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Serological evidence of *Leptospira* sp. in humans from Fernando de Noronha Island, Brazil☆

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ABSTRACT

The prevalence of leptospirosis in humans is highly variable, being influenced by climatic factors, the presence of reservoirs, occupational exposure, recreational activity, and socioeconomic conditions. The objective of this study was to estimate the prevalence of *Leptospira* sp. and identify the predominant human serovars on the island of Fernando de Noronha, Brazil, based on a microscopic agglutination test. The prevalence of anti-*Leptospira* antibodies was 1.17% (4/341; I.C. 0.46%–2.98%), with the predominance of serovars Icterohaemorrhagiae, Javanica, Mini and Louisiana. This is the first study on the occurrence of antibodies against *Leptospira* sp. in humans in Fernando de Noronha and highlights the need to implement control and prevention strategies in this island environment.

1. Introduction

Leptospirosis is a neglected zoonotic disease caused by bacteria of the genus *Leptospira*. [1], comprising more than 260 serovars of pathogenic and intermediate species and responsible for affecting more than 1 million individuals a year in developed and developing countries [2–4]. Leptospirosis causes severe symptoms such as fever, headache, vomiting, pneumonia, meningitis, uveitis, Weil's syndrome, and multiple organ failure [5,6]. These clinical signs make it difficult to diagnose other tropical febrile diseases, like dengue and chikungunya, especially in island settings where access to health services is limited and disease awareness is low [7].

In the archipelago of Fernando de Noronha, Brazil, seropositivity for anti-*Leptospira* sp. antibodies has been reported in cattle, rodents, and dogs [8], indicating that this island is home to a variety of serovars of this bacterium. These animals are potential carriers and are important in transmitting the bacteria to humans who become infected by direct or indirect contact with the urine of infected animals [9–11]. According to the Brazilian Ministry of Health, seven cases with two deaths from leptospirosis in humans were confirmed on the island of Fernando de Noronha from 2001 to 2017 [12].

The objective of this study was to determine the prevalence of *Leptospira* sp. in humans on Fernando de Noronha Island, Brazil.

2. Material and methods

2.1. Ethics aspects

The study strictly followed the ethical principles regarding research with humans and was approved by the Research Ethics Committee of Universidade de Pernambuco (n. 19344819.4.0000.5207).

2.2. Area study

The study area was the Fernando de Noronha Archipelago, which comprises 21 islands and islets and has an area of 17.017 km^2 and an

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estimated population of 3.061 [13]. It has a tropical climate comprising two seasons: one dry (August–January) and another rainy (February–July), with an average annual temperature of 26–27 °C [14].

2.3. Sampling

To determine the sample size, the finite population calculation was applied considering the population estimate of 3.061 individuals residing in Fernando de Noronha Island in 2019, according to the Brazilian Institute of Geography and Statistics [13]. Furthermore, an expected prevalence of *Leptospira* of 50% was assumed given the non-existence of previous data at a confidence interval (CI) of at least 95% with a statistical error of 5% (IC) [15]. This calculation resulted in a sample of 341 individuals.

As inclusion criteria, individuals should have been residents with a minimum stay of 6 months on the island, of both sexes, older than 2 years of age, and expressed interest in participating voluntarily in the research. Tourists were excluded from the study.

Participants were informed about the research objectives, and those who agreed to participate in the study received and signed a copy of the Informed Consent Form, according to Resolution No. 466/12 of the National Health Council [16].

2.4. Collection of blood samples

Approximately 5-mL blood samples were harvested using clot separator gel tubes (BD Vacutainer®, USA); the samples were stored at room temperature (25 °C) until visible retraction of the clot. The samples were centrifuged at 1500 ×g for 5 min, and serum aliquots were obtained and placed in microtubes, identified, and stored at -20 °C until further processing.

2.5. Serological analysis

For the detection of anti-*Leptospira* sp. antibodies among serum samples, a microscopic agglutination test (MAT) was conducted using a collection of live antigens grown on Ellinghausen–McCullough–Johnson–Harris medium, free from contamination and self-agglutination, represented by the following serogroups: *Leptospira interrogans* (serovars Bratislava, Copenhageni, Canicola, Djasiman, Grippotyphosa, Hebdomadis Hardjoprajitno, Icterohaemorrhagiae, Pomona, Pyrogenes, and Wolffi); *Leptospira borgpetersenii* (serovars Autumnalis, Ballum, Castellonis, Celledoni, Javanica, Mini, and Tarassovi); *Leptospira santarosai* (serovars Canalzone, Guaricura and Shermani); *Leptospira kirschneri* (serovars Cynopteri); and *Leptospira noguchii* (serovars Panama and Louisiana).

Positive and negative control serum samples were included in the reactions. Initially, the sera were screened at a 1:50 dilution and then subjected to a series of two-fold geometric dilutions. Samples with titers ≥1:50 were considered positive with 50% or more bacterial agglutination under a dark-field microscope. To calculate antibody prevalence, the reactive sample for one or more serovars was used, and to determine the predominant serovars, we considered those with higher titers [17,18].

Descriptive statistical analysis was used to calculate the absolute and relative frequencies of the serology results.

3. Results

The prevalence of anti-*Leptospira* antibodies in humans in Fernando de Noronha Island was 1.17% (4/341; IC 0.46–2.98%). A higher prevalence was observed in men (2.04%; 3/147) belonging to the age group of 29–68 years, and for women, the prevalence was 0.51% (1/194) in the same age range. Regarding the level of education, 75% (3/4) of the individuals with positive results had studied up to fundamental school level. The frequencies of serovars for *Leptospira* sp. are described in Table 1.

Table 1
Frequencies of serovars in serum samples from humans residing in Fernando de Noronha Island, Pernambuco, Brazil.

Titers		
Sorovar	50	100
Icterohaemorrhagiae	¼ (25%)	–
Javanica	¼ (25%)	–
Mini	–	¼ (25%)
Louisiana	–	¼ (25%)

4. Discussion

This was the first serological study of *Leptospira* sp. in humans on Fernando de Noronha Island, Brazil. The low prevalence of anti-*Leptospira* antibodies (1.17%) is similar to that found on Oahu Island (MAT/100: 1.4%) located in the EUA [19] and diverges from the prevalences found in other island environments in different regions of the world, including Barbados Islands (MAT/50: 12.5%) [20], Trinidad Island (MAT/20: 7.1%), and Tobago Island located in the Caribbean (ELISA: 20.3%) [21]; Fiji Island (MAT/50: 19.4%) [22] in the EUA; Mayotte Island in France (MAT/100: 7.8%) [23]; Andaman Island in India (MAT/100: 10.9%) [24]; and Vanuatu Island in the South Pacific Ocean (MAT/100: 11.5%), [25].

Variations in the prevalence in humans is associated with the increased spread of leptospires worldwide, especially in tropical regions where large outbreaks can occur after heavy rainfall and flooding. In addition to climatic factors, the high reservoir population, occupational exposure, recreational activities, and socioeconomic circumstances contribute to human infection with *Leptospira* sp. [9–11]

Serological analysis identified the occurrence of Icterohaemorrhagiae, Javanica, Mini, and Louisiana serovars with 25% frequency for each and MAT titers of 50 and 100. These data resemble those of other serological surveys conducted in island environments that revealed the presence of these same serovars: Icterohaemorrhagiae [21,24], Javanica [26], Mini [23], and Louisiana [25]. Most human epidemiological studies show that the most frequent serogroup is Icterohaemorrhagiae [27]. However, serological surveys conducted throughout Brazil show a large variability in serovars in different geographic locations of the country, with a high prevalence of serovar Copenhageni [28].

A recent study in Fernando de Noronha Island revealed anti-*Leptospira* antibody positivity in 28.7% of cattle and 12.7% of rodents with a predominance of Icterohaemorrhagiae serovars, followed by Djasiman and Bratislava serovars [8]. Another serological survey on the same island identified anti-*Leptospira* antibodies in 10.1% of dogs and identified the occurrence of serovars Copenhageni, Grippotyphosa, and Autumnalis [29]. This information confirms the importance of serovar Icterohaemorrhagiae and others in animals and humans on the island and indicates that cattle and rodents may contribute to the *Leptospira* transmission chain.

The natural reservoirs of leptospires are rodents, domestic animals [30–32], and wild animals [33]. Javanica and Louisiana serovars have already been identified in rodents (*Rattus rattus*and *Rattus norvegicus*) [34,35], and Mini serovar has been reported in rodents (*Rattus rattus*) in addition to cattle and dogs [23].

Regarding sex, this study showed higher positivity in men (2.04%), most of them in productive age and with a low level of education. These results corroborate other studies in island environments that indicate greater positivity in men, probably because these individuals perform some occupational activity that exposes them to the agent [2,23]. Another relevant factor for contracting the infection is the lack of information about the agent. Reports indicate that a lower education level is associated with a greater lack of knowledge about prevention

measures [2,36].

5. Conclusion

This was the first serological survey on *Leptospira* sp. in humans on Fernando de Noronha Island, Brazil. There is a low prevalence of antibodies and different serovars circulating in humans in this environment. Nevertheless, it is important to alert health authorities to make local individuals aware of the risks and harmful impact of leptospirosis.

Declaration of Competing Interest

The authors declare no conflict of interest.

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