

# A Survey of Zoonotic Pathogens Carried by Non-Indigenous Rodents at the Interface of the Wet Tropics of North Queensland, Australia

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## Summary

In 1964, *Brucella* was isolated from rodents trapped in Wooroonooran National Park (WNP), in Northern Queensland, Australia. Genotyping of bacterial isolates in 2008 determined that they were a novel *Brucella* species. This study attempted to reisolate this species of *Brucella* from rodents living in the boundary area adjacent to WNP and to establish which endo- and ecto-parasites and bacterial agents were being carried by non-indigenous rodents at this interface. Seventy non-indigenous rodents were trapped [*Mus musculus* (52), *Rattus rattus* (17) and *Rattus norvegicus* (1)], euthanized and sampled on four properties adjacent to the WNP in July 2012. Organ pools were screened by culture for *Salmonella*, *Leptospira* and *Brucella* species, real-time PCR for *Coxiella burnetii* and conventional PCR for *Leptospira*. Collected ecto- and endo-parasites were identified using morphological criteria. The percentage of rodents carrying pathogens were *Leptospira* (40%), *Salmonella choleraesuis* ssp. *arizonae* (14.29%), ectoparasites (21.42%) and endoparasites (87%). *Brucella* and *C. burnetii* were not identified, and it was concluded that their prevalences were below 12%. Two rodent-specific helminthic species, namely *Syphacia obvelata* (2.86%) and *Nippostrongylus brasiliensis* (85.71%), were identified. The most prevalent ectoparasites belonged to *Laelaps* spp. (41.17%) followed by *Polyplax* spp. (23.53%), *Hoplopleura* spp. (17.65%), *Ixodes holocyclus* (17.64%) and *Stephanocircus harrisoni* (5.88%), respectively. These ectoparasites, except *S. harrisoni*, are known to transmit zoonotic pathogens such as *Rickettsia* spp. from rat to rat and could be transmitted to humans by other arthropods that bite humans. The high prevalence of pathogenic *Leptospira* species is of significant public health concern. This is the first known study of zoonotic agents carried by non-indigenous rodents living in the Australian wet-tropical forest interface.

## Introduction

Rodents potentially carry several zoonotic pathogens such as *Leptospira*, *Salmonella*, *Brucella* and *Coxiella* (Meerburg et al., 2009). As many rodent species and humans live in close proximity, there exists potential for transmission of pathogens between them (Easterbrook et al., 2007).

North Queensland has 41 of the 59 species of native rodents recognized in Australia (Dickman et al., 2000). Three non-indigenous rodent species, namely the brown rat (*Rattus norvegicus*), the black rat (*Rattus rattus*) and the house mouse (*Mus musculus*), have been introduced into Australia by Europeans and are now widespread throughout the country (Van Dyck and Strahan, 2008). Studies on the zoonotic diseases carried by rodents in north Queensland were last done more than 30 years ago (Battey et al., 1964; Emanuel et al., 1964; Cook et al., 1966, 1967; Mesina, 1973; Campbell and Domrow, 1974; Mesina et al., 1974; Mesina and Campbell, 1975; Glazebrook et al., 1978). Few investigations have been carried out on zoonotic diseases carried by non-indigenous rodents with the last major work completed almost 40 years ago by Glazebrook (1976). This is in spite of a large number of zoonotic agents present in Australia, and that non-indigenous rodents have a widespread distribution in both urban and rural areas of Australia.

Wooroonooran is a national park in Queensland (Australia), 1367 km northwest of Brisbane, between Innisfail and Cairns. The park is one of the Wet Tropics World Heritage Area series of national parks and is a gazetted World Heritage site. The World Heritage area stretches from Townsville in the south to Cooktown in the north and contains some of the oldest surviving rainforests in the world. It also includes the parts of Australia that on average receive the most rainfall each year. The rainforests in the park contain more than 500 different tree species and a range of bird and rodent species endemic to Queensland's Wet Tropics.

Of interest is a study that was carried out on native rodents in 1964 in Jordan Creek, Wooroonooran National Park (Cook et al., 1966), from which *Brucella* was isolated. This unnamed *Brucella* strain has only recently been genotyped and found to be a unique *Brucella* species (Tiller et al., 2010). It is possible that non-indigenous rodents could have come into contact with native rodents carrying this species of *Brucella* and possibly be infected with the same agent that was isolated in 1964. *Brucella* is an important zoonosis that affects a wide range of vertebrate species and has been eradicated from domestic animals in Queensland, Australia. Having a reservoir of *Brucella* in rodents within the wet-tropical forests of Northern Australia could have important economical and public health consequences. As at the time of this study, a permit could not be obtained to sample native rodents, it was decided to examine non-native rodents in the vicinity where the original *Brucella* organism was isolated in 1964, with the view of reisolating it. At the same time, zoonotic diseases carried by non-native rodents in the boundary between domestic animal farm lands and wet-tropical forests could be studied. This interface between wild animals in the wet-tropical forest and domestic animals is important as an area where pathogens can be exchanged and where new diseases can potentially emerge and warrants more intensive investigation. Studying the diseases carried by rodents in this interface area provides a mechanism for doing this.

## Materials and Methods

Rodents were trapped daily on four household/dairy properties in the Atherton Tablelands, Northern Queensland, between 9 July 2012 and 14 July 2012 using Elliot traps. All four agricultural properties (Table 1) were located adjacent to Wooroonooran National Park, close to the area where the novel strain of *Brucella* was discovered more than four decades ago by Cook et al. (1966).

**Table 1.** Trapping locations and number and type of rodents trapped within the selected properties in Northern Queensland in 2012

Property	Site no.	Latitude	Longitude	Dist. from Jordan Ck.	<i>Mus musculus</i>	Black rat	Brown rat
1	P1	17°32'29.11"	145°41'13.56"		1	0	0
	P2	17°32'30.80"	145°41'14.93"		1	0	0
	P3	17°32'32.14"	145°41'15.72"		1	0	0
	P4	17°32'47.29"	145°41'40.2"		2	1	0
2	J1	17°31'26.18"	145°40'48.79"		14	1	0
	J2	17°31'27.91"	145°40'48.14"		8	2	0
	J3	17°31'28.13"	145°40'46.70"		4	1	0
	J4	17°31'29.44"	145°40'47.25"		7	0	1
	J5	17°31'29.42"	145°40'46.06"		1	2	0
3	L1	17°31'30.90"	145°41'19.36"		2	2	0
	L2	17°31'29.60"	145°41'20.44"		3	0	0
	L3	17°31'44.68"	145°41'24.85"		1	5	0
4		17°32'15.07"	145°41'7.40"		8	3	0
Total					53	17	1

### *Rodent sampling*

Trapping and handling procedures were done using established methods (Aplin et al., 2003; Freegard and Richter, 2009) and approved by the James Cook University (JCU), Townsville, Animal Ethics Committee (approval number A1758). The required sample size was based on an estimated prevalence for brucellosis in native rodents trapped in the region in the 1960s (Cook et al., 1966). A negative binomial simulation model was used to determine sample size to detect disease based on the assumption that 12% of rodents have Brucellosis. This calculated an estimated sample size of 92 rodents with a 100% probability of finding one rodent with brucellosis.

Traps were baited with a mixture of oats and peanut butter (Freegard and Richter, 2009) with honey and vanilla essence added to enhance attraction to the bait (Tasker and Dickman, 2002; Freegard and Richter, 2009). Traps were spaced around animal feed and dwellings where rodents were more likely to frequent, as well as along a forest path on Property 3. A total of 260 Elliot traps were deployed ( $n = 80$  on Property 1, 2 and 3 and  $n = 20$  on Property 4) with the intention of catching the greatest number of rodents over the trapping period. The number of traps was determined by their availability within the College of Marine and Environmental Sciences, James Cook University. Trapped animals were identified by a zoologist using key morphological identification characteristics (Van Dyck and Strahan, 2008; Menkhorst and Knight, 2010). Non-target, native animals were released immediately after identification, and all non-indigenous rodents were placed into clean cotton cloth bags and transported live to

the JCU Veterinary Teaching Resource Centre, in Malanda, near where the rodents were trapped. A total of 70 non-native rodents were trapped, each given a code number, sedated and then euthanized by an overdose of pentobarbital sodium injection (Lethabarb, Virbac, Milperra, NSW, Australia). Blood was drawn directly from the heart using a sterile 21-gauge needle and 2.5-ml syringe. Blood volumes obtained varied according to the size of the rodent but never exceeded 2.5 ml. Euthanized rodents were then described by their morphometric characteristics, namely sex, maturity, weight and various lengths; head-body, tail and ear pinnae. The skin was examined for the presence of ticks, fleas and lice, and a post-mortem examination was carried out where any abnormalities were recorded and organ specimens were collected for parasitological and microbiological examination.

### ***Collection of specimens***

Portions of the liver, spleen, kidney and ileum were removed aseptically and distributed in buffered peptone water (BPW) (Oxoid Group; Thermofischer Scientific, Scoresby, Victoria, Australia), brain–heart infusion (BHI) broth (Difco™, Becton-Dickinson, North Ryde, NSW, Australia) and Ellinghausen-McCullough-Johnson-Harris (EMJH) semi-solid media (Difco™, Becton-Dickinson) for the isolation of *Salmonella*, *Brucella* and *Leptospira*, respectively. Portions of each of these organs were also placed together as a pool into duplicate 2-ml sterile cryotubes (Sarstedt, Mawson Lakes, South Australia, Australia). All the above-mentioned samples were transported from Malanda, where necropsies took place, to the laboratory at James Cook University, Townsville, at 4°C. The carcass and all ectoparasites collected on the field site were preserved in containers of 10% neutral buffered formalin (10% NBF) until further examination.

On arrival at the Veterinary and Biomedical Science School, James Cook University (VBMS JCU), the BPW, BHI and EMJH media were incubated at 37°C in air, 37°C in 5% CO<sub>2</sub> and 30°C in air, respectively, and examined daily for growth. The organ pools were stored at –80°C. The blood was centrifuged, and the sera harvested and stored at –20°C.

Approximately 200 mg of an organ pool consisting of portions of spleen, liver, lymph nodes, kidney and lung from each animal was thawed rapidly and ground using sterile glass beads. The DNA was then extracted and purified using a commercial kit (Wizard® Genomic DNA Purification Kit; Promega Corporation, Alexandria, NSW, Australia), following the manufacturer's instructions. The purified DNA was then stored at –20°C prior to genomic analysis for *Leptospira* and *Coxiella*.

### ***Bacteriology***

The recommendations by the OIE (2014c) for the culture of *Brucella* were adapted as follows: a portion of the organ specimens in the BHI were streak-diluted onto 5% blood-enriched Columbia agar (BA) (Oxoid Group; Thermofischer Scientific) and incubated at 37°C in 10% CO<sub>2</sub> for up to 14 days. The organs remaining in the BHI were then incubated in the same way as the BA. Both the BHI and BA were examined daily for bacterial growth and the presence of fine, non-haemolytic translucent colonies that may indicate *Brucella* species. Where there was no growth on the BA but growth in the BHI, 100 µl of this broth was streak-diluted over a sterile BA and incubated as previously described. Suspect colonies were purified by streak-diluting a single representative colony over sterile BA. Any colonies that were Gram-negative and Stamp's modified acid fast stain positive were identified further using phenotypic tests and genotyping.

*Salmonella* species was cultured using the OIE recommended protocol (OIE, 2014d). After the pooled spleen, liver and ileum specimens had incubated in BPW for 24 hours, 1 ml of the fluid was added to 9 ml of mannitol selenite broth (Difco™, Becton-Dickinson) and incubated in air at 37°C. A brick red colour within 24–48 h of incubation indicated growth. A loopful of this growth was streaked over a plate of xylose lysine deoxycholate (XLD) agar (Difco™, Becton-Dickinson). After 24 h of incubation at 37°C, the XLD was checked for the presence of pink colonies with black centres which are typical for *Salmonella* species. Presumptive *Salmonella* colonies were streaked onto 5% blood agar and MacConkey agar (Accumedia; CellBioSciences, Heidelberg, Victoria, Australia) to purify and exclude swarming colonies that are indicative of *Proteus* species. All Gram-negative rods, non-lactose fermenters, catalase positive but oxidase and indole negative were further identified by *Salmonella* polyclonal O-antigen latex agglutination test (Oxoid; Thermofischer Scientific) and the API®20E biochemical array system (Bio-Merieux, Baulkham Hills, NSW, Australia).

Pooled biopsies of liver, spleen and kidney were cultured at 30°C in EMJH medium and checked for the first 3 days of incubation for contamination and thereafter weekly for 4 weeks for growth before being considered as negative (OIE, 2014a). One millilitre of the fluid from bottles with no visible growth was also subcultured into fresh EMJH medium weekly. Any visible growth as noted by media turbidity was subcultured onto BA and incubated at 30°C to confirm the presence of contaminants. Other indicators of contaminants were discolouration of the EMJH or non-spirochaete microscopic morphology when using a Gram's stain. Suspect cultures were examined for typical leptospiral motility and morphology using dark-field microscopy. All positives were then tested with the microscopic agglutination test (MAT) to detect the infecting serovar. The antibodies to serovars Arborea, Australis, Bulgarica, Canicola, Celledoni, Copenhageni, Grippotyphosa, Hardjo, Kremastos, Pomona, Robinsoni, Tarassovi, Topaz and Zannoni were provided by the *Leptospira* Reference Centre, Coopers Plains, Queensland, Australia. Fifty microlitre of diluted anti-sera (1 : 25 dilution in phosphate-buffered saline (PBS), pH 7.4) was mixed with 50 µl diluted culture (1 : 1 dilution in physiologically buffered water) in 96-well flat-bottomed micro-titre plates (Sarstedt). For a positive control, 50 µl of diluted control anti-sera and a known *Leptospira* serovar was mixed with equal volumes of the physiologically buffered water. The plates were shaken manually to mix the contents of the wells thoroughly and then incubated at room temperature in the dark for 2 h. Each well in the microtitre plate was read using a dark-field microscope. The reading of each well was determined in relation to the agglutination of the corresponding positive control. Every serum which gave an agglutination of at least 50% of the leptospire (as compared to positive controls) was considered positive.

A conventional PCR was used to amplify a 423-bp fragment of the *lipL32* gene of pathogenic leptospire (Levett et al., 2005). A concentration of 0.4 µmol LipL32-270F (5-CGC TGA AAT GGG AGT TCG TAT GAT T-3) and 0.4 µmol LipL32-692R (5-CCA ACA GAT GCA ACG AAA GAT CCT TT-3) was added to 12.5 µl GoTaq® Hot Start Green Master Mix, (Promega Corporation), 9.5 µl water (ddH<sub>2</sub>O) (Sigma-Aldrich Pty. Ltd., Sydney, Australia) and 1 µl of DNA extracted from the rodent organs to make a 25 µl reaction. The amplification was carried out in an Eppendorf® thermal cycler under the following conditions: 20 s at 95°C and 2 min at 94°C, followed by 40 cycles of amplification (95°C for 5 s, 67.2°C for 15 s, 72°C for 20 s), after which the reaction was stopped (72°C for 2 min). Known negative and positive sample controls were used in each PCR. The products of the PCR were separated in a 1.2% agarose gel by electrophoresis and detected by UV transillumination of the GelRed™ nucleic acid (Biotium Inc., Hayward, CA, USA).

A portion of the extracted DNA was subjected to the real-time PCR assay using published primers that amplify the outer membrane protein *comI* gene unique to *C. burnetii*, namely *comI* forward AATCGCAATACGCTGCCAAA and *comI* reverse AGCAGCGCGTCTGTGGAA (Marmion et al., 2005 and Cooper et al., 2007; OIE, 2014b). Reaction conditions for the real-time PCR assay were modified from the original description by Marmion et al. (2005). Each reaction consisted of 1× ImmoMix (Bioline, Alexandria, NSW, Australia), 0.3 μm forward primer, 0.3 μm reverse primer, 0.01 mm SYTO-9 (Invitrogen, Mulgrave, Victoria, Australia) and molecular grade water (ddH<sub>2</sub>O) (Sigma-Aldrich Pty. Ltd.) up to 19 μl. Each sample was tested in duplicate with 1 μl of DNA template. The amplification protocol consisted of an initialization step of 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 10 s and elongation 72°C for 20 s. A melt curve analysis was then performed with an increase in temperature from 72 to 95°C in 1°C increments. *Coxiella burnetii* Nine Mile clone 4 phase II (9Mi/II/C4) strain at a dilution of 1 × 10<sup>5</sup> copies/μl DNA was used as a positive control (Cooper et al., 2007). All reactions were run and analysed (quantification and melt curve analysis) using the Corbett Rotor-Gene 6000 (Qiagen) machine and software.

### ***Parasitology***

The skin and the gastrointestinal tract of each rodent were examined for ecto- and endo-parasites with a stereomicroscope. The ecto- and endo-parasites were washed in distilled water and clarified in lactophenol before morphological examination. Ecto- and endo-parasites carried by rodents were identified to genus or species level using morphological criteria (Roberts, 1960; Mawson, 1961; Domrow, 1965; Kuhn and Ludwig, 1966; Traub and Dunnet, 1972; Taffs, 1976; Centers for Disease Control and Prevention 2003).

### ***Data analysis***

Data were stored and analysed using MSEXcel 2010 (Microsoft™, Richmond, WA, USA). As the data were descriptive in nature, it was analysed for frequencies and proportions with 95% confidence intervals (Schiller et al., 2008).

## **Results**

### ***Number, types and location of rodents***

A total of 38 native and 71 non-indigenous rodents of six species were trapped on four properties. The location, species and number of non-indigenous rodent species trapped at each location are shown in Table 1. The 38 native rodents were released, and a house mouse (*M. musculus*) escaped during handling leaving a total of 70 non-indigenous rodents for the study. The predominant native species trapped was the fawn-footed melomys (*Melomys cervinipes*), which were trapped on Property 3 that borders on Wooroonooran National Park. The predominant non-indigenous species trapped were the house mouse (*M. musculus*, *n* = 53) followed by the black rat (*R. rattus*, *n* = 17) and a single brown rat (*R. norvegicus*, *n* = 1).

## Bacteriology

A non-mammalian subspecies of *Salmonella* (*Salmonella enterica* subsp. *arizonae*) was isolated from 10 (14%, 95% CI: 6, 22) of the pooled organ (spleen, liver and ileum) samples of the 70 rodents examined. *Salmonella* was found on Property 1, Property 2 and Property 3 where 17% (1/6, 95% CI: -12, 46), 18% (7/40, 95% CI: 6, 30) and 15% (2/13, 95% CI: -3, 33) of rodents were affected, respectively.

All of the suspected leptospiral growth on EMJH gave negative results with dark-field microscopy test followed by the MAT. However, many of the broths were contaminated. On the other hand, pooled organ samples tested for pathogenic *L. interrogans* by PCR-detected *Leptospira* in 28 (40%, 95% CI: 28, 52) rodents of this study. Rodents from Property 1, Property 2, Property 3 and Property 4 were positive at rates of 15% (95% CI: -12, 46), 50% (95% CI: 34, 66), 46% (95% CI: 19, 73) and 9% (95% CI: -9, 27), respectively. *Leptospira* was detected by PCR in 24 (46%, 95% CI: 32, 60) of *M. musculus* and in 4 (24%, 95% CI: 4, 44) of *R. rattus*, while no leptospires were detected in *R. norvegicus* by PCR. Leptospires were detected in 20% (95% CI: -15, 55), 58% (95% CI: 40, 76) and 67% (95% CI: 30, 104) of *M. musculus* on Property 1, Property 2 and Property 3, respectively, while 17% (95% CI: -12, 46), 29% (95% CI: 4, 62) and 33% (95% CI: 9, 27) of *R. rattus* were positive on Property 2, Property 3 and Property 4, respectively.

No *Brucella* organisms could be isolated by culture and nor were the typical *comI* genes indicative of *C. burnetii* detected in all the organ pool samples.

## Parasitology

Lice, mites or ticks were detected in 15 of 17 (88.24%) *R. rattus*. The results are shown in Table 2. With the exception of one *R. rattus*, only one ectoparasite species was identified on each animal. No ectoparasites were found on *M. musculus* or the single *R. norvegicus*.

**Table 2.** Ectoparasites collected from 17 *R. rattus* captured in north Queensland

Group	Taxon	No. infested	%	95% CI
Lice	<i>Polyplax</i> spp.	4	23.53	4.4, 44
	<i>Hoplopleura</i> spp.	3	17.65	0, 36
Mites	<i>Laelaps</i> spp.	7	41.17	17, 65
Ticks	<i>Ixodes</i> spp.	3	17.64	0, 36
Fleas	<i>Stephanocircus harrisoni</i>	1	5.88	-6, 18

Endoparasites were detected in 61 (87%) of the 70 rodents. The nematodes (*Nippostrongylus brasiliensis* and *Syphacia obvelata*) were found in all three species of rodents, whereas the cestodes were only found in *R. rattus*. Table 3 shows the percentage of each nematode species in three species of rodents. Cestodes were not identified at genus and species level because of shrinkage of parasites due to prolonged storage (about 3 months) of samples in 10% neutral buffer formalin.

**Table 3.** Endoparasites collected from non-indigenous rodents of north Queensland

	<i>Mus musculus</i> <i>n</i> = 52 (%)	<i>Rattus rattus</i> <i>n</i> = 17(%)	<i>Rattus norvegicus</i> <i>n</i> = 1(%)	Total infestation <i>n</i> = 70 (%)
<i>Syphacia obvelata</i>	1 (2%) CI: -2, 6	1 (6%) CI: -5, 17	0	2 (3%) CI: -1, 7
<i>Nippostrongylus brasiliensis</i>	44 (85%) CI: 75, 95	15 (88%) CI: 73, 103	1 (100%)	60 (86%) CI: 78, 94
Both ( <i>S. obvelata</i> and <i>N. brasiliensis</i> )	1 (2%) CI: -2, 6	0	0	1 (1%) CI: -1, 3
Total	44 (85%) CI: 75, 95	16 (94%) CI: 83, 105	1 (100%)	61 (87%) CI: 79, 95

## Discussion

Previous surveys of leptospirosis in rodents in north Queensland, using dark-field microscopy, culture and MAT, have found prevalence of 6.4% (Glazebrook and Campbell, 1977; Glazebrook et al., 1978), 11% (Battey et al., 1964) and 13.1% (Emanuel et al., 1964), while our study found a much higher apparent prevalence of 40% using PCR methods. What is interesting is we failed to find leptospirosis using dark-field microscopy, culture and MAT, and this negative finding was similar to the findings of earlier researchers (Broom and Coghlan, 1958; Ellis et al., 1981; Hathaway et al., 1982) who used dark-field microscopy examination, culture and MAT. The higher prevalence recorded in our results may thus be as a result of the better sensitivity of the PCR method than previous diagnostic tests used or possible interaction with native forest mammals that may carry leptospira. Our study looked at 14 serovars using the MAT, while the PCR method is likely to detect all serovars, including those not in our MAT panel. The results may indicate there are other serovars in these rodents not included in the panel of 14 used for the MAT, or the PCR may be detecting the serovars at lower concentrations than the MAT.

Human leptospirosis cases in northern Queensland have been associated with banana farming, dairy farming and stagnant water (Perra et al., 2002; Smythe et al., 2002; Queensland Health, 2009). A serological survey in humans from the Cairns and Hinterland health service district, an area known for dairy and banana farming, during 2008–2010 also found a high proportion of those surveyed (62.2–70.4% in 2010) had been exposed to leptospirosis (Queensland Health, 2009, 2012). These findings tie in with our study as the highest proportion of pathogenic leptospire was detected on Property 2 (sites: J1 and J4) and Property 3 (sites: L1 and L2) which had stagnant water and banana trees, respectively. It is also known that the incidence of leptospirosis is highest during the wet season in north Queensland (Derrick, 1956; Emanuel et al., 1964) and as the rodents of this study were trapped in mid July 2012, just after the peak rainfall, this may have further contributed to the high incidence of pathogenic leptospire in the study area (Bureau of Meteorology, 2012).

The percentage of rodents carrying *Salmonella* (14.29%) was considerably greater than the 3.1% of Glazebrook (1976) in north Queensland and 3.0% of Lee (1955) in Brisbane. Glazebrook (1976) and Lee (1955) found nine serotypes in four species of rodents, namely *Uromys caudimaculatus*, *R. rattus*, *Rattus sordidus* and *Rattus fuscipes/leucopus*, and four serotypes in *R. norvegicus*. The present survey isolated only *Salmonella enterica* subsp. *arizonae* in the house mouse and brown rat with most of the isolates originating from



Property 2. This subspecies of *Salmonella* is usually associated with the presence of reptiles, especially snakes, which were plentiful on the three positive properties and in the adjoining National Park. Up to 75% of snakes will carry this subspecies of *Salmonella* within their gastrointestinal tracts (Habermalz and Pietzsch, 1973). It is known to cause rare infections in people, especially children, and has been associated with the handling of infected reptiles.

Of interest was the absence of salmonellae associated with livestock such as *Salmonella enterica* serovar Dublin and even those salmonellae such as *S. enterica* serovar Typhimurium that are considered to be of rodent origin and which are highly pathogenic for humans and farm animals. Most farm-based *Salmonella* monitoring and control schemes will only target those serovars that are commonly transmitted to humans via food products such as meat or milk. A reptile serovar would thus go unreported. As this is the first recorded isolation of *S. enterica* subsp. *arizonae* in rodents and the study was limited to one sampling period, it is not known whether rodents could maintain it. However, it is feasible that subspecies *arizonae* could like other salmonellae contaminate the farm environment and be ingested by food animals (Henzler and Opitz, 1992; Liebana et al., 2003; Lapuz et al., 2008; Backhans and Fellström, 2012). Humans could be infected with the bacterium either by the consumption of infected animal products or food or drink in contact with a contaminated environment (Vanselow et al., 2007). *Salmonella* can remain localized in some farms over a 2-year period (Henzler and Opitz, 1992). Henzler and Opitz (1992) also showed the importance of limited travel patterns of mice by identifying the same phage type of *Salmonella* in mice of all ages on a poultry farm. Although the chain of transmission of salmonellae to humans has multiple sources and pathways, the fact that mice tend to remain in their home range probably reduces the risk of human exposure.

Cook et al.'s (1966) study on native rodents, in the same region, isolated brucellosis from these animals; however, no *Brucella* organisms were isolated from the non-native rodents in our study. This may be because the study was carried out on different rodent species, that the interspecies contact is less than hypothesized, that the infection is localized or that the agent is present at a low prevalence (<15%). The fact that only non-native rodents could be sampled reduced the designed sample size from 92 to 70 and hence our ability to find brucellosis as well. Although most of the samples did not yield high numbers of bacteria, some did and the heavy growths of these could have masked the much slower growing fastidious and less prevalent *Brucella*. Use of media containing the antibiotic streptomycin or PCRs with well-designed primers in future might increase the chances of detecting the rodent brucellae.

Even though *C. burnetti* was previously detected in north Queensland rodents (Cook et al., 1967), its prevalence is likely to be low as Glazebrook and Campbell (1977), and our study did not detect this intracellular bacterium. Some authors who studied this agent in rodents in parts of Europe have found that the prevalence of the agent or antibodies to the agent is generally low, but can increase up to 50% when rodents have direct contact with infected animals, especially sheep and goats (Barandika et al., 2007; Reusken et al., 2011). Cooper et al. (2011) reported a 31.1% seroprevalence in beef cattle in far North Queensland, which is considered to be high. However, our study was undertaken in a dairy, not a beef area, where prevalence of *C. burnetti* in the cattle is unknown. There may be seasonal differences in the animal carriage of this bacterium; however, most studies being based upon the detection of long-lived antibodies were inconclusive. Reusken et al. (2011) did not detect bacterial DNA in rodents in the Netherlands in the summer, but the results are not conclusive. Furthermore, the brown rat appears to be more susceptible to infection than the black rat (Reusken et al.,

2011); however, we were only able to test one brown rat in this study. The presence of tick vectors on rodents in the Wet Tropics has not been well described. *Ixodes holocyclus* was identified in 17.64% of *R. rattus* in this study. *Ixodes holocyclus* feeds off a wide host range and is also known to carry *C. burnetii* (Pluta et al., 2010; Cooper et al., 2013).

This study agrees with other researchers (Kia et al., 2009; Changbunjong et al., 2010; Kamran et al., 2012) who found that *M. musculus* harbours the lowest number of species of ectoparasites. In the present study, mites were the most common ectoparasites seen on rodents, followed by lice, ticks and fleas. *Laelaps* spp. was the dominant mite, and it is a common rodent ectoparasite throughout most of the tropical, subtropical and temperate regions of the world (Durden and Page, 1991). *Laelaps* species have been reported to bite man and can cause irritation and dermatitis (Azad, 1986). Interestingly, we only detected fleas on one *R. rattus*, which is in contrast to popular belief and the study of Changbunjong et al. (2010) who described fleas as being the dominant ectoparasite in rodents in Thailand.

Two species of sucking lice (*Polyplax* spp. and *Hoplopleura* spp.) were found in this study, but they do not parasitize humans (Kim et al., 1986). They can transmit murine typhus between rodents (Kim et al., 1973) and could ultimately be transmitted to humans by other haematophagous arthropods that do bite humans (Durden and Page, 1991).

This study differed from the study done by Mesina et al. (1974) and Glazebrook and Campbell (1977) in that the overall prevalence of endoparasites was higher, 87% compared to 48.2% and 24%, respectively. *Nippostrongylus brasiliensis* was more prevalent than *S. obvelata*, the opposite of what Mesina et al. (1974) found.

## Conclusions

The results of the current study are novel as they provide important background on the prevalence of bacteria (*Leptospira*, *Salmonella*, *Brucella* and *Coxiella*) and ectoparasites in non-native rodents living in tropical northern Queensland at the interface between human habitat and the wet-tropical wildlife populations. Previous studies have not focused on this important wet-tropical forrest: animal farming interface where exchange of pathogens between wild forrest animals and domestic animals can take place and where emerging diseases could arise. This study revealed a high prevalence of pathogenic leptospires (40%) and identified an unusual salmonella and ectoparasites that could have potential public health risks in tropical regions of the world. The study failed to find evidence of brucella or coxiella in the rodents sampled.

## Acknowledgements

The authors wish to thank Tableland Veterinary Services, Malanda, for use of their facilities and the owners of the four properties used in this study for allowing us to use their properties. The authors gratefully acknowledge Dr Jenny Elliman for providing the *Leptospira* strain (serovar Pomona) and primers, Dr Brenda Govan and the Immunology Research Group, School of Veterinary and Biomedical Sciences, JCU, for the *Coxiella burnetii* strain and primers and Mr Trevor Taylor from CSIRO Animal Health, whose idea was to try and

reisolate *Brucella* from native rodents. The authors also wish to thank Professor Lin Schwarzkopf from the school of Marine and Tropical Biology, James Cook University (JCU) for her advice; Rusaini Rusaini, Noppadol Prasertsincharoen, Orachun Hayakijkosol, Wansadaj Jaroenram and the technical staff of the School of Veterinary and Biomedical Science for technical assistance in this study.

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