

Leptospira infections in freshwater fish in Morogoro Tanzania: a hidden public health threat

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Abstract: Leptospirosis caused by spirochete bacterium of genus *Leptospira* affects humans and animals worldwide. Rodents are major reservoirs of leptospires whereas wetland and aquatic migratory birds also carry and transmit leptospires. Leptospirosis studies in fish are lacking in African countries despite favourable environment and abundant reservoirs, which can spread leptospires into aquatic habitats and infect fish. The objectives of this study were to determine presence of *Leptospira* in fish; the prevalent *Leptospira* serovars and whether are related to serovars reported in animals; and to ascertain potential public health risk. Live tilapia, catfish and eel fish (n=48) were caught in Mindu Dam in Morogoro Municipality in eastern Tanzania. Blood sample was collected using syringes and needles to obtain serum for serological detection of leptospirosis using gold standard microagglutination test utilizing local and reference *Leptospira* serovars Sokoine, Kenya, Pomona and Hebdomadis. Twenty-six fish (54.2%) were positive for serovar Kenya (29.2%) and Sokoine (25%). *Leptospira* prevalence was high in both catfish (58.3%) and tilapia fish (47.8%). Thus, different fish types are infected with *Leptospira* found in animals. Fish could be source of *Leptospira* infection to humans since tilapia and catfish are the common fish type widely consumed in Tanzania. Further study covering lakes, rivers and dams is required to better understand the prevalence of *Leptospira* in fish and actual public health threats.

Keywords: Leptospirosis, zoonosis, freshwater fish, Tanzania

Introduction

Leptospirosis is a zoonotic disease caused by spirochete bacterium of the genus *Leptospira* that affect humans and animals worldwide (Bharti *et al.*, 2003; Pappas *et al.*, 2008; WHO, 2011). Rodents are major reservoirs of *Leptospira* bacteria (Babudieri, 1958). Other wild animals and birds found in wetland areas may also carry and disseminate leptospires into the environment (Galton, 1959; Addamiano *et al.*, 1960). Leptospires can live longer in water and moist soil with pH 6–8 and have the ability to form biofilms during interaction with environmental bacteria (Barragan *et al.*, 2011). There are pathogenic, saprophytic and intermediary leptospires found in natural environment and animals hosts (Faine *et al.*, 1999; Mgode *et al.*, 2010). Humans get leptospirosis through contaminated environment and /or direct contact with infectious materials such as urine and blood from infected animal. The disease is associated with certain occupational activities such as rice and sugarcane farming, fishing and fish farming, livestock keeping, handling animal products and water sports (Waitkins, 1986; Cutler *et al.*, 2010). Clinical symptoms of leptospirosis in humans are variable (Faine, 1982), and may be mistaken with other diseases including malaria.

Leptospirosis is widely distributed in African continent that has the highest burden on global scale (WHO, 2011). The disease has been reported in humans in Gabon (Bertherat *et al.*, 1999), in small mammals from Benin (Houemenou *et al.*, 2013) and in rodents and buffalos in Kenya and Uganda (Halliday *et al.*, 2013; Atherstone *et al.*, 2013). In Tanzania, leptospirosis has been widely reported with high prevalence in rodents, cattle, dogs, pigs, and recently in bats (Machang'u *et al.*, 1997; Mgode *et al.*, 2005; Ahmed *et al.*, 2006; Mgode *et al.*, 2006; Schoonman, 2007; Schoonman & Swai, 2010; Kessy *et al.*, 2010; Mgode *et al.*, 2014). In human, a prevalence of leptospirosis of 17.5% has been reported in Morogoro in eastern Tanzania (Machang'u, 2006) and

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nine percent of hospitalized people with non-malarial fevers in northern Tanzania (Biggs *et al.*, 2011).

The high prevalence of leptospirosis in various animal hosts and humans in Africa and in Tanzania in particular, suggest that this disease could be abundant in aquatic hosts such as freshwater fish, which could maintain the pathogens and disseminate to humans. Few studies in Europe have reported leptospirosis in fish and fish farmers (Maestrone & Benjaminson, 1962; Robertson *et al.*, 1981; Barragan *et al.*, 2011). Human leptospirosis cases associated with freshwater environment especially stagnant water and fish pond areas have been reported in Serbia (Svirčev *et al.*, 2009). Such studies are lacking in Tanzania and other African countries with favourable environment for maintenance and dissemination of leptospirosis. The objectives of this study were to determine presence of *Leptospira* in freshwater fish in Mindu dam, Morogoro municipality and to establish local *Leptospira* serovars for assessing relatedness with local serovars found in the area. The study also aimed at obtaining information for promoting public awareness of the neglected zoonotic disease since fishing and extensive agricultural activities are undertaken around Mindu dam areas. People living around the dam often swim in this dam hence are likely to get infected with leptospirosis.

Materials and Methods

Study site description

Mindu dam on Ngerengere River is located 10 km from Morogoro Municipality along Morogoro-Iringa highway in eastern Tanzania. It is a man made dam supplying 80% of water in the municipality including Sangasanga and Kasanga villages located less than a kilometre away from the dam. The dam gathers water from the Uluguru mountain ranges. Crop farming and fishing are major socio-economic activities around the dam.

Fish samples and leptospiral antibodies detection

Live fish (N=48) caught in Mindu dam by fishermen in May 2003 were randomly collected for serological detection of leptospiral antibodies using microscopic agglutination test (MAT). MAT is the gold standard test for diagnosis of leptospirosis. It detects antibodies against specific causative *Leptospira* serovars with limited ability to also detect antibodies against closely related serovars. Three types of fish, namely Tilapia, Catfish and Eel fishes were obtained from the dam. Tilapia and catfish were the predominant species, which are also commonly found in local freshwater fish market. Number of samples per fish type was: Tilapia spp. (n = 23), Catfish (n = 24) and Eel fish (n=1). Blood was collected from fish using syringes and needles, and serum separated from blood by centrifugation. Sera were stored in freezer at -20°C until used in microagglutination test for serodiagnosis of leptospirosis as previously described (Cole *et al.*, 1973; Goris & Hartskeerl, 2013). Briefly, local *Leptospira* serovars isolated from animals in Tanzania, namely *Leptospira* serovar Sokoine and serovar Kenya mainly found in domestic animals and rodents were selected for use in MAT. Others serovars were reference *Leptospira* serovar Pomona and Hebdomad is also previously reported in animals in Tanzania. These serovars were cultured into fresh *Leptospira* EMJH (Ellinghausen and McCullough, modified by Johnson and Harris) culture medium incubated at 30°C for 4 to 7 days before using as live antigen in MAT. Culture purity and density was checked using darkfield microscope whereby a density of 300×10^8 leptospire/ml was considered adequate for MAT. Fish serum samples were serially diluted to 1:10 – 1:80 with phosphate buffered saline (pH 7) in U-bottomed microtitre plate to 50 µl total volumes. Live leptospire antigen (50 µl) was added to diluted serum to give final dilutions of 1:20 – 1:160 (100 µl total volume) of serum-antigen mixture. The plates with serum-antigen mixture were incubated at 30°C for 2–4 hours before examining for agglutination of leptospire and antibodies under darkfield microscope.

Ethical considerations

Live fish were handled in compliance with the Animal Research: Reporting in Vivo Experiments guidelines and the Helsinki Declaration.

Results

A total of 48 fishes were screened for leptospirosis using 4 live *Leptospira* serovars. Twenty-six fish (54.2%) were seropositive reacting with *Leptospira* serovar Kenya, serovar Sokoine and Pomona. Seropositive fish samples belonged to all three types of fish (tilapia, catfish and eel fish). The proportion of seropositivity of the three fish types is presented in Table 1.

Table 1: Leptospiral positive samples in 3 types of fish from Mindu dam, Morogoro

| Fish type | Samples tested (n) | Positive | Negative | Leptospiriosis prevalence per fish type |
|-----------|--------------------|----------|----------|---|
| Tilapia | 23 | 11 | 12 | 47.8% |
| Catfish | 24 | 14 | 10 | 58.3% |
| Eel fish | 1 | 1 | 0 | 100% |

The prevalent *Leptospira* serovars were serovar Kenya (29.2%), serovar Sokoine (25%), whereas serovar Pomona reacted with lower titres (1:20) in tilapia fish (n = 1) and catfish (n = 2) samples which were also positive for serovar Sokoine and Kenya with relatively higher titres (1:40 and 1:80). The average agglutination titres across fish types for two most reacting *Leptospira* serovars were 1:40 and 1:80. *Leptospira* serovar Hebdomadis was not detected in the fish samples (Table 2). *Leptospira* serovar Sokoine was detected in all three types of fish whereas serovar Kenya was detected in catfish and tilapia fish only.

Table 2: Prevalence of *Leptospira* serovars in 3 types of fish from Mindu dam, Morogoro

| Fish type | Samples (n) | Sokoine | Kenya | Pomona | Hebdomadis |
|--------------------------------------|-------------|---------|-------|--------|------------|
| Tilapia fish | 23 | 3 | 8 | 1* | 0 |
| Catfish | 24 | 8 | 6 | 2* | 0 |
| Eel fish | 1 | 1 | 0 | 0 | 0 |
| Total | 48 | 12 | 14 | 3 | 0 |
| <i>Leptospira</i> serovar prevalence | | 25% | 29.2% | 6.3% | |

* Three specimens that reacted with serovar Pomona were also positive for serovar Sokoine (2) and Kenya (1) suggesting cross-reaction

Leptospirosis prevalence was high in both catfish and tilapia types whereby 14 of the 24 tested catfish were leptospirosis positive (58.3%) and 11 tilapia out of 23 were positive (47.8%). The prevalence of leptospirosis in the two common fish types was relatively similar (Figure 1) with no statistical significant difference (p = 0.56, Fisher's exact test). An eel fish was also seropositive.

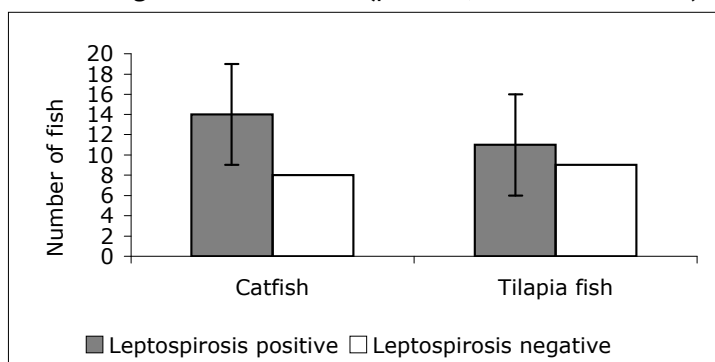


Figure 2: *Leptospira* in catfish and tilapia from Mindu dam

Leptospira serovar Kenya was detected in 14 fish samples out of 48 (29.2%) consisting of 8 tilapia fish and 6 catfish. Serovar Sokoine was detected in 12 of the 48 fish (25%) consisting of 8 catfish, 3 tilapia fish and an eel fish.

Discussion

This study reveals a high prevalence of pathogenic leptospires in three types of freshwater fish found in Mindu dam in Morogoro municipality. This is the first report on *Leptospira* in fish in Tanzania and Africa. A prevalence of 54.3% observed in common fish types largely consumed by people in this region suggest potential public health threat especially due to lack of public awareness of leptospirosis in Tanzania. People engaged with rice farming and cultivating vegetables around Mindu dam catchments area have high risk of contracting this disease as it is reported elsewhere (Waitkins, 1986; Cutler *et al.*, 2010). Other vulnerable groups include sugarcane farmers, fishermen, fresh-fish traders, swimmers and other individuals with direct and/or indirect contact with wet environment contaminated with leptospires from infected animals. Worker in these environments requires protective gears to prevent infection.

Local *Leptospira* serovars Kenya and Sokoine that are the predominant serovars in rodents and cattle in Tanzania (Machang'u *et al.*, 2004; Mgode *et al.*, 2006) are also the predominant serovars in tilapia and catfish species. This indicates relatedness or sharing of *Leptospira* pathogens between terrestrial mammals such as rodents and cattle; and aquatic living organisms like fish found in same locality. Identification of the point source of the widely circulating *Leptospira* serovars such as Sokoine found in broad range of species is necessary. This is possible through isolation in various hosts including fish and assessing the phylogenetic status of isolates. The point source or hosts that are major transmitter of leptospirosis could be targeted in control strategies.

The prevalence of leptospirosis in fish is higher exceeding the prevalence of leptospirosis in terrestrial animals such as cattle and rodents in Morogoro, which range from 10 to 20% (Machang'u, 2006). However, the higher prevalence of *Leptospira* in this study is likely to be due to the small sample of fish. *Leptospira* infection is high across freshwater fish types. *Leptospira* infection rate in different fish types (catfish and tilapia) was similar for two reacting serovars Kenya and Sokoine suggest that there is no host-specificity. A single eel fish captured in this dam was also seropositive. Although this study reports on fish specimens from a single dam, its findings may reflect the leptospirosis burden in other freshwater bodies found in Tanzania and the great lakes region of east and central Africa. Further studies with large sample size and geographic coverage, which includes Lake Tanganyika, Lake Victoria, Lake Nyasa, Lake Rukwa, Mtera and Nyumba ya Mungu dams and rivers which are the major source tilapia and catfish consumed in Tanzania is required to determine the extent of potential human and animal health risks. Isolation of *Leptospira* bacterium is needed for understanding the infecting serovars, which can be included in serodiagnosis of leptospirosis in humans and animals. The reported diversity of reservoir hosts of leptospirosis in Tanzania, namely rodents, bats, domestic animals (Machang'u *et al.*, 1997; Machang'u *et al.*, 2004; Mgode *et al.*, 2006, 2014) and the present report of *Leptospira* in fish indicate an urgent need for increasing public awareness of this emerging zoonotic disease in the country. Lack of awareness puts the vulnerable human populations engaged in irrigation farming, fishing, fish farming, livestock keeping and swimming in fresh water at high health risk.

Limitation of this study was minimal because incorporation of more *Leptospira* serovars including local serovars overcame the weakness of MAT of failing to detect serovars not included. Serovars for inclusion in MAT were carefully selected and demonstrated robustness of MAT in diagnosis of leptospirosis in diverse host species. However, the need for live *Leptospira* in MAT as antigen is limiting factor for application of this gold standard test in resource poor countries, which have highest burden in the world (WHO, 2011). Usefulness of MAT can be

strengthened by isolation and identification of *Leptospira* from different hosts and incorporation of the new isolates in the serodiagnosis of leptospirosis in humans and animals.

In conclusion this study shows high *Leptospira* infection in common freshwater fish types largely consumed by people hence indicate public health risk. To prevent or reduce leptospirosis transmission in Tanzania where the disease is largely unknown and neglected, it is recommended (i) To increase public awareness of leptospirosis to avoid its health risks. Health workers and the general public particularly those risk groups such as farmers, livestock keepers, fishermen, sewerage workers, and abattoir workers should be made aware of leptospirosis; (ii) Routine Leptospirosis diagnosis should be considered in people engaged with occupational activities that increase contact with environments likely to contain leptospires especially when malaria, typhoid fever, and dengue fever are ruled out; and (iii) Isolation of leptospires from different hosts and sources should be prioritised in order to understand the sources of infections, infecting serovars and developing a panel of serovars for use in serodiagnosis of this disease particular areas or region.

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