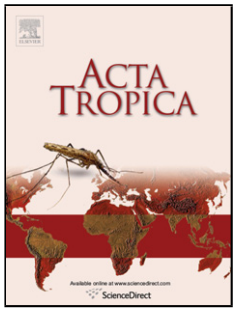
Wild fauna as a carrier of Salmonella in Reunion Island: Impact on pig farms

Claire Tessier, Laura Parama Atiana, Erwan Lagadec, Gildas Le Minter, Martine Denis, Eric Cardinale



Title: Wild fauna as a carrier of *Salmonella* in Reunion Island: impact on pig farms

Author: Claire Tessier Laura Parama Atiana Erwan Lagadec Gildas Le Minter Martine Denis Eric Cardinale

PII: S0001-706X(16)30037-7
DOI: http://dx.doi.org/doi:10.1016/j.actatropica.2016.01.027
Reference: ACTROP 3838

To appear in: *Acta Tropica*

Received date: 13-8-2015
Revised date: 24-1-2016
Accepted date: 26-1-2016

Please cite this article as: Tessier, Claire, Atiana, Laura Parama, Lagadec, Erwan, Minter, Gildas Le, Denis, Martine, Cardinale, Eric, Wild fauna as a carrier of Salmonella in Reunion Island: impact on pig farms.Acta Tropica http://dx.doi.org/10.1016/j.actatropica.2016.01.027

Wild fauna as a carrier of *Salmonella* in Reunion Island: impact on pig farms

Running Head: *Salmonella* in wild fauna in Reunion Island

Claire Tessier[a,b,c,d,e*] claire_tessier@outlook.fr, Laura Parama Atiana[b,c,d1] laura.atiana@gmail.com, Erwan Lagadec[d,f] erwan.lagadec69@yahoo.fr, Gildas Le Minter[d,f] leminterbzh@yahoo.fr, Martine Denis[g,h] martine.denis@anses.fr, Eric Cardinale[b,c,d] eric.cardinale@cirad.fr

[a]SICABAT, Société d'Intérêt Collectif Agricole d'Abattage, Saint-Pierre, La Réunion, France

[b]CIRAD, UMR CMAEE, Plateforme de Recherche CYROI, Sainte-Clotilde, La Réunion, France,

[c]INRA, UMR 1309 CMAEE, Montpellier, France

[d]CRVOI, Centre de Recherche et de Veille des maladies émergentes dans l'Océan Indien, Plateforme de Recherche CYROI, Sainte-Clotilde, La Réunion, France

[e]Université de la Réunion, Sainte-Clotilde, La Réunion, France;

[f]UMR PIMIT, Université de La Réunion, INSERM 1187, CNRS 9192, IRD 249. Plateforme de Recherche CYROI, Sainte-Clotilde, La Réunion, France

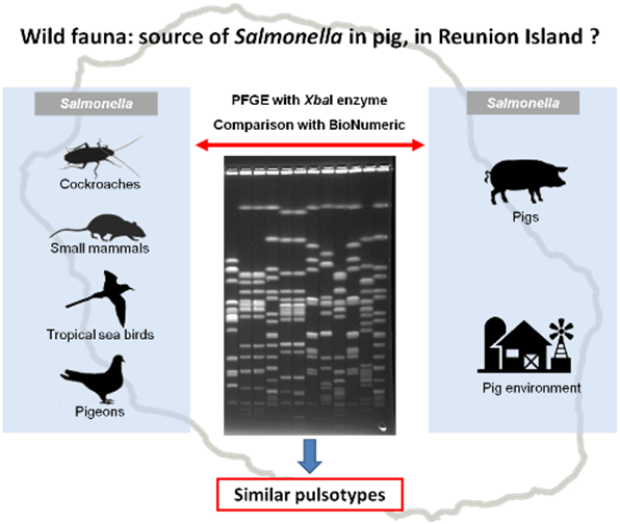
[g]Anses, Unité Hygiène et Qualité des Produits Avicoles et Porcins, Ploufragan, France

[h]Université Européenne de Bretagne, Rennes, France

[*]Corresponding author at: CIRAD, UMR CMAEE, Plateforme de Recherche CYROI, Sainte-Clotilde, La Réunion, France.

[1]Present address : Groupe Hospitalier Sud Réunion GHSR, Saint-Pierre, La Réunion, France

Graphical abstract



Highlights:

- First isolation of *Salmonella* in tenrecs and two tropical seabirds species
- High prevalence of *S.* 4,[5],12:i:- and *S*. Typhimurium in wild fauna
- High prevalence of *Salmonella* in rodents and cockroaches
- Potential involvement of rodents and cockroaches in the dissemination of *Salmonella*
- **S**trengthening rodent control and insect disinfestations in tropical pig farms

Abstract

Salmonellosis is an economic burden to the livestock industry in Reunion Island. In this study, we wanted to improve our understanding of *Salmonella* epidemiology by studying the wild fauna of Reunion Island. We assessed *Salmonella* diversity in small non-flying mammals, birds and cockroaches in order to evaluate their potential role in the epidemiology of *Salmonella*. A total of 268 samples were collected from cockroaches, small mammals and birds. The bacteriological analyses revealed that 11.7% of non-flying mammals and 25% of cockroaches tested were *Salmonella* infected; two wild bird species were also detected positive. The 128 *Salmonella* isolates were distributed in fifteen serotypes and the most predominant were *S.* 4,[5],12:i:- (21.9% of positive samples) followed by *S.* Enteritidis (15.6%), *S.* Typhimurium (15.6%), *S.* Infantis (12.5%) and *S.* Weltevreden (12.5%). A total of 27 *Xba*I profiles were identified using pulsed-field gel electrophoresis. Comparison of these *Salmonella* strains with our collection of *Salmonella* isolated from pigs and pig farm environments at the same period revealed 14 strains in common between wild fauna and pigs, especially for cockroaches. Our results suggest that wild fauna of Reunion Island could be infected by strains of *Salmonella* also isolated from pigs or pig environment. They may play a role in both persistence and spreading of *Salmonella* and therefore, could be a source of infection in pig farms. Pest control against cockroaches could be a helpful tool in the reduction of *Salmonella* infection of pigs, limiting contacts between wild fauna and both pigs and pig environment. Special attention should be paid to *S.* 4,[5],12:i:- since it was predominant in Reunion Island's wild fauna and pigs and was the third most frequently reported serotype in human salmonellosis in Europe.

Keywords*:* *Salmonella*; wild fauna; PFGE; pig; Reunion Island; tropics

1. Introduction

In the last decades, knowledge on the role of the wild fauna as reservoirs of different zoonotic pathogens has been considerably demonstrated (Bengis et al., 2004; Kruse et al., 2004). Different studies actually revealed that the wild fauna hosts different infectious agents including viruses (Wilkinson et al., 2014b) and bacteria (Lagadec et al., 2012; Cloarec et al., 1992) or macroparasites (Wilkinson et al., 2014a; Guernier et al., 2014) that can have substantial impacts on public health. Recently, European studies pointed out that *Salmonella,* a widespread enterobacteria, was the second most frequent cause of human zoonoses in 2012 (EFSA and ECDC, 2014) and pigs and pig products are one of the main sources of human salmonellosis (FCC Consortium, 2013). Furthermore, different studies also demonstrated the role of wild fauna in the maintenance and transmission of *Salmonella* in farming environments (Greig et al., 2014; Meerburg and Kijlstra, 2007; Olson and Rueger, 1950; Umali et al., 2012) However, only one study showed that *Salmonella* strains isolated from wild birds or rodents were similar to those isolated from pigs (Andres-Barranco et al., 2014).

In Reunion Island, the ARS (Agence Régionale de la Santé) notified 22% of food-borne outbreaks caused by *Salmonella* between 1996 and 2005. However, it is known that human salmonellosis is under-estimated in this small French overseas department (D'Ortenzio et al., 2008). Furthermore, prevalence of *Salmonella* in pig farms has been reported as being particularly high in Reunion Island: with more than 60% of pig batches being infected with this pathogen towards the end of the fattening period (Cardinale et al., 2010; Tessier et al., 2013b). More than 26 600 tones of pork are consumed per year (around 30 kg/inhabitant/year) and half of the pork consumed is locally produced, making the pig industry the second largest local production, after poultry. Thus, *Salmonella* is considered as a socioeconomic burden by the health authorities (D'Ortenzio et al., 2008).

Appropriate sanitary and hygiene standards are recommended to limit *Salmonella* infection in pigs. Reduction of *Salmonella* prevalence at the end of the fattening period will limit its introduction to the slaughterhouse, and thus minimize the risk to consumers (Kirchner et al., 2011; van der Wolf et al., 1999). However, *Salmonella* is able to persist in the environment for several months (Berends et al., 1996; Davies and Wray, 1996), making its exclusion from open areas almost impossible, as wild animals or insects may act as a source of reinfection (Liebana et al., 2003). Pest control is one of the recommended biosecurity measures for pig farmers; however, the role of pests in *Salmonella* epidemiology and pig infection has not been described in Reunion Island. Cockroaches, rodents, shrews, tenrecs and peridomestic birds are widely distributed in Reunion Island and are frequently observed inside or in close vicinity of pig premises (Tessier et al., 2013a). A preliminary study carried out in pig farms in Reunion Island supported the potential role of these members of the local wild fauna in *Salmonella* epidemiology (Cardinale et al., 2010).

In the present study we assessed *Salmonella* diversity in small non-flying mammals, birds and cockroaches of Reunion Island and evaluate the potential role of that wild fauna in *Salmonella* epidemiology.

2. **Material and methods**

2.1. Sample collection

From January 2011 to April 2013, cockroaches, small mammals and birds were collected throughout Reunion Island. Cockroaches (Blattodea) were collected from pig farms. For that purpose, 50 pig farms were selected randomly among the 154 members of the pig producer association in Reunion Island. Most cockroaches were collected in cleaned and disinfected fattening rooms before the introduction of a new batch of pigs. When several cockroaches were

trapped from the same site, they were pooled (between two to nine individuals per pool). Alive cockroaches were transported to the laboratory and immersed in 90% ethanol to decontaminate their external surface before being air-dried and crushed. Small mammals were trapped in different biotopes along two altitudinal transects lying on each side of the island: twelve different sites were investigated (including three pig farms). Trapping was conducted following a standardized protocol described in Guernier et al. (2014). Intestines and liver of each animal were removed aseptically in the laboratory. Finally, samples from wild birds were also collected in two different ways. Cloacal swabs were taken from rescued birds and liver and intestines of deceased birds were sampled by the Society of ornithological studies of Reunion Island (SEOR). Intestines and liver of pigeons trapped from industrial sites and removed aseptically in the laboratory were included. Samples collected in the field were transported to the laboratory in cold conditions and stored at +4°C at the laboratory for no more than 48 h before analysis.

2.2. Ethic statement

All small mammals procedures carried out in this study were performed in accordance with the European Union legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU). The ethical terms of the research protocol were approved by the CYROI Institutional Review Board (*Comité d'Ethique du CYROI* n° 114).

2.3. Salmonella isolation

The detection method was adapted from ISO 6579:2002, Annex D (International Organization for Standardization, 2007) with minor modifications. Samples were diluted 1:10 in buffered peptone water (BPW) and homogenised with stomacher before incubation. Swabs were pre-enriched with 10 ml of BPW respectively. After incubation at 37°C for 18 h ± 2 h, one ml of

BPW broth was inoculated onto 10 ml of Müller–Kauffmann Tetrathionate-Novobiocin broth (MKTTn; bioMérieux, Marcy l'Etoile, France) and three drops of 100 µl each of BPW broth were inoculated on a Modified Semisolid Rappaport Vassiliadis agar (MSRV; bioMérieux, Marcy l'Etoile, France) plate. MKTTn and MSRV cultures were incubated at 37°C for 24 h and 41.5°C for 24 to 48 h, respectively. MKTTn cultures and MSRV plates with a characteristic halo of migration were then streaked on both Xylose-Lysine-Desoxycholate Agar (XLD; bioMérieux, Marcy l'Etoile, France) and Rambach Agar (RA; Merck KGaA, Darmstadt, Germany). After 24 h at 37°C, four typical colonies per sample (2 colonies per selective enrichment when possible) were purified and biochemically identified as *Salmonella* by assays on Kligler-Hajna medium, urea-indole broth and o-nitrophenyl-β-D-galactopyranose (ONPG) disks (bioMérieux, Marcy l'Etoile, France). Suspected *Salmonella* colonies were confirmed using the Rapid'One system based on biochemical tests (Oxoid, Dardilly, France). *Salmonella* isolates were stored at -80°C in a peptone glycerol broth.

2.4. Genetic characterization and serotyping

Pulsed-Field Gel Electrophoresis (PFGE) using *Xba*I restriction endonuclease was performed on all the isolates using the CHEF-DR III system (Bio-Rad, France), according to the standard PulseNet protocol (Ribot et al., 2006). For some of the isolates from which the extracted DNA was not stable, we used HEPES buffer instead of Tris buffer at each step preceding the DNA restriction analysis and we added 100 µM thio-urea to the running buffer as previously described (Liesegang et al., 2002). *Salmonella enterica* serotype Braenderup H9812 digested by *Xba*I was used as molecular marker (Hunter et al., 2005). DNA patterns were analyzed with BioNumerics® software (V 6.5, Applied Maths, Sint-Martens-Latem, Belgium). Each PFGE pattern with a difference of at least one band from a known pattern type was considered to be a new pattern

(Tenover et al., 1995). Similarities between profiles based on band positions, were calculated using Dice's coefficient, with a maximum position tolerance of 1% and an optimization of 1%. Strains were clustered by the unweighted pair-group method using the arithmetic mean (Struelens, 1996).

Kerouanton et al. (2007) demonstrated that all isolates with the same *Xba*I pattern could be attributed to the same *Salmonella* serotype given the strong relationship between *Xba*I pattern and *Salmonella* serotype. We therefore serotyped one isolate per PFGE pattern in the French National Reference Laboratory of *Salmonella* (Anses, Ploufragan, France) according to the Kauffmann-White scheme (Grimont and Weill, 2007) using a slide agglutination test for the determination of somatic O and phase 1 and 2 flagellar antigens with standard antisera (BioRad, Marnes la Coquette, France; bioMérieux, Bruz, France).

2.5. Isolate collection

For the genotypic comparison, we considered one isolate per PFGE profile and per sample collected from wild birds, small mammals and cockroaches. We also considered *Salmonella* isolates collected from *Salmonella*-positive pig farms (pool of faeces or pair of gauze socks of pens) investigated during the same period (Tessier et al., 2013b). Out of these 50 investigated pig farms, a collection of 127 isolates was taken into account, still considering one isolate per PFGE profile and per farm.

3. **Results**

3.1. Isolation of Salmonella *in wild fauna*

A total of 268 samples from wild fauna (cockroaches, birds and small mammals) were collected from 37 different sites (including 24 pig farms) throughout Reunion Island (Figure 1 and Table 1).

Cockroaches have been collected from 24 out of the 50 pig farms selected. We collected 185 cockroaches pooled into 44 samples (mean of 4 cockroaches per pool) and 25% (11/44) of the samples were *Salmonella*-positive. Nine out of the 24 pig farms (37.5%) had *Salmonella*-infected cockroaches.

One hundred and sixty-two small mammals belonging to five species including *Mus musculus*, *Rattus* spp., *Suncus murinus* and *Tenrec ecaudatus* were trapped throughout different sites in Reunion Island and 11.7% (19/162) of the total samples were *Salmonella*-positive. While all the samples of mice (*Mus musculus)* were *Salmonella*-negative, 9 rats (2 from the same pig farm and 7 from different natural sites) out of the 121 rats trapped (20 from pig farms and 101 from natural sites) were *Salmonella*-infected, representing 6.7 % (9/134) positive rodents. In addition, *Salmonella* was isolated from 21.7 % (5/23) of the shrews (*Suncus murinus*) tested. Only five tenrecs (*Tenrec ecaudatus*) were trapped, all from the same biotope and interestingly they were all *Salmonella*-positive. Small mammals were sampled from 13 sites and 5 of them were *Salmonella*-positive (with at least one positive-sample) (Figure 1 and Table 1).

In total, we also tested 62 birds belonging to four species including *Columba livia, Phaethon lepturus, Pterodroma baraui* and *Puffinus lherminieri*. Only two samples collected from white-tailed tropicbird (*Phaethon lepturus*) and shearwaters (*Puffinus lherminieri*) were *Salmonella*-infected.

3.2. Distribution of Salmonella serotypes in wild fauna

From the 32 *Salmonella*-positive samples, 128 strains could be isolated. A total of 15 *Salmonella* serotypes were identified (Figure 2). The most predominant serotype was *S.* 4,[5],12:i:- detected in 21.9% of positive samples followed by *S.* Enteritidis (15.6%), *S.* Typhimurium (15.6%), *S.* Infantis (12.5%) and *S.* Weltevreden (12.5%). The serotype *S.* 4,[5],12:i:- was isolated from birds, rodents (rats) and cockroaches. Cockroaches, shrews and rats were shedding six, seven and four different serotypes, respectively. In cockroaches, *S.* 4,[5],12:i:- was the most predominant (36.4%), followed by *S.* Typhimurium (27.3%), *S.* Livingstone (18.2%) and *S.* Weltevreden (18.2%). The most predominant serotype in shrews was *S.* Infantis (60% of positive samples) whereas in rats, it was *S.* Kisangani (22.2%), *S.* Typhimurium (22.2%) and *S.* Weltevreden (22.2%). Birds and tenrecs were shedding only one serotype, *S.* 4,[5],12:i:- and *S.* Enteritidis, respectively.

3.3. Salmonella characterization

Out of 32 *Salmonella*-infected samples, one isolate per sample and per *Xba*I profile was considered giving a total of 37 isolates (Figure 3); two isolates from wild birds (one from Audubon's Shearwater and one from White-tailed Tropicbird), 13 from cockroaches and 22 from small mammals (11 from rats, 5 from tenrecs and 6 from shrews). The 37 isolates were distributed in 27 *Xba*I profiles with 1, 11 and 16 *Xba*I profiles for birds, cockroaches and small mammals, respectively with a single *Xba*I profile shared between cockroaches and small mammals. Interestingly, one *Xba*I profile was detected in shrews and a rat trapped at the same site.

The 127 isolates selected from pig faeces and pen floors in pig farms in Reunion Island (Tessier et al., 2013b) were distributed into 83 *Xba*I profiles. Fourteen *Xba*I profiles (36 isolates) were common between wild fauna and pig samples: nine from cockroaches, two from rats, one

from tenrecs, one from birds and one from both cockroaches and rats (Figure 3). Most *Salmonella* isolates from wild fauna (67.6%) were identical to isolates from pig farm environment. All isolates from birds (100%) and 92.3% of isolates from cockroaches were identical to isolates from pig farms. Six serotypes detected either in rats or shrews (*S.* Aberdeen, *S.* Brancaster, *S.* Hofit, *S.* Infantis, *S.* Kibusi and *S.* Uganda) were not detected elsewhere. It is noted that the two rats which were trapped from farms were infected with different serotypes and pulsotypes from those detected in the farm.

4. **Discussion**

We have showed that the wild fauna of Reunion Island was possibly infected by *Salmonella* highlighting that it could play a potential role in the epidemiology of *Salmonella*. Proportions of positive samples between the different species of wild fauna and the different sites (farms, industrial or natural sites, rescue center) were not easily comparable because of different methods of trapping; however, this study demonstrated that wild fauna shared the same *Salmonella* serotypes and pulsotypes also isolated from pig and pig environment, suggesting that pests may introduce *Salmonella* into local pig farms or be contaminated from these *Salmonella*.

In our study, 25% of samples of cockroaches were detected *Salmonella*-positive. It is significantly higher than it has been reported elsewhere (Devi and Murray, 1991). Preliminary measures for decontamination of cockroach exoskeletons before testing ensured that our results reflected only internal bacterial carriage. The observed elevated prevalence may result from sample pooling, which has been shown to be optimal for *Salmonella* detection however but this result is not indicative of individual infection of cockroaches (Arnold et al., 2005; Arnold et al., 2014). Furthermore, all samples were collected from pig farms across Reunion Island, where presence of *Salmonella* is known to be frequent (Cardinale et al., 2010; Tessier et al., 2013b). *Salmonella*-positive cockroaches were detected in 37.5% of the farms that had cockroaches,

indicating a significant circulation of *Salmonella* in this insect. Similar PFGE profiles obtained from *Salmonella* strains were detected both in cockroaches and pigs, suggesting cross-contaminations. Although infection could be bidirectional, pig infection may occur either directly after consumption of infected wild fauna or indirectly after contact with infected faeces (Kilonzo et al., 2013; Meerburg et al., 2006; Rose et al., 2000). Due to their feeding behaviour, cockroaches have a great potential to disseminate faecal bacteria, potentially contaminating pig feed or water via excretions or regurgitations (Olson and Rueger, 1950; Fathpour et al., 2003; Ahmad et al., 2011; Chaichanawongsaroj et al., 2004). Furthermore, *Salmonella* is known to persist on cockroaches for up to 10 months (Fathpour et al., 2003).

We reported that 11% of the tested small mammals (6 % of rodents, 21 % of shrews and 100% of tenrecs) were infected by *Salmonella*. Other studies have reported positive results in these species to be variable, ranging from 0% to 47% for rodents and 10% to 76% for shrews (Meerburg and Kijlstra, 2007; Andres-Barranco et al., 2014; Kilonzo et al., 2013; Singh et al., 1980; Joseph et al., 1984; Nkogwe et al., 2011). *Salmonella* was not detected in house mouse (*Mus musculus*) despite evidence of infection in this species in other studies (Kilonzo et al., 2013; Shimi et al., 1979), however this may be a result of the small number of specimens available. Unexpectedly, all the tested tenrecs (*Tenrec ecaudatus*) were infected by the same *S.* Enteritidis pulsotype but there were only five samples trapped from the same site. The tenrec is endemic to the Mascarene Islands and has elsewhere been identified as an important reservoir of bacterial infection (Tortosa et al., unpublished results). To date, no case of *Salmonella* infection has been reported in this species. However, the *S.* Enteritidis pulsotype was displayed by both tenrecs and pigs, suggesting that it will be interesting to carry out further studies on a more representative sampling in order to elucidate its role in regional *Salmonella* epidemiology. A large diversity of PFGE profiles was detected from other small mammals, but 3 profiles were similar to those isolated from pigs, suggesting cross-contaminations as well. Besides, some *Salmonella* serotypes

were only recovered from small mammals such as *S.* Brancaster, *S.* Infantis *S.* Kibusi and *S.* Aberdeen; for instance the same *S.* Infantis pulsotype was isolated from rats and shrews confirming other sources of contamination such as broiler chicken flocks (Henry et al., 2013) ocellated lizards (Martinez et al., 2011) and pet birds (Seepersadsingh and Adesiyun, 2003).

Only two birds were detected *Salmonella*-positive. All birds were collected from industrial sites or from the bird rescue Center (SEOR), where no elevated risk of *Salmonella* infection was expected. Comparable low positive results have been observed in healthy wild birds in other countries (Andres-Barranco et al., 2013; Brittingham et al., 1988; Gaukler et al., 2009; Krawiec et al., 2015). Different studies have highlighted that *Salmonella* detection is strongly influenced by sampling locality, with higher proportion being observed at sites in proximity to infected areas, such as pig farms (Andres-Barranco et al., 2014; Andres-Barranco et al., 2013; Cizek et al., 1994). Furthermore, different methods of sampling were used which could lead to different sensitivity of *Salmonella* detection. Although several studies have previously isolated *Salmonella* from seabirds (Stoddard et al., 2008; Palmgren et al., 2000), we provide the first evidence of *Salmonella* infection in two species of tropical seabirds, *Phaethon lepturus* and *Puffinus lherminieri*. Elsewhere, *Salmonella* shedding has been reported in pigeons (Gargiulo et al., 2014; Gong et al., 2014), but all the samples tested in our study were negative. Despite similar strains were isolated from seabirds and pigs, the low detection of *Salmonella* did not suggest that seabirds play a major role in pig infection. But it would be indicative to look at the small sparrows that are often seen in the pig houses.

The serotype *S.* 4,[5],12:i:- was the most predominant, and was isolated from birds, rodents and cockroaches confirming a broad distribution of this serotype within Reunion Island's wild fauna. Despite the predominance could be explained by our sampling strategy, these results could also be related to the emergence of this serotype in the pig and poultry production in Reunion Island (Tessier et al., 2013a; Tessier et al., 2013b; Henry et al., 2015; Cardinale et al., 2010) .This

observation is in contrast with other studies, which have scarcely detected *S.* 4,[5],12:i:- in wild animal communities (Andres-Barranco et al., 2014; Smith et al., 2002). In Europe also, *S.* 4,[5],12:i:- has emerged over the last two decades (EFSA Panel on Biological Hazards (BIOHAZ), 2010) and was the third most frequently reported serotype in human salmonellosis in 2012 (EFSA and ECDC, 2014).

Here, we have demonstrated that birds, small mammals and cockroaches are carriers of *Salmonella* in Reunion Island, and thus that the local wild fauna could play a role in the epidemiological cycles of several different *Salmonella* subtypes. This is particularly true in the context of mixed or high density farming of pigs and poultry, which is commonplace in Reunion Island. Serological and molecular evidence seemed to corroborate the dual role of wild fauna in both maintenance and spreading of *Salmonella* in the environment, which may either result from direct physical contact with the bacterium or by established infection in wild animal or insect populations. High *Salmonella* detection and strong similarity between *Salmonella* strains isolated from cockroaches, small mammals (rodents and shrews) and pigs could suggest that thorough insect disinfestation procedures and pest control plans should be effective in reducing *Salmonella* re-introduction into pig farms. Although implementation of correct sanitation measures will go a long way to reducing infection levels, the added complication of pest-origin *Salmonella* re-introduction will make this challenging, especially in Reunion Island due to its (i) unique ecology, (ii) tropical climate (optimal for both pest densities and *Salmonella* growth) and (iii) high density of pig and poultry farms.

Acknowledgements

This epidemiological investigation was funded by Region Reunion and the *Association Nationale de la Recherche Technique* (ANRT). Small mammal capture was financed by an ongoing program, FEDER-POCT LeptOI (N°32913). We thank the *Direction de*

l'Environnement, de l'Aménagement et du Logement (DEAL Reunion) for issuing permits, the *Parc National de La Reunion* and the *Office National des Forêts* who allowed trapping in protected area. The authors are grateful to staff of the Society of ornithological studies of Reunion Island (SEOR) and Rentokil Pest Control for sampling of birds and small mammals. The authors also thank local pig farmers for their active cooperation. The authors want to thanks Claire Bernard, from Cirad (UMR CMAEE) for her help in map design, David Wilkinson and Beza Ramasindrazana (UMR PIMIT) for having carefully read the manuscript and Annaëlle Kerouanton (Anses, UHQPAP) for her advises on PFGE and BioNumerics. We also thank the anonymous reviewer for his comments and suggestions on the earlier version of the manuscript. He significantly contributed to its amelioration.

The authors declare that they have no conflict of interest.

References

Ahmad, A., Ghosh, A., Schal, C. and Zurek, L. 2011. Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community. BMC Microbiol, 11**,** 23.

Andres-Barranco, S., Vico, J. P., Garrido, V., Grillo, M. J., Samper, S., Gavin, P., Herrera-Leon, S. and Mainar-Jaime, R. C. 2013. Epidemiology of subclinical salmonellosis in wild birds from an area of high prevalence of pig salmonellosis: phenotypic and genetic profiles of *Salmonella* isolates. Zoonoses Public Health, 60**,** 355-65.

Andres-Barranco, S., Vico, J. P., Garrido, V., Samper, S., Herrera-Leon, S., de Frutos, C. and Mainar-Jaime, R. C. 2014. Role of wild bird and rodents in the epidemiology of subclinical salmonellosis in finishing pigs. Foodborne Pathog Dis.

Arnold, M. E., Cook, A. and Davies, R. 2005. A modelling approach to estimate the sensitivity of pooled faecal samples for isolation of *Salmonella* in pigs. J R Soc Interface, 2**,** 365-72.

Arnold, M. E., Gosling, R. J., Martelli, F., Mueller-Doblies, D. and Davies, R. H. 2014. Evaluation of the sensitivity of faecal sampling for detection of monophasic *Salmonella* Typhimurium and other *Salmonella* in cattle and pigs. Epidemiol. Infect.**,** 1-11.

Bengis, R. G., Leighton, F. A., Fischer, J. R., Artois, M., Morner, T. and Tate, C. M. 2004. The role of wildlife in emerging and re-emerging zoonoses. Rev Sci Tech, 23**,** 497-511.

Berends, B. R., Urlings, H. A. P., Snijders, J. M. A. and Van Knapen, F. 1996. Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. Int. J. Food Microbiol., 30**,** 37-53.

Brittingham, M. C., Temple, S. A. and Duncan, R. M. 1988. A survey of the prevalence of selected bacteria in wild birds. J Wildl Dis, 24**,** 299-307.

Cardinale, E., Maeder, S., Porphyre, V. and Debin, M. 2010. *Salmonella* in fattening pigs in Reunion Island: Herd prevalence and risk factors for infection. Prev Vet Med, 96**,** 281-285.

Chaichanawongsaroj, N., Vanichayatanarak, K., Pipatkullachat, T., Polrojpanya, M. and Somkiatcharoen, S. 2004. Isolation of gram-negative bacteria from cockroaches trapped from urban environment. Southeast Asian J Trop Med Public Health, 35**,** 681-4.

Cizek, A., Literak, I., Hejlicek, K., Treml, F. and Smola, J. 1994. *Salmonella* contamination of the environment and its incidence in wild birds. Zentralbl Veterinarmed B, 41**,** 320-7.

Cloarec, A., Rivault, C., Fontaine, F. and Le Guyader, A. 1992. Cockroaches as carriers of bacteria in multi-family dwellings. Epidemiol. Infect., 109, 483-90.

D'Ortenzio, E., Weill, F.X., Ragonneau, S., Lebon, J.A., Renault, P., Pierre, V., 2008. First report of a Salmonella enterica serovar Weltevreden outbreak on Reunion Island, France, August 2007. Euro surveill 13.

Davies, R. H. and Wray, C. 1996. Studies of contamination of three broiler breeder houses with *Salmonella* Enteritidis before and after cleansing and disinfection. Avian Dis., 40**,** 626-33.

Devi, S. J. and Murray, C. J. 1991. Cockroaches (*Blatta* and *Periplaneta* species) as reservoirs of drug-resistant salmonellas. Epidemiol. Infect., 107**,** 357-61.

EFSA and ECDC 2014. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. EFSA Journal 2014, 12**,** 312.

EFSA Panel on Biological Hazards (BIOHAZ) 2010. Scientific opinion on monitoring and assessment of the public health risk of "*Salmonella* Typhimurium-like" strains. EFSA Journal 2010, 8**,** 48.

Fathpour, H., Emtiazi, G. and Ghasemi, E. 2003. Cockroaches as reservoirs and vectors of drug resistant *Salmonella* spp. Iran Biomed J, 7**,** 35-38.

FCC Consortium 2013. Final Report : Analysis of the costs and benefits of setting a target for the reduction of *Salmonella* in pigs at slaughterhouse level for The European Commission Directorate-General for Health and Consumers SANCO/E2/2009/SI2.534057.

Gargiulo, A., Russo, T. P., Schettini, R., Mallardo, K., Calabria, M., Menna, L. F., Raia, P., Pagnini, U., Caputo, V., Fioretti, A. and Dipineto, L. 2014. Occurrence of enteropathogenic bacteria in urban pigeons (*Columba livia*) in Italy. Vector Borne Zoonotic Dis, 14**,** 251-5.

Gaukler, S. M., Linz, G. M., Sherwood, J. S., Dyer, N. W., Bleier, W. J., Wannemuehler, Y. M., Nolan, L. K. and Logue, C. M. 2009. *Escherichia coli*, *Salmonella*, and *Mycobacterium avium* subsp. *paratuberculosis* in wild European starlings at a Kansas cattle feedlot. Avian Dis., 53**,** 544-51.

Gong, J., Zhang, J., Xu, M., Zhu, C., Yu, Y., Liu, X., Kelly, P., Xu, B. and Wang, C. 2014. Prevalence and fimbrial genotype distribution of poultry *Salmonella* isolates in China (2006 to 2012). Appl. Environ. Microbiol., 80**,** 687-93.

Greig, J., Rajic, A., Young, I., Mascarenhas, M., Waddell, L. and LeJeune, J. 2014. A scoping review of the role of wildlife in the transmission of bacterial pathogens and antimicrobial resistance to the food chain. Zoonoses Public Health.

Grimont, P. A. D. and Weill, F.-X. 2007. Antigenic formulae of the *Salmonella* serovars. Pasteur Institute, Paris, France: World Health Organization Collaborating Centre for Reference and Research on *Salmonella*.

Guernier, V., Lagadec, E., LeMinter, G., Licciardi, S., Balleydier, E., Pages, F., Laudisoit, A., Dellagi, K. and Tortosa, P. 2014. Fleas of small mammals on Reunion Island: diversity, distribution and epidemiological consequences. PLoS Negl Trop Dis, 8**,** e3129.

Henry, I., Chemaly, M., Weill, F. X., Granier, S., Lalande, F., Courtillon, C., Salvat, G. and Cardinale, E. 2015. Epidemiological analysis of *Salmonella enterica* serovar

Typhimurium and serovar 1,4,[5],12:i:- isolates determined by pulsed field gel electrophoresis and antibiotic susceptibility: comparison of isolates from broiler chickens, humans and the environment in Reunion Island. The Open Veterinary Science Journal, 9**,** 10-18.

Henry, I., Granier, S., Courtillon, C., Lalande, F., Chemaly, M., Salvat, G. and Cardinale, E. 2013. *Salmonella enterica* subsp. *enterica* isolated from chicken carcasses and environment at slaughter in Reunion Island: prevalence, genetic characterization and antibiotic susceptibility. Trop Anim Health Prod, 45**,** 317-26.

Hunter, S. B., Vauterin, P., Lambert-Fair, M. A., Van Duyne, M. S., Kubota, K., Graves, L., Wrigley, D., Barrett, T. and Ribot, E. 2005. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. J. Clin. Microbiol., 43**,** 1045-50.

International Organization for Standardization 2007. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp. AMENDMENT 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

Joseph, P. G., Yee, H. T. and Sivanandan, S. P. 1984. The occurrence of *Salmonellae* in house shrews and rats in Ipoh, Malaysia. Southeast Asian J Trop Med Public Health, 15**,** 326-30.

Kerouanton, A., Marault, M., Lailler, R., Weill, F. X., Feurer, C., Espie, E. and Brisabois, A. 2007. Pulsed-field gel electrophoresis subtyping database for foodborne *Salmonella enterica* serotype discrimination. Foodborne Pathog Dis, 4**,** 293-303.

Kilonzo, C., Li, X., Vivas, E. J., Jay-Russell, M. T., Fernandez, K. L. and Atwill, E. R. 2013. Fecal shedding of zoonotic food-borne pathogens by wild rodents in a major agricultural region of the central California coast. Appl. Environ. Microbiol., 79**,** 6337-44.

Kirchner, M., Marier, E., Miller, A., Snow, L., McLaren, I., Davies, R. H., Clifton-Hadley, F. A. and Cook, A. J. C. 2011. Application of variable number of tandem repeat analysis to track *Salmonella enterica* ssp. *enterica* serovar Typhimurium infection of pigs reared on three British farms through the production cycle to the abattoir. J Appl Microbiol, 111**,** 960-970.

Krawiec, M., Kuczkowski, M., Kruszewicz, A. and Wieliczko, A. 2015. Prevalence and genetic characteristics of *Salmonella* in free-living birds in Poland. BMC Vet Res, 11**,** 15.

Kruse, H., kirkemo, A. M. and Handeland, K. 2004. Wildlife as source of zoonotic infections. Emerging Infect. Dis., 10**,** 2067-72.

Lagadec, E., Gomard, Y., Guernier, V., Dietrich, M., Pascalis, H., Temmam, S., Ramasindrazana, B., Goodman, S. M., Tortosa, P. and Dellagi, K. 2012. Pathogenic *Leptospira* spp. in bats, Madagascar and Union of the Comoros. Emerging Infect. Dis., 18**,** 1696-8.

Liebana, E., Garcia-Migura, L., Clouting, C., Clifton-Hadley, F. A., Breslin, M. and Davies, R. H. 2003. Molecular fingerprinting evidence of the contribution of wildlife vectors in the maintenance of *Salmonella* Enteritidis infection in layer farms. J Appl Microbiol, 94**,** 1024-9.

Liesegang, A., Davos, D., Balzer, J. C., Rabsch, W., Prager, R., Lightfoot, D., Siitonen, A., Claus, H. and Tschape, H. 2002. Phage typing and PFGE pattern analysis as tools for epidemiological surveillance of *Salmonella enterica* serovar Bovismorbificans infections. Epidemiol. Infect., 128**,** 119-30.

Martinez, R., Sanchez, S., Alonso, J. M., Herrera-Leon, S., Rey, J., Echeita, M. A., Moran, J. M. and Garcia-Sanchez, A. 2011. *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* prevalence in an ocellated lizard (*Timon lepidus*) research center in Spain. Foodborne Pathog Dis, 8**,** 1309-11.

Meerburg, B. G., Jacobs-Reitsma, W. F., Wagenaar, J. A. and Kijlstra, A. 2006. Presence of *Salmonella* and *Campylobacter* spp. in wild small mammals on organic farms. Appl. Environ. Microbiol., 72**,** 960-962.

Meerburg, B. G. and Kijlstra, A. 2007. Role of rodents in transmission of *Salmonella* and *Campylobacter*. J Sci Food Agric, 87**,** 2774-2781.

Nkogwe, C., Raletobana, J., Stewart-Johnson, A., Suepaul, S. and Adesiyun, A. 2011. Frequency of detection of *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. in the faeces of wild rats (*Rattus* spp.) in Trinidad and Tobago. Vet Med Int, 2011**,** 686923.

Olson, T. A. and Rueger, M. E. 1950. Experimental transmission of *Salmonella* Oranienburg through cockroaches. Public Health Rep, 65**,** 531-40.

Palmgren, H., McCafferty, D., Aspan, A., Broman, T., Sellin, M., Wollin, R., Bergstrom, S. and Olsen, B. 2000. *Salmonella* in sub-Antarctica: low heterogeneity in *Salmonella* serotypes in South Georgian seals and birds. Epidemiol. Infect., 125**,** 257-62.

Ribot, E. M., Fair, M. A., Gautom, R., Cameron, D. N., Hunter, S. B., Swaminathan, B. and Barrett, T. J. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis, 3**,** 59-67.

Rose, N., Beaudeau, F., Drouin, P., Toux, J. Y., Rose, V. and Colin, P. 2000. Risk factors for *Salmonella* persistence after cleansing and disinfection in French broiler-chicken houses. Prev Vet Med, 44**,** 9-20.

Seepersadsingh, N. and Adesiyun, A. A. 2003. Prevalence and antimicrobial resistance of *Salmonella* spp. in pet mammals, reptiles, fish aquarium water, and birds in Trinidad. J Vet Med B Infect Dis Vet Public Health, 50**,** 488-493.

Shimi, A., Keyhani, M. and Hedayati, K. 1979. Studies on salmonellosis in the house mouse, *Mus musculus*. Lab Anim, 13**,** 33-4.

Singh, S. P., Sethi, M. S. and Sharma, V. D. 1980. The occurrence of *salmonellae* in rodent, shrew, cockroach and ant. Int J Zoonoses, 7, 58-61.

Smith, W. A., Mazet, J. A. and Hirsh, D. C. 2002. *Salmonella* in California wildlife species: prevalence in rehabilitation centers and characterization of isolates. J Zoo Wildl Med, 33, 228-35.

Stoddard, R. A., DeLong, R. L., Byrne, B. A., Jang, S. and Gulland, F. M. 2008. Prevalence and characterization of *Salmonella* spp. among marine animals in the Channel Islands, California. Dis Aquat Organ, 81, 5-11.

Struelens, M. J. 1996. Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clin. Microbiol. Infect., 2, 2-11.

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. and Swaminathan, B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol., 33, 2233-9.

Tessier, C., Atiana, L., Cardinale, E. and Denis, M. Year. Longitudinal investigation of *Salmonella* spp. from farm to fork in the pig industry in Reunion Island. *In:* 10th International Conference on the Epidemiology and Control of Biological, Chemical and Physical Hazards in Pigs and Pork,, Septembre 9-12, 2013 2013a Portland, United-States. 106-109.

Tessier, C., Parama Atiana, L., Denis, M. and Cardinale, E. Year. Prevalence and risk indicators associated with *Salmonella* infection in farrow-to-finish farm in Reunion Island. *In:* 10th International Conference on the Epidemiology and Control of Biological, Chemical and Physical Hazards in Pigs and Pork, 2013b Portland, United-States. 110-113.

Umali, D. V., Lapuz, R. R., Suzuki, T., Shirota, K. and Katoh, H. 2012. Transmission and shedding patterns of *Salmonella* in naturally infected captive wild roof rats (*Rattus rattus*) from a *Salmonella*-contaminated layer farm. Avian Dis., 56**,** 288-94.

van der Wolf, P. J., Bongers, J. H., Elbers, A. R., Franssen, F. M., Hunneman, W. A., van Exsel, A. C. and Tielen, M. J. 1999. *Salmonella* infections in finishing pigs in The Netherlands: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection. Vet. Microbiol., 67**,** 263-75.

Wilkinson, D. A., Dietrich, M., Lebarbenchon, C., Jaeger, A., Le Rouzic, C., Bastien, M., Lagadec, E., McCoy, K. D., Pascalis, H., Le Corre, M., Dellagi, K. and Tortosa, P. 2014a. Massive infection of seabird ticks with bacterial species related to *Coxiella burnetii*. Appl. Environ. Microbiol., 80**,** 3327-33.

Wilkinson, D. A., Melade, J., Dietrich, M., Ramasindrazana, B., Soarimalala, V., Lagadec, E., le Minter, G., Tortosa, P., Heraud, J. M., de Lamballerie, X., Goodman, S. M., Dellagi, K. and Pascalis, H. 2014b. Highly diverse morbillivirus-related paramyxoviruses in wild fauna of the southwestern Indian Ocean Islands: evidence of exchange between introduced and endemic small mammals. J Virol, 88**,** 8268-77.

Figure Captions

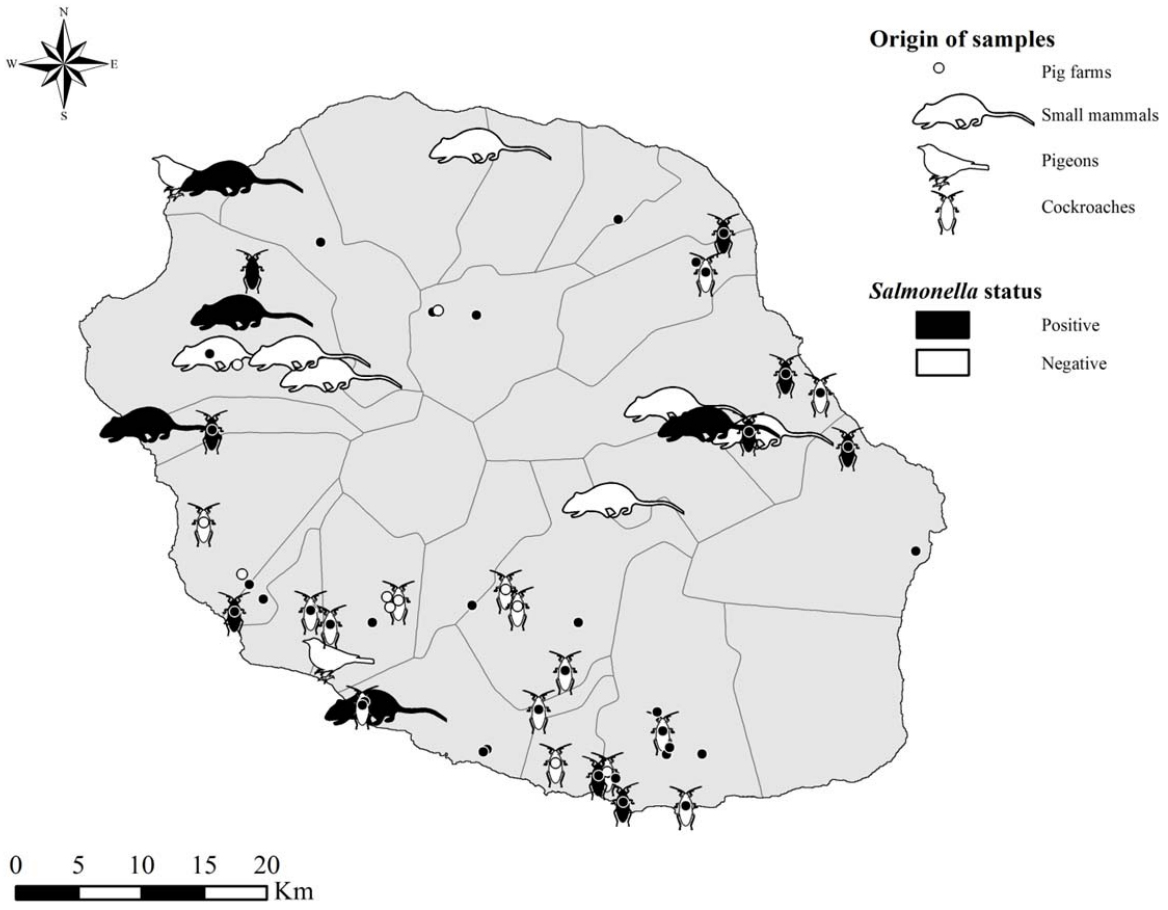


Figure 1. Localisation of the different sites sampled in Reunion Island and *Salmonella* status, January 2011 – January 2013. Pig farms (n=50) screened in a previous study are presented by a black spot if positive and a white spot if negative. Seabirds were not presented on the map since they were sampled at the rescue center.

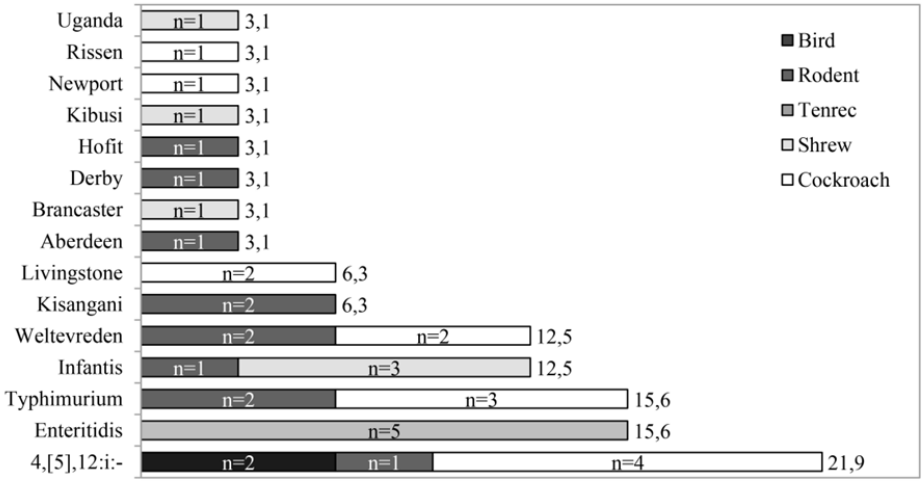
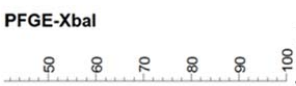


Figure 2. Number of *Salmonella* spp. positive samples (n=268) in wild fauna according to serotypes and origin of samples. The percentage (%) of each serotype and the number of positive samples per species is indicated respectively on the right and inside of the bars.



*Xba*I profile	*Salmonella* serotypes	Number of isolates per origin				
		Bird	Cockroach	Small Mammal	Pig	Total
KIS02	Kisangani			1[c]		1
KIS01	Kisangani			1[c]	1	2
DER02	Derby			1[c]		1
INF01	Infantis			2[d]/ 1[c]		3
INF02	Infantis			1[d]		1
KIB01	Kibusi			1[d]		1
LIV08	Livingstone		1			1
LIV02	Livingstone		1		7	8
WEL01	Weltevreden		2[b]		6	8
WEL09	Weltevreden			1[c]		1
WEL05	Weltevreden			1[c]		1
ABE01	Aberdeen			1[c]		1
HOF01	Hofit			1[c]		1
UGA01	Uganda			1[d]		1
NEW01	Newport		1		1	2
TYP13	Typhimurium			1[c]		1
TYP05	Typhimurium			1[c]	1	2
TYP08	Typhimurium		1		1	2
TYP06	Typhimurium		2[b]		1	3
MON15	4,[5],12:i:-		1[b]		3	4
MON19	4,[5],12:i:-	2[a]			2	4
MON05	4,[5],12:i:-		1		1	2
MON10	4,[5],12:i:-		1		1	2
MON09	4,[5],12:i:-		1[b]	1[c]	4	6
RIS02	Rissen		1[b]		6	7
BRA01	Brancaster			1[d]		1
ENT01	Enteritidis			5[e]	1	6
Total		2	13	22	36	73

Figure 3. Dendrogram of *Xba*I profiles of *Salmonella* spp. isolated from wild fauna in Reunion Island and from a selection of strains from pig farms. The number of isolates of each origin (bird, cockroach, small mammals and pig) was shown in the right side. [a]: isolated from White-tailed tropicbird and Audubon's shearwater, [b]: isolated from cockroach in farm with pig isolates sharing the same *Xba*I profile, [c]: isolated from rat, [d]: isolated from shrews, [e]: isolated from tenrecs.

Tables

Table 1. Number of collected samples and number of *Salmonella*-positive samples in wild fauna of Reunion Island.

Type of samples	No. samples	No. (%) of positive samples	95% CI
Insects – Cockroach	**44**	**11 (25)[a]**	**12.2-37.8**
Small mammals	**162**	**19 (11.7)**	**6.8-17.7**
Mouse (*Mus musculus)*	13	0 (0)	0-20.6
Rat (*Rattus rattus, Rattus norvegicus)*	121	9 (7.4)	2.8-12.1
Shrew (*Suncus murinus)*	23	5 (21.7)	9.9-43.7
Tenrec (*Tenrec ecaudatus)*	5	5 (100)	-
Wild birds	**62**	**2 (3.2)**	**1.1-11.2**
Audubon's Shearwater (*Puffinus lherminieri)*	10	1 (10)	2.2-44.5
Barau's Petrel (*Pterodroma baraui)*	19	0 (0)	0-14.6
Pigeon (*Columba livia)*	30	0 (0)	0-9.5
White-tailed Tropicbird (*Phaethon lepturus)*	3	1 (33.3)	4.4-90.6

[a]samples of pooled cockroaches