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# First epidemiological data on pathogenic leptospires isolated on the Azorean islands

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**Abstract.** Insectivores (*Erinaceus europaeus*) and rodents (*Rattus rattus*, *R. norvegicus* and *Mus musculus*) from different islands of the Azores Archipelago were found to carry three distinct *Leptospira interrogans* s.l. serovars (*copenhageni*, *icterohaemorrhagiae* and *ballum*) which have never been previously investigated there. The house mouse and the black rat were the major *Leptospira* reservoirs showing isolation rates ranging from 0% for both species (in Graciosa) to 88% and 33%, respectively (in São Miguel). This study also showed that the majority of the animals with positive kidney cultures exhibited specific agglutinins against the isolated strains of *Leptospira*. The observed isolation rates in the different islands, with a very interesting island variation in prevalence, suggest that small mammals, serving as sylvatic reservoirs of pathogenic leptospires, may represent an important risk to the health of humans and livestock, particularly in the islands of Terceira and São Miguel.

**Key words:** Azorean small mammals, Epidemiology, Insular syndrome, Isolates, Leptospirosis, Zoonosis

**Abbreviations:** GMT = geometric mean titre; MAT = microscopic agglutination test; MCAs = monoclonal antibodies; sv = serovar; s.l. = sensu lato

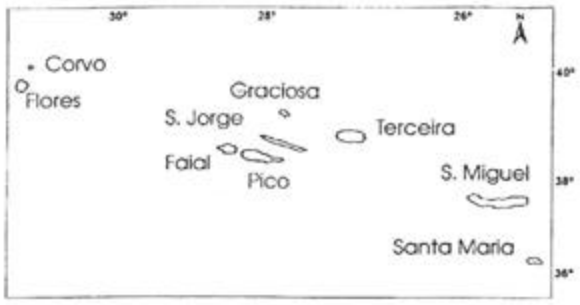
## Introduction

Leptospirosis is a zoonotic disease caused by pathogenic spirochetes that are worldwide distributed. Small mammals, especially rodents, are the main reservoirs. Since the majority of commensal species are ubiquitous and opportunistics, and produce large numbers of offspring, they may play a major role in the dispersion and transmission of leptospires directly to humans or livestock.

In the Azores Archipelago, the occurrence of human leptospirosis was already clinically recognized through the evidence of symptoms similar to those of Weil's syndrome but could not be confirmed due to the lack of adequate laboratory support. Only recently the first serological evidence of both human [1, 2] and bovine infections [3, 4] have been recorded for Terceira Island and thus, little is known about the epidemiology of leptospirosis on the Archipelago. The predominant serogroup then reported in hospitalized cases (3 in 1986 [1] and 9 in 1989 [2]) was Icterohaemorrhagiae whereas the most important group in bovines was Sejroe (serovars *saxkoebing* and *hardjo*) accounting for the majority of positive seroreactions (11 in 12). The recent isolation of two *hardjo* strains from slaughtered cattle in Terceira island (2 out of 20 animals) confirms for the first time the existence of renal carriers for this serovar in Azorean bovines (M. Collares-Pereira, personal communication). To clarify the epidemiology of leptospirosis on the Azorean Islands insight is needed into the occurrence of locally circulating strains of *Leptospira* and their reservoir-hosts. Also, the obtention of local isolates is always very important for serosurveys considering their strongest antigenic reactivity when compared to the reference serovars [5]. We describe in this paper the isolation and antigenic typing of Azorean strains and their ecological relationships.

## Materials and methods

*Azores: geography, geophysics and climate.* The Azores is a group of North Atlantic islands administrated independently of the metropolitan territory of Portugal. Lying about 1,584 km west of Lisbon, the Archipelago is formed by nine volcanic islands situated on the mid Atlantic ridge (Map 1). Nowadays over 260,000 people live on these islands




**Map 1.** Azores Islands.

[6] which cover a total area of 2,340 km$^2$. About 50% of the inhabitants live on the islands of São Miguel and Terceira.

Vegetation was originally luxuriant, but has been strongly modified by human interference mainly through agriculture and cattle breeding. Most of the autochthonous vegetation areas of the Archipelago (dominated by Azorean or Macaronesian endemic arboreous species) are restricted to high altitudes.

The climate of the Azores is characterized by low thermal amplitude and high precipitation and humidity. The mean annual temperature is 17.5 °C, with a maximum (25 °C) in August and a minimum (10 °C) in February. The mean annual rainfall ranges from 752.3 mm in Santa Maria (airport) and 2,385.9 mm in Terceira (Carvão) [7]. At high altitudes, a 'cloud-zone forest', where the rainfall is high, leaves the air saturated with moisture and the soil water-logged [6].

*Mammal species.* The Azorean terrestrial mammal fauna comprises two native species of bats (*Myotis myotis* and *Nyctalus azoreum*) and accidentally or deliberately introduced species, either wild (one insectivore, *Erinaceus europaeus*; one lagomorph, *Oryctolagus cuniculus*, three rodents – the house mouse, *Mus musculus*, the black rat, *Rattus rattus*, and the brown rat, *R. norvegicus*, and one carnivore, the weasel, *Mustela nivalis*) or feral (two carnivores, the ferret, *Mustela furo*, and the domestic cat, *Felis catus*).

*Trapping.* Over a three year period, three expeditions were conducted in June/July to the islands of São Miguel and Terceira (1993–95), Flores (1994–95) and Graciosa, Pico and São Jorge (1995) by a multi-disciplinary research team.

The intention of this account was to obtain a larger representation of the studied population in the three different insular groups (western, central and eastern) by investigating at least one island from each group. The delay in sampling the western island of Flores was due to the interest of studying the two crowded islands where there was previous clinical information on human leptospirosis cases.

In each island, the sampling sites were chosen considering altitude, vegetation and man's use of landscape. Using Longworth, Sherman and 'Firobins' live-traps, a total of 46 (in 1993), 113 (in 1994) and 124 (in 1995) animals were trapped, consisting of 14 *E. europaeus*, 23 *R. norvegicus*, 87 *R. rattus*, 157 *M. musculus*, 1 *Mustela nivalis* and 1 *M. furo*. Rodents, particularly *R. rattus* and *M. musculus*, were the predominant species and showed a wide dispersion, either in man-made or natural habitats. Both mustelids were captured in Terceira, in 1993.

All animals were sexed, aged and measured (weight and lengths of head and body, tail, hind-foot and ear). They were grouped into two age classes – adults-subadults and juveniles – according to their body size and breeding condition. Large and scrotal testes in males and perforated vagina, pregnancy and lactation conditions in females have been considered to indicate sexual activity and, therefore, an adult animal. The two *Rattus* species were identified according to their distinctive karyotype or skull morphology.

Animals were taken to the laboratory and killed with ether as soon as possible after their capture. Blood samples and organs were collected immediately after death, being aseptically removed and treated for serology and culturing, respectively.

*Serology and culturing.* A blood sample was obtained from the heart or the orbital vein of each animal and centrifuged. Only about 70% of the sera was available for *Leptospira* purposes, because of the simultaneous research on other zoonotic agents within the project. The sera were stored at –20 °C until examination by the microscopic agglutination test (MAT) using a standard microtitre technique [8]. Nineteen serogroups were represented by a battery of reference serovars used as live culture antigens; these were *australis* (Australis serogroup); *autumnalis* (Autumnalis); *ballum*, *arborea* and *castellonis* (Ballum); *bataviae* (Bataviae); *canicola* (Canicola); *celledoni* (Celledoni); *cynopteri* (Cynopteri); *djasiman* (Djasiman); *grippotyphosa* (Grippotyphosa); *hebdomadis* (Hebdomadis); *copenhageni* and *icterohaemorrhagiae* (Icterohaemorrhagiae); *javanica* (Javanica); *louisiana* (Louisiana); *mini* (Mini); *panama* (Panama); *mozdok* and *pomona* (Pomona); *pyrogenes* (Pyrogenes); *hardjo*, *saxkoebing*, *sejroe* and *wolffi* (Sejroe); and *tarassovi* (Tarassovi).

Sera were screened at a dilution of 1:40 and those which were reactive at this dilution were further titrated to the end point. Animals with titres equal to or higher than 1:40 were regarded as positive in this study.

Each kidney was homogenized in a sterile Stomacher plastic bag containing 10 to 30 ml of a 1% bovine serum albumin diluent (BSA, Sigma). After leaving the suspension to settle down at room

temperature for at least one hour, each supernatant was cultured using a serial dilution technique (1:10) in liquid EMJH (Difco, refs. 0794-01 and 0795-72) [9, 10] and finally cultured in semi-solid EMJH containing noble agar (0.1%; Sigma), 5-fluoro-uracil (200 µg/ml; Sigma) and rabbit serum (5%; Izasa) (only two to three drops of each dilution). The cultures were incubated at 30 °C for up to 20 weeks. Reading and culture manipulation were carried out as described in the literature [5, 8].

*Characterization of isolates.* Isolates were subcultured into liquid EMJH medium to get a density suitable for use in the microscopic agglutination test (MAT) with 23 standard antisera (group sera) to determine serogroup affinities [11].

The identification is based on serological criteria of evaluating agglutination of a *L. interrogans* s.l. culture ($2 \times 10^8$ cells/ml) with an equal volume of hyperimmune reference anti-rabbit serum at increasing dilutions and was carried out in the Institute of Hygiene and Tropical Medicine, in Lisbon.

For serovar identification with monoclonal antibodies (MCAs), all the isolates of the first and second year were sent to the Royal Tropical Institute in Amsterdam; those concerned with the last expedition were only identified at serogroup level. As for DNA analysis a much smaller number of strains (only those of the first year) was sent to Pasteur Institute in Paris (France) and in Noumea (New Caledonia). We will report here on the MCA results.

*Production of monoclonal antibodies* (MCAs). Monoclonal antibodies were produced according to standard techniques as described earlier [12]. Briefly, BALB/c mice were immunized by injecting (i.p.) live leptospires of the following serovars: fusion 12 serovar *copenhageni* (serogroup Icterohaemorrhagiae, strain Wijnberg), fusion 52 serovar *mankarso* (serogroup Icterohaemorrhagiae, strain Mankarso), fusion 70 serovar *copenhageni* (serogroup Icterohaemorrhagiae, strain M 20), fusion 89 serovar *ndambari* (serogroup Icterohaemorrhagiae, strain Ndambari) and fusion 74 serovar *castellonis* (serogroup Ballum, strain Castellon 3).

Monoclonal antibodies of the first four fusions were used to characterize the reference strains for serovars of the Icterohaemorrhagiae serogroup and the Azorean isolates. MCA F12C3 was highly specific for serovars *icterohaemorrhagiae* and *copenhageni*. MCAs F52C1 and F70C7 both were serogroup specific monoclonals. MCA F70C14 was specific for *icterohaemorrhagiae* and F70C24 specific for *copenhageni*. MCA F89C12 reacted to the whole Icterohaemorrhagiae serogroup serovars except for serovar *icterohaemorrhagiae* [13–15].

Four monoclonal antibodies derived from fusion number 74 were used to identify the reference strains for Ballum group serovars *arborea*, *ballum* and *castellonis* and the Azorean isolates. MCA F74C1 was positive for all of them. MCAs F74C4 and F74C12 were positive for *ballum* and *castellonis*, while MCA F74C7 was serovar specific.

*Statistical analysis.* Statistical analysis of data for association between variables was undertaken by the $\chi^2$ test with Yates's correction for continuity whenever the values in a fourfold table were fairly small [16]; the results were considered significant for $p$ values $< 0.05$. Only the most abundant species in captures, *R. rattus* and *M. musculus*, have been considered for this purpose. The geometric mean titre (GMT) of serological reactions was determined according to Sokal & Rohlf [17] and represented as a reciprocal of the value.

## Results

Leptospires were isolated from 79 small mammals (27.9%) with the following species distribution: *E. europaeus* (3 positives in 14, 21.4%), *R. norvegicus* (5 in 23, 21.7%), *R. rattus* (18 in 87, 20.7%) and *M. musculus* (53 in 157, 33.8%) (Table 1). Reservoirs, particularly rodents, were found in different habitats and altitudes, ranging from sea level up to 750 meters. The two carnivores showed both cultural and serological negative results.

No significant statistical differences ($p > 0.05$) in infection rates between males and females were

**Table 1.** Sex and age distribution and positivity rate (%) of culturally positive (Pos) animals in the whole population (1993–1995)

| Wild mammal | Sex | | Age | |
|---|---|---|---|---|
| | Males Pos/N (%) | Females Pos/N (%) | Adults-subadults Pos/N (%) | Juveniles Pos/N (%) |
| *Erinaceus europaeus* | 3/8 (37.5) | 0/6 | 3/11 (27.3) | 0/3 |
| *Rattus norvegicus* | 5/14 (35.7) | 0/9 | 4/17 (23.5) | 1/6 (16.7) |
| *Rattus rattus* | 11/51 (21.6) | 7/36 (19.4) | 17/70 (24.3) | 1/17 (5.9) |
| *Mus musculus* | 30/87 (34.5) | 23/70 (32.9) | 50/136 (36.8) | 3/21 (14.3) |
| Total | 49/160 | 30/121 | 74/234 | 5/47 |

observed in *R. rattus* ($\chi^2$ = 0.038, 1 df) or *M. musculus* ($\chi^2$ = 0.023, 1 df) and similarly no significant difference ($p > 0.05$) was found between age classes of *R. rattus* ($\chi^2$ = 2.823, 1 df). However the percentage of isolated leptospires in *M. musculus* was significantly higher in adults-sub-adults than in juveniles ($\chi^2$ = 4.110, 1 df; $0.05 < p < 0.01$). Consequently, both sexes of either species were combined together in further analysis and the age differences amongst house mice were taken into consideration.

The highest isolation rates of *Leptospira interrogans* s.l. infection occurred in São Miguel (53.1%) and Terceira (45.8%) islands; there were no positive cultures in Graciosa (Table 2). A significant difference on the frequency of isolates between the islands of Flores, Pico and São Jorge was respectively confirmed with the island of São Miguel but not Terceira, which was only significantly different from Flores (Table 3). All isolates were identified at serogroup level as belonging to Ballum (n = 63; 79.7%) or Icterohaemorrhagiae (n = 16; 20.3%) groups. Within the first group, 45 isolates were typed with monoclonal antibodies as serovar *arborea*; they were obtained from *M. musculus* (n = 38) and *R. rattus* (n = 7) (Table 4). The five Icterohaemorrhagiae group isolates, which were typed at serovar level as *copenhageni* or *icterohaemorrhagiae* serovars, were respectively obtained from *E. europaeus* (n = 3) and *R. rattus* (n = 2) (Table 5). The remaining eleven

**Table 2.** Isolation rate of *Leptospira* infection in wild mammals from six Azorean islands

| Wild mammal | São Miguel Pos/N (%) | Terceira Pos/N (%) | Flores Pos/N (%) | Graciosa Pos/N (%) | Pico Pos/N (%) | São Jorge Pos/N (%) |
|---|---|---|---|---|---|---|
| *Erinaceus europaeus* | 3/11 (27.3) | 0/3 | * | * | * | * |
| *Rattus norvegicus* | 2/3 (66.7) | 2/5 (40.0) | 0/7 | – | 0/2 | 1/6 (16.7) |
| *Rattus rattus* | 6/18 (33.3) | 12/53 (22.6) | 0/6 | 0/4 | 0/2 | 0/4 |
| *Mus musculus* | 15/17 (88.2) | 35/44 (79.5) | 2/43 (4.7) | 0/47 | 1/6 (16.7) | – |
| Total | 26/49 (53.1) | 49/107 (45.8) | 2/56 (3.6) | 0/51 (0.0) | 1/10 (10.0) | 1/10 (10.0) |

* Species not reported yet.

**Table 3.** Chi-square values (1 df) of *Leptospira* isolation frequencies between islands

| | Terceira | Flores | Pico | São Jorge |
|---|---|---|---|---|
| São Miguel | $\chi^2$ = 0.450<br>($p$ = 0.5025)<br>NS | $\chi^2$ = 30.249<br>($p$ = 3.808E-08)<br>S | $\chi^2$ = 4.591<br>($p$ = 0.0321)<br>S | $\chi^2$ = 4.591<br>($p$ = 0.0321)<br>S |
| Terceira | | $\chi^2$ = 28.551<br>($p$ = 9.132E-08)<br>S | $\chi^2$ = 3.437<br>($p$ = 0.0637)<br>NS | $\chi^2$ = 3.437<br>($p$ = 0.0637)<br>NS |
| Flores | | | $\chi^2$ = 0.201<br>($p$ = 0.6536)<br>*NS* | $\chi^2$ = 0.201<br>($p$ = 0.6536)<br>*NS* |
| Pico | | | | $\chi^2$ = 0.556<br>($p$ = 0.4561)<br>NS |

NS = not significant; S = significant.

**Table 4.** Agglutination titres of monoclonal antibodies with Ballum serogroup reference strains and Azorean isolates

| Serovar/strain | Monoclonal antibodies | | | |
|---|---|---|---|---|
| | F74C1 | F74C4 | F74C12 | F74C7 |
| *arborea*/Arborea | 1:5120 | negative | negative | negative |
| *ballum*/Mus 127 | 1:10240 | 1:5120 | 1:2560 | 1:1280 |
| *castellonis*/Castellon 3 | 1:2560 | 1:5120 | 1:80 | negative |
| Azorean isolates (45×) | 1:1280 to 1:5120 | negative | negative | negative |

**Table 5.** Agglutination titres of monoclonal antibodies with two Icterohaemorrhagiae group reference strains and Azorean isolates

| Serovar/strain | F12C3 | F52C1 | F70C7 | F70C14 | F70C24 | F89C12 |
|---|---|---|---|---|---|---|
| *copenhageni*/M20 | 20480 | 5120 | 81920 | 80 | 40960 | 2560 |
| Azorean isolates (3×) | 20480 to 40960 | 1280 to 5120 | 40960 to 81920 | negative | 20480 to 40960 | 640 to 5120 |
| *icterohaemorrhagiae*/RGA | 10240 | 1280 | 40960 | 40960 | 160 | negative |
| Azorean isolates (2×) | 10240 to 20480 | 640 to 2560 | 40960 | 40960 | 40 to 320 | negative |

Icterohaemorrhagiae group strains were isolated from *R. rattus* (n = 5), *R. norvegicus* (n = 4) and *M. musculus* (n = 2). Such potential carriers for both serogroups were widely spread, in Terceira and São Miguel, either in man-made or natural habitats. As far as the other islands are concerned, there was one Icterohaemorrhagiae isolate in Pico (from a house mouse), in São Jorge (from a brown rat) and in Flores (together with one Ballum isolate; both from house mice).

From 74 animals with positive kidney cultures, all but 10 (13.5%) showed specific agglutinins with MAT titres ranging from 1:40 to 1:10,240 against Ballum (GMT = 385) and Icterohaemorrhagiae (GMT = 762) serogroup antigens. Of the remaining 136 (64.8%) serum samples from animals with negative kidney cultures, only 18 (13.2%) reacted predominantly against Ballum and Icterohaemorrhagiae strains at titres between 1:40 and 1:640 (GMTs = 145 and 94, respectively); there was one *E. europaeus* and one *R. norvegicus* with agglutination titres against Australis (1:40) and Sejroe (1:320), respectively.

The serological results largely concurred with the results obtained by culture.

## Discussion

The widespread occurrence of *Leptospira* infection in Azorean small mammals is evidenced in the present work. As far as we know, this is indeed the first report of bacteriological and serological findings in wild mammals of the Archipelago.

The current study shows the presence of *Leptospira interrogans* s.l. wild reservoir-hosts, particularly, in the islands of São Miguel (53.1%) and Terceira (45.8%). The obtained bacteriological rates deserve a special attention when compared to the much lower *Leptospira* prevalences recently reported in small mammals from other geographic regions, namely, 21% for *Rattus* spp., in Barbados [18] and 3% for *R. norvegicus* in the UK [19]. Three rodent species (*R. rattus*/*R. norvegicus*/*M. musculus*) appeared to be of main importance as natural leptospiral hosts in the Azores. The isolation of Ballum (sv. *arborea*) and Icterohaemorrhagiae (sv. *icterohaemorrhagiae*) serogroup strains in these wild carriers and their wide distribution is consistent with other findings elsewhere and also with the known affinity of these leptospires to these free-living species in natural and human introduced foci [20].

Moreover, the high number of Ballum isolates in house mice (*M. musculus*), particularly in adults-subadults, reflects the high degree of parasitic adaptation of the infecting strain (*arborea*) to this host. Indeed, Ballum infection in Azorean mice was almost entirely restricted to sexually-mature animals as was earlier reported by Hathaway, in New Zealand, for serovar *ballum* [21]. In this case, the association of transmission with sexual behavior, following the onset of puberty, ensures the perpetuation of a particular serovar, even in free-living populations at low density. This pattern of endemic infection in house mice was noticeable on both islands of São Miguel and Terceira, where the significant bacteriological rate of *arborea* infection (81.9%, i.e., 50 out of 61) was confirmed by a similar high rate of seroreactors to *arborea* (88.5%, i.e., 46 out of 52). It is also worthy to mention that, as a consequence of the use of recently isolated (local) antigens in the serological test, the specific seropositivity may actually have increased the prevalence of *Leptospira*.

The black rat (*R. rattus*) occurs in all the island habitat types whereas *R. norvegicus* is restricted to garbage dumps and other man-made habitats, e.g., urban areas. A higher isolation rate for serovar *arborea* was also more evident in *R. rattus* (12.6%) than in *R. norvegicus* (4.3%), which is a well confirmed preferential carrier for Icterohaemorrhagiae (17.4%), its main serogroup [18]. It is presumed that the higher prevalence of *arborea* infection in black rats in habitats they share with house mice is a result of house mice to rat transmission. This study showed that the black rat may become an important maintenance-host population too for serovar *arborea*, as was already reported in New Zealand for serovar *ballum* [21] and in the Caribbean islands for serogroup Autumnalis [22].

In the islands, available serological evidence of

Icterohaemorrhagiae and Ballum infections in the majority of human hospital cases, most of which rural workers, suggests that their distribution is largely related to the presence of rats and house mice, favoring the transmission. These two species are the most abundant among mammals on the islands and are predominantly carriers of respectively Icterohaemorrhagiae and Ballum svs.

In both islands of Terceira and São Miguel, the serological response to Ballum and Icterohaemorrhagiae renal infections was consistent with the evidence of higher geometric mean titres (around 1:450) than those obtained in culturally negative animals (around 1:146). This immune reaction, quoted sometimes as not being characteristic of infection in a maintenance host, could be explained by an hyper-antigenic stimulation of the immune system in the presence of highly susceptible rodent populations, when compared with those from mainland (M. Collares-Pereira, personal communication), and/or with optimal climatic conditions of transmission, namely moderate temperature and high humidity.

The hedgehog, *E. europaeus*, seems to be an important carrier of sv. *copenhageni* (Icterohaemorrhagiae group) in São Miguel, although it is often carrying Australis svs. [20, 23]. Possibly, larger samples could have shown different findings.

Not surprisingly, few reservoir-host species and few *Leptospira* serovars occurred on the islands in comparison with mainland Portugal. The reduced diversity of serovars (3 against 6 in mainland) [24] seems to be correlated with the minor diversity of rodents and insectivores observed in the islands (four species against twenty-one on mainland). These results can be analyzed in view of the 'insular syndrome' theory [25, 26], which predicts a niche expansion of native or introduced species in consequence of a higher adaptability favored by a reduced natural predation pressure, together with a restricted interspecific competition. In fact, only three commensal rodents have colonized the islands. Without being restricted to man-made environment, they were also found in natural areas that in mainland Portugal are inhabited by non-commensal species. The niche expansion of these few rodent species, together with the favorable climatic conditions, stimulates the widespread occurrence of few *Leptospira* serovars with increased rates of bacteriological infection.

Another interesting aspect is the difference in *M. musculus* infection rates between Terceira (80%) and São Miguel (88%), on the one hand, and Flores (5%) and Graciosa (0%) on the other hand. Graciosa is the most dry island of the Archipelago and the unfavorable climatic conditions probably reflect poor *Leptospira* transmission. Although this possibility is unproved, no better explanation is presently forthcoming, since our results are based on a single field sampling (June 1995). Besides, the evidence of Icterohaemorrhagiae seroreactors among local cattle (M. Collares-Pereira, personal communication) demands further analysis.

The case of Flores is different and more difficult to explain. The human impact on the ecosystems is quite the same in Terceira, São Miguel and Flores, at least in the core of the islands. Most of the natural ecosystems have been replaced by stands of *Cryptomeria japonica*, *Pittosporum undulatum* and eucalyptus or by cattle meadows fenced with stone walls or *Hydrangea macrophylla* hedges. Many natural laurel forests were also transformed into a shrubland dominated by *Erica azorica*. Nevertheless, major differences among islands are noticeable at their periphery where all the cities and villages are concentrated. This belt area is more sunny, a little bit drier and, above all, more hot than the centre. These conditions are more favorable to the house mouse, involving perhaps the development of higher densities of rodents and therefore increasing the number of contacts between individuals. In Flores however, the belt area is very narrow and not densely populated by humans (32 inhabitants/km$^2$ over more than 100 in the islands of São Miguel and Terceira). This situation leads to lower densities of the house mouse which, in association with the quick increase in altitude towards the center of the island and consequently with very low temperatures, prevents their widespreading. Also, few inhabitants involves much less economic trade and thus a lower probability for house mice and rats, *Leptospira* infected or not, to reach such a remote island.

It should be noticed that the results obtained in a few selected communities are not necessarily representative at an Archipelago level. Nevertheless, this survey points out the occurrence of leptospires in the Azores Archipelago. With a warm, wet climate and an important density of small mammals, particularly rodents, the islands surely provide a good environment for the survival of leptospires outside their hosts. Rats, being abundantly present in plantations, forests and urban areas, along with house mice, constitute the most important wild carriers of leptospires. Therefore, it is necessary to consider these reservoir-hosts as potential hazards for the health of man and domestic animals, especially in the two densely populated islands of São Miguel and Terceira.

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