Tracking animal reservoirs of pathogenic *Leptospira*: the right test for the right claim.

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Abstract.

Leptospirosis, caused by a pathogenic *Leptospira* bacteria, is the most prevalent zoonosis worldwide and in this context has been extensively investigated through a One Health framework. Diagnosis of human leptospirosis includes molecular and serological tools, with serological Microscopic Agglutination Test (MAT) still being considered as a gold standard. Mammals considered as biological reservoirs include species or populations that are able to maintain chronic infection and shed the bacteria via their urine in the environment. *Leptospira* bacteria are often investigated using the same diagnosis tool, serological MAT. However, MAT testing of putative animal reservoirs can lead to mis-interpretations as it can signal previous infection and not necessarily bring in robust information regarding the capacity of such sero-positive animals to maintain chronic infection. We use previously published data and present new results on introduced and endemic small mammals to show that MAT should not be used for the identification of reservoirs. By contrast, serological data are informative on the level of exposure of animals occupying a specific environment. Finally, we present a sequential methodology to investigate human leptospirosis in a One Health framework that associates molecular detection in humans and animals, together with MAT of human samples using *Leptospira* isolates obtained from reservoir animals occurring in the same environment.

Introduction

Leptospirosis is claimed as the most widespread bacterial zoonosis worldwide causing over one million human cases and nearly 60,000 deaths per year (Costa *et al.* 2015a). Despite its medical and veterinary importance, the burden of the disease remains underestimated in numerous countries, stimulating epidemiological investigations conducted in a One Health framework and aiming to identify the major drivers of the disease (Vinetz *et al.* 2005; Smythe and Chappel 2012; Allan *et al.* 2015). Leptospirosis is caused by pathogenic bacteria belonging to the genus *Leptospira* (family Leptospiraceae), which have been historically classified using antigenic determinants through Microscopic Agglutination Test (MAT) (Martin and Pettit 1918) and Co-Agglutination Absorption Technique (CAAT), allowing to define over 20 serogroups and 300 serovars (Levett 2001; Picardeau 2013). Molecular tools have been more recently developed and have revealed a high genetic diversity of *Leptospira* (Picardeau 2013; Saito *et al.* 2013; Bourhy *et al.* 2014) with several additional species later uncovered by genomic approaches (Guglielmini *et al.* 2019; Vincent *et al.* 2019).

The main biological cycle of pathogenic *Leptospira* involves wild or domestic animals acting as reservoirs through the shedding of the bacteria via their urine in the environment (Ko, Goarant and Picardeau 2009). Humans get mostly (but not only, see Bulach *et al.* 2006) infected through indirect contact with a contaminated environment. Although virtually all mammal species can get infected by these pathogenic bacteria, some requirements are needed to consider them as reservoirs (Babudieri 1958), and indeed only a limited number of species have been definitively shown to support chronic maintenance of the bacteria in their kidneys. Rodents are considered as the main reservoirs but other mammals such as bats (Dietrich *et al.* 2015), invasive (Cosson *et al.* 2014; Costa *et al.* 2015b) or endemic (Dietrich *et al.* 2014; Lagadec *et al.* 2016) small terrestrial mammals as well as cattle (Barragan *et al.* 2016; Guernier *et al.* 2016) have been identified as important reservoirs of *Leptospira*. The multiplication of pathogenic bacteria in animal reservoirs has been examined in experimental infections of mice under laboratory controlled conditions (Ratet *et al.* 2014). Using bioluminescent *Leptospira*, authors showed that a systemic infection associated with weight loss can occur within three days following intra peritoneal infection. Thereafter, within a week, bacteria become rapidly invisible while animals return to a body weight that is hardly distinguishable from that of control animals. Then, a bioluminescent signal of *Leptospira* appears in two spots, corresponding to kidneys where bacteria actively divide leading to a glowing signal persisting for months while systemic infection has apparently irreversibly vanished (Ratet *et al.* 2014).

Hence, the fate of pathogenic *Leptospira* appears to be different in reservoir and incident hosts, with a systemic infection followed by renal colonization in the former contrasting with a general absence of renal colonization in the latter. The separation between reservoir and incident hosts may be not that clear cut and depends on different parameters. Indeed, experimental infections have shown that survival of infected animals and shedding of bacteria depend on the bacterial strains, the infecting bacterial dose, the vertebrate species concerned, as well as the routes of infection (Ratet *et al.* 2014; Matsui *et al.* 2015; Wunder *et al.* 2016; Gomes-Solecki, Santecchia and Werts 2017). For instance, experimental infection of Golden hamsters considered as models of acute infection may lead to chronic shedding in the few animals surviving the infection (Cordonin *et al.* 2019). However, the colonization of renal tubules, which is typical of animal reservoirs, has a considerable immunological consequence: pathogenic *Leptospira* organized in biofilms in the lumen of renal tubules (Ristow *et al.* 2008) remain hidden from the immune system. Since the duration of sero-positivity following infection is not well known (Lloyd-Smith *et al.* 2007), the immunological signature detected in sera may not reflect the *Leptospira* that are chronically shed by the reservoir animal.

Beside its use in *Leptospira* classification, MAT is considered as the reference test for leptospirosis diagnosis in incident hosts (humans and domestic animals), as it allows detecting host antibodies testifying to current, recent, and past infections (Levett 2001; Musso and La Scola 2013). MAT has also been widely used for the investigation of animal reservoirs (Roberts *et al.* 2010; Desvars *et al.* 2012; Assenga *et al.* 2015; Andersen-Ranberg, Pipper and Jensen 2016; Rodrigues *et al.* 2016; Sigaud *et al.* 2009) but some studies indicate that MAT does not definitively verify the carrier status of a given animal species (Ellis, O'Brien and Cassells 1981; Miraglia *et al.* 2008; Libonati, Pinto and Lilenbaum 2017; Sant'anna *et al.* 2017). In the present work, we present further support for these latter observations and argue that the use of MAT may lead to misconclusions regarding the importance of investigated animal species as reservoirs.

To support our purposes, we focused on animal species known as pathogenic *Leptospira* reservoirs on Southwestern Indian Ocean (SWIO) islands. This region is home to a wide diversity of mammals, many being endemic, as well as introduced rodents (family Muridae) and shrews (family Soricidae). The typing of *Leptospira* excreted by mammals in this region has demonstrated high levels of *Leptospira*-host specificity (Dietrich *et al.* 2014, 2018; Gomard *et al.* 2016; Lagadec *et al.* 2016). Indeed, the region is home to a large diversity of bats from seven different families that represent multiple colonizations of the region and endemic terrestrial mammals of the family Tenrecidae and subfamily Nesomyinae (each

representing separate adaptive radiations), which appear to be the exclusive reservoirs of specific pathogenic *Leptospira*, providing interesting biological circumstances to address the power of MAT for investigating leptospirosis epidemiology.

Three animal species from the SWIO region, known to host distinct bacterial lineages/species, were included in the present investigation. Using published molecular and serological data together with original results, we demonstrate that *Leptospira* serological signatures are not necessarily connected to the *Leptospira* excreted by animal reservoirs. We demonstrate the shortfalls of MAT used alone for the identification of *Leptospira* animal reservoirs and discuss the utility of MAT for clarifying leptospirosis epidemiology.

Materials and methods

Ethical considerations.

Biological materials screened in the present study were sampled in the context of a research program for which permits numbers and IACUC acceptance have been presented elsewhere (Dietrich *et al.* 2018).

Animal sampling, Leptospira *serological, and molecular data.*

Three mammal species from the SWIO were included in the study: *Mormopterus acetabulosus* (Molossidae), an insectivorous bat species endemic to Mauritius Island; *Tenrec ecaudatus* (Tenrecidae), an omnivorous terrestrial mammal species endemic to Madagascar and introduced to several SWIO islands, including Reunion Island and Mayotte; an invasive rodent species, *R. rattus* (Muridae) sampled both on Reunion Island and on Mayotte (Table 1). Most of these species were previously investigated for *Leptospira* infection through molecular and/or serological methods by different researcher groups (Desvars *et al.* 2012, 2013; Pagès *et al.* 2015; Guernier *et al.* 2016; Lagadec *et al.* 2016; Dietrich *et al.* 2018). In addition, we produced serological data through MAT for the *M. acetabulosus* samples. The same individual specimens were previously investigated for *Leptospira* infection through molecular methods (Dietrich *et al.* 2018). MAT was performed essentially as previously described (Biscornet *et al.* 2017) using 18 *Leptospira* strains and screening most serogroups reported in both human cases and animals on SWIO islands (Table S1). A serum was considered as positive when the MAT titer was ≥ 1:100.

Results

Bats

Serotyping of *Mormopterus acetabulosus* samples through MAT indicates that 20.0% (6/30) of specimens were seropositive, with sera agglutinating Panama and Pyrogenes serogroups (Table 2 and S2). Using nucleic acids extracted from the kidneys of the same individual specimens, Dietrich et al. (2018) reported that 73.3% (22/30) of the animals tested positive through Real-Time Polymerase Chain Reaction (RT-PCR), showing poor agreement between MAT and RT-PCR (Kappa test = 0.17). More specifically, the six MAT-positive bats were also positive by RT-PCR while, most importantly, 66.6% of the remaining MAT-negative bats (16/24) tested positive by RT-PCR. The sequencing of RT-PCR-positives specimens (also positive in MAT) confirmed that *M. acetabulosus* harbors a *Leptospira* bacterial sequence falling within the *Mormopterus*-borne *Leptospira* monophyletic clade embedded in *L. borgpetersenii* (Dietrich *et al.* 2018) (Table 2).

Rats

Introduced populations of *Rattus rattus* are present on both Mayotte and Reunion Island, but molecular and serological screenings highlight striking differences between these two islands (Table 2). On Mayotte, three serogroups have been previously reported, namely Mini, Pyrogenes, and Grippotyphosa, whereas on Reunion Island the main detected serogroups correspond to Icterohaemorrhagiae, Canicola, Sejroe, Mini, and Cynopteri (Desvars *et al.* 2012, 2013). The molecular investigations of *R. rattus* on both islands confirms sharp inter island differences, with Reunion Island animals harboring strictly *L. interrogans* (Guernier *et al.* 2016), while on Mayotte this rodent may harbor either of three distinct *Leptospira* species (*L. interrogans*, *L. borgpetersenii*, and *L. kirschneri*) (Lagadec *et al.* 2016).

On Reunion Island, a study investigated an outbreak of human leptospirosis after a triathlon and included 10 *R. rattus* that were incidentally trapped at the site where the sporting event took place a few weeks before the event. Five out of the 10 sampled rats tested positive by PCR based on kidney samples. The sequencing of the positive samples revealed only *L. interrogans* (Pagès *et al.* 2015; Guernier *et al.* 2016). Of note, two of the PCR-positive specimens were positive through MAT, whereas the remaining PCR-positive rats were sero-negative.

Tenrecs

On Reunion Island, three serogroups have been reported in *Tenrec ecaudatus*: Icterohaemorrhagiae usually detected with high titers, while Canicola and Bataviae

serogroups are agglutinated with low titers (Desvars *et al.* 2013a; Sigaud *et al.* 2009) (Table 2). Although these serogroups have been detected with moderate to high prevalence on Reunion Island, *T. ecaudatus* is not considered as a *Leptospira* reservoir on that island since renal carriage could not be demonstrated through two independent studies (Desvars *et al.* 2013; Guernier *et al.* 2016) (Table 2). This absence of infection on Reunion Island contrasts with the situation on Mayotte, where *T. ecaudatus* was identified as the exclusive reservoir of *L. mayottensis*, a pathogenic species commonly associated with human leptospirosis on that island (Lagadec *et al.* 2016) (Table 2).

Discussion

Microscopic Agglutination Test (MAT) has been, and still is, considered the gold standard for leptospirosis diagnosis in humans. A meta-analysis has calculated the mean prevalence in reservoir mammals using the data published in 300 papers including eight different taxonomic orders (Andersen-Ranberg, Pipper and Jensen 2016). MAT and PCR were given an equivalent weight in that analysis, and the nature of the screened samples, *i.e.* blood (for MAT and PCR) or kidney/urine (for PCR only) was not taken into consideration. Hence, acute/passed infections and chronic kidney carriage were not distinguished in that study, as has been done in several others. In the bat samples screened in the present study, we demonstrate a poor agreement between data from serological and molecular analyses. Similar findings were also reported on a fruit bat species, *Pteropus alecto* (Pteropodidae), from Australia (Cox, Smythe and Leung 2005), which indicated poor agreement between PCR (detection on kidneys) and serological data; these results underlined that a carrier status for this species could not be shown based on serology. In Brazil, studies have reported limited or absence of agreement between PCR (detection in urine) and serological results in livestock animals or asymptomatic dogs (Hamond *et al.* 2014; Sant'anna *et al.* 2017).

The bat species investigated herein belongs to the genus *Mormopterus*, which includes within the SWIO region two other species, *M. francoismoutoui* and *M. jugularis*, endemic to Reunion Island and Madagascar, respectively. Recently, the research on these three molossid bats has shown that they shelter pathogenic *Leptospira* clustering into a single monophyletic *L. borgpetersenii* clade (Gomard *et al.* 2016; Dietrich *et al.* 2018). Interestingly, the screening of *M. acetabulosus* specimens through MAT reveals that sera agglutinate two distinct serogroups, *i.e.* Panama, and Pyrogenes. Although there is poor congruence between

serogroups and genomospecies, members of Panama serogroup can be found in two species, *L. noguchii* and *L. inadai* (Levett 2001), but not in *L. borgpetersenii*. This suggests that the Panama serogroup signature results from independent systemic infections, which have cleared out without leading to renal colonization.

Although the molecular and serological analyses from *Rattus* and *Tenrec* were not all obtained from the same specimens, the results presented herein hardly support any agreement of data obtained from molecular and serological work. Interestingly, the investigation of these two mammal genera on Mayotte and Reunion Island highlight the importance of independently evaluating the reservoir status of a given mammal species on different islands, as *Rattus* do not shelter the same *Leptospira* species. The investigation of *Tenrec* is more compelling. While on Mayotte *T. ecaudatus* is the exclusive carrier of the recently described *L. mayottensis*, investigations on Reunion Island showed that up to 81% of individual *Tenrec* were seropositive (mostly reacting against Icterohaemorragiae) but not one individual showed evidence for chronic kidney infection (Desvars *et al.* 2013; Guernier *et al.* 2016). On Reunion Island, *T. ecaudatus* is therefore not a reservoir of *Leptospira* and Icterohaemorragiae serogroup revealed through MAT should be considered as evidence of animal exposure to the *Leptospira* actually present in the environment.

In our tested samples, some individuals were positive through PCR but negative through MAT. These discrepancies may result from past infections and subsequent kidney colonization followed by titer decay and eventually seronegativation, as previously reported in animal reservoirs (Lloyd-Smith *et al.* 2007) and incidental hosts (Blackmore, Schollum and Moriarty 1984; Lupidi *et al.* 1991; Cumberland *et al.* 2001). We propose that conflicting results of known reservoirs, such as bats testing positive through MAT but negative through PCR using kidney tissues and/or urine, are best explained by animals that experienced past infections in which *Leptospira* did not colonize the kidneys. This absence of colonization might be related to a low infecting dose and/or from an infecting bacterial genomospecies that is unable to establish persistent renal colonization in a specific vertebrate species. This assumption is based on the existence of host-*Leptospira* molecular determinants required for renal colonization, an hypothesis substantiated by experimental infections in which bat-borne and Tenrec-borne *Leptospira* were not able to lead to chronic infection in rats (Cordonin *et al.* 2020).

Finally, the biological setting of SWIO islands brings further evidence of problems using MAT for the identification of *Leptospira* reservoirs. Several studies have used MAT on samples of wild animals to address their role in the epidemiology of leptospirosis. As demonstrated here, this serological technique is very useful as it opens a window on environmental exposure to *Leptospira*. However, even though it is clearly important to address the diversity and intensity of *Leptospira* exposure in an environmental setting, we emphasize that MAT data from investigated animals cannot lead to any robust conclusion regarding their role as a reservoir. Such investigations, carried out in a One Health framework require bacterial genotyping using kidney or urine samples so that bacteria excreted by mammal reservoirs can be compared to those identified in acute human cases. Ultimately, a thorough investigation of leptospirosis following a One Health framework would require (i) PCR screening of urine or kidney tissues from putative animal reservoirs, (ii) isolation of *Leptospira* from identified reservoirs, and (iii) the inclusion of these isolates in a MAT panel used to screen human sera collected from persons living or having a professional/recreational activity in the investigated environment. Such investigations would allow not only identifying animal reservoirs in a specific environmental setting, but highlighting those bacterial species/lineages of major medical concern.

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References

Allan KJ, Biggs HM, Halliday JEB *et al.* Epidemiology of Leptospirosis in Africa: A Systematic Review of a Neglected Zoonosis and a Paradigm for 'One Health' in Africa. *PLoS Negl Trop Dis* 2015;**9**:e0003899.

Andersen-Ranberg EU, Pipper C, Jensen PM. Global patterns of Leptospira prevalence in vertebrate reservoir hosts. *J Wildl Dis* 2016;**52**:468–77.

Assenga JA, Matemba LE, Muller SK *et al.* Predominant leptospiral serogroups circulating among humans, livestock and wildlife in Katavi-Rukwa ecosystem, Tanzania. *PLoS Negl Trop Dis* 2015;**9**:e0003607.

Babudieri B. Animal reservoirs of leptospires. *Ann N Y Acad Sci* 1958;**70**:393–413.

Barragan V, Chiriboga J, Miller E *et al.* High Leptospira Diversity in Animals and Humans Complicates the Search for Common Reservoirs of Human Disease in Rural Ecuador. *PLOS Negl Trop Dis* 2016;**10**:e0004990.

Biscornet L, Dellagi K, Pagès F *et al.* Human leptospirosis in Seychelles: A prospective study confirms the heavy burden of the disease but suggests that rats are not the main reservoir. *PLoS Negl Trop Dis* 2017;**11**:e0005831.

Blackmore DK, Schollum LM, Moriarty KM. The magnitude and duration of titres of leptospiral agglutinins in human sera. *N Z Med J* 1984;**97**:83–6.

Bourhy P, Collet L, Brisse S *et al.* Leptospira mayottensis sp. nov., a pathogenic Leptospira species isolated from humans. *Int J Syst Evol Microbiol* 2014:ijs.0.066597-0.

Bulach DM, Zuerner RL, Wilson P *et al.* Genome reduction in Leptospira borgpetersenii reflects limited transmission potential. *Proc Natl Acad Sci U S A* 2006;**103**:14560–5.

Cordonin C, Turpin M, Bascands J-L *et al.* Three Leptospira Strains From Western Indian Ocean Wildlife Show Highly Distinct Virulence Phenotypes Through Hamster Experimental Infection. *Front Microbiol* 2019;**10**, DOI: 10.3389/fmicb.2019.00382.

Cordonin C, Turpin M, Bringart M *et al.* Pathogenic Leptospira and their animal reservoirs: testing host specificity through experimental infection. *Sci Rep* 2020;**10**:7239.

Cosson J-F, Picardeau M, Mielcarek M *et al.* Epidemiology of leptospira transmitted by rodents in southeast Asia. *PLoS Negl Trop Dis* 2014;**8**:e2902.

Costa F, Hagan JE, Calcagno J *et al.* Global Morbidity and Mortality of Leptospirosis: A Systematic Review. *PLoS Negl Trop Dis* 2015a;**9**:e0003898.

Costa F, Wunder EA Jr, De Oliveira D *et al.* Patterns in Leptospira Shedding in Norway Rats (Rattus norvegicus) from Brazilian Slum Communities at High Risk of Disease Transmission. *PLoS Negl Trop Dis* 2015b;**9**:e0003819.

Cox TE, Smythe LD, Leung LK-P. Flying Foxes as Carriers of Pathogenic Leptospira Species. *J Wildl Dis* 2005;**41**:753–7.

Cumberland P, Everard CO, Wheeler JG *et al.* Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979-1989. *Eur J Epidemiol* 2001;**17**:601–8.

Desvars A, Naze F, Benneveau A *et al.* Endemicity of leptospirosis in domestic and wild animal species from Reunion Island (Indian Ocean). *Epidemiol Infect* 2013;**141**:1154–65.

Desvars A, Naze F, Vourc'h G *et al.* Similarities in Leptospira Serogroup and Species

Distribution in Animals and Humans in the Indian Ocean Island of Mayotte. *Am J Trop Med Hyg* 2012;**87**:134–40.

Dietrich M, Gomard Y, Lagadec E *et al.* Biogeography of Leptospira in wild animal communities inhabiting the insular ecosystem of the western Indian Ocean islands and neighboring Africa. *Emerg Microbes Infect* 2018;**7**:57.

Dietrich M, Wilkinson DA, Benlali A *et al.* Leptospira and Paramyxovirus infection dynamics in a bat maternity enlightens pathogen maintenance in wildlife. *Environ Microbiol* 2015, DOI: 10.1111/1462-2920.12766.

Dietrich M, Wilkinson DA, Soarimalala V *et al.* Diversification of an emerging pathogen in a biodiversity hotspot: Leptospira in endemic small mammals of Madagascar. *Mol Ecol* 2014;**23**:2783–96.

Ellis WA, O'Brien JJ, Cassells J. Role of cattle in the maintenance of Leptospira interrogans serotype hardjo infection in Northern Ireland. *Vet Rec* 1981;**108**:555–7.

Gomard Y, Dietrich M, Wieseke N *et al.* Malagasy bats shelter a considerable genetic diversity of pathogenic Leptospira suggesting notable host-specificity patterns. *FEMS Microbiol Ecol* 2016, DOI: 10.1093/femsec/fiw037.

Gomes-Solecki M, Santecchia I, Werts C. Animal Models of Leptospirosis: Of Mice and Hamsters. *Front Immunol* 2017;**8**, DOI: 10.3389/fimmu.2017.00058.

Guernier V, Lagadec E, Cordonin C *et al.* Human Leptospirosis on Reunion Island, Indian Ocean: Are Rodents the (Only) Ones to Blame? *PLOS Negl Trop Dis* 2016;**10**:e0004733.

Guglielmini J, Bourhy P, Schiettekatte O *et al.* Genus-wide Leptospira core genome multilocus sequence typing for strain taxonomy and global surveillance. *PLoS Negl Trop Dis* 2019;**13**:e0007374.

Hamond C, Martins G, Loureiro AP *et al.* Urinary PCR as an increasingly useful tool for an accurate diagnosis of leptospirosis in livestock. *Vet Res Commun* 2014;**38**:81–5.

Ko AI, Goarant C, Picardeau M. Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nat Rev Microbiol* 2009;**7**:736–47.

Lagadec E, Gomard Y, Minter GL *et al.* Identification of Tenrec ecaudatus , a Wild Mammal Introduced to Mayotte Island, as a Reservoir of the Newly Identified Human Pathogenic Leptospira mayottensis. *PLOS Negl Trop Dis* 2016;**10**:e0004933.

Levett PN. Leptospirosis. *Clin Microbiol Rev* 2001;**14**:296–326.

Libonati H, Pinto PS, Lilenbaum W. Seronegativity of bovines face to their own recovered leptospiral isolates. *Microb Pathog* 2017;**108**:101–3.

Lloyd-Smith JO, Greig DJ, Hietala S *et al.* Cyclical changes in seroprevalence of leptospirosis in California sea lions: endemic and epidemic disease in one host species? *BMC Infect Dis* 2007;**7**:125.

Lupidi R, Cinco M, Balanzin D *et al.* Serological follow-up of patients involved in a localized outbreak of leptospirosis. *J Clin Microbiol* 1991;**29**:805–9.

Martin L, Pettit A. Sero-diagnostic de la spirochaetose icterohaemorrhagique. *Bull Mém Société Médicale Hôp Paris* 1918.

Matsui M, Roche L, Soupé-Gilbert M-E *et al.* Experimental Hamster Infection with a Strain of Leptospira borgpetersenii Ballum Isolated from a Reservoir Mouse in New Caledonia. *Am J Trop Med Hyg* 2015:14–0462.

348 Miraglia F, Moreno AM, Gomes CR *et al.* Isolation and characterization of Leptospira
349 interrogans from pigs slaughtered in São Paulo State, Brazil. *Braz J Microbiol Publ Braz*
350 *Soc Microbiol* 2008, DOI: 10.1590/S1517-838220080003000017.

351 Musso D, La Scola B. Laboratory diagnosis of leptospirosis: A challenge. *J Microbiol*
352 *Immunol Infect* 2013;**46**:245–52.

353 Pagès F, Larrieu S, Simoes J *et al.* Investigation of a leptospirosis outbreak in triathlon
354 participants, Réunion Island, 2013. *Epidemiol Infect* 2015:1–9.

355 Picardeau M. Diagnosis and epidemiology of leptospirosis. *Médecine Mal Infect* 2013;**43**:1–9.

356 Ratet G, Veyrier FJ, Fanton d'Andon M *et al.* Live Imaging of Bioluminescent Leptospira
357 interrogans in Mice Reveals Renal Colonization as a Stealth Escape from the Blood
358 Defenses and Antibiotics. *PLoS Negl Trop Dis* 2014;**8**, DOI:
359 10.1371/journal.pntd.0003359.

360 Ristow P, Bourhy P, Kerneis S *et al.* Biofilm formation by saprophytic and pathogenic
361 leptospires. *Microbiol Read Engl* 2008;**154**:1309–17.

362 Roberts MW, Smythe L, Dohnt M *et al.* Serologic-based investigation of leptospirosis in a
363 population of free-ranging eastern grey kangaroos (Macropus giganteus) indicating the
364 presence of Leptospira weilii serovar Topaz. *J Wildl Dis* 2010;**46**:564–9.

365 Rodrigues TCS, Santos ALQ, Lima AMC *et al.* Anti-Leptospira spp. antibodies in Crotalus
366 durissus collilineatus kept in captivity and its zoonotic relevance. *Acta Trop*
367 2016;**158**:39–42.

368 Saito M, Villanueva SYAM, Kawamura Y *et al.* Leptospiraidonii sp. nov., isolated from
369 environmental water. *Int J Syst Evol Microbiol* 2013;**63**:2457–62.

370 Sant'anna R, Vieira AS, Grapiglia J *et al.* High number of asymptomatic dogs as leptospiral
371 carriers in an endemic area indicates a serious public health concern. *Epidemiol Infect*
372 2017:1–3.

373 Sigaud M, Caceres S, Picard M *et al.* Tailless tenrec (Tenrec ecaudatus)□: natural
374 maintenance host of leptospires? *Bull Société Pathol Exot* **102**:19–20.

375 Smythe LD, Chappel RJ. Leptospirosis – importance of a One Health approach. *Microbiol*
376 *Aust* 2012;**33**:154–6.

377 Vincent AT, Schiettekatte O, Goarant C *et al.* Revisiting the taxonomy and evolution of
378 pathogenicity of the genus Leptospira through the prism of genomics. *PLoS Negl Trop*
379 *Dis* 2019;**13**:e0007270.

380 Vinetz JM, Wilcox BA, Aguirre A *et al.* Beyond Disciplinary Boundaries: Leptospirosis as a
381 Model of Incorporating Transdisciplinary Approaches to Understand Infectious Disease
382 Emergence. *EcoHealth* 2005;**2**:291–306.

383 Wunder EA, Figueira CP, Santos GR *et al.* Real-Time PCR Reveals Rapid Dissemination of
384 Leptospira interrogans after Intraperitoneal and Conjunctival Inoculation of Hamsters.
385 *Infect Immun* 2016;**84**:2105–15.

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Table 1. Animal species used in the present study and the associated publications for *Leptospira* investigations.

Animal species	Islands	Serological data	Molecular data
Mormopterus acetabulosus	Mauritius	Present study	Dietrich *et al*. (2018)
Tenrec ecaudatus	Mayotte	NA	Lagadec *et al*. (2016)
	La Réunion	Desvars et al. (2013a)	Desvars *et al*. (2013a); Guernier *et al*. (2016)
Rattus rattus	Mayotte	Desvars et al. (2012)	Desvars *et al*. (2012)
	La Réunion	Desvars *et al*. (2012, 2013a)	Desvars *et al*. (2012, 2013a); Guernier *et al*. (2016)

NA: not available.

Table 2. Comparison of serological and molecular *Leptospira* data obtained from the investigated animal species.

Animal species	Islands	Serological data (MAT)		Molecular data (RT PCR)	
		Positive animals (%)	**Detected serogroups (titer)**	**Positive animals (%)**	***Leptospira* spp.**
Mormopterus acetabulosus	Mauritius	20.0% (6/30)	Panama (1:100 – 400) (n=5) Pyrogenes (1:200) (n=1)	73.3% (22/30)	*Lb* (n=8)
Tenrec ecaudatus	Mayotte	*NA*	*NA*	27.0% (10/37)	*Lm* (n=8)
	Reunion	13.2% (5/38)	Icterohaemorrhagiae (1:200 – 800) (n=3) Canicola (1:100) (n=1) Bataviae (1:100) (n=1)	0.0% (0/35) 0.0% (0/35)	- -
Rattus rattus	Mayotte	11.2% (14/125)	Mini (1:100 – 400) (n=7) Pyrogenes (1:200) (n=1) Grippotyphosa (1:100 – 1,600) (n=3) Co-agglutinations (n=3)	29.8% (42/121) 15.9% (46/289)	*Lb* (n=9), *Li* (n=7), *Lk* (n=2), *Lm* (n=2) *Lb* (n=13), *Li* (n=3), *Lk* (n=5)
	Reunion	78.8% (52/66)	Icterohaemorrhagiae (1:100 – 3,200) (n=22) Canicola (1:100 – 400) (n=7) Sejroe (1:100 – 1:200) (n=2) Mini (1:100) (n=1) Cynopteri (1:3,200) (n=1) Co-agglutinations (n=19)	65.8% (50/76) 38.5% (214/562)	*NA* *Li* (n=201)

Lb: L. borgpetersenii, *Li:* L. interrogans, *Lk*: L. kirschneri, *Lm*: L. mayottensis.
MAT: Microscopic Agglutination Test, RT PCR: Real-Time Polymerase Chain Reaction.
NA: not available

Table S1. Details of *Leptospira* strains used for the Microscopic Agglutination Test on *Mormopterus acetabulosus*.

Species	Serogroup	Serovar	Strain
L. biflexa	Semaranga	Patoc	Patoc I (Paris)
L. borgpetersenii	Ballum	Castellonis	Castellon 3
	Sejroe	Hardjobovis	Sponselee
	Sejroe	Sejroe	M 84
	Tarassovi	Tarassovi	Perepelicin
L. interrogans	Australis	Australis	Ballico
	Autumnalis	Autumnalis	Akiyami A
	Bataviae	Bataviae	Van Tienen
	Canicola	Canicola	Hond Utrecht IV
	Hebdomadis	Hebdomadis	Hebdomadis
	Icterohaemorrhagiae	Copenhageni	Wijiberg
	Icterohaemorrhagiae	Icterohaemorrhagiae	-
	Pomona	Pomona	-
	Pyrogenes	Pyrogenes	Salinem
L. kirschneri	Cynopteri	Cynopteri	3522C
	Grippotyphosa	Grippotyphosa	Moskva V
	Mini	undertermined	200803703
L. noguchii	Panama	Panama	CZ 214K

Table S2. Details of bat samples, *Mormopterus acetabulosus* from Mauritius, used in the present study. The table includes the (a) Microscopic Agglutination Test (MAT) results and *Leptospira* molecular data obtained from the present study and the work of Dietrich et al. (2018) respectively. *RT-PCR: Real-Time Polymerase Chain Reaction, CT: Cycle Threshold, FMNH: specimen deposited in the Field Museum of Natural History*

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Specimen ID No.	ID extraction	Sex	Age	Date (day/month /year)	Site	Serology results		Molecular results		
						MAT	*Leptospira* serogroup (MAT titer)	RT - PCR	CT	*Leptospira* species
FMNH 213456	152	F	A	14/12/10	Palma Cave (Palma)	(-)	-	**(+)**		*L. borgpetersenii* related
FMNH 213457	153	F	A	14/12/10	Palma Cave (Palma)	(-)	-	(-)	-	
FMNH 213458	154	F	A	14/12/10	Palma Cave (Palma)	(-)	-	**(+)**		
FMNH 213459	155	M	A	14/12/10	Palma Cave (Palma)	(-)	-	(-)	-	
FMNH 213461	157	M	A	14/12/10	Palma Cave (Palma)	(-)	-	(-)	-	
FMNH 213463	159	M	A	14/12/10	Palma Cave (Palma)	(-)	-	**(+)**		
FMNH 213465	161	M	A	14/12/10	Palma Cave (Palma)	(-)	-	**(+)**		*L. borgpetersenii* related
FMNH 213466	162	M	A	14/12/10	Palma Cave (Palma)	**(+)**	Pyrogenes (1:200)	**(+)**		*L. borgpetersenii* related
FMNH 213467	163	F	A	14/12/10	Palma Cave (Palma)	(-)	-	**(+)**		
FMNH 213470	166	M	A	14/12/10	Palma Cave (Palma)	(-)	-	**(+)**		
FMNH 213471	167	M	A	14/12/10	Palma Cave (Palma)	(-)	-	**(+)**		
FMNH 213472	168	F	A	15/12/10	Twilight Cave (Roches Noires)	(-)	-	(-)	-	
FMNH 213475	171	F	A	15/12/10	Twilight Cave (Roches Noires)	(-)	-	(-)	-	
FMNH 213477	173	M	A	15/12/10	Twilight Cave (Roches Noires)	(-)	-	(-)	-	

FMNH 213474	170	F	A	15/12/10	Twilight Cave (Roches Noires)	(-)	-	**(+)**		
FMNH 213479	175	F	A	15/12/10	Twilight Cave (Roches Noires)	(-)	-	**(+)**		
FMNH 213481	176	F	A	15/12/10	Twilight Cave (Roches Noires)	(-)	-	**(+)**		
FMNH 213483	179	M	A	15/12/10	Twilight Cave (Roches Noires)	(-)	-	(-)	-	
FMNH 213485	181	F	A	15/12/10	Caverne Trois Bras (Moulin à Vent)	**(+)**	Panama (1:100)	**(+)**	42,00	
FMNH 213486	182	F	A	15/12/10	Caverne Trois Bras (Moulin à Vent)	(-)	-	(-)	-	
FMNH 213487	183	F	A	15/12/10	Caverne Trois Bras (Moulin à Vent)	(-)	-	**(+)**	34,00	*L. borgpetersenii* related
FMNH 213489	185	F	A	15/12/10	Caverne Trois Bras (Moulin à Vent)	(-)	-	**(+)**	37,00	
FMNH 213490	186	F	A	15/12/10	Caverne Trois Bras (Moulin à Vent)	(-)	-	**(+)**	35,00	
FMNH 213491	187	F	A	15/12/10	Caverne Trois Bras (Moulin à Vent)	(-)	-	**(+)**	38,00	
FMNH 213493	189	F	A	15/12/10	Caverne Trois Bras (Moulin à Vent)	**(+)**	Panama (1:200)	**(+)**	34,00	
FMNH 213494	190	F	A	15/12/10	Caverne Trois Bras(Moulin à Vent)	**(+)**	Panama (1:400)	**(+)**	35,00	
FMNH 213495	191	F	A	15/12/10	Caverne Trois Bras (Moulin à Vent)	(-)	-	**(+)**		
FMNH 213503	199	F	A	16/12/10	Camp Thorel Cave (Camp Thorel)	**(+)**	Panama (1:400)	**(+)**	40,00	
FMNH 213504	200	M	A	16/12/10	Camp Thorel Cave (Camp Thorel)	(-)	-	**(+)**	34,00	
FMNH 213508	204	M	A	16/12/10	Camp Thorel Cave (Camp Thorel)	**(+)**	Panama (1:400)	**(+)**	37,00	