The role of fruit bats in the transmission of pathogenic leptospires in Australia

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Although antileptospirosis antibodies and leptospiral DNA have been detected in Australian fruit bats, the role of such bats as infectious hosts for the leptospires found in rodents and humans remains unconfirmed. A cohort-design, replicated survey was recently conducted in Far North Queensland, Australia, to determine if the abundance and leptospiral status of rodents were affected by association with colonies of fruit bats (*Pteropus conspicillatus* spp.) via rodent contact with potentially infectious fruit-bat urine. In each of four study areas, a 'colony site' that included a fruit-bat colony and the land within 1500 m of the colony was compared with a 'control site' that held no fruit-bat colonies and was >2000 m from the nearest edge of the colony site. Rodents were surveyed, for a total of 2400 trap-nights, over six sampling sessions between September 2007 and September 2008. A low abundance of rodents but a high carriage of leptospires in the rodents present were found to be associated with proximity to a fruit-bat colony. For example, means of 0.4 and 2.3 fawn-footed melomys (*Melomys cervinipes*) were collected/100 trap-nights at sites with and without fruit-bat colonies, respectively ($P<0.001$), but the corresponding prevalences of leptospiral carriage were 100% and 3.6% ($P<0.001$). Such trends were consistent across all of the sampling sessions but not across all of the sampling sites.

Leptospires were not isolated from fruit bats by culture, and the role of such bats in the transmission of leptospires to rodents cannot be confirmed. The data collected do, however, indicate the existence of a potential pathway for transmission of leptospires from fruit bats to rodents, via rodent contact with infectious fruit-bat urine. Fruit bats may possibly be involved in the ecology of leptospires (including emergent serovars), as disseminators of pathogens to rodent populations. Stringent quantitative risk analysis of the present and similar data, to explore their implications in terms of disease prevalence and wildlife population dynamics, is recommended.

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Human leptospirosis, a globally significant disease, is recognised as an important re-emerging zoonosis in the Australasian region (Lau *et al.*, 2010). Pathogenic

Leptospira serovars have been detected in several types of wild mammal, with rodents considered central to the transmission of leptospires to humans (Turk *et al.*, 2003).

In 2004, the leptospiral status of rodents on banana farms in the Tully and Innisfail regions of Far North Queensland, Australia, was investigated (Rivera, 2005). Of the animals tested, 11.4% were found positive for *Leptospira*, by kidney or urine culture or by serology. Culture revealed that one of the *Mus domesticus* investigated was infected with *L. borgpetersenii* serovar Arborea (a previously exotic but emerging serovar) and serology indicated the presence of *Leptospira interrogans* serovar Cynopteri (a serovar generally found in bats) in a common bush rat (*Rattus fuscipes*). In Australia, the source of rodent infection with these serovars remains to be determined.

The role of bats as reservoirs or maintenance hosts for pathogenic leptospires is not well understood, although some studies have been undertaken to elucidate the routes of transmission. Matthias *et al.* (2005), for example, evaluated the prevalence of leptospiral carriage among micro-bats in the Peruvian Amazon. Of the 589 bats investigated, by culture and molecular typing, 3.4% showed leptospiral colonization in their kidneys, the prevalence of such infection apparently being affected by habitat, location and bat species. A genetically diverse group of leptospires was found in the bat populations around Iquitos, including *L. interrogans*, *L. kirschneri*, *L. borgpetersenii*, *L. fainei* and two previously undescribed leptospiral species (Matthias *et al.*, 2005). Although *L. kirschneri* and *L. interrogans* had already been isolated from bats by Emanuel *et al.* (1964), Matthias *et al.* (2005) were the first to describe *L. borgpetersenii* infection in bats. The detection in bats of leptospiral species such as *L. interrogans* serovar Icterohaemorrhagiae and *L. borgpetersenii* (Matthias *et al.*, 2005), which are typically maintained by peridomestic rats, indicated the possibility of a bat–rodent infection cycle.

Smythe *et al.* (2002) detected antileptospiral antibodies in Australian bats, including evidence of the presence of the previously exotic *L. interrogans* serovar Cynopteri in fruit bats (Pteropodidae), and suggested that fruit bats may introduce exotic serovars of leptospires to Australia. In a subsequent study in Australia, 11% of the renal samples from fruit bats investigated by Cox *et al.* (2005) were found positive for leptospires, and urine samples from 39% of the fruit bats tested were found positive for leptospiral DNA in a real-time assay based on a TaqMan PCR. It therefore appears that fruit bats shed leptospires in their urine, although the transmission of leptospires from fruit bats to other mammals has not been confirmed.

To understand the ecology of leptospirosis, and to evaluate the risk of leptospires to public health, it is crucial to identify the prevalent serovars, their maintenance hosts, and all of their transmission routes, at least at a regional level. In the present study, in the light of the emergence of exotic serovars of *Leptospira* in Queensland and the associated health risks (Slack *et al.*, 2006), the possibilities for the transmission of exotic, pathogenic *Leptospira* serovars from fruit bats to rodents were evaluated. The main objectives of the present study were to explore the distribution (at ground level, where rodent contact is probable) of fruit-bat urine around a fruit-bat colony and investigate how a fruit-bat colony may affect the abundance, species distributions and leptospiral carriage of the local rodents.

ANIMALS AND METHODS

Sampling

STUDY SITES AND SAMPLING PLAN

In an observational study employing a cohort design, rodent populations living within 1500 m of fruit-bat colonies (at ‘colony sites’) were compared with those in neighbouring sites without such colonies (‘control

sites'), in terms of abundance, species composition and leptospiral carriage.

At each of four study areas (in or around Gordonvale, Innisfail, Mareeba and Tully, all in Far North Queensland, Australia), a 'colony site' that included an active colony of spectacled fruit bats (*Pteropus conspicillatus*) as well as the land within 1500 m of the colony was compared with a 'control site' that, although similar to the colony site in terms of vegetation density, height and composition (Kearney *et al.*, 2007), held no fruit-bat colonies and was >2000 m from the nearest edge of the colony site. Movement of rodents between each colony site and the nearby, paired control site was considered negligible, as the paired sites were chosen so that they were separated by a physical barrier, such as road or creek, and by minimum distance that was greater than the home range of most species of native rodent (Laurance, 1994).

At each of the eight study sites, a trap grid — consisting of 50, 51 × 11 × 34-cm, aluminium, Elliott traps (Elliott Scientific, Upwey, Australia) set at 30-m spacing over a 150 × 300-m rectangle — was used to sample the rodent populations. Six trapping sessions were conducted between September 2007 and September 2008 (see Table 1), with rodents trapped over four consecutive nights in each session. Traps were set and baited in the evenings, before sunset, and checked in the mornings, after sunrise. Each was washed with water after each trapping night. The bait used was a mixture of peanut butter, rolled oats, honey, plain flour and vanilla essence. Trapping was conducted in accordance with the guidelines of The University of Queensland Animal Ethics Committee (permit SAS/388/06/CRC) and the relevant state and national-park regulations, including those of Queensland Parks and Wildlife Services (WISP 04118306), the Aboriginal Rainforest Council and the relevant local and regional councils.

In Far North Queensland, a wet season typically runs from November to April, with

the rest of the year (May–October) considered the dry season.

GROUND-LEVEL URINE-GRADIENT ANALYSIS

The fruit-bat roost at Gordonvale was selected for this investigation because of its location on an enclosed, private property in the township. It is a permanent roost with a mean population of 60,000 bats (Shilton *et al.*, 2008). Three plastic sheets, each measuring 2.6 × 3.6 m, were placed so that one was directly underneath the roost, another 2 m outside the edge of the roost, and the third 10 m outside of the edge of roost (in the same direction, relative to the roost, as the second sheet). On three consecutive mornings during the 2007 dry season (8–10 August), the sheets were put down early in the morning, before the bats had returned from their night-time feeding activities (i.e. before 'fly-in') and were examined soon after fly-in was complete (usually just after sunrise; Chua, 2003). Urine collected on each sheet was transferred into 15-ml, plastic, Falcon tubes (Bacto Laboratories, Liverpool, Australia) using disposable polyethylene pipettes (Thermo Fisher Scientific, Scoresby, Australia). Only samples from visually distinct pools of yellow fluid were collected, to avoid the collection of any condensate.

RODENT SAMPLING

Each trapped rodent was euthanized so that a cross-section of the cortex of one of its kidneys could be excised, flame-sterilized and transported in semi-solid medium [Ellinghausen–McCulloch–Johnson–Harris (EMJH) medium made with 1.5% (w/v) agar and 0.09% (w/v) sodium pyruvate] to the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Brisbane, Australia (the 'reference laboratory').

In addition, a blood sample — a volume equivalent to ≤5% of the body weight of a rodent weighing <25 g (typically 100 µl from a 20-g animal), collected into a

TABLE 1. The climate in the four study areas, and the sizes of the four fruit-bat colonies investigated, during each of the six sessions of rodent trapping

Sampling month	Site	Temperature (°C)		Mean monthly rainfall (mm)	No. of bats in colony*
		Mean minimum	Mean maximum		
September 2007	Gordonvale	18.7	28.4	32.8	60,000
	Innisfail	17.8	26.7	78.5	800
	Mareeba	15.5	28.7	7.5	300
	Tully	16.2	27.6	38.2	400
December 2007	Gordonvale	23.7	31.6	171.6	73,250
	Innisfail	23.0	30.7	289.3	12,000
	Mareeba	20.5	31.6	59.7	9000
	Tully	22.5	31.5	193.5	700
March 2008	Gordonvale	23.1	31.6	171.6	40,000
	Innisfail	22.6	30.7	289.3	11,000
	Mareeba	20.1	31.6	59.7	40,000
	Tully	22.5	31.5	193.5	400
May 2008	Gordonvale	23.1	30.7	388.7	35,000
	Innisfail	22.6	29.7	553.3	9000
	Mareeba	20.1	29.7	133.2	30,000
	Tully	21.9	30.7	397.6	500
July 2008	Gordonvale	17.1	25.9	30.7	60,000
	Innisfail	15.9	23.9	159.6	12,000
	Mareeba	13.5	24.8	11.5	30,000
	Tully	13.6	24.9	31.4	500
September 2008	Gordonvale	18.9	27.9	34.3	60,000
	Innisfail	19.9	28.2	88.2	10,000
	Mareeba	17.5	30.2	13.9	0
	Tully	18.9	29.3	52.9	500

*Estimated as the mean count at fly-out over four consecutive evenings.

non-heparinized microhaematocrit tube (VetQuip, Castle Hill, Australia) from a retro-orbital sinus, or approximately 2 ml, collected, into a microfuge tube (Bacto Laboratories), by cardiac puncture with 3-ml syringe fitted with a 25-gauge needle, from a larger animal — was also collected from each rodent. Each blood sample was allowed to clot so that serum could be collected, within 3 h of blood collection, by centrifuging at $3000 \times g$ for 1 min.

Urine collected by cystocentesis was also cultured in semi-solid EMJH medium.

Finally, compression smears were made from 0.1-mm-thick transverse sections of each of 137 kidney samples, fixed (in a mix of triaryl methane dye and methanol; Thermo Fisher Scientific) and air-dried.

On each trapping night, the size of each colony was estimated (Table 1) by counting

the bats at 'fly-out' (Westcott and McKeown, 2004; Shilton *et al.*, 2008).

The rodents caught were identified in the field, using a field guide (Menkhorst and Knight, 2001) and morphometric data. Samples of each species identified were photographed and (after the fieldwork) the photographs were compared against reference specimens to check that the field identifications were correct. Skull morphology was used to distinguish between *Melomys cervinipes* and *Me. burtoni* (at the Queensland Museum of Natural History, Brisbane). Although *R. fuscipes* and *R. leucopus* were tentatively distinguished on the basis of teat number (females) and body size (Lidicker and Laurance, 1990), ear-tissue samples were collected from rats identified as *R. fuscipes*/*R. leucopus* and stored in 100% ethanol until they could be

used in a PCR-RFLP-based assay that allows the two species to be distinguished (Rivera, 2005).

Detection of Leptospires

At the reference laboratory, kidney and urine cultures were subcultured into fresh EMJH medium, incubated at 28–30°C, and examined weekly, for 13 weeks, by dark-field microscopy. Any culture that showed growth of non-*Leptospira* bacteria on microscopic examination was passed through a sterile, 0.2-µm-pore membrane filter before being subcultured into fresh medium. Cultures contaminated by fungi that could not be eliminated using such filtration were discarded. Any culture found positive for leptospires was subcultured into 8 ml EMJH medium, incubated until it contained at least 10⁸ cells/ml, and then investigated further.

Each leptospiral isolate recovered was tested against a panel of antisera (see Table 2), in serum agglutination tests (SAT; Turner, 1970), to identify its most probable leptospiral serogroup. The serovar of each isolate was characterised in cross absorption agglutinin tests (CAAT; Cerqueira and Picardeau, 2009). Each new isolate was also cryopreserved, using a 1-ml aliquot of an EMJH culture mixed with 100 µl dimethyl sulphoxide and stored in liquid nitrogen, at –200°C.

The rodent sera were checked for *Leptospira*-specific antibodies in microscopic agglutination tests (MAT; Ahmad *et al.*, 2005). In these tests, a rodent with serum that agglutinated 50% or more of the leptospires when diluted to at least 1:50 was considered to have a leptospiral infection (Faine *et al.*, 1999).

SILVER STAINING

The Warthin–Starry method of silver staining (Young, 1969) was used to reveal leptospires in the kidney smears. Positive control slides were prepared using cross-sections of kidney tissue from a laboratory

mouse that had been experimentally infected with *L. interrogans* serovar Zanoni (confirmed by culture) at the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Brisbane.

QUANTITATIVE REAL-TIME PCR

DNA was extracted from samples of rodent kidney tissue and urine using a commercial kit (High Pure PCR template preparation kit; Roche Scientific, Penzberg, Germany) according to the manufacturer's instructions. DNA was eluted in a final volume of 200 µl. Real-time PCR was performed, in version 1 of the LightCycler thermocycler (Roche Scientific), using the modified TaqMan method described by Slack *et al.* (2007). Test samples giving cycle-to-threshold (Ct) values of 5–40, 41–45 and >45 cycles were considered positive, 'equivocal' and negative, respectively. Each equivocal sample was re-run (Slack *et al.*, 2007). For discrepant analysis, PCR was performed on serum samples, to detect new or double infections.

Data Analysis

A rodent found positive for leptospires, by culture or the PCR-based investigation of kidney or urine, was considered to be a carrier. A rodent found positive for leptospires by MAT only was considered seropositive (but not necessarily a carrier) because *Leptospira*-specific antibodies can be detected in convalescent sera by MAT and positivity in an MAT is indicative of past or new infection but not necessarily of renal shedding (Faine *et al.*, 1999). Prevalences of carriage and seroprevalences were calculated separately, for each rodent species, at control and colony sites.

The statistical significance of each of the between-site and between-season variations observed in the prevalences of carriage was explored using the Marascuilo procedure, which was developed for the comparison of proportion in multiple groups, for non-parametric data (Zwick and Marascuilo,

TABLE 2. The reference species, their corresponding serovars, and the specific strains used as the reference antisera panel for the microscopic agglutination tests (MAT)

No.*	Species	Serovar	Strain
1	<i>Leptospira interrogans</i>	Zanoni	zanoni
2	<i>L. interrogans</i>	Hardjo	hardjoprajitno
3	<i>L. interrogans</i>	Robinsoni	robinson
4	<i>L. borgpetersenii</i>	Arborea	arborea
5	<i>L. weilii</i>	Celledoni	celledoni
6	<i>L. weilii</i>	Topaz	lt772
7	<i>L. interrogans</i>	Kremastos	kremastos
8	<i>L. interrogans</i>	Szwajizak	szwajizak
9	<i>L. interrogans</i>	Australis	ballico
10	<i>L. interrogans</i>	Pomona	pomona
11	<i>L. interrogans</i>	Grippotyphosa	moskva v
12	<i>L. interrogans</i>	Copenhageni	m 20
13	<i>L. interrogans</i>	Canicola	hond utrecht iv
14	<i>L. interrogans</i>	Medanensis	hond hc
15	<i>L. interrogans</i>	Bataviae	swart
16	<i>L. interrogans</i>	Djasiman	djasiman
17	<i>L. borgpetersenii</i>	Javanica	veldrat bataviae 46
18	<i>L. borgpetersenii</i>	Tarassovi	perepelitsin
19	<i>L. kirschneri</i>	Cynopteri	3522 c
20	<i>L. interrogans</i>	Bulgarica	nikolaev
21	<i>L. noguchii</i>	Panama	cz214
22	<i>L. santarosai</i>	Shermani	1342 K

*Numbers 1–15 are found in Australia whereas numbers 16–22 are found in Asia and are exotic to Australia.

1984). Pooled values for seroprevalence and prevalence of carriage, from the colony and control sites, were used for this analysis [with no attempt to make comparison between the rodent species encountered and with two bandicoot species (*Perameles nasuta* and *Isodon macrurus*) included in the analysis as rodents].

The abundance of rodents was estimated using a relative abundance index (RAI; expressed as animals caught/trap-night), produced by dividing the number of individuals caught by the relevant number of trap-nights (i.e. 2400 for the study, 1200 for all control or colony sites, and 200 for each site/month). Mean RAI for each trapping session were calculated.

Trap success was calculated as a percentage, by dividing the number of rodents captured by the total number of traps set and multiplying by 100.

Trap success, relative abundance and prevalence of carriage in the control and

colony sites were compared using χ^2 tests. A Wilcoxon signed-rank test was performed to test differences in the abundances of individual rodent species at the paired colony and control sites.

A relative-abundance-of-carrier-animals index (RACI) was calculated by multiplying the proportion of the rodents tested found to be carrier animals by the relevant mean RAI, for each species and each sampling session. Mean RACI, across the sampling sessions, were also evaluated.

Spearman's rank coefficients were calculated to test for correlation between the density of the fruit-bat colony and rodent abundance, as well as between the volume of fruit-bat urine collected at ground level and the distance from the colony.

The statistical significance of the differences seen, in prevalences, between the colony and control sites was evaluated using χ^2 tests with Yates' correction (for prevalences of >5%) or a phi coefficient of

association (for lower prevalences). Relative risk ratios for leptospiral carriage associated with proximity to a fruit-bat colony (i.e. within a colony site) *versus* otherwise (i.e. within a control site) were calculated for each rodent species and for the overall rodent populations.

RESULTS

Ground-level Urine Gradient

A distinct negative correlation was observed between volume of fruit-bat urine at ground level and distance from the fruit-bat colony, with a Spearman's rank correlation (ρ) of 0.73 ($P=0.03$).

Relative Abundance

Overall, 213 rodents were caught across the eight sites over the 12 months and 2400 trap-nights, 173 at the control sites and 40 at the colony sites (Table 3). Overall trap success was 8.9%, with significantly lower trap success at the colony sites than at the

control sites [3.3% *v.* 14.4%; $\chi^2=6.42$; degrees of freedom (df)=6; $P=0.012$]. The relative abundance of rodents at control sites was much higher than that at colony sites (14.4 *v.* 3.3 rodents caught/100 trap-nights; $\chi^2=85.03$; df=6; $P<0.001$). The most abundant rodent species in all four study areas (in terms of the numbers of specimens caught) were *R. fuscipes*, *Me. cervinipes* (fawn-footed melomys or mosaic-tailed rat), *Me. burtoni* (grassland melomys) and *R. sordidus* (cane-field rat), with RAI (for colony and control sites combined) of 2.3, 1.3, 1.3 and 1.1 animals/100 trap-nights, respectively. Table 3 gives the RAI and RACI for each of the 12 rodent species that were caught. For all 12 species, the mean RAI in the dry season was significantly higher than that recorded in the wet season. The mean RAI and (S.E.) for *R. fuscipes* (the most abundant rodent species), for example, were 10 (0.82) animals/100 trap-nights in the dry season and 4.3 (0.89) animals/100 trap-nights in the wet season ($F=10$; $P=0.03$). The mean RAI and (S.E.) for *R. fuscipes* in the four study areas — 11 (0.7)

TABLE 3. The mean relative abundance indices (RAI; in rodents/100 trap-nights) and the mean relative abundance of carrier animal indices (RACI; in rodents/100 trap-nights) for the rodents caught in colony and control sites

Rodent species	Control sites				Colony sites				All sites			
	No. of specimens:				No. of specimens:				No. of specimens:			
	Tested	Found to be carrier	RAI	RACI	Tested	Found to be carrier	RAI	RACI	Tested	Found to be carrier	RAI	RACI
<i>Rattus fuscipes</i>	43	12	3.5	1.0	14	4	1.1	0.3	57	16	2.3	0.6
<i>Melomys burtoni</i>	28	10	2.3	0.8	4	3	0.3	0.2	32	13	1.3	0.5
<i>Melomys cervinipes</i>	28	1	2.3	0.0	5	5	0.4	0.4	33	6	1.3	0.2
<i>Rattus sordidus</i>	25	0	2.0	0.0	2	0	0.1	0.0	27	0	1.1	0.0
<i>Mus musculus</i>	18	6	1.5	0.5	3	3	0.2	0.2	21	9	0.8	0.3
<i>Rattus rattus</i>	12	9	1.0	0.7	6	2	0.5	0.1	18	11	0.7	0.4
<i>Rattus leucopus</i>	5	1	0.4	0.0	0	0	0.0	0.0	5	1	0.2	0.0
<i>Uromys caudimaculatus</i>	6	0	0.5	0.0	0	0	0.0	0.0	6	0	0.2	0.0
<i>Perameles nasuta</i>	0	0	0.0	0.0	2	1	0.1	0.0	2	1	0.0	0.0
<i>Rattus tunneyi</i>	3	0	0.2	0.0	0	0	0.0	0.0	3	0	0.1	0.0
<i>Rattus lutreolus</i>	5	0	0.4	0.0	2	0	0.1	0.0	7	0	0.2	0.0
<i>Isodon macrourus</i>	0	0	0.0	0.0	2	0	0.1	0.0	2	0	0.0	0.0
All collected	173	39	14.4	3.2	40	18	3.3	1.5	213	57	8.8	2.3

animals/100 trap-nights in Gordonvale, 15 (0.63) in Innisfail, 15 (0.72) in Mareeba and 14 (0.68) in Tully — were similar ($F=15$; $P<0.001$).

No statistically significant correlations were observed between the density of the fruit-bat colony in a colony site and the abundance of any rodent species at that site. The relevant Spearman's rank correlation (ρ) for *R. fuscipes*, for example, was only 0.063 ($P=0.46$). Wilcoxon signed-rank tests indicated a significant difference in abundance, for individual rodent species, between the colony and control sites ($W=72$; $N=12$; $z=2.8$; $P<0.05$).

Seroprevalence and Carriage

All 57 rodents found infected with leptospires (*Me. burtoni*, *Me. cervinipes*, *Mu. musculus*, *Perameles nasuta*, *R. fuscipes*, *R. sordidus*, *R. rattus* and *R. leucopus*) had

kidney tissue that was PCR-positive for leptospiral DNA (Table 4). Four of the 57 were also found positive by culture, and 11 of the 57 were also found seropositive by MAT. Of the 57 infections, 39 were from control sites and 18 from colony sites (Table 4).

The percentage of the collected rodents that showed any evidence of exposure to leptospires (i.e. positivity by culture, PCR and/or MAT) was almost twice as high at colony sites than at the control sites (42.5% *v.* 23.6%; $\chi^2=6.33$; $df=12$; $P=0.01$). The prevalences of carriage showed a very similar trend (45.0% at colony sites *v.* 22.5% at control sites; $\chi^2=7.25$; $df=12$; $P=0.007$). Two *R. sordidus* from control sites, which were found seropositive in the MAT but negative by culture and PCR (Tulsiani *et al.*, 2010), were the only seropositive specimens not considered to be carriers. Compared with the animals caught in the dry season,

TABLE 4. Demonstration of leptospiral carriage among the rodents trapped at colony and control sites

	No. of samples found positive/tested and (% of tested samples found positive) by:						Serovars identified by:	
	Urine and kidney culture	PCR kidney	PCR serum	Microscopic agglutination test (MAT)	Silver impregnation	Any culture or PCR	Culture	MAT
SITE								
Control	2/97 (2.0)	39/97 (40.2)	0/173 (0.0)	9/173 (5.2)	0/97 (0.0)	39/173 (22.5)	Australis (<i>N</i> =2)	Australis (<i>N</i> =7), Zanoni (<i>N</i> =1) and Szwajizak (<i>N</i> =1)
Colony	2/40 (5.0)	18/40 (45.0)	0/40 (0.0)	4/40 (10.0)	0/40 (0.0)	18/40 (45.0)	Zanoni (<i>N</i> =1) and Australis (<i>N</i> =1)	Arborea (<i>N</i> =1), Szwajizak (<i>N</i> =2) and Australis (<i>N</i> =1)
Statistical comparison	$\Phi=0.08$; degrees of freedom (<i>df</i>)=1; $P=0.58$	$\chi^2=7.5$; <i>df</i> =6; $P<0.05$	–	$\chi^2=6.0$; <i>df</i> =6; $P=0.04$	–	$\chi^2=7.25$; <i>df</i> =12; $P=0.007$	–	–

the rodents caught in the wet season appeared no more or less likely to show evidence of exposure to leptospires ($\chi^2=0.001$; $df=1$; $P=0.97$). Although leptospiral carriage appeared to be almost twice as likely among the rodents at the colony sites than among the rodents at the control sites (relative risk ratio=1.97; $P=0.009$), comparisons between two study areas, using the Marascuilo procedure, did not consistently show this trend. The significant difference in carriage between colony and control rodents was consistent between Gordonvale and Tully ($\chi^2=0.07$; $df=3$; $P=0.99$), Gordonvale and Mareeba ($\chi^2=5.0$; $df=3$; $P=0.17$) and Mareeba and Innisfail ($\chi^2=0.33$; $df=3$; $P=0.95$) but not between Gordonvale and Innisfail ($\chi^2=8.4$; $df=3$; $P=0.03$), Tully and Innisfail ($\chi^2=14.5$; $df=3$; $P=0.002$) or Mareeba and Tully ($\chi^2=8.5$; $df=3$; $P=0.03$). Conversely, the observation of relatively high carriage in rodents at colony sites was consistent across the six sampling sessions. When carriage for rodents in September 2007 was, for example, compared with carriage for rodents in May 2008, the Marascuilo procedure indicated no significant difference ($\chi^2=0.02$; $df=5$; $P=0.99$).

DISCUSSION

Rodents have long been associated with human leptospirosis, as reservoir or maintenance hosts (Emanuel *et al.*, 1964). The recent detection and isolation of exotic and emergent serovars of leptospires in Australian rodents (Holt *et al.*, 2006; Anon., 2009) raises questions on the source of the infections. The possibility that, in Australia, rodents might be infected by contact with leptospires shed by fruit bats (Smythe *et al.*, 2002; Cox *et al.*, 2005) was explored in the present study.

The colony and control sites investigated in the present study were selected such that the presence/absence of a fruit-bat colony was the principal difference between them.

When comparisons were made between rodents at the control and colony sites, two distinct trends were observed, with the colony sites showing lower rodent abundance but higher prevalence of leptospiral carriage among the rodents that were present. Since each control site appeared similar, in terms of its understorey and tree vegetation, elevation and soil conditions, to the colony site with which it was paired (data not shown), these trends may be largely, if not entirely, attributable to the presence of a fruit-bat roost in each colony site. The roosting activities of fruit bats can cause considerable damage to vegetation, via the weight of the bats on branches and the bats' faecal droppings (www.derm.qld.gov.au/wildlife-ecosystems/wildlife/living_with_wildlife/flyingfoxes/index.html). The native rodents of Australia, particularly the arboreal and semi-arboreal species, require an adequate understorey for shelter, food and protection. Bat colonies may also attract predators, such as wedge-tailed eagles (*Aquila audax*), goannas (*Varanus* spp.) and pythons (*Morelia* spp.; Nelson, 1965; Slip and Shine, 1988), that readily feed on both bats and rodents. It is, however, impossible to exclude the possibility that rodents were relatively rare in the colony sites investigated in the present study because of factors unrelated to fruit bats, such as micro-level, topographic differences between the colony and control sites. Such potential inconsistencies, and the possibility that the colony sites are not representative of fruit-bat roosts in general, need to be considered before the present results are extrapolated. Further investigation into the short- and long-term effects of the presence of a fruit-bat colony on the suitability of a site for rodent habitation needs to be conducted.

The overall trap success (8.9%) and rodent abundance at control sites (14.4 rodents/100 trap-nights) were considerably different to those reported in other studies in Queensland. Rivera (2005), for example, reported a trap success of 16% and an

abundance of 4.14 rodents/100 trap-nights, while Laurance (1994) recorded a trap success of 29.0%. Overall, the abundance of each rodent species caught in the present study was lower than the corresponding value reported by Rivera (2005). In the present study, even though rodent abundance appeared generally low in the control sites, mean abundance in the colony sites was markedly lower (except for the values for the two bandicoot species, *P. nasuta* and *I. macroris*). The difference in rodent abundance, between colony and control sites, was found to be statistically significant in a Wilcoxon signed-rank test ($P < 0.05$), when rodent species and the presence of a fruit-bat colony were treated as nominal variables and rodent abundance as a measurable variable. The Wilcoxon signed-rank test was, however, based on the assumption that rodents within each study area are representative of a source population. Since the two study sites (colony and control) within each study area were selected to ensure that interaction between the rodent populations from each site was unlikely, the results from the Wilcoxon signed-rank test were not considered valid.

The overall seroprevalence recorded in the present study (6.1%), indicating current or previous leptospiral infection of the collected rodents, was also much lower than the 43% (Rivera, 2005) and 80% (Emanuel *et al.*, 1964) previously reported among rodents from Far North Queensland. Although the overall prevalence of carriage observed in rodents associated with colony sites was higher than that in the rodents caught at control sites, the RACI did not reflect this trend for every rodent species. For instance, the RACI for *R. fuscipes* at the control sites was higher than that recorded at the colony sites (0.3 *v.* 0.9 animals/100 trap-nights; $\chi^2 = 0.002$; $df = 6$; $P = 0.9$) whereas the RACI for *Me. cervinipes* at control sites was markedly lower than that recorded at the colony sites (0.0 *v.* 0.4 animals/100 trap-nights; $\chi^2 = 20.43$; $df = 6$; $P < 0.001$). The RACI is, however, based on

a combination of the detected prevalence of infection of a rodent species and the relative abundance of that species. Since the abundance data on which the present RACI were based included animals that were not tested for infection, the RACI could have been influenced by the effects of infection on the probability of being trapped (and species effects). This, combined with the relatively low trap success, meant that the RACI could not be used to predict the prevalence of leptospiral infection in all of the rodent species accurately, and emphasis was instead placed on the prevalences of leptospiral carriage.

The surprisingly low overall abundance of rodents and prevalence of leptospiral carriage recorded in the present study may be attributable to seasonality in rodent abundance and the limited sensitivity of the methods used to check for carriage. Seasons play a crucial role in rodent studies, with population densities directly affected by rainfall and the amount of food available (Aplin *et al.*, 2003). In general, the rodents of western Australia are more abundant in the wet season than during the dry season (Bradley *et al.*, 1987). This cyclic pattern of seasonality is also documented for the leptospires in Queensland, which survive in the environment better in the wet season, when the humidity and temperatures are almost optimal, than in the dry season (Derrick *et al.*, 1954; Rivera, 2005). The seroprevalences of leptospires in the domestic and wild mammals in this state may also fluctuate by up to 15% from one year to the next (Elder *et al.*, 1986). In the present study, surprisingly, the rodents caught in the dry season were no less likely to show evidence of exposure to leptospires than the rodents caught in wetter months. The present sampling sessions were conducted throughout a year, to check for seasonal variation, but only for a 13-month period. It remains unclear, therefore, whether the current data reflect an unusual year (there was a tropical cyclone in the study region in 2006) or not. The relatively small number of

rodents sampled may have prevented accurate estimations of rodent abundance (Cross *et al.*, 1998) and prevalence of leptospiral carriage. Estimates of the minimum sample size required, based on the model proposed by Cannon and Roe (1982) for a population of 100 individuals and an a-priori estimate of prevalence of 1%, with a reliability boundary of 5%, indicate that at least 14 rodents needed to be tested to determine a prevalence estimate with 95% confidence. In the present study, only *R. fuscipes* (57 animals caught), *Me. burtoni* (32), *Me. cervinipes* (33), *R. sordidus* (52), *Mu. musculus* (21) and *R. rattus* (18) could be used to determine prevalences for individual species accurately and then only by pooling across all sampling sessions and sites (Table 3). Pooled values, across all rodent species, had to be used to determine trends in exposure and carriage between colony and control sites.

Although serology is the principal method used to confirm a diagnosis of leptospirosis in humans, the detection of antileptospiral antibodies does not, in itself, indicate renal shedding or carriage status (Cerqueira and Picardeau, 2009). The PCR-based detection of leptospiral DNA in a serum sample is indicative of the presence of leptospires in the bloodstream, which, in carrier animals, indicates either a new infection (in which the leptospires have not yet colonized their host's kidneys) or a second infection with a non-endemic serovar (Slack *et al.*, 2007). A rodent that was sampled in the acute phase of a leptospiral infection would usually be found MAT-negative but have PCR-detectable leptospiral DNA in its kidneys and/or urine (Faine *et al.*, 1999). In the present study, the PCR-based detection of leptospiral DNA in a urine and/or kidney sample (or the culture-based detection of viable leptospires in such samples) was accepted as the final indicator of carrier or infectious status (Table 4). Serum samples from the collected rodents were also checked for leptospiral DNA, to test for discrepancies in the results, but no serum was found

PCR-positive for such DNA (and the results of such testing were excluded from the final estimates of incidence and prevalence). The PCR-based testing of samples of kidney tissue and urine did not give any discrepant results and the results of this testing were consequently analysed and presented together. No leptospires were detected in the silver-stained impression smears made from kidney samples, perhaps because the leptospiral cells that were present had been distorted by the smearing, the smears were over-stained and/or the silver staining was not sufficiently specific (Turner, 1970). As only four strains of leptospires were successfully isolated from the collected rodents, molecular epidemiology and the phylogenetic comparison of *Leptospira* isolates from colony and control sites were not considered useful.

Each diagnostic test for leptospirosis has inherent limitations. The serological tests, for example, tend to have subjective endpoints, while genotypic tests generally offer objective and quantitative results but, unlike culture, do not result in isolates that can be further analysed. In the present study, serology (which is the mainstay of *Leptospira* diagnosis) only revealed a small proportion of the carrier rodents, highlighting the need to tailor the detection methods used to the study requirements.

Urination forms part of the social grooming behaviour of fruit-bats while they are in their day colonies (MacKenzie and Field, 2004) and data from the present study indicate that substantial volumes of fruit-bat urine can accumulate on the ground, proximal to such colonies. Leptospires can survive for up to 6 weeks in soil and water, and the free leptospires in such habitats are considered important in the ecology of leptospirosis (Smith and Self, 1955). In the present study, although rodents were found to be relatively rare near to fruit-bat colonies, there remains the potential for the transmission of leptospires from fruit bats to the ground-dwelling rodents that do live near to fruit-bat colonies (Caley and Ramsey, 2001).

The present results include indirect evidence for such transmission, with the prevalence of leptospiral carriage and risk of exposure to leptospires both appearing higher among the rodents at the colony sites than among their counterparts at the control sites. Although fruit-bat-rodent transmission may occur relatively infrequently, it could allow *Leptospira* strains to be introduced into rodent populations, where they could be amplified and further distributed. Australian fruit bats, which have long and often international flight ranges, may disseminate novel, emergent or exotic strains more broadly, across or between rodent populations via the latter's exposure to urine at colony sites. Further research in this area should concentrate on transmission modelling, focussing on the fitting of density, frequency or asymptotic models (McCallum *et al.*, 2001). Future studies need to include large-scale investigations that allow the relative importance of individual rodent species, in bat-rodent transmission, and any associations between particular leptospiral serovars and each bat or rodent species to be explored. Methods to enhance isolation success would also be beneficial, so that advanced comparative characterisation of the isolates being transmitted from fruit bats and/or among the rodents close to fruit-bat colonies can be performed.

Over the last few decades, there have been significant changes to the ecology of Australian fruit bats, which may be attributed to the loss and/or fragmentation of suitable habitat (Constantine, 2003), changes in climatic conditions (Grant *et al.*, 1997) and external threats such as the management of colonies by human intervention (Tidemann, 2003). Biodiversity and ecosystem threats have been suggested as causes of pathogen spill-over from wildlife reservoirs into the human ecosystem (Macdonald and Laurenson, 2006; Pongsiri *et al.*, 2009), including the spill-over of zoonotic and re-emerging viruses from Australian fruit bats (MacKenzie and Field, 2004). Data from the current study

support the possibility of transmission of leptospires to and between rodent populations, as a result of contact with infectious fruit-bat urine. With increasing overlap in bat and domestic habitats, particularly in peri-urban settings, the potential threat to human health posed by such transmission may well be growing. An improved understanding of the shedding of leptospires by fruit bats, the interactions between populations of fruit bats, rodents, domestic livestock and humans that might have an impact on the ecology and epidemiology of leptospirosis, and the associated risks to public health is clearly required.

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