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ORIGINAL ARTICLE

Leptospira Species in Feral Cats and Black Rats from Western Australia and Christmas Island

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Leptospirosis is a neglected, re-emerging bacterial disease with both zoonotic and conservation implications. Rats and livestock are considered the usual sources of human infection, but all mammalian species are capable of carrying *Leptospira* spp. and transmitting pathogenic leptospires in their urine, and uncertainty remains about the ecology and transmission dynamics of *Leptospira* in different regions. In light of a recent case of human leptospirosis on tropical Christmas Island, this study aimed to investigate the role of introduced animals (feral cats and black rats) as carriers of pathogenic *Leptospira* spp. on Christmas Island and to compare this with two different climatic regions of Western Australia (one island and one mainland). Kidney samples were collected from black rats ($n = 68$) and feral cats ($n = 59$) from Christmas Island, as well as feral cats from Dirk Hartog Island ($n = 23$) and southwest Western Australia ($n = 59$). Molecular (PCR) screening detected pathogenic leptospires in 42.4% (95% confidence interval 29.6–55.9) of cats and 2.9% (0.4–10.2) of rats from Christmas Island. Sequencing of cat- and rat-positive samples from Christmas Island showed 100% similarity for *Leptospira interrogans*. Pathogenic leptospires were not detected in cats from Dirk Hartog Island or southwest Western Australia. These findings were consistent with previous reports of higher *Leptospira* spp. prevalence in tropical regions compared with arid and temperate regions. Despite the abundance of black rats on Christmas Island, feral cats appear to be the more important reservoir species for the persistence of pathogenic *L. interrogans* on the island. This research highlights the importance of disease surveillance and feral animal management to effectively control potential disease transmission.

Keywords: *Felis catus, Leptospira, Rattus*, zoonosis

Introduction

Leptospirosis is a re-emerging zoonosis of global importance. Recently classified as a neglected tropical disease (WHO 2003, Hartskeerl et al. 2011), it is one of the most widespread zoonoses (Pappas et al. 2008, Zavitsanou and Babatsikou 2008, Krøjgaard et al. 2009, Desvars et al. 2011), occurring on every continent with the exception of Antarctica (Adler and de la Peña Moctezuma 2010). Symptoms of leptospirosis in people range from nonspecific influenza-like illness to multiorgan failure, with 1.7 million cases of the disease reported annually worldwide (Krøjgaard et al. 2009, Hartskeerl et al. 2011, Chiriboga et al. 2015); however, due to the nonspecific symptoms, many cases of leptospirosis go undiagnosed, and therefore, the true incidence is expected to be higher (Meerburg et al. 2009, Lau et al. 2010, Azócar-Aedo et al. 2014).

Twenty species of *Leptospira* have been described in three clusters (saprophytic, pathogenic, and intermediate pathogenic) with more than 250 known serovars (Zavitsanou and Babatsikou 2008, Ko et al. 2009, Hartmann et al. 2013). Globally, rodents and domestic mammals, including cattle (*Bos taurus*), pigs (*Sus scrofa*), and dogs (*Canis lupus familiaris*), are considered the most important reservoir hosts for this bacteria with respect to zoonotic potential; however, many mammal species are capable of acting as hosts (Faine et al. 1999, WHO 2003). Reptiles and amphibians are also capable of transmitting leptospires (Everard et al. 1983, 1988, 1990, Gravekamp et al. 1991, Calle et al. 2001), as are migratory birds, carrying contaminated soil on their legs (Faine et al. 1999, Guerra 2009). Within Australia, *Leptospira* spp. have been detected in domestic animals and native mammal species (Milner et al. 1981, Dickeson and Love 1993, Perolat et al. 1998, Cox et al. 2005).

Although the role of rats in the transmission of leptospires is well recognized, the role of cats in the transmission of *Leptospira* spp. in different environments is not well described, and yet is of interest, given the close association between cats and humans (Jamshidi et al. 2009).

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Approximately 64% of human leptospirosis cases reported in Australia between 2000 and 2015 originated in Queensland, typically within the tropics (Australian Government Department of Health 2015). Increased reports of leptospirosis in tropical regions reflect the favorable conditions that support transmission indirectly via contact with contaminated soil and/or water (Ricaldi and Vinetz 2006, Lau et al. 2010, Desvars et al. 2011, Hartskeerl et al. 2011). However, *Leptospira* transmission cycles can persist in a wide range of environmental conditions and the diverse range of potential host and reservoir species means that leptospirosis poses both a public and animal health issue.

A confirmed, autochthonous case of leptospirosis in a person on Christmas Island (Dr Julie Graham, personal communication) prompted further investigation into the role of invasive animals as carriers of *Leptospira* as part of a larger project concerning these species conducted on Christmas Island (CHI), Dirk Hartog Island (DHI), and southwest Western Australia (swWA). The three locations have distinct climatic characteristics, but all have conservation significance due to recent declines in native fauna. Only one of five originally endemic mammal species remains on CHI, where there are no livestock present (other than feral chickens) and two invasive mammal species (cats and black rats). Three of 13 endemic mammal species remain on DHI along with the introduced house mouse (*Mus musculus*). At the time of this study, there were only a small number of sheep and goats remaining on DHI. Reptiles (both endemic and invasive) and birds (endemic, migratory, and invasive) are present on both CHI and DHI. Southwest Western Australia includes agricultural, urban, and forested areas with a wide diversity of endemic and introduced reptiles, amphibians, birds, and mammals (including cats and black rats).

The aim of this study was to explore the role of two invasive animals (cats and black rats) as carriers of pathogenic *Leptospira* spp. in three locations with a close wildlife and/or human interface.

Methods

Study locations

Samples were collected from three geographically and climatically distinct locations: Christmas Island, Dirk Hartog Island, and mainland southwest Western Australia (Fig. 1). Christmas Island is an Australian Territory located in the Indian Ocean (10° 29′ S, 105° 38′ E) ~360 km south of the Indonesian capital Jakarta and experiences a tropical climate. Dirk Hartog Island is a large arid inshore island (25°50′ S, 113°05′ E) located to the west of Shark Bay off the Western Australian coast. The southwest of Western Australia is a large ecoregion, located south of a line from Geraldton (28° 46′ 28″ S, 114° 36′ 32″ E) to Esperance (33° 51′40″ S, 121° 33′ 31″ E) with a predominately Mediterranean climate (hot dry summers, cool wet winters).

Sample collection

Cat cadavers were collected from CHI ($n = 59$) in 2011, DHI ($n = 22$) from 2012 to 2013, and swWA ($n = 59$) from 2012 to 2013; black rats ($n = 68$) were also collected concurrently from CHI. Cats from CHI and DHI were sourced from the Department of Parks and Wildlife management programs and cats from swWA were shot during community-coordinated culling programs from 12 locations (Red Card for Rabbits and Foxes). Trapping protocols from CHI and DHI have been described previously by Algar et al. (2014) and Deller et al. (2015), respectively.

All cadavers were placed individually in sealed plastic body bags immediately after death and stored at −20° C until necropsy was conducted. Head-body (HB) lengths were measured and weights recorded for each animal to calculate a body condition index (= weight/HB length) (Rodríguez and Carbonell 1998, Vervaeke et al. 2005). Kidney tissue was collected at necropsy and preserved in 70% ethanol.

DNA extraction and PCR conditions

DNA was extracted from kidney tissue of cats and rats using the Qiagen spin columns for blood and tissue kit according to the manufacturer's instructions (Qiagen, USA). A nested PCR *Leptospira* protocol to identify and differentiate between the presence of pathogenic (615 bp product), and saprophytic/intermediate (316 bp product) *Leptospira* spp. from the 23S rDNA region was conducted according to Kositanont et al. (2007). External primers used were LepF1 (5′-GTTACCAAGCACAAGATTAG-3′) and LepR1 (5′-TAGTCCCGATTACATTTTC-3′). Internal forward primers used were PU1 (5′-TATCAGAGCCTTTTAATGG-3′) and SU1 (5′-TTTAGGGTTAGCGTGGTA-3′) and the reverse primer was again LepR1. A 50 μL reaction mixture was used to amplify 5 μL of template DNA with 5 μL of 10 × PCR buffer, 8 μL of 25 mmol/L $MgCl_2$, 1 μL of 20 pmol of ea primer, 200 μmol/L of ea dNTP, and 1 U of Taq polymerase. Thermocycling conditions include an initial denaturation step of 96°C for 5 min, and then, 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 50°C for 50 s, and extension at 72°C for 1 min (the extension time increased by 3 s for each cycle). The final elongation step was 72°C for 7 min.

Leptospira interrogans serovar Pomona (WHO/FAO/OIE 2015) was used as a positive control and PCR-grade water was used as a negative control. Amplified DNA fragments were visualized on a 1.5% agarose gel by electrophoresis.

DNA purification and sequencing

Sequencing of PCR-positive samples was conducted to confirm the presence of pathogenic species. PCR products were purified using the tip elution method described in Yang et al. (2013). The purified DNA was then sequenced using an ABI prism Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions on an Applied Biosystems 3730 DNA Analyzer. Sequencing results were compared against available sequences in GenBank using BLAST search (http://blast.ncbi.nah.gov/Blast.cgi).

Statistical analyses

Prevalence confidence intervals were calculated using the exact binomial method (Graat et al. 1997). Cats were categorized for age based on weight (kitten <1.0 kg; juvenile 1–2.4 kg; adult >2.5 kg) as previously described by Algar et al. (2014). All cats in this study were either juvenile or adult. Statistical analyses were performed using the software SPSS

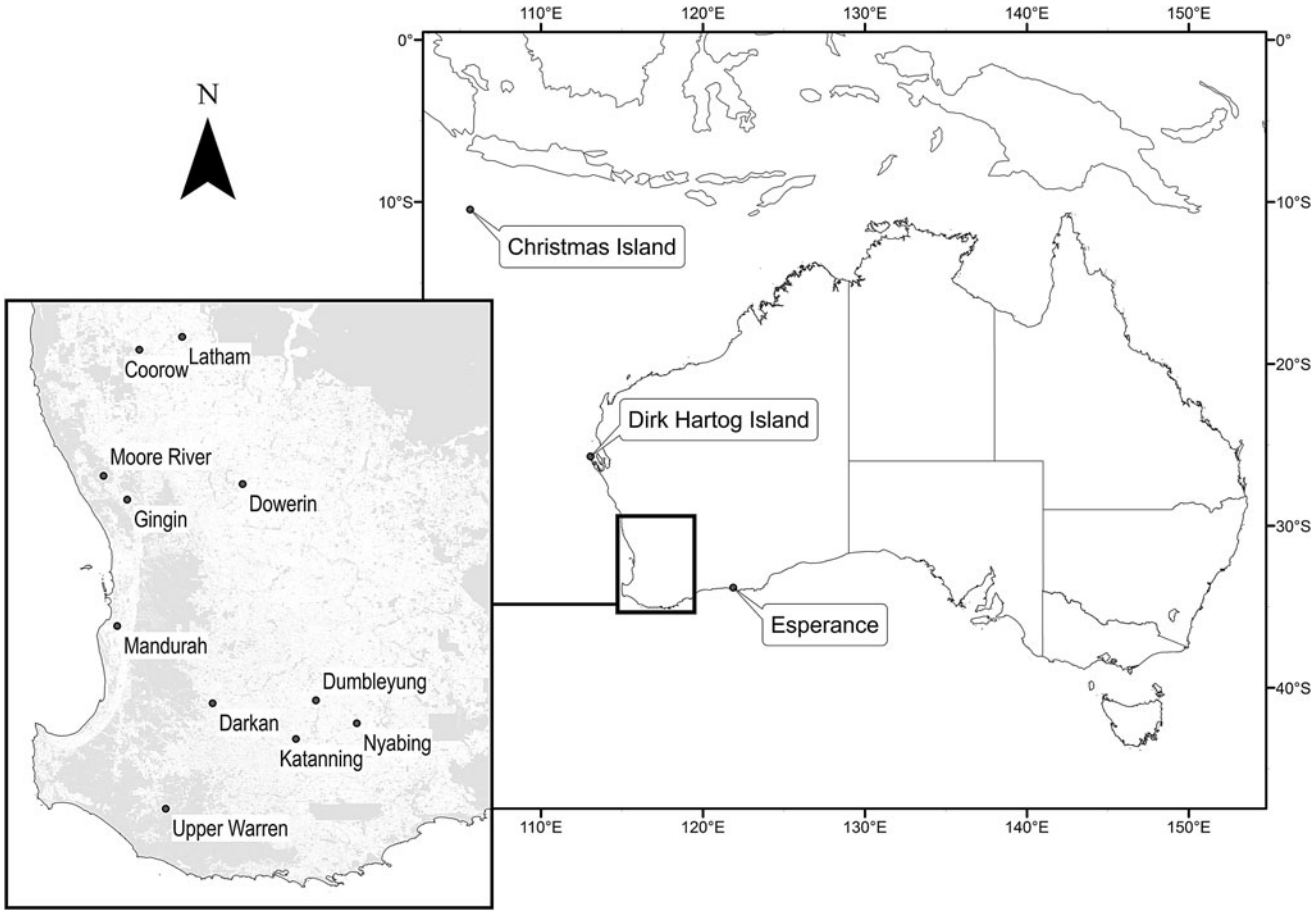

FIG. 1. *Leptospira* sampling locations.

Statistics version 21 (IBM). Associations between the presence of *Leptospira* spp. in cats and sex (male or female; one degree of freedom) were analyzed with Pearson's chi-square two-sided test as all cells had an expected count greater than 5. Associations between the presence of *Leptospira* spp. in cats and age category (juvenile or adult; one degree of freedom) were analyzed using Fisher's two-sided exact test as two cells had an expected count less than 5. Associations between body condition index and the presence of *Leptospira* spp. in cats were analyzed using a univariate general linear model, with body condition index as the dependent variable and sex, age, and presence of *Leptospira* spp. included as fixed factors. Statistical analyses were not performed on rodent *Leptospira* due to the low prevalence.

Results

Kidney tissue samples were PCR positive for *Leptospira* spp. for feral cats (42.4%; 95% CI 29.6–55.9) and black rats (2.9%; 0.4–10.2) on CHI, but not for cats in swWA or DHI (Table 1). No saprophytic/intermediate *Leptospira* spp. were detected.

Sequencing of *Leptospira*-positive samples from cats ($n=24$) and rats ($n=2$) showed 100% similarity for *L. interrogans.* All samples equally aligned with seven pathogenic serovars: Hardjo (CP013147 and CP012603), Manilae (CP011934 and CP011931), Bratislava (CP011410), Linhai (CP006723), Copenhageni (NR_076199 and AE016826), Canicola (X14249), and Lei (NR_076199, CP001221, and AE010300). Despite aligning with these seven serovars, sequencing was not able to discriminate the actual infective serovar or whether animals were harboring one or a combination of serovars. Sequences identified have been deposited in GenBank under the following accession numbers: KU991650–KU991655 and KY230168–KY230186.

No significant associations between host sex (Pearson's chi-square two-sided test, $p=0.346$) and age category (Fisher's exact test, $p=1.000$) with the presence of pathogenic *Leptospira* spp. were identified in feral cats from CHI. There was no

TABLE 1. *LEPTOSPIRA* SPP. DETECTED IN CATS AND RATS FROM THREE GEOGRAPHICAL REGIONS

	SwWA		*CHI*	
	Felis catus	*DHI* F. catus	F. catus	Rattus rattus
Leptospira spp.				
Samples (*n*)	59	22	59	68
Positive samples (*n*)	0	0	25	2
Prevalence (%)	0	0	42.4	2.9
95% Confidence interval	0.0–6.1	0.0–15.4	29.6–55.9	0.4–10.2

association between the presence of pathogenic *Leptospira* spp. and body condition index (univariate general linear model Leptospira main effect, $p = 0.843$).

Discussion

To the best of the authors' knowledge, this is the first report of *Leptospira* spp. being introduced in animals on CHI. The identification of *L. interrogans* in feral cats and black rats on CHI (a tropical environment) is further supported by a recently reported autochthonous case in a human working on the island (Dr Julie Graham, personal communication). In contrast, *Leptospira* spp. were not identified in cats from DHI (arid environment) or swWA (a temperate/Mediterranean environment). This was consistent with the notion that environmental factors influence *Leptospira* spp. prevalence, and therefore, the public and veterinary health risks associated with pathogenic *Leptospira* spp. transmission from feral animals.

Although swWA and DHI have a greater number of potential mammalian reservoir host species present, it is likely that the prevailing climatic conditions in arid (DHI) and temperate (swWA) environments are less conducive for the survival and transmission of *Leptospira* spp. compared with tropical CHI. Leptospires are excreted via infected urine; a higher temperature and humidity in tropical environments are favorable for the longer persistence of leptospires in the environment (Hartskeerl and Terpstra 1996, Bharti et al. 2003, Hartskeerl et al. 2011). This is reflected in the higher case notification rate for (human) leptospirosis for (tropical) north Queensland, compared to the remainder of Australia (Pappas et al. 2008).

This study utilized PCR to detect the presence of *Leptospira* spp. DNA within host kidney tissue. Serological tests have been traditionally used to investigate the level of exposure within a population; however, diagnostic sensitivity can be low as some leptospire serovars are shed in urine by animals with low to no detectable serological titer levels (Shophet 1979). Utilization of molecular screening techniques can increase sensitivity (Schreier et al. 2013), and molecular (PCR) analyses using kidney tissue are considered an effective screening tool because leptospires can clear from all organs aside from renal tubules in susceptible hosts (Athanazio et al. 2008). Furthermore, there is evidence that leptospires do not colonize the kidneys of noncarrier species (Hartskeerl and Terpstra 1996, Cox et al. 2005). Therefore, if cats were an incidental host or noncarrier species, minimal *Leptospira* spp. detection would be expected in kidney samples (Hartskeerl and Terpstra 1996, Faine et al. 1999).

The source of infection in the 2013 human case of leptospirosis on CHI was not confirmed. The patient was a fly-in-fly-out employee on the island (*e.g.*, lived and worked on CHI for a period of time and then returned to his hometown for a number of days of rest). The infection was presumed to have been locally acquired as the patient had been on CHI for 28 days before the onset of symptoms. Indirect transmission via contaminated water (waterfalls used for recreation) was considered most likely based on the patient's history (Dr Julie Graham, personal communication). An increased risk for human leptospirosis has been associated with higher rates of contact with reservoir hosts in rural areas (Ghneim et al. 2007, Hartmann et al. 2013). Lau et al. (2010) reported a higher risk of *Leptospira* transmission on islands where multiple risk factors coexist in a smaller (restricted) area (*e.g.*, favorable climatic conditions, poor sanitation, stagnant water, and/or abundance of reservoir hosts). Human–human transmission of *Leptospira* spp. has been demonstrated, but is considered rare (Adler and de la Peña Moctezuma 2010).

Rats have been generally considered the most common reservoir host for *Leptospira* spp. However, this study unexpectedly revealed a higher prevalence in cats than rats on CHI. Cats were introduced to CHI at settlement in 1888 (Tidemann 1994) and feral populations established soon thereafter. Black rats were thought to have been introduced accidentally from the SS Hindustan in the late 19th century (Andrews 1900).

Feline leptospirosis is likely underdiagnosed or underreported as clinical presentations are highly variable, if present at all (Azócar-Aedo et al. 2014). Cats in this study were feral and had not received any veterinary care. Cats are presumed to become infected by ingesting infected reservoir hosts, and predator–prey transmission between cats and rats is thought to be an important transmission route (Hartmann et al. 2013). However, the *Leptospira* prevalence in black rats on CHI was low (2.9%), and only one other alternative mammalian prey species (flying fox; *Pteropus melanotus natalis*) is present on CHI. It is not known whether the flying fox population on CHI is infected or susceptible to *Leptospira* infection (Cox et al. 2005). Pathogenic *Leptospira* spp. have been reported in house mice (*Mus musculus*) (Vanasco et al. 2003), but there are conflicting reports about the presence of these species on CHI. The most recent CHI Biodiversity Conservation plan (Anonymous 2014) accounts for house mice on the island, however, numbers and distribution are currently unknown and no confirmed observations have been made recently (David Algar and Dion Maple, personal communication). Furthermore, a concurrent dietary study of feral cats conducted on CHI did not identify either house mice or flying foxes in their gastrointestinal tracts, while rats represented 27% of their diet (Hayes 2011).

It is possible that rats infected with *Leptospira* are more susceptible to predation by cats thus leaving a majority of uninfected rats in the population. This suggests that the cat–rat transmission route cannot be excluded on CHI, however, an alternative transmission pathway may also exist on the island. Both horizontal and vertical transmission routes have been reported, and *L. interrogans* infection in cats on CHI may be attributed to inhalation, invasion through skin abrasions and mucous membranes, or via the genital tract (Hartskeerl and Terpstra 1996, WHO 2003, Hartskeerl et al. 2011). Transmission via aquatic environments is unlikely due to cats' natural aversion to water and contaminated soils represent a more likely transmission route. With a higher population density and higher rates of contact between hosts on islands, there would be an inevitable higher rate of exposure and direct transmission of leptospires between feral cats.

Apart from climatic conditions favoring survival of leptospires in tropical environments, the higher prevalence observed on CHI compared with DHI and swWA could be related to abundance of suitable mammalian reservoir host species. This lack of diversity may contribute to the loss of the ''dilution effect'' (*i.e.*, a reduced number of competent reservoir hosts that can ''absorb'' pathogens within the environment, resulting in increased prevalence and disease risk) (Allan et al. 2003, Mills 2006, Wynwood et al. 2014). Increased species diversity and richness may regulate the

abundance of competent reservoir hosts, which in turn reduces the probability of the pathogen encountering a susceptible host (Keesing et al. 2006).

It is widely accepted that *Leptospira* spp. are transmitted exclusively by mammals; however, *Leptospira* spirochetes have previously been identified in the kidneys of birds (Everard et al. 1985, Jobbins and Alexander 2015). This begs the questions as to the role of birds in the transmission (indirect and/or direct) of *Leptospira*. Direct transmission from birds to cats could be possible on CHI, given that birds accounted for ~30% of feral cat diet (predominantly feral chickens) in one study (Hayes 2011). Indirect transmission by birds is also possible through the shedding of leptospires and subsequent contamination of soil and water in the environment. However, the role of birds in the transmission of leptospirosis is largely unknown and further research is required to investigate this.

Apart from public health risks, the presence of pathogenic *Leptospira* spp. in both cats and rats on CHI (although small risk from rats) suggests a potential disease risk to the remaining flying fox population. It is currently unknown if the flying fox population on CHI harbors *Leptospira* spp., or, if they do, whether transmission occurs between the flying foxes and feral cats. Previous research suggests that some serovars may not impact flying fox populations negatively (Cox et al. 2005), but flying foxes can be infected as accidental or incidental hosts that may be associated with clinical disease and can be fatal (Faine et al. 1999, WHO 2003, Hartmann et al. 2013).

In conclusion, this study identified pathogenic *Leptospira* (*L. interrogans*) in cats and rats on tropical CHI, but not cats from the arid DHI or Mediterranean/temperate swWA. Although cats have not previously been considered important carriers or reservoir hosts of *Leptospira* spp. and leptospirosis is not usually considered in the differential diagnosis of feline disease (Dickeson and Love 1993, Lilenbaum et al. 2004, Arbour et al. 2012), this study suggests that feral cats play a role in disseminating leptospires within the tropical environment on CHI. Ongoing monitoring of *Leptospira* spp. in feral cats (as well as native and invasive fauna) will further improve our understanding of the public and veterinary health risks.

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Author Disclosure statement

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