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Seroepidemiology of leptospirosis among febrile patients in a rapidly growing suburban slum and a flood-vulnerable rural district in Mozambique, 2012–2014: Implications for the management of fever

Policarpo Ribeiro[a], Nilesh Bhatt[a], Sádia Ali[a], Vanessa Monteiro[a], Edmilson da Silva[b], Ilana Teruszkin Balassiano[c], Carolina Aquino[d], Nilsa de Deus[a], Onelia Guiliche[a], Argentina Felisbela Muianga[a], Pascoal Jorge Alho[a], Virginia Lara Evaristo[a], Ivan Manhiça[e], Marco Medeiros[d], Eduardo Samo Gudo[a,*]

[a] Instituto Nacional de Saúde, Ministry of Health, Maputo, Mozambique
[b] Laboratório Tecnologia Diagnóstico Bio-Manguinhos, Fiocruz, Rio de Janeiro, Brazil
[c] Laboratório de Zoonoses Bacterianas, Serviço de Referência Nacional para Leptospirose, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil
[d] Laboratório Tecnologia Recombinante, Bio-Manguinhos, Fiocruz, Rio de Janeiro, Brazil
[e] Direcção Nacional de Saúde Pública, Ministry of Health, Maputo, Mozambique

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ABSTRACT

Objective: Leptospirosis is one of the most widespread zoonoses in the world and is caused by spirochetes of the genus *Leptospira*. In Mozambique, the disease is largely ignored and its epidemiology is unknown. The objective of this study was to investigate the occurrence of leptospirosis in febrile patients.
Methods: This cross-sectional study was performed between July 2012 and September 2015 among febrile patients. A total of 373 paired serum samples were drawn from febrile patients; 208 were from Caia District Hospital (rural setting) in Sofala Province and 165 were from Polana Caniço General Hospital (suburban setting) in Maputo City. Samples were initially screened using an in-house ELISA for IgM and IgG antibodies. Double positive samples were confirmed using a microagglutination test (MAT).
Results: Of the 373 febrile patients, five (1.3%) had acute leptospirosis (MAT $\geq$400) and 38 (10.2%) had a presumptive infection (IgM-positive/MAT <400). While most of the patients with a presumptive infection lived in the rural setting (84.2%, 32/38), the majority of patients with acute infections (60%, 3/5) and with negative results (60.3%, 199/330) lived in the suburban setting ($p = 0.000$). Contact with rodents was significantly higher in patient with acute leptospirosis (100%, 5/5) than in those with a presumptive infection (39.5%, 15/38) or negative results (41.8%, 138/330) ($p = 0.031$). Four out of the five patients (80%) with acute leptospirosis were treated with antimalarial drugs although malaria results were negative. The prevailing serogroup, according to MAT results, was Australis (40%; 4/10), followed by Icterohaemorrhagiae (30%, 3/10).
Conclusions: This study found that leptospirosis is prevalent among Mozambicans, and most cases are misdiagnosed as malaria.

Introduction

Leptospirosis is a zoonosis caused by the pathogenic strains of *Leptospira spp*, which have emerged as a leading cause of infection worldwide (Costa et al., 2015; Levett, 2001; Pappas et al., 2008). Leptospirosis is considered one of the most widespread zoonoses globally (Costa et al., 2015). The spectrum of the disease ranges from asymptomatic to severe and life-threatening disease with a high mortality rate (Levett, 2001; Pappas et al., 2008). It is estimated that more than one million cases and more than 50 000 deaths occur worldwide each year (Costa et al., 2015; Torgerson et al., 2015). The greatest burden of the disease is reported in developing countries and tropical settings (Costa et al., 2015; Levett, 2001; Pappas et al., 2008).

* Corresponding author at: Instituto Nacional de Saude, Ministry of Health, Av. Eduardo Mondlane 1008, Ministry of Health Main Building, 2nd floor, PO Box 264, Maputo, Mozambique. Fax: +258 21 311038.
E-mail address: esamogudojr@gmail.com (E.S. Gudo).

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A total of 13 pathogenic strains of *Leptospira spp* have been described so far using DNA homology (Adler and de la Pena Moctezuma, 2010; Allan et al., 2015), comprising more than 250 serovars organized into 25 serogroups (Allan et al., 2015; Levett, 2001). Rodents are the common reservoirs of Leptospira, but many other wild and domestic animals have been identified as potential reservoirs (Allan et al., 2015; de Vries et al., 2014). Transmission to humans occurs through direct contact with the urine of an infected animal or through contact with contaminated water or soil (Adler, 2010; Levett, 2001).

Leptospirosis has traditionally been considered to occur in rural areas, where contact with domestic or wild animals or with the contaminated environment during occupational activities, such as grazing and farming, are frequent (Lacerda et al., 2008; Sethi et al., 2010). However, in recent decades the disease has expanded rapidly into urban areas, as demonstrated by the recent outbreaks of urban leptospirosis in people living in the poor suburban slums in Brazil (Ko et al., 1999). These impoverished suburban slums are characterized by poor sanitation, high vulnerability to floods, the accumulation of solid waste, and an abundance of rodents, which together create the environmental conditions for the occurrence of outbreaks and epidemics (Allan et al., 2015; Ko et al., 1999; Reis et al., 2008). Other factors that greatly contribute to leptospirosis epidemics are climate change and increases in rainfall (Lau et al., 2010; McIver et al., 2016).

Sub-Saharan Africa is at particularly high risk of leptospirosis, as the rate of expansion of unplanned and impoverished urban slums in this region (Lau et al., 2010; UNFPA, 2007) and vulnerability to extreme climatic events (Kula et al., 2013) is one of the highest in the world. However, in many Sub-Saharan African countries, including Mozambique, data on the burden of leptospirosis are scarce (Allan et al., 2015; de Vries et al., 2014).

The suburban slums in Mozambique are expanding rapidly (Cunguara et al., 2012) and the frequency of floods has increased significantly in recent decades (Ambiental, 2005), which places Mozambique at high risk of leptospirosis. Moreover, no information exists in Mozambique on the prevailing serogroups and serovars of the pathogenic species of *Leptospira*. The lack of data on the epidemiology of leptospirosis in Mozambique represents a major obstacle for the implementation of interventions to prevent the infection and improve the care of infected people. The aim of this study was to determine the prevalence and distribution of strains and the risk factors for leptospirosis in febrile patients in a large suburban slum and in a flood-vulnerable rural district in Mozambique.

Methods

Study design and population

A cross-sectional study was conducted between July 2012 to September 2015 among acute febrile patients attending outpatient clinics at Caia District Hospital and Polana Caniço General Hospital (Figure 1).

The protocol was approved by the national bioethics committee (Ref. 221/11 for patients recruited in Polana Caniço General Hospital; Ref. 316/12 for patients recruited in Caia District Hospital). Paired acute and convalescent blood samples were collected from each patient. Written consent was requested from all eligible patients before enrolment.

The climate in Mozambique is tropical, with a rainy season from November to April, and a dry season from May to October. Polana Caniço General Hospital is a health facility in the Mavalane health area, in Kamavota District, in the suburban area of Maputo City. Kamavota District is characterized by poor sanitation, the accumulation of solid and liquid waste, and high vulnerability to floods. Most households are precarious and often overcrowded. The main source of income is the informal market and small businesses (Maputo, 2010).

Caia District Hospital is situated in Caia District, a rural area in Sofala Province. The district is in a valley situated along the Zambezi River, the largest river in Mozambique. Caia is one of the districts most vulnerable to floods in Mozambique. Livestock-keeping and small-scale farming are the main sources of income in Caia District (Estatal, 2005).

Sample collection and handling

Both acute and convalescent samples were collected from each patient. The acute samples were collected during the enrolment visit and the convalescent samples were collected on average 21 days later. Samples were then transported to Brazil for testing at the Laboratory of Diagnostics Technology, and the National Reference Center for Leptospirosis, both at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Case definition

Presumptive leptospirosis was defined as the presence of an acute febrile illness and positive results for IgM ELISA and/or a microagglutination test (MAT) antibody titer of <400. A confirmed case of acute infection with leptospirosis was defined as the presence of an acute febrile illness and MAT antibody titer ≥ 400 in a single sample (Adler and de la Pena Moctezuma, 2010; de Vries et al., 2014; Lau et al., 2010).

Laboratory testing

Malaria testing

Malaria diagnosis was performed using a rapid diagnostic test (RDT) (SD Bio Line, Korea). All RDT-positive samples were confirmed by smear microscopy and Giemsa staining.

Diagnosis of leptospirosis

Leptospirosis was tested at the Laboratory of Diagnostics Technology, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. Serum samples from all participants who returned for the convalescent visit were initially screened using an ELISA. MAT was performed at the National Reference Center for Leptospirosis, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

ELISA

The ELISA was performed using an in-house assay, which was standardized using two purified proteins from the species *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130: the protein LigA at a concentration of 1.6 mg/ml and the protein LigB at a concentration of 2.3 mg/ml. IgM antibodies were measured in the acute samples of all patients who returned for the convalescent visit and IgG antibodies were measured in their corresponding convalescent samples (see Figure 2). The sensitivity and specificity of the IgM ELISA were 88% and 99%, respectively, and for the IgG ELISA were 81% and 95%, respectively.

MAT

Convalescent serum samples from all patients that were double positive for IgM/IgG antibodies against Leptospira were tested by MAT (see Figure 2). Standard strains of Leptospira were grown in liquid Ellinghausen–McCullough–Johnson–Harris medium (EMJH). A total of 19 reference *Leptospira spp* serovars were used for the MAT, in accordance with World Health Organization (WHO) recommendations (WHO, 2003) (see Supplementary material, Table S1 in the online version, at DOI: 10.1016/j.ijid.2017.08.018).