Feral cats do not play a major role in leptospirosis epidemiology on Reunion Island

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Abstract

Although previous studies have reported Leptospira carriage in kidneys and urine of cats, the role of these animals in leptospirosis epidemiology remains poorly understood. Using molecular methods, we investigated Leptospira renal carriage in 172 feral cats from Reunion Island, an oceanic geographically isolated island located in the South West Indian Ocean. Only one out of the 172 analysed specimens tested positive for Leptospira DNA through quantitative real-time polymerase chain reaction. Using this positive sample, we could obtain sequences at three Leptospira loci (rrs2, lipL32 and lipL41) allowing to report for the first time Leptospira borgpetersenii naturally infecting cats. Comparisons with bacterial sequences from both acute human cases and animal reservoirs revealed similarities with Leptospira sequences previously reported on Reunion Island. However, the low prevalence (0.6%) reported herein does not support any major role of feral cats in leptospirosis epidemiology on Reunion Island, contrasting with results recently reported on another Indian Ocean Island, Christmas Island. The significance of these discrepancies is discussed.

Leptospirosis is a widespread re-emerging infectious disease caused by pathogenic bacteria belonging to the genus Leptospira (Spirochaetales, Leptospiraceae) [1]. Pathogenic Leptospira are maintained in the renal tubules of animal reservoirs, which contaminate the environment through their urine. Human infection occurs either through contact with the animal’s urine or contaminated environment [1]. It is estimated that leptospirosis causes over 1 million human cases per year, leading to nearly 60 000 fatal cases [2]. The disease incidence is higher in subtropical regions [3] probably due to environmental conditions (increased humidity and temperature) favourable to Leptospira maintenance and transmission.

Leptospirosis represents a major burden in the South West Indian Ocean (SWIO) region, with some islands such as Seychelles displaying amongst the highest incidence worldwide [3, 4]. In this context, considerable efforts have been made to characterise leptospirosis epidemiology in the region. Recently, molecular studies comparing Leptospira sequence types obtained from acute human cases and animal reservoirs have identified a number of probable important reservoirs [4–7]. These studies have shown that beside rats, other animals play a significant role in Leptospira epidemiology including tenrecs, a family of small insectivorous mammals endemic to Madagascar, as well as introduced mammals such as cows, mice and dogs [4, 6, 7]. However, for some Leptospira lineages infecting humans, the animal reservoir(s) still remain(s) to be identified.

The reported presence of pathogenic leptospiral DNA in the urine of cats [6, 8, 9] strengthens the need for an investigation of this potential reservoir. Leptospira carriage has been previously reported in stray cats on Reunion Island [10] although no sequences were produced and hence precluded any molecular comparison with bacterial strains characterised from human acute cases and animals. Recently, feral cats have been reported as important carriers of pathogenic Leptospira on Christmas Island [11], a true oceanic Island located in the Eastern Indian Ocean. These data stimulate the need for investigating feral cats as reservoirs of pathogenic Leptospira on Reunion Island.

This study was carried out in the frame of the LIFE+ Pétrels project (http://www.petrels.re), a conservation project aiming at protecting two endemic and endangered seabird species of...
Feral cats are known to predate the Barau’s Petrel (Pterodroma baraui) and Mascarene Petrel (Pseudobulweria aterrima). Feral cats are known to predate the Barau’s Petrel (eggs, chicks, juveniles and adults) [12] and removal of feral cats in nesting areas is a major conservation action implemented locally by the LIFE+ Pétrès partners in collaboration with a local non-governmental organisation called AVE2M. The protocols of research were approved by the CYROI institutional ethical committee, certified by the French Ministry of Higher Education and Research (NoAPAFIS#6916-20151 00213267087 v6). Cats were trapped between July 2015 and December 2016 using live traps at six different sites from 110 to 2850 m elevation, in disturbed and preserved areas. The periods of sampling covered the two local seasons: cool–dry season from July to October and hot–wet summer from November to June. The animals were then euthanised by the veterinary clinic of Saint Louis. Different tissues samples were taken (heart, stomachs, kidneys and blood) and stored at −80 °C until laboratory analyses.

For each animal, total nucleic acids were extracted from a small piece of kidney using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer’s recommendations. Leptospira detection was performed on each DNA extract by using a specific protocol of quantitative real-time polymerase chain reaction (qPCR) targeting the 16S gene (rrs2) of pathogenic Leptospira [13]. On each qPCR-positive sample, Leptospira was genotyped using a multilocus sequence typing (MLST) (pubmlst.org, scheme# 3) encompassing six genes (secY, adk, rrs2, icdA, lipL32 and lipL41) [14] and optimised in order to characterise the Leptospira diversity actually circulating in the SWIO region [15]. Each PCR product was visualised under UV light after migration on a 3% agarose gel containing 1X GelRed™ (Biotum Inc., Hayward, CA, USA). The PCR products were sequenced on both strands (Genoscreen, Lille, France) by using the corresponding set of primers and all sequences were deposited in GenBank (MH820176–MH820178).

A total of 172 feral cats were tested for pathogenic Leptospira renal carriage of which only one tested positive (cycle threshold for qPCR: 37.6) yielding a prevalence of 0.6%. The single positive animal corresponded to an adult female sampled in a disturbed mountain rainforest. Full MLST was attempted on this sample but a sequence could be obtained for only three out of the six MLST loci, rrs2, lipL32 and lipL41, showing that the infecting Leptospira species diversity actually circulating in the SWIO region. Each PCR product was visualised under UV light after migration on a 3% agarose gel containing 1X GelRed™ (Biotum Inc., Hayward, CA, USA). The PCR products were sequenced on both strands (Genoscreen, Lille, France) by using the corresponding set of primers and all sequences were deposited in GenBank (MH820176–MH820178).

The implication of cats in leptospirosis epidemiology remains poorly investigated and most of the available studies are based on serological approaches (Microscopic Agglutination Test) (see in [16]), which bring in information on the actual exposure of animals to Leptospira but certainly not on their role as a biological reservoir. Indeed, studies reported the absence of congruence between serology and PCR results in different mammal species which indicated that the serology is not a relevant tool to predict the reservoir status of given species [8, 17, 18]. To our knowledge, only scarce molecular identification of Leptospira infecting cats is available, with two Leptospira species being previously reported, namely Leptospira interrogans and Leptospira kirschneri [8, 11]. We report for the first time L. borgpetersenii naturally infecting cats. On Reunion Island, L. borgpetersenii has been reported in cows and mice and rarely in human acute cases [6]. The detection of an identical lipL32 sequence in the single positive cat and in a house mouse, which is typically a cat prey, supports a previously proposed hypothesis of infection through predation [19]. However, our data do not allow fully addressing such hypothesis, which would require gathering additional molecular data, including access to full genomes.

Altogether, the presence of pathogenic leptospiral DNA in the urine of cats confirms previous studies suggesting that these animals are a potential source of contamination for humans. However, more investigations are necessary to determine the epidemiological importance of this reservoir in the disease, including the isolation of Leptospira from cats’ urine. In the context of Reunion Island, although we detected identical Leptospira sequences in the single positive cat and in acute human cases on three loci, the low prevalence allows us rejecting any major role of feral cats in the local epidemiology of leptospirosis. These results are strikingly different from those reported on Christmas Island, where the prevalence is indeed high in cats [11]. Hence, this work supports previous published studies showing distinct transmission chains in the different islands of the SWIO region [4, 6, 7]. Altogether, these data pinpoint the importance of a proper molecular assessment of Leptospira prevailing at each specific environment in order to establish the role of major...
involved biological compartments and optimise the design of preventive measures.

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**Conflict of interest.** None.

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