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SHORT COMMUNICATION

Leptospirosis risk increases with changes in species composition of rat populations

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Abstract Rats are major reservoirs of leptospirosis and considered as a main threat to biodiversity. A recent introduction of *Rattus rattus* to the island of Futuna (Western Polynesia) provided the opportunity to test if a possible change in species composition of rat populations would increase the risk of leptospirosis to humans. We trapped rodents on Wallis and Futuna and assessed *Leptospira* carriage in 357 rodents (*Rattus norvegicus*, *R. rattus*, *Rattus exulans*, and *Mus domesticus*) from 2008 to 2012. While *Leptospira* prevalence in rodents and the composition of rat populations on Futuna fluctuated with rainfall, the biomass of *Leptospira*-carrying rodents has been continuously rising from 2008 to 2012. Our results suggest that the introduction of *R. rattus* increases the risk to humans being infected with leptospirosis by rats.

Keywords Invasive species · *Leptospira* · Population dynamics · *Rattus*

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Introduction

Leptospirosis is widespread in the Pacific Islands (Berlioz-Arthaud et al. 2007; Victoriano et al. 2009), but with over 1,000 annual cases per 100,000 inhabitants (up to 53 confirmed cases per year in 4,200 inhabitants), the island of Futuna (14°17′ S, 178°08′ W) has the highest prevalence worldwide (Centre National de Référence de la Leptospirose 2010). Rats are the most important reservoirs of leptospirosis (Victoriano et al. 2009) and are considered as a major threat to biodiversity (Towns et al. 2006). Derne et al. (2011) hypothesized that leptospirosis risk increases with decreasing diversity of species within an ecological community. Following this hypothesis, the risk of leptospirosis could increase where a newly introduced rat species reduces native biodiversity. Therefore, a recent introduction of *Rattus rattus* to Futuna, discovered in 2008 (Theuerkauf et al. 2010), provided the opportunity to evaluate if a possible change in species composition of rat populations could increase the risk of leptospirosis to humans.

Methods

During five periods from 2008 to 2012, we estimated rodent abundance by intensive lethal trapping with Ka Mate Traps (Theuerkauf et al. 2010) on Futuna (Table 1) and calculated standardized abundance indices following Theuerkauf et al. (2011). We sampled a total of 286 rodents on Futuna and compared them with 15 samples that we took on Alofi (14° 20′ S, 178°02′ W), an uninhabited island 2 km from Futuna, and with 56 samples from Wallis (13°17′ S, 176°12′ W), which is a populated island (Table 1). As prevalence of *Leptospira* in rats increases with their age (Krøjgaard et al. 2009; Perez et al. 2011), we exclusively sampled kidneys

from adult (females that have already reproduced, males with enlarged testes) rodents (48 % females and 52 % males) and stored them after dissection in the field in tubes containing 95 % ethanol at room temperature until brought back to the laboratory (up to 3 weeks).

We aseptically dissected a ca. 20 mg piece of the cortical region of each kidney and immersed it overnight in 2 ml sterile Milli-Q water. We then replaced the water by 50 μl sterile phosphate buffer saline, DNA lysis buffer, and proteinase K from the QIAamp DNA mini kit (Qiagen). We extracted DNA from kidneys using QIAamp DNA mini kit following the manufacturer's recommendation. We screened the extract using a real-time PCR that detects all known pathogenic *Leptospira* species (Stoddard et al. 2009) and assessed the absence of PCR inhibition as described previously (Perez et al. 2011). The genotyping scheme was based on polymorphism of partial DNA sequence of the genes *secY* and *lfb1* following Perez and Goarant (2010) and Perez et al. (2011). We genotyped the first 29 *Leptospira*-positive kidney samples from Futuna (9 of 42 positive *Rattus norvegicus*, 6 of 17 *R. rattus*, and 14 of 25 *Rattus exulans*) and the only positive sample from Wallis (*R. exulans*) to identify the *Leptospira* genotype carried by rodents. Because all tested samples carried the same genotype, we did not genotype additional individuals.

Results and discussion

The sum of standardized indices of rat abundance on Futuna varied between 20 and 35 rats per 100 corrected trap nights (Fig. 1). This is about 50 % lower than that of Wallis (Table 1), but higher than in New Caledonia (Rouys and Theuerkauf 2003), where comparable abundances are only reached during hot, rainy seasons (Perez et al. 2011), when weather conditions are similar to those on Wallis and Futuna. All 30 genotyped *Leptospira*-positive specimens were *Leptospira interrogans*. The DNA sequences (genes *lfb1* and *secY*) were identical and compatible with the serovars Icterohaemorrhagiae or Copenhageni, both belonging to serogroup Icterohaemorrhagiae, the most prevalent serogroup identified in human cases in Futuna (Morisse et al. 2006).

The prevalence of *Leptospira* in *R. norvegicus* and *R. rattus* fluctuated during the sampling period, whereas it was stable or insignificantly declined in *R. exulans* (Fig. 1). Over the five periods, the mean prevalence of *Leptospira* in *R. norvegicus* was higher than that in *R. exulans* (paired *t* test, $p=0.034$), whereas it was intermediate in *R. rattus* and not significantly different from the other two species. In contrast, only one *R. exulans* carried *Leptospira* on Wallis, corresponding to a mean prevalence of only about 2 % (Table 1). No infected rat was found on Alofi, and although the sample size was small, it is likely that the island, which has no open water bodies, is free of leptospirosis. It is, however, difficult to explain why rodents of Futuna carry much more *Leptospira* than they carry on Wallis. One possible explanation might be that the technique to grow taro (*Colocasia esculenta*) differs. While fields are irrigated on Wallis by ditches, people on Futuna flood their fields. These large water bodies might facilitate the spread of *Leptospira* among rats and from rats to humans. The high rate of leptospirosis in younger men (Yvon 2008), who usually maintain the fields in Futuna, would support this assumption.

Mean body mass of *R. norvegicus* was 234 g (SD=103 g, $n=13$); of *R. rattus*, 153 g (SD=53 g, $n=13$); and of *R. exulans*, 49 g (SD=16 g, $n=39$). Because the larger rat species had higher prevalence of *Leptospira* and urine production (thus probably also *Leptospira* excretion) is proportional to body mass (Pass and Freeth 1993), we calculated the total biomass of infected rats over the study period (Fig. 1). This comparison revealed that the biomass of infected rats (all species) has been continuously increasing on Futuna from 2008 to 2012, with the exception of the November 2011 sampling period, when samples were taken right after a long (8 months) dry period (Fig. 1). To control for seasonal variation, we only used the three

Table 1 Human population density (in 2008), trapping effort, number of rodents sampled for analysis, abundance of rodents (individuals per 100 corrected trap nights), and prevalence of *Leptospira* in rodents on the three main islands of Wallis and Futuna from 2008 to 2012

	Futuna	Wallis	Alofi
Inhabitants (with island size)	109 km^{-2} (46 km^2)	123 km^{-2} (75 km^2)	0 km^{-2} (18 km^2)
Trap nights	20,316	741	200
Number of samples (with abundance of species present on the island)	102 *R. norvegicus* (9)		1 *R. norvegicus* (2)
	53 *R. rattus* (1)	14 *R. rattus* (14)	
	131 *R. exulans* (18)	41 *R. exulans* (26)	14 *R. exulans* (11)
	0 *M. domesticus* (<1)	1 *M. domesticus* (<1)	
Prevalence of *Leptospira* (with SD)	42.8 % (16.2 %) *R. norvegicus*		0 % *R. norvegicus*
	36.4 % (18.0 %) *R. rattus*	0 % *R. rattus*	
	18.8 % (3.4 %) *R. exulans*	2.4 % (15.6 %) *R. exulans*	0 % *R. exulans*
	M. domesticus not tested	0 % *M. domesticus*	

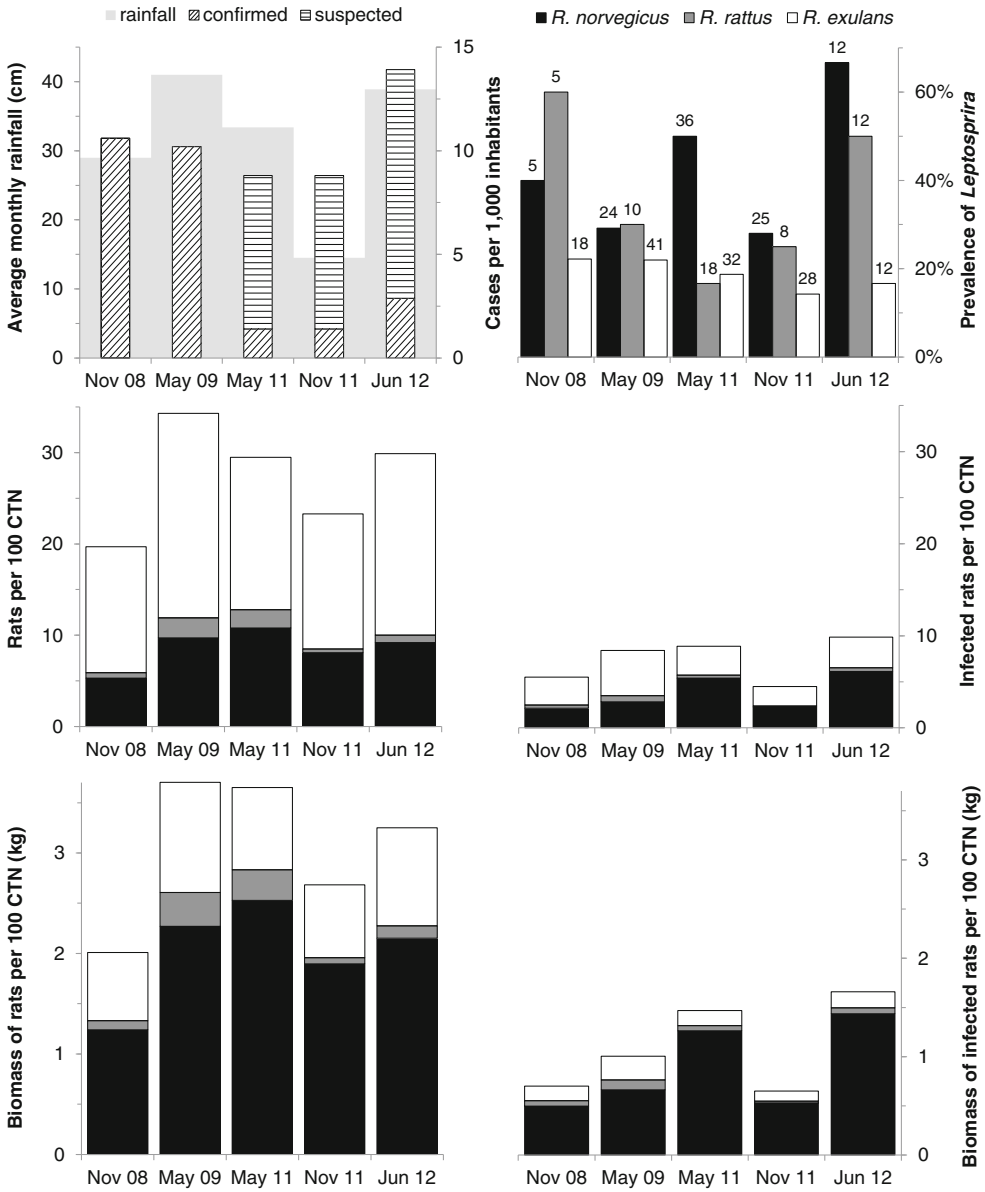


Fig. 1 Confirmed and suspected cases (data of 2008–2009 from the Centre National de Référence de la Leptospirose (2010) and of 2011–2012 provided by the hospital of Futuna, pers. comm.) of leptospirosis in humans (per 1,000 inhabitants) during the year of sampling (for 2012 extrapolated based on the data of January–May), average monthly rainfall during the 6 months preceding the time of sampling, prevalence of *Leptospira* in rats (with sample size), number of rats per 100 corrected trap nights (CTN), number of *Leptospira*-infected rats per 100 CTN, biomass of rats, and biomass of infected rats on Futuna during five sampling periods from 2008 to 2012

May/June periods to assess if leptospirosis risk increased. Excluding the November samplings, the biomass of infected rats significantly increased during the study period (linear regression, $p=0.045$), even though the average prevalence of *Leptospira* ($p=0.408$) and the abundance of rats ($p=0.274$) did not change. This means that the risk being infected with *Leptospira* by rats increased on Futuna. Unfortunately, the surveillance system for human leptospirosis in Wallis and Futuna has been less intensive since 2009 (only a part of cases are tested in the laboratory), preventing a thorough comparison of human leptospirosis incidence over the study period (see Fig. 1). Nevertheless, the increased biomass of infected rats means that there is a higher risk that the leptospirosis incidence in humans increases as soon as the environmental conditions favor the transmission of *Leptospira* between rats and humans (i.e., during wet periods). The increase of (confirmed and suspected) cases of leptospirosis in 2012 would support this assumption.

Comparing the five sampling periods, the prevalence of *Leptospira* in the three rat species was neither correlated to their abundance ($R=0.195$, $p=0.753$, post hoc power=0.78) nor to their total biomass ($R=0.105$, $p=0.867$, power=0.87), suggesting that the prevalence was not density dependent. At the species level, prevalence in *R. rattus* was also neither correlated to the abundance of infected rats ($R=0.339$, $p=0.577$, power=0.68) nor to the total biomass of infected rats ($R=0.301$, $p=0.623$, power=0.70). In contrast, prevalence was correlated to the abundance of infected rats and the total biomass of infected rats in *R. norvegicus* ($R=0.927$, $p=0.023$, power=0.79, and $R=0.930$, $p=0.022$, power=0.80, respectively) and *R. exulans* ($R=0.883$, $p=0.047$, power=0.78, and $R=0.902$, $p=0.036$, power=0.78, respectively).

This means that prevalence might not be an appropriate statistical proxy for the abundance of *Leptospira* in recently introduced rat species and that the biomass of infected rats is a better parameter to infer leptospirosis risk by rats.

We suggest that assessing prevalence in rodents alone might not be indicative of *Leptospira* carriage if the abundance or biomass of each species is not included in the analyses. We, therefore, recommend including rodent census in any leptospirosis risk assessment. On Futuna, already a minor change in rat species composition increased the abundance and biomass of infected rats. In the future, a possible impact of the recently introduced *R. rattus* on biodiversity and a possible change in rat population composition may result in a further increase of leptospirosis risk for humans. We, therefore, suggest that rat eradication or at least rat control should be implemented to minimize human leptospirosis burden on this exceptionally impacted island.

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