The Prevalence of Haemoparasites in Rodents and Shrews Trapped from Domestic and Peridomestic Houses in Morogoro Municipality, Tanzania. A Hidden Public Health Threat

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SUMMARY

A total of 70 small mammals were captured from domestic and peri-domestic houses in Morogoro municipality to determine the prevalence, relative abundance and diversity of haemoparasites. Tramping was carried out using Shermans and locally made live traps bated with a mixture of peanut butter and maize bran. Blood samples were collected from supraorbital vein in the captured animals. Thick and thin smears were made and screened for infectious agents of public health importance that included *Babesia* spp., *Plasmodium* spp., *Trypanosoma* spp. and *Bacillus* spp.Two rodent species from captured samall mammals were identified as *Rattus rattus* (Roof rat) being the most dominant specie followed by *Mastomys natalensis* (Farm rat). Four blood protozoan species were found infecting the rodent population namely, *Plasmodium* spp. (n=6/70, 8.57%), *Babesia* spp. (n=5/70, 7.14%), *Bacillus* spp. (n=2/70, 2.86%) and *Trypanosoma* spp. (n=3/70, 4.29%). The relative abundance of the *Rattus rattus* was estimated to be (n=60/70, 85.7%) while that of *Mastomys natalensis* was (n=10/70, 14.3%). There was no any *Shrew* spp. that were captured in the trapping sites. The diversity of haemoparasites in the study area was 1.01. *Plasmodium* spp. infections as well as that of *Trypanosoma* spp were observed in both sexes; however, infections were higher in sub-adult rats. Malaria and sleeping sickness remain as a serious health threat and yet a vaccine is not yet available. Mosquitoes that are biting rodents also bite humans in their houses. So spreading of *Plasmodium* spp and *Trypanosoma* spp from rodents to humans is inevitable. Every year, many people suffer from malaria and sleeping sickness and die as a consequence of these diseases. In most cases children in Africa under the age of five die from Malaria while people of all ages die from sleeping sickness in the tsetse fly infested areas. The public health implications of these findings require communitywide rodents control strategies with strong emphasis on community participation in order to prevent rapid spread of rodent population.

Keywords: Haemoparasites, Rodents, Malaria, Sleeping sickness, Public health, Morogoro Municipality

INTRODUCTION

Rodents are small mammals, characterized by the presence of diastema, continuous gnawing and constant growth of their long incisor teeth. In classification, both the rodents and shrews are in the Kingdom Animalia, Phylum Chordata, and Class Mammalian. The rodents are in the order Rodentia whereas the shrews are in the order Soricomorpha and Scandentia (tree shrews) (Kingdon, 1997).

Rodents are the most successful mammals. They show great diversity in their ecology, morphology, physiology, behavior and life history strategies. The order Rodentia comprises the largest mammalian species (Kingdon, 1997). Rodents are adapted to wide range of environments (Nowak, 1999). They are highly successful mammals in different environments all over the world. Their success is probably due to their smaller size, having short breeding cycle and wide variety of food items. The way rodents select their habitats mainly depend on the vegetation type and life history strategies (Fitzherbert *et al*., 2007). The distribution and abundance of rodents are influenced by environmental factors such as nature and density of vegetation, climatic conditions, disease, predication and habitat utilization by humans (Johnson and Horn, 2008). Absence of sufficient food and ground cover largely determine the number of individual rodents in a certain area. The loss of ground vegetation leads to decreasing rodents' diversity but increasing predation risk (Hoffmann and Zeller, 2005). Rodents live in different micro habitats. Some rodents spend their entire life in the underground tunnel systems. Others such as the ground squirrels dig extensive burrow systems used for resting and caring for their young, whereas few are arboreal. Some are gliders and others are adapted for semi-aquatic life (Nowak, 1999; Wright *et al*., 2002). Rodents often respond rapidly to changes in habitat structures such as plant composition and ground cover (Leis *et al*., 2008). Their diversity tends to be lower in open habitats; this is due to reduction of habitats and food resources (Silva *et al*., 2005).

Shrews are tiny insectivorous mammals, distantly related to the mole, yet distinct from it. Unlike rodents,

shrews have very sharp, spike-like teeth. Shrews are very tiny; most shrews are smaller than a mouse (Kingdon, 1997). Through the food-web relationships, rodents and shrews directly influence population levels of insect pests and disease carriers such as gypsy moths and deer ticks, as well as certain regionally rare hawks and owls. Peridomestic animals are the ones living in and around human habitations, rat is example of peridomestic animal. Rodents by their nature and design make excellent vehicles for harbouring and rapidly transporting disease causing agents. Rodents are well adapted to living with or in close proximity to humans hence man are quite vulnerable to the potential spread of any pathogens carried by rodents. Even without parasites, rodents can rapidly transmit deadly germs excreted in their urine and feces. In addition, rodents shed their hairs daily and lose an entire coat twice a year. In this way, millions of rodent hairs and hair fragments possibly containing pathogens that could also be deposited into our environment that is shared by human beings (Barnett, 2002).

Haemoparasites (blood parasites) are those parasites which pass most of their lives usually in the vascular system of vertebrates and mammals. Some of these are *Babesia, Theileria, Borrelia, Trypanosomes,* and *Plasmodium* species (Battersby *et al.,* 2002*). Babesia* is an apicomplexan parasite that infects red blood cells, transmitted by ticks (Nowell, 1969). *Babesia* species infect livestock worldwide, also wild and domestic vertebrate animals and occasionally humans where it is causing a disease known as babesiosis (Despommier and Dickson, 1995). *Trypanosoma* is a genus of Kinetoplastids; class Kinetoplastida, a monophyletic group of unicellular parasitic flagellate protozoa (Hamilton *et al*., 2004). In an invertebrate host they are generally found in the intestine, but normally occupy the bloodstream or an intracellular in the mammalian host. Trypanosomes are unicellular flagellated protozoa found in all classes of vertebrates, and include the agents of human sleeping sickness and Chagas disease (Stevens *et al*., 1999a). Members of the genus *Bartonella* (including the reclassified genus Grahamella) are Gram-negative cocco-bacillary proteobacteria and obligate parasites of erythrocytes (Birtles*et al*., 1995); several species of which are associated with human disease (Breitschwerdt and Kordick, 2000).

Haemoparasites have generally been shown to cause destruction of red blood cells resulting in anaemia, jaundice, anorexia, weight loss and infertility. Parasitic diseases have debilitating impact on human and animal health worldwide particularly in developing countries (Ellis *et al*., 2003).

Haemoparasites of small rodents and shrews have received relatively little attention in Tanzania. Thus, little data exists about the role of small mammals in disseminating vector-transmitted blood parasites. Studies that have investigated blood parasitic fauna in other parts of the world have only focused on their occurrence and prevalence in single study areas, thus making it difficult to compare parasites of small mammals living in diverse habitats. In Africa, these rodent-borne zoonoses either cause diseases, which go undiagnosed or are misdiagnosed due to lack of information on the prevalence of the causative agents (Begon 2003). Infections with zoonotic haemoparasites are widespread in wild rodents (Korbawiak *et al*., 2005). They include *Borrelia*, *Trypanosomes*, *Bacilli*, *Plasmodia* and *Coccobacilli* species (Silayo 1992; Gratz 1997; Juha *et al*., 2003; Powelczyk *et al*., 2004). In humans, these pathogens are responsible for many rodent-borne diseases including plague, toxoplasmosis, leptospirosis, leishmaniasis and hemorrhagic fevers (Machang'u *et al*., 1997; Kilonzo *et al*., 2005; Makundi *et al.*, 2008; Laudisoit *et al*., 2009). While some haemoparasites of small rodents are crucial models in the study of human diseases, others may cause serious zoonoses. For instance, murine rodent *Plasmodia* has shed useful insights on the biology and behaviour of human malaria parasites while *Babesia microti*, a tick-transmitted parasite of rodents causes human babesiosis. Rodents impose economic damages and significant costs in the public health system. Rodents could be as reservoir hosts for vector borne parasites and study on their zoonotic haemoparasites has medical and veterinary importance.

Given the importance of rodents and shrews in terms of transmission of disease-causing agents, including parasites, to humans, studying the potential for transmission of these agents in each geographic region is essential for health. The continued interaction between house rodents and other wildlife increases the chance of transmission of zoonotics from the wild to communities or from communities to the wild. Due to the difference in prevalence of small mammals' parasitic infections in different parts of Tanzania, this study aimed to investigate the haemoparasites in small mammals trapped from domestic and peridomestic houses in relation to parasitic zoonosis. Therefore, this study will provide more baseline information that will help to fill the knowledge gaps and update the scanty information available and reaching sound decision on matters pertaining to prevalence of haemoparasites that are of public health importance in humans and animals.

MATERIALS AND METHODS

Study site

The study was carried in Morogoro Municipality. The coordinates of the study area were 6°85'S and 37°65'E.

Morogoro is a city in the eastern part of Tanzania, 196 km west of Dar es Salaam, the country's largest city and commercial centre, and 260 km east of Dodoma, the country's capital city. The climate here is tropical. In winter, there is much less rainfall than in summer. December is the warmest month of the year. The temperature in December averages 26.9 °C. In July, the average temperature is 21.5 °C. It is the lowest average temperature of the whole year. There is a difference of 178 mm of precipitation between the driest and wettest months. The average temperatures vary during the year by 5.4 °C. (Source: Climate-Data Organization).

Rodents and shrews trapping

Animals were captured using Sherman and box traps baited with peanut butter. A total of 70 small mammals were trapped and used in the study. Trapping of small mammals was carried out inside houses and in peridomestic areas using HB-Sherman live traps and locally made live- traps. Thirty Shermans and 30 box traps were set in houses for three consecutive nights at strategic points to increase the capture rate. In peridomestic areas, 20 box and 20 Sherman traps were placed in trap lines located 4 meters apart. For the large rodents like Cricetomys, havarhart traps baited with green maize and ripe banana were used. Traps were inspected every morning and the captured animals were transported to SUA Pest Management Center (SPMC) laboratory for identification and sample collection.

Sample collection

Live captured rodents were anaesthetized with chloroform. Sex of the animal was recorded. Blood sample was drawn from the supra orbital vein using a glass capillary.

Thick blood smears

A smear was made using one drop of whole blood on the centre of microscopic slide and allowed to air dry. It was then fixed with methanol for 3 minutes.

Thin blood smears

One small drop was placed near one end of a microscopic slide, spreader was placed with its edge touching the drop and inclined so that the drop runs along less than 90^{o}. The spreader was dragged forward on the slide. The obtained smear was allowed to air dry and fixed with methanol for 3 minutes.

Staining

The blood smears were stained by 10% Giemsa stain for 30 minutes was then washed with running tap water and allowed to dry. The slides were examined under ordinary light microscope at 100x magnification objective lens under oil emersion. The haemoparasites were identified using the information and structures on parasitized red blood cells (WHO, 1991).

Data analysis

The prevalence of haemoparasites in rodents was estimated between species, and habitat variations. The prevalence of the haemoparasites was tested and expressed in percentage (Okeke *et al*., 2013). Prevalence (N) =N1/N2*100/1, where N=percentage prevalence, N1=Number of host infected, N2=Total number of hosts examined for the blood parasites. Data was presented in tables and bar charts.Species richness and diversity was computed by using Shannon-Weaver Diversity Index (Shannon and Weaver, 1949). The species diversity is important to know which species is highly distributed and was calculated by using the formula $H' = \Sigma$ (Pi) ln (pi). pi = ni/N ni = number of individuals of species pi = relative abundance of species $H' = - \sum pi[\ln (pi)]$ N = total number of individuals of all species H = Shannon Diversity Index. Hmax=maximum diversity possible.

RESULTS

Rodent species captured

A total of 70 rodents were examined: Rattus*rattus* from domestic sites and *Mastomys natalensis* from the peridomestic sites. *Rattus rattus* had the highest relative abundance whereas *M. natalensis* had the least as indicated in Table 1.

Table 1: Species composition and relative abundance of the live-captured rodents in the study area

Species of rodents	Total catch	Relative abundance (%)
Mastomys natalensis	10	14.3
Rattus rattus	60	85.7
Total (2 species)	70	100

Diversity of rodent species in the study area.

The diversity was calculated basing on the number of rodent species and individual captures in every species captured. Table 2 has the summary of the diversity.

Table 2: Diversity of rodent species in the study area

Number of sample	Pi=sample/SUM	ln(Pi)	Pi*ln (Pi)
10	0.14	-1.97	-0.30
60	0.86	-0.15	-0.13
SUM=70			SUM=-0.43

If Sample values for the rodent's species (S) = 10, 60, and the total number of species (N) = 2. The sum of the sample values = 10+60 = 70 and the diversity (H') =0.43. Therefore, the diversity of the rodent species in the study area was estimated to be 0.43. After computing species diversity, species richness was also determined as follows:
Hmax = ln (N) = ln (2) =0.693, Evenness=H'/Hmax =0.43/0.693 = 0.62. Therefore, the species richness of the rodent species in the study area was estimated to be 0.62.

Prevalence of Haemoparasites in the Captured Rodents

Bacillus species, schizonts of *Plasmodium* species, *Babesia* species and *Trypanosma* species were found in both *R. rattus* and in *M. natalensis*. Out of 70 rodents only 16 were infested with parasites (Table 3). None of the shrews were captured.

Table 3: Prevalence of *Bacillus, Babesia, Plasmodium* and *Trypanosomes*species

Species (spp)	Rodent spp examined (n)	N(%) of *Bacillus* infection	N(%) of *Babesia* infection	N (%) of *Plasmodium* infection	N (%) of *Trypanosomes* infection
M. natalensis	10	1 (10)	1 (10)	1 (10)	1 (10)
R. rattus	60	1 (1.7)	4 (6.7)	5 (8.3)	2 (3.3)

Table 4: Prevalence of haemoparasites in the captured rodents

Haemoparasites	Number of samples	No. of rodents infected	Prevalence (%)
Plasmodium species	70	6	8.6
Babesia spp	70	5	7.2
Bacillus spp	70	2	2.9
Trypanosma spp	70	3	4.3
Total	70	16	22.9

Distribution of parasite in captured rodents

Bacillus, *Babesia, Plasmodium* and *Trypanosma* species were found in *M. natalensis* and *R. rattus. Bacillus* spp prevalence was 1.67% and 10%, *Babesia* spp prevalence was 6.67% and 10% whereas that of *Plasmodium* spp was 8.33% and 10% in *R. rattus* and *M.natalensis,* respectively. Schizonts were the main blood stage parasite forms in the two rodent species (Table 4).

Diversity of Haemoparasites in the Study Area

Sample values infected with haemoparasites (S) = 6, 5, 2 and 3 respectively. Total number of Haemoparasites species (N) = 4 and the sum of the sample values was calculated as: SUM=6+5+2+3 =16

Therefore, the diversity of the blood parasites in the study area was estimated to be 1.33**,** where
Hmax = ln (N) = ln (4) = 1.47, and
Evenness = H'/Hmax = 1.33/1.47 = 0.91 respectively

Table 5: Diversity of haemoparasites in the study area

Number of sample infected	Pi=sample/sum	ln(Pi)	Pi*ln(Pi)
6	0.46	-0.78	-0.36
5	0.31	-0.97	-0.37
2	0.13	-1.90	-0.28
3	0.19	-1.66	-0.32
SUM=16			SUM= 1.33
H'=1.33			

Total Prevalence of haemoparasites in the study area.

The total prevalence was calculated from the total number of rodents infected (N1) =16 and the total number of rodents examined for the haemoparasites (N2) =70 using the formula: Total percentage prevalence =N1/N2*100% =16/70*100% =22.86%. Therefore, the total prevalence (%) of haemoparasites in the study area was 22.86%.

DISCUSSION

Species composition and relative abundance were observed in this study. A total of 2 species of rodents were recorded during this investigation. The relative abundance of *M. natalensis* (14.3%) from the present investigation was the least in terms of number and diversity. According to Boutin (1990), the presence of good quality and quantity of food in a given habitat may have significant effect on the time of reproduction and body growth rate of rodents. Food shortage and reduced ground cover might be the main reasons for the declined number of rodents in the peridomestic sites (Delany, 1964). The reduction of *M. natalensis* might be food and other factors such as the nature and density of vegetation. In the perodomestic areas the habitat was utilized by humans and there was no dense vegetation to favour this specie of rodent. It has been also observed that *M. natalensis* were trapped and captured in houses as they were searching for food (cereals) due to the shortage of food in the environments (Katakweba *et al*., 2012). *Rattus rattus* accounted for 85.7% of the captures and was the most abundant and widely occurring species of the total captured rodents. *Rattusrattus* is the most widespread and numerous rodents in the domestic sites of Morogoro municipality. This might be attributed to the diverse feeding habit of the species. Based on the statistical data obtained, the relative abundance of rodents showed far more marked significant variation across domestic and peridomestic sites. This could be mainly attributed to population fluctuation in some species and capturing of some species only in one season. Furthermore *R. rattus* is the main specie of rodents that is solely found in house as the name of roof rats. Having this abundance of this type of rodent is of its normal ecological nature. Rodents species (*R. rattus*, and *M. natalensis*) captured in this study belong to the cosmopolitan commensal rodents that are often found in close association with people in dense settlements.

The bacillus species were also encountered in this study though at lower frequencies. However, this finding cannot be considered conclusive due to the small sample size of rodents studied. The presence of the bacillus species in rodent blood smears was not totally unexpected since rodents are known to be carriers of various bacteria in their blood, including the agent of plague *Yersinia pestis* (Kilonzo, 1997). In plague endemic areas, the presence of bacillus species in the blood of commensal rodents can be a cause of concern for the public health. The close proximity between human with rats in housing areas also have been including *T. rhodesiense, T. gambiense, T. brucei, T. congolense, T. vivax* and *T. suis* (Silayo 1992; Juha infecting rats i.e.; *R. norvegicus* in Sri Lanka (Sannasuriya *et al*., 1999), *Rattus* and *Bandicota* species identified to contribute significantly to the spread of many zoonotic diseases. Rats being closely associated with human serve as high potential for zoonotic infections to human (SitiShafiyyah *et al*., 2012). The spread of disease from rodents to humans is mainly facilitated by ectoparasites found on rodents like fleas, ticks and lice.

The schizonts found in this study were identified as *Plasmodium* because of the absence of white food vacuoles, multiple infected cells, extracellular forms, and pleomorphic forms. *Plasmodium* species prevalences were greater in *R.rattus* than in *M. natalensis.* The presence of *Plasmodium* spp was previously also reported in rats (Kreier *et al*., 1972; Makokha *et al*., 2011). Makokha *et al*. (2011), reported low prevalence of *Plasmodium* species infections with 6.8% and 3.7% in *Praomysjacksoni* and *Mastomys* species, respectively. Ramakrishnan and Prakash (1950), identified *Plasmodium berghei* as the species infecting *R. norvegicus* and *R. rattus* and noted the morphological characteristics of *P. berghei* in rats while Kreier *et al*. (1972), reported on the relationship between erythrocyte morphology and parasitization of *Plasmodium* species on rats.The malaria illness in humans have been reported in many countries in the world which cause high mortality and morbidity rate to humans that consequently have great impact to public health and are of major economic importance to the community. The haemoparasites (*Plasmodium* and *Babesia* species) observed in the blood cell of the rodents is expected. These genera of haemoparasites are those commonly found associated with rodents (Opara and Fagbemi, 2008). Katakweba *et al*. (2013), found few plasmodium spp in *R. rattus* captured from houses (3/760 = 0.4%) compared to the present study. This could be explained by the seasonal variations between the two studies. The overall prevalence of babesiosis in the study area was relatively low (7.14%). This may be due to geographical difference and distribution of tick vector in the study area.

Many species of *Trypanosomes* have been isolated from different rodent species. *T. lewisi* in the blood of a large proportion of individuals of *R. rattus* raises a public health concern because of the commensal nature of this species. This is supported by a study of Katakweba *et al*. (2012) who reported 45.2% (211/467) prevalence of *T. lewisi*in *R. rattus* in Tanzania. Another study by Katakweba *et al.* (2013), also reported in *R. rattus* the prevalence of 17.6% (134/760) of *Trypanosomes* spp. *Rattus rattus* is thought to be a potential reservoir and vector of human or animal pathogenic trypanosomes, 2003; Powelczyk *et al*., 2004). *Trypanosoma lewisi* infections have been recorded throughout the world in Thailand and Tanzania (Jittapalapong *et al*., 2008; Katakweba *et al.,* 2012)*, R. novegicus*in Brazil (Linardi

and Botelho, 2002), black rats in Niger, West Africa (Dobigny *et al*., 2011), free living rats in Poland (Karbowiak and Wita, 2001), small rodents of Kakamega Forest in Western Kenya (Makokha *et al*., 2011), in northern Iraq (Molan and Hussein, 1988) and in Ibadan (Akinboade *et al*., 1981). A *Trypanosoma lewisi* - like haemoflagellate was also reported in a single *Rattus tiomanicus* during a field study of small wild mammals in Central Pahang in Peninsular Malaysia (Yap *et al*., 1977). Higher *T. lewisi* infections in male compared to female rats was observed by Linardi and Botelho (2002), and attributed this to ecological and behavioral conditions. Male rats are territorial with a wider home range. This behavior exposes the hosts to *Xnopsilla cheopis* flea infestation (Linardi *et al*., 1985) and therefore, *Trypanosoma* infection in humans and animals biten by fleas that had fed blood from *R. rattus.*

This study has shown that infestation of rodents by haemoparasites is of serious zoonotic importance. Rodent and rodent-borne parasites may become more serious in human population, zoonotic transmission of these rat-borne parasites are exacerbated in communities where standards of environmental and personal hygiene are not maintained. At the root of rodent-human interactions lies structural poverty and poor housing infrastructure and lack of basic amenities encourage colonization by rodents and increase the frequency and intensity of rodent-human contact. Rodents prefer buildings with good cover in surrounding areas; where vegetation reaches right up to the walls of the building, which ideally for them should have soft floors, broken brickwork and the like, and be untidy. Under such conditions control, particularly with rodenticides, is virtually impossible. The two species of rodents documented in this study and their haemoparasite fauna are of veterinary and medical importance. According to Pini*et al.*(2003), their involvement in the epidemiology of new and emerging infectious diseases of epidemic importance cannot be put aside. This finding is therefore a critical step to estimating and assessing the status of rodent infestation in the Morogoro municipality.

There was certain limitations in this study. Firstly, the study sample was small and therefore may not have been adequately sized to accurately measure haemoparasites species prevalence and diversity of all rodents in the study area.

Thus, the absence of statistical findings about other haemoparasites in this study may not necessarily mean the absence of parasites or correlations in the study area, and larger cohort studies are therefore needed. Secondly, this study was done during the minor rainy season. An extension of the study to include both major and minor rainy seasons would have provided a clearer interpretation of the influence of rainy seasons on malaria and sleeping sickness parasites. The species evenness for the haemoparasites in the study area was approximately equal to be equal to 0.91. However, in an ecological study designed to measure species diversity, a wildlife biologist might count the number of individuals of all species present in an area and calculate the diversity index for the area. Comparison of the diversity index to that of other areas or observing an area over time will provide insight into the health of an ecosystem.

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