Trypanosomes Infection in Rodents and their Zoonotic Potential from Ruaha Ward in Kilosa District, Tanzania

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Abstract

Zoonotic haemoparasites are among of the public health problems that affect human population and are capable of being transmitted from wildlife reservoirs. Study on trypanosomes infection in rodents from Ruaha ward in Kilosa district, Tanzania was carried out on March 2020. The total of 99 individuals of rodents were captured from different localities in Ruaha, using Sherman live traps. Blood samples were collected from supraorbital vein of captured individuals, both thick and thin smears were made, dried and stained with Giemsa at the ratio of 1:10. After washing and drying they were observed under microscope at 100 magnifications with oil immersion for trypanosomes infection. Out of 99 rodents captured there were, Rattus rattus 22 (22.22%), Mastomys natalensis 72 (72.73%), and Aethomys chrysophilus 5 (5.05%). Among the captured rodents, 62 (62.63%) were males and 37 (37.37%) were females. Rattus rattus appeared to be predominant species in resident areas, while Mastomys natalensis followed by Aethomys chrysophilus bieng dominant in fallow and cultivated land areas. The infectious agent (Protozoa) belonging to genus Trypanosoma was found infecting the rodent population. Rattus rattus (n=3/99, 3.03%) were shown to have high prevalence compared to Mastomys natalensis (n=1/99, 1.01%), meanwhile Aethomys chrysophilus (n=0/99, 0.00%) were found not infected with any trypanosomes. The overall prevalence of trypanosomes were (n=4/99, 4.04%), however, captured female rodents were not infected. It is concluded that zoonotic agent (Trypanosoma spp.) are prevalent to rodents in Ruaha ward, hence it is recommended that more survey of trypanosomes infections in rodents are crucial for disease surveillance as the way toward ending Trypanosomiasis by 2030.

Keywords: Prevalence, Rodents, Zoonoses, Public health, Trypanosoma spp.

Introduction

Rodents are the most frequent and important mammals on the earth, because they tend to adapt themselves with different locations and environmental changes (Seifollahi *et al.,* 2016). However, rodents are hazardous, as they can amplify pathogen from environment and from reservoirs of (zoonotic) disease (Gratz 1994; Meerburg *et al.,* 2009), and they spread the diseases through two different ways as described by Meerburg *et al.* (2009). These two ways include, direct route that rodent can transmit pathogen to human (example, by biting or breath on germs that are present in rodent excrements), and indirect route, in the sense that rodents can serve as amplifying host of pathogens and can bring into direct contact with humans by means of ectoparasitic arthropod vectors (mites, ticks, flies).

The infections with zoonotic haemoparasites are widespread in wild rodents (Korbawiak *et al.,* 2005; Katakweba *et al.,* 2012), they include Borrelia, Trypanosomes, Bacilli, Plasmodia and Coccobacilli (Silayo 1992; Gratz 1997; Juha *et al.,* 2003; Powelczyk *et al.,* 2004; Katakweba *et al.,* 2012). There are five groups of protozoan haemoparasites recognized, one group of euglenozoan flagellates including trypanosomes

and four groups of apicomplexan parasites (O'Donoghue, 2017), where they tend to infect all terrestrial vertebrate groups including rodents.

Trypanosomes parasites are of medical and veterinary importance which mainly transmitted by tsetse fly (Glossina genus) to a wide range of vertebrate hosts (Morrison, 2011), where rodents are considered as reservoirs of these pathogens (Meerburg *et al.,* 2009; Seifollahi *et al.,* 2016). In addition, according to Han *et al.* (2015), out of 2277 extant rodent species studied only 217 species were found to be reservoirs harboring 66 zoonoses caused by viruses, bacteria, fungi, helminthes and protozoa, also they found 79 species of rodents were hyper reservoirs carrying between 2 and 11 zoonoses.

Trypanosomiasis have unbearable impact on human and animal health worldwide particularly in developing countries (Swallow *et al.,* 2000). Most outbreaks of rodent borne diseases in humans are commonly related to socio-economic deficiencies such as poor housing, poverty and overcrowding (Katakweba *et al.,* 2012), that are more prevalent in developing counties like Tanzania. Given the damage of rodents to humans and economic loss and due to health importance, parasitological studies on rodents seem necessary (Seifollahi *et al.,* 2016).

Rodents are well adapted to leave with or in close proximity to humans hence man are quite vulnerable to the potential spread of any pathogens carried by rodents (Dada, 2016). The close association between rodents and humans especially in rural areas facilitated the spread of zoonotic agents (Hamed *et al.,* 2003; Gholipoury *et al.,* 2016). In many settlements there is a relatively little awareness that rodents can transmit diseases, consequently little emphasis is directed towards the management of rodents and associated disease vectors (Katakweba *et al.,* 2013). Some rodent species such as Mastomys natalensis play more important role in distribution of zoonotic diseases like bubonic plague (Bastos *et al.,* 2005)

Human activities that change the ecosystem of rodents' living place have an important role in the epidemiology of zoonotic disease. These activities among others are agricultural fields and agroforest which provide food and shelter; yet, these are the most critical factors that influence rodent's distribution (Hieronimo *et al.,* 2014).

Generally, there is under-reporting of rodent-borne zoonoses and insufficient attention is paid to the diagnosis of these important diseases in sub-Saharan Africa (Katakweba *et al.,* 2012). As far as there is frequent rodent-human interactions, could increase the potential for contracting some zoonotic diseases like Trypanosomiasis in which (at critical stage) its treatment requires drugs that can cross the blood-brain barrier to reach the parasites. Therefore, this study was conducted to collect some informative data about trypanosomes infections in rodents from rural areas, where rodents are in close association with human settlements.

Materials and methods

Study Site

The study was conducted at Ruaha in Kilosa district Morogoro, Tanzania. Kilosa which is located at latitude 5º55ˈ to 7º53ˈS and longitude 36º30ˈ to 37º30ˈE, and its elevation is variable, Ruaha is situated at elevation 400 M above the sea level. The area is consisted with Ukaguru and Rubeho Mountains which is part of Eastern Arc Mountains (EAMs) that is recognized as global biodiversity hotspot and the mountains lies along western side of the district. The area experience bi-modal rainfall with short rains range from November to January, and long rains range from March to May with its peak in April. Annual mean rainfall of the area ranges from 1000 mm to 1400 mm in Southern part of the district, and temperature of the district varies with altitude. The mean annual temperature is 25ºC with coldest month (19º) and hottest month (30ºC) being July and March, respectively.

Study Design and Rodent Trapping

A cross sectional study design were used whereby different areas were selected and designed for trapping rodents within the ward. The traps were placed in 30 human residence (inside houses), fallow and cultivated areas. Rodents were captured by using Sherman LFA live traps (7.5*9.0*23.0 cm: HB Sherman

traps, Inc, Tallahossee, FL) and locally made live-traps (wire cage), whereby peanut butter which mixed with maize flour was serves as bait Three Sherman traps and two local traps were placed inside houses for three consecutive nights, at strategic points (corridors and kitchen) in order to increase capture rate. In both fallow and cultivated areas, traps were placed in line transect whereby each line trap had 10 traps that were situated 7 m apart within the line and 5 m apart between line traps. Traps were inspected every morning to collect and identify the captured rodents using (Kingdon, 1997), as described by (Mulungu *et al.,* 2008; Herbreteau *et al.,* 2011). Captured rodents were identified for sex (male or female) whereby perforated and unperforated vagina indicate female active and not active females, respectively while with scrotal or abdominal as male active and not active, respectively.

Blood Sample Collection and Smears Preparation

Captured rodents were anaesthetized by Ethyl ether soaked in cotton wool and 1-2 mls of blood were drawn from supraorbital vein using a glass capillary. Thin and thick blood smears were prepared from each blood sample on microscopic glass slides. The smears were left to dry in air for 5 minutes, and then fixed with methanol for 3 minutes. Each capillary was used for one animal only in order to reduce the chance of transmitting haemoparasites from one animal to another and to ensure accurate results (Ameen *et al.,* 2012). The blood smears were immersed in 10% Giemsa stain solution (1:10 dilution) for 30 minutes (Katakweba *et al,* 2012). Then smears were then flushed with water (tap water) to remove excess stain for 10 second, and allows to dry completely before fixation into pure methanol for 1 minutes then dried again (Olubunmi, 2013; Dada, 2016). The blood smears were examined under the light microscope (Olympus CX21) at 100x magnification with oil immersion and the haemoparasites were identified using the information and structures on parasitized red blood cells (WHO, 1991).

Haemoparasites identification

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Data Analysis

Data obtained was recorded and entered in Microsoft Excel, whereby Trypanosomes infections was compared between rodent species and sex. The estimation of prevalence was done through the formula; Prevalence (N)=N1N2*100%, Where N=Percentage prevalence, N1=Number of rodents infected, N2=Total number of rodents examined for the parasite.

Results

Captured rodents

All 99 rodents were captured from different localities around Ruaha ward, including; *Rattus rattus* 22 (22.22%), *Mastomys natalensis* 72 (72.73%), and *Aethomys chrysophilus* 5 (5.05%). Among the captured rodents, 62 (62.63%) were males and 37 (37.37%) were females as indicated in Table 2. Out of three species of rodents captured, *Rattus rattus* was dominant species in resident areas, while *Mastomys natalensis* and *Aethomys chrysophilus* were dominant in fallow and cultivated land (Table 1).

Table 1: Species composition and relative abundance of rodents captured

Rodent species	Total captured	Sex		Relative abundance (%)
		Male	Female	
Rattus rattus	22	18	4	22.22
Mastomys natalensis	72	40	32	72.73
Aethomys chysophilus	5	4	1	5.05
Total (3-species)	**99**	**62**	**37**	**100**

Table 2: Localities where rodents were captured/trapped for detection of trypanosomes infection

Species	Inside house	Fallow land	Cultivated land
R. rattus	22	-	-
M. natalensis	2	21	49
A. chrysophilus	-	-	5
Total	**24**	**21**	**54**

Haemoparasites detection

There was a very low prevalence of trypanosomes in the study area. Out of 99 blood samples screened, only 4 (4.04%) samples were positive for trypanosomes infection, whereas 95 (95.96%) samples were not infected (Table 3). Regarding the sex, all of infected rodents were male (100%) compared to that of female (0%). The detected *Trypanosoma* spp. in rodents is mainly zoonotic in nature.

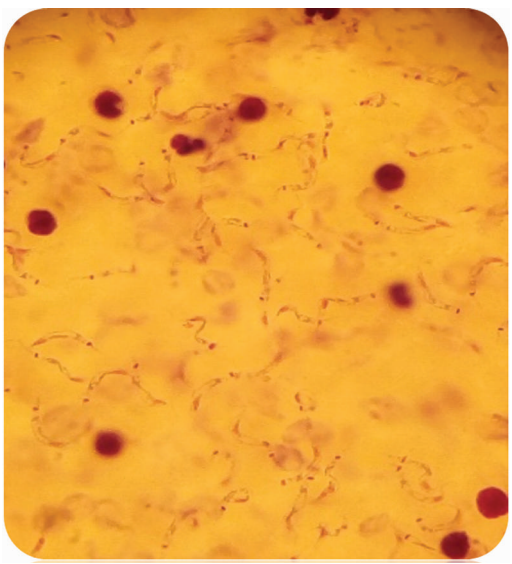

Plate 1: Trypanosoma spp. viewed in thick blood smear

Discussion

The present study was carried out to determine the trypanosomes infection in rodents and their zoonotic potential from Ruaha in Kilosa district, Tanzania. The rodent species captured in this study are often found in close association with people in dense settlements. Rattus rattus (22.22%) shows moderate abundant and were captured inside houses. This finding is in line with Belmain *et al.* (2002), that R. rattus is rarely to be trapped in the bush or in farmers' fields, and the species appears to be predominantly confined to areas of human settlement due to their nesting behaviour. Also, similar finding has been reported by Katakweba *et al.* (2013). The availability of food in human settlements provide an ideal environment for rodents' infestation particularly *Rattus rattus*.

Mastomys natalesis and *A. chrysophilus* were captured from cultivated area and fallow land. However, few Mastomys natalensis were found in houses, this concurrent with katakweba *et al.* (2012). Meanwhile within cultivated areas were high amount of food and guaranteed availability of water, hence, push growth rate of these two species. This also have been described by Boutin, (1990) and elsewhere. Mastomys natalensis (72.73%) shown to be more abundant compare to other species captured, this attributed to their nesting behaviour. Belmain *et al.* (2002), reported similar finding that *M.*

Table 3: Prevalence of trypanosome infection in captured rodents

Species	No. captured	No. infested	Prevalence (%)
Rattus rattus	22	3	3.03
Mastomys natalensis	72	1	1.01
Aethomys chrysophilus	5	0	0
Total	**99**	**4**	**4.04**

natalensis show more prevalent in the area where houses are isolated from each other (500-1500 m), this means areas with fallow and cultivated land. Also, Hubbard. (1972) and, Timbuka and Kabigumila. (2006), reported that Mastomys has generally been regarded as the most adaptable and the most widespread rodent in East Africa.

The importance of adequate housing and sanitation for the maintenance of health has long been a topic of scientific and public health policy discussion. In this study houses with disrepair indicators and poor sanitary conditions accounted for higher rodent captured when compared with those without evidence of disrepair. Therefore, the current study agreed with Dada (2016), that crowded, unsanitary and dilapidated housing conditions exacerbate rodent infestation.

This study has demonstrated the presence of trypanosomes in the blood smears of rodents, with *R. rattus* accounting for the majority of positive cases. This phenomenon has been reported elsewhere (Katakweba *et al.,* 2012; Katakweba *et al.,* 2013). The Trypanosome spp. observed in the blood smears of rodents was expected, since this genera of haemoparasites are commonly found associated with rodents as described by Dada, (2016). In addition, the current study shows higher infestation on male rodents than females. This concurrent with Linardi and Botelho, (2002). Furthermore, this study concurrent with Katakweba *et al.,* (2013), that presence of the trypanosomes in the blood of a large number of R. rattus raises a public health question whether this commensal rat could be a potential reservoir and vector of human or animal pathogenic trypanosomes such as *T. rhodesiense*, *T. gambiae* or others. The observed trypanosomes were not further characterized to determine their species or pathogenic significance in infected animals.

The current study shows that the overall prevalence of trypanosomes to be relatively low (4.04%), with 3.03% and 1.01% prevalence in *R. rattus* and *M. natalensis* respectively. Low prevalence may be attributed to geographical difference and distribution of vector in the study area. Also, Abdullah *et al.* (2019), reported that occurrence of haemoparasite is determined by abundance of the vector responsible for the transmission. Further, environmental factors (rainfall, temperature, relative humidity) have effects on the occurrence of vectors responsible for transmission on the area.

Rodent infestations in dense urban settlements are expected and unfortunately human health risk is not known. In the current study commensal rodents (*M. natalensis* and *R. rattus*) were found infected. Unfortunately, it is reported that world urban population is set to raise by 2.1 billion by 2030 (Taylor *et al.,* 2008), and by 89 million Tanzania in particular by 2035 (MNRT, 2020), such population explosions will inevitably favour commensal rodents. With ongoing urbanization in Ruaha ward the situation creates high risk of not only trypanosomes transmission but also other rodent-borne zoonotic disease transmission.

Therefore, this study is not totally conclusive due to small samples collected from the area of study because of time limit, meanwhile this study was conducted during wet season so, there is open room for further investigation on characterizing trypanosomes spp. in rodents across seasons.

Conclusion

This study shows Trypanosomes infection in commensal rodents (*R. rattus* and *M. natalensis*), whereby *R. rattus* was highly infected and in term of sex, males were mostly infected. Despite of the small sample size included, nevertheless the study suggests that *R. rattus* act as main rodent reservoir of trypanosomes parasites in the area. Also, it is revealed that houses with disrepair indicators and poor sanitary conditions accounted for higher rodent captured when compared with those without evidence of disrepair. So far, human-rodent interaction facilitates not only trypanosomes transmission but also other rodent-borne parasites which may become more serious in human health. Therefore, this finding is a critical step in estimating and assessing the status of rodent infestation in the study area. In view of this, community wide rodents control strategies with strong emphasis on community participation must be employed to prevent rapid spread of rodent population. As reported by

Belmain *et al.,* (2008) that involving the local communities in management of rodents has been shown to be effective in reducing commensal rodent infestations.

Recommendations

As the way toward ending trypanosomiasis (to zero transmission) by 2030, more surveys of trypanosomes in rodents are crucial for disease surveillance and control. Also, better planning of urban (and developing rural) housing scheme is much encouraged to reduce human-rodent interaction.

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