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Prevalence of leptospirosis and toxoplasmosis: A study of rodents and shrews in cultivated and fallow land, Morogoro rural district, Tanzania

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Abstract: Leptospirosis and toxoplasmosis are among understudied zoonotic diseases that are also not diagnosed routinely in Tanzania. Humans get leptospirosis and toxoplasmosis through contact with an environment contaminated with *Leptospira* bacteria and *Toxoplasma* protozoa from reservoir hosts, which are rodents and cats, respectively. The objective of this study was to determine the prevalence of *Leptospira* and *Toxoplasma* infections in rodents and shrews in Mikese area of Morogoro Rural District in eastern Tanzania. A total of 89 rodents and one shrew from cultivated and fallow land were tested for leptospirosis using six *Leptospira* serovars: Sokoine, Kenya, Canicola, Lora, Hebdomadis and Pomona. Toxoplasmosis was determined in 46 rodents brain smears. The prevalence of leptospirosis was 25.8%, and *Leptospira* serovar Sokoine was the most prevalent serovar (16.9%). *Toxoplasma* was detected in one rodent (2.17%) individual while three rodent individuals had *Toxoplasma*-like parasites hence were considered suspect positive. Findings suggest potential existence of human leptospirosis which needs to be further investigated. Public awareness of leptospirosis and toxoplasmosis should be promoted and their diagnosis considered in patients in health care facilities.

Keywords: Leptospirosis, toxoplasmosis, land use, zoonosis, rodents, shrews, Tanzania

Introduction

Rodents are reservoirs of various emerging infectious diseases including leptospirosis caused by *Leptospira* bacterium and toxoplasmosis caused by *Toxoplasma gondii* (Jones *et al*., 2001). These diseases are among the understudied rodent borne zoonotic diseases in Africa. Previous studies in rodents in Tanzania including Morogoro urban areas show a high prevalence of leptospirosis. Prevalence of leptospirosis in cattle in pastoral areas of Tanzania has been reported as 5.6% (Machang'u *et al*., 1997) while among cattle sampled at livestock market as 51% (Swai & Schoonman, 2012). A prevalence of leptospirosis of 37% has been reported in dogs in Moshi (Machang'u *et al*., 1997). A study among patients with fever admitted at a hospital in northern Tanzania reported a leptospirosis prevalence of 9% (Biggs *et al*., 2011).

A high prevalence of toxoplasmosis (52.2%) has been reported among livestock keepers in Tanga, north-eastern Tanzania (Swai & Schoonman, 2009). A recent study in Mwanza in northern Tanzania has shown that 30.9% of pregnant women were infected with Toxoplasma (Mwambe *et al*., 2013), whereas in Dar es Salaam a prevalence of 35% has been reported (Doehring *et al*., 1995). Relatively higher prevalence of toxoplasmosis has been reported elsewhere in the world. The prevalence of toxoplasmosis in child-bearing women in rural Sudan is even higher ranging between 60.7% and 87.3% (Mohamed *et al*., 2009). In the USA it is reported that 85% of childbearing women have toxoplasmosis (Jones *et al*., 2001). This shows that toxoplasmosis is an important zoonosis. Humans get leptospirosis and toxoplasmosis through contact with environment contaminated with *Leptospira* and *Toxoplasma* pathogens mainly from rodents and cats, respectively (Babudieri, 1958; Frenkel & Ruiz, 1981; Hill & Dubey, 2002).

Human and other animals such as rodents excluding cats are intermediate hosts of *Toxoplasma gondii*. Intermediate host animals infected with *T. gondii* changes their normal

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behaviour towards predators. For example, rodents infected with *T. gondii* tend not to fear cats (House *et al*., 2011; Ingram *et al*., 2013; Flegr *et al*., 2002; Flegr *et al*., 2011; Berdoy *et al*., 2000), which are natural enemies of rodents. Toxoplasmosis infections in rodents may increase rodent-predators associations and increase public health threats especially in areas where rodents are eaten across Tanzania, Africa, Asia and Latin America (Ter Meulen *et al*., 1996; Khiem & Chien, 2003; Assogbadjo *et al*., 2005; Barragán *et al*. 2007).

There is limited information on the prevalence of both leptospirosis and toxoplasmosis in rodents and other intermediate host animals in Tanzania. This study therefore was carried out to: (i) determine the prevalence of leptospirosis and toxoplasmosis in rodents and shrews in Morogoro rural, Tanzania; and (ii) to compare the prevalence of the disease in animals from cultivated and fallow land to establish any association between disease prevalence with habitat type.

Materials and Methods

Study site

This study was carried out in Mikese village (37°53'60' E; 6°46'S) located in Morogoro Rural District in Tanzania some 30 kilometres east of the Morogoro Municipality, along the Morogoro - Dar es Salaam highway. The annual rainfall ranges from 700 to 1000mm. The short rainy season starts in mid-October and ends in December while the long rains start in February and ends in mid May. The dry season extends from June to October. The annual average maximum and minimum temperatures are 26°C and 21°C, respectively. Soils are acidic *lithosols* and *ferralitic latosols* with deeper deposits of *ferruginous* sandy clay. Mikese villagers depend mostly on rain-fed agriculture and maize is the main crop. Other crops cultivated include beans, soybeans and horticultural crops.

Animal trapping

Live rodents and shrews were captured in cultivated and fallow land fields measuring 70 x 70m each from August 2012 through May 2013. Each field consisted of seven parallel lines, 10m apart, with seven trapping stations per line, also 10m apart, making a total of 49 trapping stations per grid. One Sherman LFA live trap (8 x 9 x 23cm, H.B. Sherman Traps Inc., Tallahassee, FL, U.S.A.) was placed at each trapping station and all were set for three consecutive nights at intervals of four weeks. Traps were baited with peanut butter mixed with maize bran/maize flour, set in the afternoon, and inspected in the morning. All captured animals were taken for subsequent analyses (removal trapping). The distance between these two fields was approximately 2 km.

Processing of captured animals

All the captured animals were taken to the field laboratory, sex determined, and identified to species level as described by Kingdon (1997). Blood samples were collected from orbital vein and through heart puncture after anaesthetizing the animals with di-ethyl ether. Serum samples for serological detection of *Leptospira* antibodies were separated from blood by centrifugation. Rodent brain was preserved in 80% ethanol for preparation of brain impression smears for determination of *Toxoplasma* pathogens.

Detection of Leptospira infection

Antibodies against *Leptospira* were determined using microscopic agglutination test (MAT) (Cole *et al.*, 1973; Goris & Hartskeerl, 2013). Six *Leptospira* serovars were selected for use as live antigen in MAT. These included reference serovars and local serovars isolated from cattle and rodents in Morogoro areas, namely, serovar Sokoine, Kenya, Lora, Canicola (Machang'u *et al*., 2004; Mgode *et al*., 2006; Ahmed *et al*., 2006), Hebdomadis and Pomona. The selected serovars were grown into fresh *Leptospira* Ellinghausen and McCullough, modified by Johnson and Harris (EMJH)

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culture medium for 4-7 days while monitoring for growth density and contamination using a darkfield microscope.

Detection of Toxoplasma parasites

A total of 46 rodents were examined for toxoplasmosis. Brain impression smears were made onto clean microscope slides and left to dry at room temperature. Brain suspension splash smears were prepared by putting the brain into a clean test tube containing phosphate buffered saline (pH 7.0) and homogenized using sterile glass rods to obtain brain suspension. A drop of brain suspension was transferred using sterile Pasteur pipette onto clean microscope slides. The smears were left to dry in air and thereafter stained with 1:10 Giemsa solution for 30min. Smears were washed with running tap water and dried in air before examining for *Toxoplasma* parasites under light microscope (1000 x magnification).

Data analysis

The prevalence of the disease in animals from cultivated and fallow land was compared through descriptive analysis (Microsoft Excel, 2013) to determine whether there was association between disease prevalence with habitat type by examining the proportions of positives animals from the two fields out of the total positives. A *Leptospira* serovar Sokoine that was most prevalent in both sites was used to determine this association.

Results

Rodents and shrews

A total of 89 rodents belonging to five rodent genera and one shrew genus were collected from cultivated and fallow land (Table 1). *Mastomys natalensis* was the most predominant species contributing to 79% of total captures.

Table 1: Composition of rodent species and shrews in the study area

Type	Genus/species	Number	Percent composition
Rodents	*Mastomys natalensis*	71	78.88
	Lemniscomys sp.	6	6.66
	Acomys sp.	6	6.66
	Gerbilliscus vicinus	5	5.55
	Nannomys sp.	1	1.11
Shrews	*Crocidura* sp.	1	1.11
Total		90	

Prevalence of Leptospirosis

Twenty-three rodents out of 89 and one shrew tested positive against *Leptospira* serovars Sokoine, Lora, Kenya, Canicola and Hebdomadis. The overall prevalence of leptospirosis in rodents determined using six *Leptospira* serovars was 25.8%. *Leptospira* serovar Sokoine was more prevalent in these sites (Table 2) with 15 leptospirosis positive animals coming from both cultivated and fallow land. Eight of the 15 positive animals for serovar Sokoine were from cultivated land (53.3%) and the remaining 7 were from fallow land (46.6%). Other serovars reacted with fewer animals whereas serovar Pomona was not detected. A single shrew (*Crocidura* sp.) was positive to serovar Canicola.

Majority of the leptospirosis positive rodents had low antibodies titres (1:20 and 1:40) which are below the current adopted cut-off point of 1:160. A *Crocidura* sp. (shrew) was only positive to serovar Canicola with low titre (1:20). Such a low titre was also observed from one rodent species against serovar Canicola. A relatively high titre (1:80) was found against serovar Sokoine and Lora in two rodents. The distribution of antibodies levels in rodents against the six *Leptospira* serovars is shown in Table 3.

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Table 2: Prevalence of six *Leptospira* serovars in rodents

Leptospira serovars tested	Number of animals	Leptospirosis positive (1:20 – 1:80 titres)*	Serovar prevalence
Sokoine	89	15	16.9%
Lora	89	8 (3)	9%
Kenya	89	2 (1)	2.2%
Canicola	89	2 (1)	2.2%
Hebdomadis	89	1	1.1%
Pomona	89	0	0.0

***** Number of specimens in brackets reacted with more than one *Leptospira* serovar including serovar Sokoine (cross reactions) hence are not included in overall prevalence (23 positive animals out of 89).

Table 3: Antibody titres of rodents (n = 89) and shrew (n = 1) tested with MAT including six *Leptospira* serovars

	Leptospira serovars					
Titre	**Sokoine**	**Lora**	**Kenya**	**Canicola**	**Hebdomadis**	**Pomona**
1:20	13	4	2	2	1	0
1:40	1	3	0	0	0	0
1:80	1	1	0	0	0	0
Total	15	8	2	2	1	0

The predominant rodent species *M. natalensis* contributed more to leptospirosis positive animals: *M. natalensis* (n=18), *Lemniscomys sp.* (n=3), *Acomys sp.* (n=2), and one *Crocidura* sp. (shrew). Serovar Sokoine was detected most in *M. natalensis* (n=12), *Lemniscomys* sp. (n=2) and *Acomys* (n=1), whereas serovar Lora was also detected most in *M. natalensis* (n=6), and in 1 *Acomys* and *Lemniscomys* sp.

Prevalence of Toxoplasmosis

There was one positive rodent out of 46 examined for *Toxoplasma* parasites in rodent brain smears (2.17%). Three other rodents were suspects requiring further analysis with other methods to confirm the positivity. The rodent with *Toxoplasma* was *Lemniscomys* sp. collected from fallow land. Despite their abundance, *M. natalensis* (Table 1) was negative for toxoplasmosis. A *Crocidura* sp. (shrew) was also negative for toxoplasmosis.

Discussion

Findings of this study show a high prevalence of leptospirosis in rodents and shrews from both cultivated and fallow areas. Eight rodents with leptospiral antibodies were from fallow land whereas seven were from cultivated land. This relatively equal distribution of positive rodents and shrews assessed using serovar Sokoine which was most prevalent in both sites suggests that rodents and shrews movements are not restricted in these sites and they could efficiently transmit diseases in both areas as was expected. *Leptospira* serovar Sokoine was the most prevalent in positive rodents. This serovar has been isolated in cattle and rodents from Morogoro areas (Mgode *et al*., 2006; Ahmed *et al*., 2006) hence these findings suggest that serovar Sokoine is potentially the widely circulating *Leptospira* in animals in this region.

Other serovars which reacted in the MAT were *Leptospira* serovars Lora also reported in rodents from Morogoro areas; serovar Kenya from African giant rats, serovar Canicola which reacted with a *Crocidura* is specific for dogs, whereas serovar Hebdomadis has wide range of hosts and serovar Pomona is common in pigs. Seropositivity for the five *Leptospira* serovars was characterized by low antibody titres with two animals demonstrating a relatively higher titre, which is also below the 1:160 cut-off point adopted from European study (Goris *et al*., 2012). These

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results suggest urgent need of reviewing the cut-off-point of leptospirosis diagnosis by microscopic agglutination test in Tanzania and other countries in Africa. The need for establishing the actual cut-off titres for this region is especially important due to differences environmental factors and diversity of reservoir hosts.

Lower titres may suggest chronic leptospirosis infections demonstrated by low levels of IgG that can be below the detection threshold of MAT, or infection with *Leptospira* serovars related to the reacting serovars included in the MAT. Serovars belonging to same serogroup tend to cross react and levels of cross-reactions depict their relatedness (Dikken & Kmety, 1978; Faine, 1982). Serovar Sokoine belongs to serogroup Icterohaemorrhagiae widely reported in eastern and central Africa (Faine *et al*., 1999).

Serovar Sokoine cross reacts with several members of *L. kirschneri* species serogroup Icterohaemorrhagiae such as serovar Mwogolo, Ndahambukuje and Ndambari first described in the Democratic Republic of Congo (Faine *et al*., 1999). These findings calls for further studies including PCR detection of leptospiral DNA (Mgode *et al*., 2005) and isolation of leptospires from rodents in Mikese area to enhance identification of the causative serovars. Such information is necessary for future surveillance and development of list of local *Leptospira* serovars for human leptospirosis diagnosis.

Low prevalence of serovars Kenya, Canicola, Hebdomadis and absence of serovar Pomona in this study suggest host-specificity. For example, serovar Kenya which is common in Morogoro is widely found in African giant pouched rats (*Cricetomys* sp.) (Kranendork *et al*., 1968; Machang'u *et al*., 2004) was not investigated in this study.

The prevalence of toxoplasmosis in the study area is generally low. However, this could be an underestimate considering the crude method used (Giemsa staining) and relatively small sample size. Further studies involving robust serological and molecular detection of *T. gondii* antibodies and DNA (Desmonts *et al*., 1980; Homan *et al*., 2000; Burg *et al*., 1989) are needed to establish actual prevalence.

It can be concluded that leptospirosis is harboured by different species of rodents and shrew species found in Mikese. The rodents and shrew from this area have high prevalence of leptospirosis and a relatively lower prevalence of toxoplasmosis. There is a need to increase public awareness of rodent-borne diseases such as leptospirosis and toxoplasmosis that are likely to be transmitted to humans engaged in agricultural activities in this area. The burden of leptospirosis in humans and domestic animals needs to be determined considering that people engaged with farming in the study area come from different parts of Tanzania including areas where rodents are eaten as food source that increase risks of rodent-borne infections. Further investigation on prevalence of toxoplasmosis should be carried out using serological or molecular methods that are robust than microscopic examination of *T. gondii*. The diagnosis of leptospirosis, toxoplasmosis and other neglected zoonoses should be given priority in Tanzania to improve public health.

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