Factors associated with clinical leptospirosis: a population-based case-control study in the Seychelles (Indian Ocean)

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**Background**
In Western countries, leptospirosis is uncommon and mainly occurs in farmers and individuals indulging in water-related activities. In tropical countries, leptospirosis can be up to 1000 times more frequent and risk factors for this often severe disease may differ.

**Methods**
We conducted a one-year population-based matched case-control study to investigate the frequency and associated factors of leptospirosis in the entire population of Seychelles.

**Results**
A total of 75 patients had definite acute leptospirosis based on microagglutination test (MAT) and polymerase chain reaction (PCR) assay (incidence: 101 per 100 000 per year; 95% confidence interval [CI] : 79–126). Among the controls, MAT was positive in 37% (past infection) and PCR assay in 9% (subclinical infection) of men aged 25–64 with manual occupation. Comparing cases and controls with negative MAT and PCR, leptospirosis was associated positively with walking barefoot around the home, washing in streams, gardening, activities in forests, alcohol consumption, rainfall, wet soil around the home, refuse around the home, rats visible around the home during day time, cats in the home, skin wounds and inversely with indoor occupation. The considered factors accounted for as much as 57% of the variance in predicting the disease.

**Conclusion**
These data indicate a high incidence of leptospirosis in Seychelles. This suggests that leptospires are likely to be ubiquitous and that effective leptospirosis control in tropical countries needs a multifactorial approach including major behaviour change by large segments of the general public.

**Keywords**
Leptospirosis, risk factors, epidemiology, tropical countries, Seychelles, Indian Ocean

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Leptospirosis is a worldwide zoonosis and usually human contamination occurs after contact with water and soil containing urine of infected rats and other animals.1,2 The severity of this disease varies from mild to rapidly fatal. Mild forms are often misdiagnosed or ignored and severe forms are characterized by hepatic involvement, acute renal failure and haemorrhagic syndrome.2,3 Because leptospirosis has protean clinical manifestations, biological tests are essential for diagnosis, such as microagglutination test (MAT) and, more recently, polymerase chain reaction (PCR) assay.4–6

In temperate climates, leptospirosis is relatively uncommon with incidence rates reported to range from 0.05 to 0.5 per 100 000 in countries such as the United States, Switzerland or France.7–9 Nowadays the disease mostly occurs in these countries among farmers and those indulging in water-related leisure activities.10–13 In contrast, incidence rates can be up to 1000 times higher in tropical countries, where the warm and wet climate provides a favourable environment for the survival of leptospires. Incidence ranged, for example, from 18 to 128 in Barbados, New Caledonia or Hawaii.14–16 Previous retrospective studies in Seychelles also showed high incidence of leptospirosis (40–60 per 100 000).17,18

Although environmental and behavioural factors are universally acknowledged to relate to human leptospiral contamination,1 studies to identify risk factors in tropical areas have rarely been prospective,16 were not population-based and used retrospective data.19–22 We therefore conducted investigations on leptospirosis in Seychelles to examine the clinical
presentation and risk factors of the disease. The clinical results have been described separately. Briefly eight serogroups were identified with Icterohaemorrhagiae (31%) and Hurstbridge (20%) being the most frequent. Influenza-like forms accounted for 37% of the cases while jaundice, acute renal failure and pulmonary haemorrhage occurred in 52%, 28% and 19%, respectively. Case fatality was 8%.

In this paper we report the results of a prospective population-based case-control study to determine the incidence of acute leptospirosis in the population, the prevalence of subclinical leptospirosis in the healthy population, and the environmental and behavioural factors associated with acute leptospirosis in an endemic tropical setting.

Subjects and Methods

Study area and population
The study area covered the entire population of the Seychelles, The Seychelles consist of more than 110 islands situated in the Indian Ocean, 1800 km east of Kenya and 1800 km north of Mauritius. Temperature ranges from 24 to 31°C, humidity from 75 to 82%, and rainfall averages 1500 to 2200 mm per year. The population was 74,331 inhabitants in 1994 of whom 89% lived on the main island. Occupation in the general population was distributed as follows (men/women; in per cent): farmer and fisherman 7.3/1.6, unskilled manual 43.7/43.9, skilled manual 5.4/2.4, unskilled non-manual 11.1/29.7, skilled non-manual 15.3/16.9.

Study design
The study was a population-based matched case-control study conducted between 1 April 1995 and 31 March 1996. All the physicians of the country were repeatedly informed about the study and requested to refer all patients suspected of having leptospirosis to one of the two main hospitals of the country which are the only establishments where these patients could be admitted. A detailed questionnaire was administered at the time eligible cases were admitted to hospital in the presence of relatives of the patients in most cases. For eligible controls, the questionnaire was administered at home or working place and blood collected at the same time. Questions assessed demographic, social, educational, professional, occupational, environmental, and behavioural variables. Average rainfall over the 14 days preceding the administration of the questionnaire was obtained for all cases and controls using data on daily rainfall from the National Meteorological Services. Rainfall for each individual corresponded to measurements obtained in the nearest of the 10 considered meteorological stations. For the eligible cases, a blood sample was obtained within 24 hours of hospital admission and a second sample 2–4 weeks later except for patients who died before collection of the second sample. For the eligible controls, only one blood sample was obtained. Sera were immediately stored at −20°C. MAT and PCR assay were performed after all eligible cases and eligible controls had been enrolled in the study. Informed consent was obtained from all subjects and the study approved by the Ministry of Health of Seychelles.

Case definition
Eligible cases were all patients admitted to hospital during the 12-month study period who had fever of unknown origin and/or any of the following symptoms and signs: myalgia, tender liver, meningism, bleeding tendency, jaundice, acute renal failure, and radiological lungs infiltrates which could not be accounted for by a definite diagnosis other than leptospirosis. Definite cases (hereafter referred as ‘cases’) were the eligible cases who fulfilled the laboratory criteria corresponding to acute leptospirosis: a MAT seroconversion (i.e. a negative first serum sample and a titre ≥1:400 in the second sample), a fourfold or greater increase in MAT titre, or a positive PCR assay in one serum sample. A clinical description of these cases has been reported elsewhere. Renal failure was considered for creatinine over 130 μmol/l, jaundice for bilirubin over 30 μmol/l and pulmonary haemorrhage in case of concomitant haemoptysis and pulmonary infiltrates on the chest x-ray.

Control definition
For each eligible case, one eligible control matched for age, sex and occupation was randomly selected from the general population (definite cases, and subsequently, definite controls could be determined only after the data for all participants had been collected). Eligible controls were matched to eligible cases according to sex, age and occupation as these variables are known to strongly relate to leptospirosis incidence. Matching for age was made with a 5-year tolerance and for occupation within eight categories: farmers, manual jobs requesting low, intermediate or high qualification, non-manual jobs requesting low, intermediate or high qualification, and students. Eligible controls aged 20 and above were extracted from a list of 1067 subjects aged 25–64 randomly selected from census data and who participated in a community-based survey of cardiovascular risk factors in 1994. For the 10 eligible cases aged less than 20, eligible controls were picked at random locations of the main island. Eligible controls were selected and investigated at the time eligible cases were enrolled in the study (within 15 days), so that cases and controls were additionally partially matched for period, consequently for rainfall, which is another variable associated with increased leptospirosis incidence. Eligible controls with positive PCR assay were considered to have current subclinical infection and those with MAT titres ≥1:100 to have evidence of past leptospiral infection. Eligible controls with negative PCR and MAT were considered to have no current or past leptospirosis (although a few of these subjects could have experienced past leptospiral infection followed by a seronegativation) and were selected as definite controls (hereafter referred as ‘controls’).

Laboratory methods
The microagglutination test (MAT) was performed using standard methods including a battery of 24 live antigens (provided by the WHO Collaborating Centre for Leptospirosis, Institute Pasteur, Paris, France) representing the main pathogenic serogroups of Leptospira. According to the standard interpretation, a titre ≥100 was considered positive. The PCR assay was performed with the amplification of a 331 bp-fragment designed from the 16S rRNA gene of Leptospira spp. followed by a dot blot hybridization. PCR assay was performed in standardized reference conditions as in previous studies. DNA purified from 10^2 cells from the reference strain of Leptospira interrogans was used as positive control. Two negative controls (extraction and amplification steps) were included in each PCR run. All
MAT and PCR analyses were performed at the Leptospira Laboratory, Institute Pasteur, Noumea, New Caledonia.

All exposure variables were defined before the study started and after having been tested during a 3-month pilot phase. Selected exposure variables (except those used for matching cases and controls) are listed in Table 2 and labelled in a generally self-explanatory form. ‘Activities in forests’ refers to occupations often taking place in forests; ‘gardening’ refers to activities during leisure time; ‘indoor occupation’ refers to those taking place mainly indoors. Many people with any occupation also cultivate a vegetable garden outside working hours. Variables often considered as risk factors for leptospirosis\(^1,16\) such as working with rainwater catchment systems, in fresh water immersed fields or with animal tissues are not relevant for the Seychelles.

Analysis

Exact binomial 95% confidence intervals (CI) were calculated for incidence estimates. Difference in frequency of the considered variables between cases and controls was tested using the \(\chi^2\) statistic. Logistic regression was performed to examine the associations between leptospirosis and the considered factors. Conditional logistic regression was carried out using the matched pairs of cases and controls. Unconditional logistic regression adjusted for the matched variables (age, sex and occupation) was carried out using all (unmatched) cases and controls. Stepwise multivariate logistic regression including age, sex, job and all considered variables was carried out on unmatched data to identify the strongest independent correlates of acute leptospirosis. Univariate logistic regression was carried out to examine the association between major complications of the disease (renal failure, jaundice or pulmonary haemorrhage, coded as 1 or 0) and the variables. All \(P\)-values are two-tailed and those below 0.05 were considered to indicate statistical significance. No adjustment was made for multiple comparisons.

Analyses were conducted with Stata for Windows, version 5.0 (Stata Corporation, College Station, Texas, USA).

Results

Selection of cases and controls

The distribution of eligible and definite cases and controls across categories of matching variables is shown in Table 1. The arbitrary selection of the categories of age chosen in the Table explains slight discrepancies in the numbers of cases and controls within age categories. Among the 125 eligible cases who had clinical criteria for leptospirosis, 75 also met the laboratory criteria for the case definition. Among the 125 eligible controls, 65 had both negative MAT and PCR assay and therefore met all criteria for the control definition. Among the 75 cases and 65 controls available, 38 original pairs of matched cases and controls remained.

Incidence of acute leptospirosis

Table 1 shows that 75 cases had definite acute leptospirosis, which corresponds to an incidence of 101 per 100 000 per year (95% CI: 79–126 per 100 000). Most cases were among males with manual occupations. Six fatalities occurred (case-fatality rate: 8.0%). All six fatalities occurred within 48 hours of admission.
to hospital, had a post-mortem examination compatible with the diagnosis of leptospirosis, and had PCR assay positive for leptospirosis.

**Prevalence of leptospiral infection among eligible controls**

Among the 125 eligible controls, 60 had positive MAT and/or PCR assay (Table 1). The majority (35 out of 60) of eligible controls with positive MAT and/or PCR assay were among men aged 25–64 with manual occupations due to the matched study design. Within this category, 37.3% (28/75) had evidence of past leptospiral infection (positive MAT with negative PCR) and 9.3% (7/75) had evidence of current leptospiroemia (positive PCR). Similar proportions of infection seemed to occur in other categories although the size of these groups was too limited for a significant estimation. None of the eligible controls with positive MAT reported past clinical leptospirosis and none of the controls with positive PCR expressed symptoms of an acute infectious disease when blood was collected or was admitted later to hospital for acute leptospirosis.

**Association between leptospirosis and selected characteristics**

Distribution of selected characteristics among cases and controls is shown in Table 2. Table 3 shows the odds ratios relating leptospirosis to these selected characteristics using logistic regression analysis with either matched or unmatched data. Using matched data, leptospirosis was directly associated with rainfall, gardening, wet soil around the home, cats at home, rats visible around the home, walking barefoot near the home, skin wounds, and alcohol consumption, and inversely with indoor occupation. Marginal associations were found with activities in forests and refuse not collected by public services (i.e. garbage kept or processed around the home). Odds ratios of similar magnitude were found using unmatched data (admittedly that unmatched analysis of matched data may introduce a form of selection bias).
bias). Acute leptospirosis did not consistently relate to population density or regions (data not shown). A few variables were marginally associated with one of the three complications (renal failure, jaundice or pulmonary haemorrhage) and only one (pigs around home, OR: 3.1–3.3, \( P = 0.034–0.044 \)) was associated with all three complications. These findings may, however, have only limited significance considering that these analyses had only marginally statistical significance, were done a posteriori and were not adjusted for multiple comparison.

Multivariate backward stepwise logistic regression indicated that the strongest independent correlates of acute leptospirosis were, by decreasing odds ratios, gardening, cats at home, skin wound, drinking home brews, wet soil around home, refuse not collected by public services, home built with corrugated iron and, inversely, indoor occupation (Table 3). This model could explain 57% of the variance in predicting acute leptospirosis status. Forward or backward stepwise multivariate models gathered almost identical results. Table 4 shows that a large variance could be ‘explained’ by selected single common factors (gardening, wet soil, skin wounds) and that the combination of all three accounted for as much as 37%.

**Discussion**

**Incidence of acute leptospirosis**

The systematic inclusion of all patients with suspected leptospirosis during the one-year study period permitted an accurate estimation of the incidence of the disease. With 101 cases per 100 000 (95% CI: 79–126), the incidence was higher in the Seychelles than in other tropical countries (13 in Reunion, 18 in Barbados, 41 in French Polynesia except in Hawaii (128)). This high incidence in Seychelles may result from the
particularly high detection rate of cases in this study due to the prospective surveillance strategy for identifying cases, awareness of leptospirosis among local physicians, well-developed primary health care, easy access to medical services as health care is free of charge to all inhabitants, and the use of PCR assay to diagnose cases (a few of which could have been missed if diagnosis had relied on MAT). In addition, the significance of our results is enhanced by the choice of stringent biological diagnosis criteria for the definition of serological confirmed cases.

Prevalence of current subclinical and past leptospiral infection

The population-based selection of controls and the matched study design permitted the estimation of the prevalence of current subclinical and past leptospiral infection in selected categories of the general population. For example, as many as 9% of healthy adult males with manual occupations had evidence of current leptospiraemia (positive PCR) and 37% had evidence of past leptospiral infection (positive MAT) with none of them reporting past or current symptoms related to leptospirosis. These findings indicate that a substantial proportion of the population in this tropical environment experiences leptospiral infection, with many infections remaining subclinical or undiagnosed. In other studies in tropical countries, prevalence rates were found to be 21.9% in Trinidad and 18.5% in Barbados in subjects 5 years and over randomly selected from the general population.29 In non-tropical countries, a 16% seroprevalence was found in a series of 1150 patients attending a sexually transmitted disease clinic in Baltimore;30 seroprevalence rates in healthy Italian men aged 30–39 ranged from 0% in dry southern regions to 40% in the Veneto, a humid northern region where the disease is endemic,31 and seroprevalence was higher than 21% in Spanish workers with environmental exposure (crayfishing and rice workers) in the marsh zone of the Guadalquivir river.32 Positive PCR is not incompatible with asymptomatic forms of leptospirosis or during the long-term follow-up in some patients. Indeed leptospires have been isolated up to several months after the acute episode from blood33 or cerebrospinal fluid,34 and detected by PCR in urines35 and blood36 of patients a long time after acute infection. In addition, delayed detection by PCR in aqueous humour in a patient with unilateral uveitis was reported.36 These previous studies demonstrate that the presence of leptospires in body fluids or target organs may last much longer than is generally acknowledged.

The high seroprevalence in the entire population is not incompatible with a clinically expressed disease concentrating in selected population groups. Indeed, Duval et al. showed that men with an outdoor occupation more often have a clinical disease and were preferentially exposed to the icterohaemorrhagiae serovar (often implicated in severe leptospirosis) while subjects with different occupations may be exposed to different and possibly less virulent strains.19 Alternatively, individuals with outdoor occupations may contract more (subclinical) leptospiral infections during their lifetime, hence increasing their chance of expressing clinical disease.

Association between acute leptospirosis and matching variables

The concentration of the disease in adult males with manual occupations—who are likely to be more exposed to the environment—is consistent with other reports.12,13,15,25,29,37 However, several cases also occurred among housewives (four cases), students (four cases) and workers with mostly indoor activities (six cases with a non-manual job).

Association between acute leptospirosis and other selected variables

By matching for variables already known to be associated with leptospirosis (age, sex, occupation and, implicitly, rainfall), the study design permitted to investigate efficiently other factors associated with the disease. Overall, the variables considered in the regression analyses accounted for as much as 57% of the variance in predicting the acute disease. In particular, significant associations were found for factors increasing exposure to environment such as activities in forest, gardening, wet soil around home, refuse not collected by public services (hence favouring rats density), living in a house built with corrugated iron, and having a kitchen accessible to rats. Leptospirosis was also associated with behaviour such as washing clothes or bathing in the river and walking barefoot outside the home, and to other conditions such as skin wounds (hence favouring leptospiral penetration). A weak relation between rainfall and leptospirosis was found, which may relate to the partial matching for period, to the absence of a well-defined rainfall season and to the inability of a global measure of rainfall to encompass localized phenomena such as humidity, soil characteristics, microclimate specificity in the exact places of contamination. However, no substantial association was found for population density, geographical area, contact with pigs and dogs, and using untreated water at home. Noticeably, a strong association was found between cats at home and acute leptospirosis. This association may, however, have been biased as people in the Seychelles often have cats in an attempt to control rat density. Controversial data are available in the literature: cats have been believed to facilitate the infection,1,38 while a protective role for cats was found in another study.39 The positive association between alcohol intake and acute leptospirosis could be partly confounded by the respondents’ occupation (‘outdoor’ occupation was associated with both alcohol intake and leptospirosis). Only weak associations were found between acute leptospirosis and variables assessing rat density around or inside home, in contrast to other reports.40
An association between rat density and leptospirosis may have been attenuated in this study for several reasons: the high rat density in all areas of the country (hence low variability of this variable), the discrepancy between rat trails (contaminated with infected urine) and the actual habitat of rats, and the poor precision in rat density as reported by individuals. Although a few variables represent the same behavioural and environmental constructs and may be used interchangeably to predict leptospirosis, several of the variables showed independent effects. For example, independent association was found for gardening, wet soil and skin wound, which is consistent with the fact that leptospires survive in humid soils for several months and contamination occurs through skin abrasion or wounds. Accumulating numbers of these and other behavioural or environmental factors therefore confer a commensurately increasing risk of getting the disease.

Public health implications

The findings of this study have implications for clinical and public health management of leptospirosis in tropical countries. As leptospirosis is common and often severe in tropical countries (e.g. 1–2% of total mortality in Seychelles over the last years), the diagnosis should be systematically considered in febrile patients. However, the large prevalence of positive serological findings on single blood samples in healthy individuals stress the need to utilize well-recognized criteria for the definite diagnosis of acute leptospiral infections. The risk factors identified support the view that prevention of leptospirosis needs to be multifactorial, particularly in tropical areas where leptospires are ubiquitous and where outdoor activities bring along several risk factors for leptospiral infection. In these tropical countries, health education is therefore of paramount importance and should stress the need to apply all available measures relevant to risky activities, e.g. to wear footwear (and possibly protective gloves) when gardening, particularly when the soil is wet, and when there are skin wounds on the limbs. Mass vaccination is not considered as there is currently not sufficient evidence for the possible efficiency of this strategy.

Although rodent control is a useful preventive measure, focusing only on this approach would wrongly convey to the public the message that prevention can be achieved by this approach only. Instead, our study suggests that major behaviour change by large segments of the general public will be needed for effective and sustained prevention and control of leptospirosis in tropical countries. This approach contrasts with strategies in non-tropical countries where the disease is mostly related to specific professional or leisure occupations and where control can subsequently be achieved by more specific and less demanding measures.

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