Has Madagascar Lost Its Exceptional Leptospirosis Free-Like Status?

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Abstract

Background
Leptospirosis is a widespread but underreported cause of morbidity and mortality. It has rarely been reported in either humans or animals in Madagascar.

Methods
We conducted a cross-sectional survey of the inhabitants in Moramanga, Madagascar, in June 2011, to estimate the prevalence of human infection using the microscopic agglutination test (MAT). This activity was carried out as part of a workshop implemented by the Pasteur Institute of Madagascar, focusing on surveillance with a one week field study and targeting the health staff of the district level.

Results
In total, we sampled 678 inhabitants from 263 households. The sex ratio (M/F) was 0.65 and the mean age 26.7 years. We obtained a value of 2.9% for the first recorded seroprevalence of this disease in the human community of Moramanga. Questionnaire responses revealed frequent contacts between humans and rodents in Moramanga. However, activities involving cattle were identified as a risk factor significantly associated with seropositivity (OR=3).

Conclusion
Leptospirosis remains a neglected disease in Madagascar. This study highlights the need to quantify the public health impact of this neglected disease in a more large scale, in all the country and to establish point-of-care laboratories in remote areas.
Introduction

Leptospirosis is a worldwide zoonotic infection for which incidence is highest in tropical regions [1,2], constituting a major public health problem in developing countries. Humans are usually infected by contact with urine of an infected host, contaminated drinking water or soil, or infected animal tissue. Notorious reservoirs are rodents, but reservoirs include a variety of wild and domestic animals, livestock, and insectivores.

Leptospira are conventionally divided into two species, the pathogenic Leptospira interrogans and the saprophytic Leptospira biflexa. More than 60 serovars have been described in the latter and over 250 serovars in 25 serogroups are recognized in L.interrogans.

It has long been a matter of concern for public health in the southwestern Indian Ocean [3–5]. On the islands close to Madagascar, incidence ranges of 5/100,000 for Reunion Island, 9/100,000 for Mayotte and 101/100,000 for the Seychelles have been reported [5]. The environmental and socioeconomic conditions of Madagascar, with its tropical climate, rice and sugar cane agriculture, livestock farming, slums and the presence of the notorious commensal rodents Rattus norvegicus (brown rat) and R. rattus (black rat), would be expected to favor leptospirosis transmission [6]. However, despite the similarity between conditions on Madagascar and on other nearby islands, the disease has rarely been reported in either humans or animals on Madagascar, where diagnosis is based solely on indirect evidence obtained through antibody detection. A study carried out in the Toliara district in 1968 detected both human and animal leptospirosis. Silverie et al. reported that 51% of patients with clinically suspected disease were seropositive for serogroups Tarassovi, Grippotyphosa, Hebdomadis and Australis, and that the seroprevalence of Leptospira was 46% in cattle and 8% in pigs [7]. However, subsequent efforts to detect the infection failed to confirm these results. In a survey conducted in Antananarivo, on 2646 serum samples from subjects with no symptoms suggestive of leptospirosis, agglutinating antibodies against the Icterohaemorrhagiae, Grippotyphosa and Canicola serogroups were found in only five samples [8]. In a subsequent study, 105 workers with occupational exposure to Leptospira underwent serological screening; antibodies were found in only one worker, and their titer was low [9].

Animal surveys have reported an absence of seropositivity in dogs, sheep, donkeys, horses, cattle and pigs from other sites [10]. No pathogenic strains were obtained from bacteriological cultures of kidney samples from 55 R. rattus and 50 Pteropus rufus (Madagascan flying fox) collected at Marovitsika-Anjiro (100 km north of Antananarivo)[8]. A more recent PCR-based study detected no kidney carriage in 115 rats, 50 zebu cattle and 13 pigs from various sites [9].

The first direct evidence of widespread leptospiral carriage in small mammals in Madagascar was obtained in 2009 [11]. Rates of infection, calculated from the frequencies of positive PCR results, were highest in Moramanga (54%), Toliara (48%) and Mahajanga (47.4%). The 10 isolates obtained from nine rats were all identified as species L. interrogans serogroup Canicola serovar Kuwait and all had identical partial rrs and secY sequences[11]. However, the geographic restriction of seropositivity in Rattus species to the Moramanga district remains unexplained. The epidemiological features of leptospirosis in this setting require further investigation. We therefore carried out a survey in Moramanga in 2011, to estimate the prevalence of specific antibodies in humans.

Methods

Setting

This study was conducted in Moramanga, a low-income semi-urban area, on the central eastern region of Madagascar. This area was selected because it was here that the DNA carriage in
Rattus species (54%) was highest in 2009 [11]. The survey was carried out over the course of one week in June 2011, at the start of the cold season, as part of a workshop for the training of health district staff in the use of surveillance tools.

Sampling
A cross-sectional study was conducted between May 31st 2011 and June 4th 2011, in all the neighborhoods of Moramanga. Households were selected at random for study in each neighborhood. All family members were included in the study, for each household.

Data collection
A predesigned, semi-structured and validated questionnaire was used for data collection during house-to-house visits. Participants were asked to provide sociodemographic information, and information about the nature of their employment, potential contact with animals whilst at work, the type of contact with animals, the presence of rats at home and in the workplace, types of contact and environmental factors (Table 1).

Microscopic agglutination tests (MAT)
We performed the standard MAT reference test as described by Faine et al. [12], using a battery of 15 serovars of Leptospira spp. as antigens. All strains were maintained in liquid Ellinghausen-McCullough-Johnson-Harris medium (Biorad 55954) at 28°C for one week before testing.

MAT titers are reported as the reciprocal of the largest dilution resulting in the agglutination of at least 50% of the live bacterial antigen, and titers of at least 1:100 were considered positive [13]. The strains were added to serially diluted serum samples in 96-well flat-bottom microtiter plates, which were then incubated at room temperature for two hours. Agglutination was then evaluated by dark-field microscopy at 10 or 20X magnification. The Leptospira species included in the antigen panel are listed in Table 2. Serovars for which 50% agglutination was achieved with the highest sample dilution were considered to be probable predominant serovars. Serum samples were considered to be MAT-negative if no agglutination was detected.

Statistical analysis
Data analyses were performed with R software [14]. For all statistical tests, a p-value less than 0.05 was considered to denote statistical significance. Qualitative variables are expressed as percentages. Groups were compared in Fisher’s exact tests for categorical variables and quantitative variable were compared in nonparametric tests (Kruskal-Wallis tests).

Ethics statement
Written informed consent was obtained from each adult participant and from the parent or guardian of each child before enrollment. The study was approved by the National Ethics Committee of the Ministry of Health of Madagascar.

Results
In total, 263 households and 813 inhabitants participated in the study. Blood samples were obtained from 678 subjects. Serum was obtained from these samples and subjected to the microscopic agglutination test (MAT) for antibodies. The median number of inhabitants per household was 3 (range: 1–10). In total 477 of the subjects studied (59%) were female, and 334 (41%) were male. The mean age of the study subjects was 25.7 years (95% CI: [24.6–27.1]) and the median age was 22 years (range: 6 months to 87 years).
Table 1. Characteristics of the study population in Moramanga, 2011, and risk factors for leptospirosis.

|                              | All N = 813 | Sampled N = 678 | Positive N = 20 | p   |
|------------------------------|-------------|-----------------|-----------------|-----|
| Sex                          |             |                 |                 |     |
| male                         | 334 (41)    | 266 (39)        | 7 (35)          | 0.82|
| female                       | 477 (59)    | 410 (61)        | 13 (65)         |     |
| Age                          |             |                 |                 |     |
| median range                 |             | [0.5–87]        | [0.5–87]        | 17  |
| Presence of rats in the household | 691 (85)   | 585 (86)        | 16 (80)         | 0.33|
| Noise in the ceiling          | 492 (72)    | 414 (71)        | 10 (63)         | 0.45|
| Food eaten                   | 582 (84)    | 499 (85)        | 14 (87)         | 0.99|
| Rat feces in the rooms       | 462 (67)    | 404 (70)        | 12 (75)         | 0.78|
| Water used for washing       |             |                 |                 |     |
| River or pond                | 174 (21)    | 42 (21)         | 5 (25)          | 0.58|
| Tap                          | 345 (43)    | 297 (44)        | 9 (45)          | 0.99|
| Well                         | 343 (42)    | 285 (42)        | 9 (45)          | 0.83|
| Drinking water               |             |                 |                 |     |
| River or pond                | 35 (4)      | 30 (4)          | 1 (5)           | 0.60|
| Tap                          | 390 (48)    | 332 (49)        | 9 (45)          | 0.82|
| Well                         | 394 (49)    | 320 (47)        | 10 (50)         | 0.82|
| Work setting                 |             |                 |                 |     |
| Office                       | 527 (65)    | 448 (66)        | 11 (55)         | 0.33|
| Workshop                     | 17 (2)      | 16 (2)          | 0 (0)           | -   |
| In the fields                | 114 (14)    | 98 (15)         | 3 (15)          | 0.99|
| Outdoors, but not in the fields | 95 (12)   | 75 (11)         | 4 (20)          | 0.26|
| Presence of rats in the workplace | 486 (60)   | 418 (62)        | 8 (40)          | 0.07|
| Noise in the ceiling          | 312 (66)    | 269 (66)        | 3 (38)          | 0.15|
| Food eaten                   | 345 (72)    | 296 (72)        | 6 (75)          | 0.99|
| Rat feces in the rooms       | 278 (58)    | 252 (62)        | 3 (38)          | 0.21|
| Contact with animals         |             |                 |                 |     |
| Rats                         | 552 (96)    | 453 (95)        | 14 (93)         | 0.51|
| Dogs                         | 230 (32)    | 186 (31)        | 8 (42)          | 0.31|
| Cats                         | 229 (31)    | 194 (32)        | 6 (32)          | 0.99|
| Pigs                         | 156 (21)    | 133 (22)        | 1 (5)           | 0.09|
| Cattle                       | 89 (12)     | 75 (12)         | 6 (31)          | 0.02|
| Bats                         | 26 (4)      | 25 (4)          | 0 (0)           | -   |
| Leisure activities           |             |                 |                 |     |
| Fishing in the river         | 157 (19)    | 141 (21)        | 2 (10)          | 0.39|
| Swimming in the river        | 262 (32)    | 226 (33)        | 6 (30)          | 0.81|
| Hunting                      | 20 (2)      | 10 (1)          | 0 (0)           | -   |
| Last febrile illness         |             |                 |                 |     |
| Currently                    | 54 (7)      | 46 (7)          | 2 (10)          | 0.63|
| Last month                   | 107 (13)    | 93 (14)         | 3 (15)          | 0.75|
| Last 12 months               | 45 (6)      | 39 (6)          | 2 (10)          | 0.32|
| More than one year           | 186 (23)    | 163 (24)        | 2 (10)          | 0.18|

(Continued)
In total, 410 (61%) of the subjects from whom blood samples were collected were female and 266 (39%) were male. The mean age of these subjects was 26.7 years (95% CI: [25.4–28.1]) and their median age was 23 years (range: 6 months to 86 years). The characteristics of the total study population did not differ significantly from those of the population of subjects from whom blood samples were obtained (Table 1).

MAT results were positive for 20 of the 678 subjects from whom samples were collected; seroprevalence was therefore estimated at 2.9% (95%CI: [1.9%-4.7%]). MAT detected antibodies against the Icterohaemorrhagiae (n = 16), Panama (n = 3) and Canicola (n = 2) serogroups. Antibodies against both the Icterohaemorrhagiae and Canicola serogroups were found in one individual.

Contact with cattle was the only risk factor identified in univariate analysis (OR: 3.4 95% CI: [1.03–10.03], p-value = 0.02; Table 1).

**Discussion**

The primary aim of this survey was to demonstrate the existence of exposure to leptospirosis in the human population of Moramanga, as suggested by the recent finding of an estimated DNA carriage of 54% in the mammalian population of the area [11]. The prevalence of leptospirosis in the inhabitants of Moramanga was estimated at 2.9%. Based on our recent findings, Madagascar seems to have lost its exceptionally low leptospirosis prevalence status among the islands.
of the Indian Ocean. The estimated prevalence of leptospirosis was higher than that for Re-
union Island, which was estimated at about 1.1% in 1991 [15] and at less than 1% in 2006 [3].
However, the prevalence of leptospirosis in Moramanga seems to be similar to that in some
areas of Reunion Island with high rainfall. Duval et al. [15] reported differences in prevalence
as a function of rainfall, from 0.7% on the west coast to 3.1% in south-eastern areas. However,
the prevalence of leptospirosis appeared to be lower in Moramanga than in the Union of Com-
oros. A recent study reported estimated prevalences of 3.4% in Anjouan, 4.2% in Grande-
Comore and 10.3% in Mohéli [16].

Previous investigations in Madagascar failed to detect leptospirosis in humans. Lhuiller
et al. [8] reported a low seroprevalence in the inhabitants of Antananarivo. However, in 1968,
Silverie et al. reported a prevalence of 51% in selected patients [17] with clinical symptoms,
but this result cannot be compared with that reported here since this new study was a cross-
sectional community-based study. Furthermore, these new results are not representative of all
the country because limited to the population in Moramanga.

The prevalence of the different Leptospira serogroups in human populations depends
strongly on the local reservoir hosts and the strains they carry [18]. The only risk factor identi-
fied in this study was contact with cattle. Bovine leptospirosis has already been suspected in
Madagascar [19], but the renal carriage of Leptospira in cattle has never been documented [9].
The ecology of leptospirosis in this region of Madagascar, including the range and importance
of various reservoir animals, is, therefore, probably not completely understood. The rat is rec-
ognized as the major reservoir host for the bacterium, but recent data from Reunion Island in-
dicate that almost all mammals can act as a source of contamination. Pathogenic Leptospira
spp. (L. borgpetersenii, L. interrogans) were recently found in bats in Madagascar and the
Union of Comoros [20]. A better knowledge of animal leptospirosis is therefore essential, to
improve assessments of the risk to humans. The study area for this investigation was therefore
selected on the basis of results for surveillance in mammals [11].

Acute leptospirosis has never been reported in Madagascar. In humans, clinical leptospirosis
has diverse manifestations, but it generally causes a febrile illness that is often difficult to distin-
guish from other acute influenza-like fevers, such as dengue, influenza, chikungunya and ma-
laria during its early stages [1]. A lack of appropriate laboratory facilities is the principal reason
for the failure to diagnose leptospirosis in Madagascar, highlighting the need for point-of-care
laboratories in remote areas [21].

Madagascar is a large island and our cross-sectional study was restricted to Moramanga district.
This is undoubtedly a limitation for conclusion about the all country leptospirosis status but is an
important first step to go further in the description of the epidemiological profile of the country.

Conclusion

Information about leptospirosis in Madagascar remains scare. Risk assessment should take
into account not only the diagnosis of incident cases in humans, but improvements in our
knowledge of animal risks and the correspondence between human seroprevalence data and
different climate areas and seasons.

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Author Contributions

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