

Research Article

Leptospirosis in Wild Mice: An Ongoing Concern for the Azorean Islands

Camarinho R^{1,2*}; Nhampule Y¹; Garcia PV^{1,3}; Rodrigues AS^{1,2}

¹Faculty of Sciences and Technology, University of the Azores, Portugal

²IVAR – Instituto de Vulcanologia e Avaliação de Riscos, University of the Azores, Portugal

³CE3C – cE3c, Centre for Ecology, Evolution and Environmental Changes /Azorean Biodiversity Group, University of the Azores, Portugal

***Corresponding author: Camarinho R**

IVAR – Instituto de Vulcanologia e Avaliação de Riscos, University of the Azores, Rua da Mãe de Deus, Apartado 1422, 9501-801 Ponta Delgada, Açores, Portugal.

Tel: +351 296650460; Fax: +351 296650100

Email: ricardo.ad.camarinho@uac.pt

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Introduction

Leptospirosis (also called Weil's disease, after the person who first described the pathogenic organism in 1886) is a bacterial zoonosis caused by the spirochetes of the genus *Leptospira* [8]. This disease affects Human and animal populations worldwide, and the infection can occur through direct contact with urine or tissues of infected animals, or indirectly through exposure to infected wet soil or water [14]. In animals, leptospirosis can cause abortion, stillbirth, weakness among neonates, reduced milk production in dairy farm animals, and death. In humans, clinical manifestation of the disease may vary from flu-like symptoms to multiple organ failure and death with some reports of diarrhoea, vomiting, myalgia and kidney failure [14,5]. Certain professions such as abattoir workers, banana cutters, cattle farmers and sewer workers, have increased risk of leptospirosis. Similarly, outdoor activities such as swimming, hunting and fishing, also present higher risk. The occurrence of this disease is strongly influenced by climate, indigenous fauna and agricultural practices [6]. The organism survives well in warm, moist conditions, and

Abstract

Background: Leptospirosis is a worldwide zoonotic infectious disease that affects Human and animals worldwide with increased risk in regions with temperate climates. Such is the case of the Azorean islands (a Portuguese archipelago with 9 islands) where this disease is considered endemic. Rodents of these islands have been considered the reservoir species for *Leptospira* and regulating measurements have been previously developed to control and prevent this disease in the archipelago. This study aims to determine a more recent infection rate of leptospirosis in the house-mouse (*Mus musculus*).

Methodology & Findings: This retrospective study covering a period of 8 years (2011 to 2018), was performed by live capturing *Mus musculus* in 6 locations of S. Miguel Island (the most populated island of the archipelago). After euthanasia and necropsy of the mice, kidneys were surgically removed and processed in standard histological routine, and slides were stained with modified Warthin-Starry silver stain for spirochetes and scanned for the presence of Leptospire. It was possible to determine that from the 133 wild mice captured, 89 were positive for leptospirosis, representing a 66.91% infection rate.

Conclusion: This study reveals that leptospirosis still represents an increased risk to Human lives and an uncalculated economical loss to the dairy farms of the Azorean islands.

Keywords: Leptospirosis; *Mus musculus*; Azores; Histological identification

hence the disease is endemic to many tropical countries. Such is the case of the Azores: a nine island archipelago located in the North Atlantic Ocean (Figure 1). Around 242.000 people live in these islands, which cover around 2340 km². On account of the tropical temperate climate, leptospirosis has been a permanent concern and considered an endemic problem [15], particularly in the island of S. Miguel and Terceira (on which around 70% of Azoreans reside), on account of increased cattle infection and several fatal human cases [5,7].

Rodents have previously been established as the main wild reservoirs and carriers of *Leptospira* [3,11] and although they exist in all Azorean islands, their populations haven't received enough attention [9]. Nonetheless a survey of Collares-Pereira (1997) determined a very high infection rate (88%) of *Leptospira* in the house mouse (*Mus musculus*), with other species of rodents with smaller infection rate [black Rats (*Rattus rattus*) – 33%; and brown rats (*Rattus norvegicus*) – 67%]. Facing

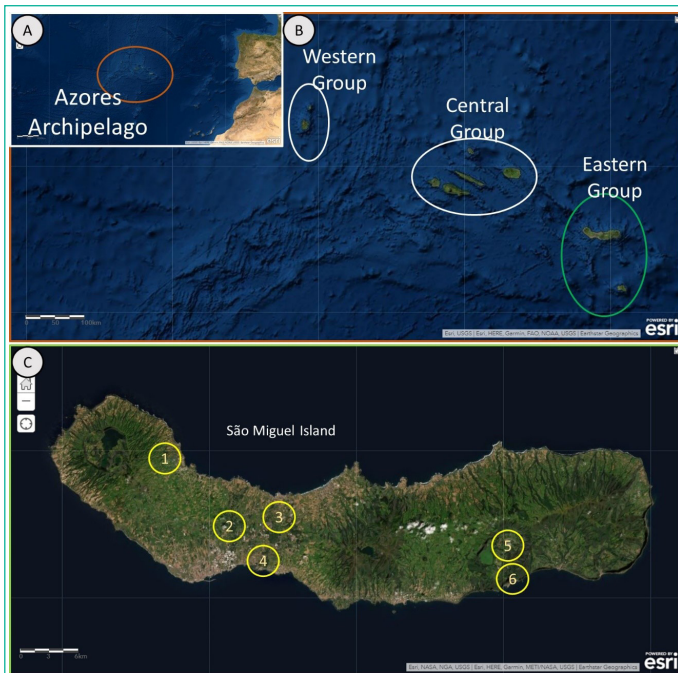


Figure 1: Map of the Azores Archipelago. A- Location of the Azores archipelago in the Atlantic Ocean; B- Geographical groups of the Azores archipelago; C- Map of S. Miguel Island and study areas; 1- An organic farm from Santo Antonio Village; 2- Leisure area of Pinhal da Paz; 3- Leisure areas of Rabo de Peixe Village; 4- Conventional farm from the city of Lagoa; 5- Rural areas of Furnas Village; 6- Rural areas of Ribeira Quente Village. Basemap aerial view backgrounds by ESRI ArcGIS online. "World Imagery" [basemap]. "World Imagery Map". Last updated 11/06/2020. <https://www.arcgis.com/home/item.html?id=10df2279f9684e4a9f6a7f08febac2a9>. Attribution information to both ESRI and other data providers shown in the figure.

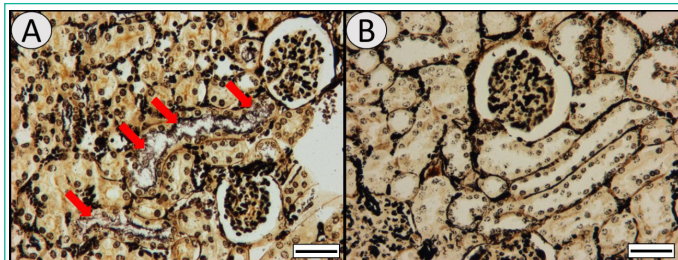


Figure 2: Histochemical stain for spirochetes of kidney histological sections of captured mice and processed according to the modified protocol of Warthin-Starry (1988). The red arrows point to spirochetes. A- Mice positive for Leptospirosis; B - Mice negative for Leptospirosis. Scale bar = 25µm.

high infection rates in cattle and loss of human lives, the Azorean Government proposed a project to reduce human cases and reduce infection rates of wild and domestic animals. The proposed actions included a better understanding of the ecology of Leptospirosis and their reservoir species, improvement of farming techniques and infrastructures to ultimately allow a sustained reduction of Leptospirosis. Although the absolute eradication of Leptospirosis is an impossible task [16], proper prevention and control can reduce the infection rates in both humans and domestic animals [12].

The aim of this study was to access the infection rates of *Leptospira* in the most common house mouse (*Mus musculus*) for the island of São Miguel.

Material and Methods

Ethical Statements

All procedures were carried out in strict accordance with

the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123: directive 2010/63/EU) and the Portuguese Law Decree (DL 113/2012). This study was approved by the University of Azores ethics committee (10/2020).

Source of Specimens

A total of 133 wild *Mus musculus* were live captured from 2011 to 2018 throughout the Island of S. Miguel in 6 locations [Figure 1: 1- An organic farm from Santo Antonio Village (n=18); 2- Leisure area of Pinhal da Paz (n=26); 3- Leisure areas of Rabo de Peixe Village (n=31); 4- Conventional farm from the city of Lagoa (n=23); 5- Rural areas of Furnas Village (n=18); 6- Rural areas of Ribeira Quente Village (n=17)]. Only wild mice (males and females) with more than 10 grams were selected for this analysis. The selection of locations took in consideration the choice of two different farms, two leisure areas and two villages, and their separate locations in order to maximize the analysed area of S. Miguel.

After live-capture, wild mice were housed no longer than necessary until euthanasia. Euthanasia was performed using Isoflurane and followed by necropsy. Right and left kidneys were surgically removed and fixed in 4% buffered formaldehyde and followed standard histological routine for paraffin embedding.

Histopathology

All histological blocks were depleted through serial 4 µm sections, making several slides per individual ensuring a thorough inspection. Histological diagnosis was done on all slides stained with Warthin-Starry silver staining technique [13], modified for the use of a microwave oven by Churukian and Schenk (1988), and scanned for the presence of Leptospirosis (Figure 2).

Results and Discussion

In this retrospective study covering a period of 8 years, it was possible to determine that from the 133 wild mice captured, 89 were positive for leptospirosis, representing a 66.91% infection rate. Per locations, the infection rate was: 1- An organic farm from Santo Antonio Village - 77.78%; 2- Leisure area of Pinhal da Paz - 69.23%; 3- Leisure areas of Rabo de Peixe Village - 74.19%; 4- Conventional farm from the city of Lagoa - 43.47%; 5- Rural areas of Furnas Village - 66.67%; 6- Rural areas of Ribeira Quente Village - 70.59% - (Figure 1).

The relatively poor sensitivity of histological stain methods for leptospires has been previously reported [1,10], and although further developments throughout the years of the histological techniques enhanced the staining quality [2], the results presented in this study are most likely an under-representation of Leptospirosis infection rate, further demonstrating an unknown risk for the Human and animal populations of S. Miguel Island.

Regarding the presented results, the lowest infection rate is in the conventional farming area from the city of Lagoa (43.47%), which is more likely due to the increased usage of conventional pesticides that most likely prevent and control the leptospires in the ground, where it is known to endure up to 6 weeks in moist and warm conditions [14]. Also, the usage of rodenticide is more common in these types of farming. Contrarily, the highest infection rates were found in the organic farm from Santo Antonio, where pesticides are forbidden, and chemical rodent control is kept to a minimum. Although the

farms represent an increased risk to farmers, the leisure areas can represent an even greater risk due to the increased number of people visiting those places, specially Pinhal da Paz (69.23%), where children are allowed extended playtime on the grass and structures of the place. Although saving Human lives is the primary concern, this study also highlights the welfare of cattle and other domestic or farm animals. Given that the main sector of the Azorean's economy is the dairy industry, contributing 30% to national milk production, and no study has ever been performed to calculate cattle leptospirosis infection rates, there are unaccounted economical losses that should be addressed in the near future. Although the previous Azorean project by Collares-Pereira (2009) sought to control and contain the spread of leptospirosis, this retrospective study urges the need of an enforcement in the several areas suggested, such as vaccination, rodent populational control and the use of proper protective gear when dealing with potentially infected humans or animals, in order to prevent loss of Human lives and the uncalculated economical losses to the dairy farms that are the major economy of the Azorean Islands.

Conclusion

The result of this study reveals that although the leptospirosis infection rate may be under-represented, it still represents a high risk for Human lives and an uncalculated economical loss in the Azorean islands. Additionally, vaccination and rodent controls measurements should be required in order to control leptospirosis. Lastly, further studies should be conducted in this area to contain leptospirosis threat and also calculate the possible economical losses.

Author Statements

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