | |
| *Praomysdelectorum* | 5 | 0(0.0) | | |
| *Mus* sp. | 2 | 0(0.0) | | |
| *Arvicanthis nairobe* | 6 | 0(0.0 | | |
| **Total** | **105** | **5(4.8)** | | |
| **Sex** | | | | |
| Males | 61 | 3(2.9) | 0.008 | 0.930 |
| Females | 44 | 2(1.9) | | |
| **Total** | **105** | **5(4.8)** | | |

**Table 5. Prevalence of *Trypanosoma* sp. in rodents regarding to habitat types and wards**

| Variable | Sample size | Infected no. (%) | Chi-square | P-value |
|---|---|---|---|---|
| **Habitat type** | | | | |
| Fallow land | 19 | 0(0.0) | | |
| Crop fields | 47 | 2(1.9) | | |
| Indoor | 19 | 2(1.9) | | |
| Plantation forest | 13 | 1(1.0) | 2.965 | 0.0705 |
| Bush | 4 | 0(0.0) | | |
| Near Natural forest | 3 | 0(0.0) | | |
| **Total** | **105** | **5(4.8)** | | |
| **Wards** | | | | |
| Manolo | 20 | 2(1.9) | | |
| Shume | 39 | 0(0.0) | 3.837 | 0.280 |
| Nahasey | 24 | 2(1.9) | | |
| Yaeda Ampa | 22 | 1(1.0) | | |
| **Total** | **105** | **5(4.8)** | | |

## 4. DISCUSSION

The present study captured a total of 105 rodent species with 9 species recorded.*M. natalensis* and *R. rattus* dominated the total capture with more than 70% of the total capture. Indeed, predation and availability of the food might influence the increase in the relative density of individuals captured. Furthermore, density of the species, nature of trap, nature of vegetation, type of habitat and number of days for trapping might affect the total number of captured individuals. Our results are concurring with the study done by katakweba et al. [25], katakweba, [6] and Mulungu et al. [4] where by *M. natalensis* and *R. rattus* dominated the total capture. Most of relatively large number of captured individuals were from crop field followed by fallow land and indoor whereas the bushes, plantation forest and near natural forest habitats had very low number. Indeed, this may be explained due to the fact that the nature of the vegetation found in this habitat may influence the distribution as well as abundance of the individual species, nature of the vegetation in turns affect the food and shelter availability in respected habitats. Furthermore, the study period coincide with harvesting period where by the crop fields had plenty crops such as maize, yams and beans.

Indubitably, the higher abundance of *M. natalensis* in various habitats in the areas might be due to the fast reproductive cycle, having large litter size, availability of various excellent quality and quantity food options and their ecological generalist behavior [31,25]. *R. rattus* were only exclusively captured indoors on almost all districts. The present findings concur with the study conducted by Kilonzo el al. [32] and Laudisoit et al. [33]. This invasive commensal rodent can outcompete other species, the present findings shows all the captured individuals were indoor, although *R. rattus* can occupy various large range of habitats, such as the forests, inside buildings and areas with densely profound litter cover amongst others [28,34,35]. *P. delectorum* and *Lophuromys* spp. were captured from the forest, this in fact due to their forest specialist behavior and edge dwellers. Mulungu et al. [4] reported the highest abundance of these species in forests. *A. nairobe, Mus* sp. *Acomys* sp. and *Grammomys* sp. were captured in relative low numbers compared to others specie, these species have been reported to occurs in relative low numbers in Tanzania [16,17].

We recorded the Trypanosomiasis infection prevalence of 4.8%, our findings are in agreement with the recently study conducted by Samiji et al. [26] in Morogoro, Tanzania, who recorded the overall prevalence of 4.04%. In addition, our findings are also in agreement with the study conducted by Katakweba, [6] who reported the prevalence of 4.29% in rodents from domestic and peri-domestic areas of Morogoro Municipality, Tanzania. Katakweba et al. [25] recorded the 4.1% prevalence in the study that involved six different regions of Tanzania namely Kilimanjaro, Mtwara, Mbeya, Singida, Tanga, Dodoma and Morogoro. Our findings are in disagreement with the study conducted by [24] in Central Tanzania with high prevalence of 22.7%, with the same study reporting low prevalence of 1.33% in Swaziland. Dahesh and Mikhail, [36] recorded the high prevalence of 24.7% in captured rodents in selected areas of Egypt. Prevalence of 21.7%, 21.3% and 20% has been recorded in Brazil, Venezuela and Italy by Linardi and Botelho, [37], Herrera and Urdaneta-Morales, [38] and De Carnieri and Castellino, [39] respectively.

The low prevalence of *Trypanosoma* sp. encountered in our present study might be due to low number of sampled animals compared to other studies. Furthermore, the decrease in vector abundance in plague endemic area due to continuous control measures against the flea vectors that are responsible for transmitting the *Yersinia pestis* could results into low transmission rate of the *Trypanosoma* sp. in the area [27].Moreover, rodents have shown to be immune to challenge dose of *Trypanosoma* infection hence this could also attribute to the low prevalence found during our study period.

Despite of not being significance differences between host species and *Trypanosoma* sp. prevalence, *M. natalensis* and *R. rattus* were recorded to have equal percentage (1.9%) of infection. Indeed, *M. natalensis* and *R. rattus* have been reported to be infected by different *Trypanosoma* species in different parts of the world. For instance in Tanzania they have been reported to be infected by *Trypanosoma* species by Katakweba et al. [24], Katakweba et al. [25], Katakweba, [6] and Samiji et al. [26]. In addition, it has been reported in Uganda, Egypt, Brazil, Mali and Australia by Linardi and Botelho, [37], Salzer et al. [12], Schwan et al. [13], Thompson et al. [40] and . Dahesh and Mikhail, [36]. Votýpka et al. [41] showed a high diversity of trypanosomes in small mammals of sub-Saharan

mammals. *M. natalensis* and *R. rattus* are more adapted to domestic and peri domestic areas, hence they are frequently interacting with humans and this could increase the risk of infections to humans [26].Also due to their behavior and their abundance in different habitats they are in high risk of getting infected by the parasite and it is known that they frequently harbors more flea vectors [17].

Although, sex was not a significant factor to the prevalence, three males were found to be infected in contrast to the two infected females. Indeed, sex-biased parasitism was shown to favor the males, indeed, males are known to be more infected by parasites due to several factors, males have large body size hence increases the probability of being infected [42]. Furthermore, males cover large territory, in turns the movements within and across territories are increased which predispose them to risk of infection, in addition, hormones such as testosterone have been shown to lower immunity hence increase also the risk of infection [43,44,45]. Samiji et al. [26] reported that all the infected rodents with Trypanosomes were males, none of the females were infected.

Habitat including crop field, plantation forest and indoors were shown to harbor the infected captured rodents, with indoor and crop field having relative high number of infected rodents than plantation forest although the results was not differed significantly. Crop fields during the study field was having plenty of foods such as maize, yams and beans which in fact contributed to high abundance of the rodents. Rodents that were captured indoor (*R. rattus*) two of them was found to be infected by *Trypanosoma* sp. this indeed implies the risk of humans to get infected is much more highly since these rodents occurs within the same households, indeed, they are sharing the same house with humans [6,26]

When compared the *Trypanosoma* sp. infection between the wards, Manolo ward in Lushoto district was having two rodents that were infected while Nahasey and Yaeda Ampa wards in Mbulu district had two and one rodents infected respectively, although these findings weren't statistically significant. The presence of *Trypanosoma* sp. infection in rodents captured in the two districts have clearly shown the possibility of people to get infected by these parasites, hence it alerts the public on risk that could be attributed by the rodents as well as their flea vectors in these areas.

## 5. CONCLUSION

The present study has confirm the presence of *Trypanosoma* sp. infection in rodents, particularly the *M. natalensis*, *R. rattus* and *L. kilonzoi* captured in plague endemic foci of Mbulu and Lushoto districts in Northern Tanzania. Trypanosomiasis is still a public health threat in most of Sub-Saharan countries, hence the awareness should be raised coupled with continuous surveillance and need of imposing control and prevention measures in the study area and elsewhere.

## CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

Ethical clearance was obtained from the Institutional Ethical Committee of the Sokoine University of Agriculture with reference number SUA/DPRTC/186/VOL IV.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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