DIAGNÓSTICO DE *Mycobacterium leprae* EM TATUS (*Dasypus novemcinctus*) E SUA CORRELAÇÃO COM A PROXIMIDADE DAS FONTES DE ÁGUA NO DISTRITO DE RIVE, ESPÍRITO SANTO-BRASIL*

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RESUMO

Tatus (*Dasypus novemcinctus*) há muito tempo são considerados reservatórios do *Mycobacterium leprae.* Os seres humanos são os principais reservatórios da hanseníase, e a água e o solo também são considerados como reservatórios não humanos da hanseníase. As fontes de água são associadas com os habitats dos tatus. O objetivo deste estudo foi investigar a possível correlação entre fontes de água e infecção do *M. leprae* em tatus, utilizando o teste de ELISA, o programa ArcGIS 9.0 e analíse estatísitica. A maioria dos tatus que estavam próximos as fontes de água foram negativos para o teste de ELISA. Após a análise estatística, o estudo não encontrou correlação entre tatus infectados e a proximidade com as fontes de água.

Palavras-chave: *Mycobacterium leprae*, tatus, fontes de água, hanseníase.

DIAGNOSIS OF *Mycobacterium leprae* IN ARMADILLOS (*Dasypus novemcinctus*) AND THE CORRELATION WITH WATER SOURCE PROXIMITY IN RIVE COUNTY, ESPÍRITO SANTO STATE-BRAZIL

ABSTRACT

Armadillos (*Dasypus novemcinctus*) have been for long time recognized as reservoir of *Mycobacterium leprae*. The human beings are the main reservoir for leprosy, and water and soil also have been recognized as a non-human leprosy reservoir. The water sources are considered to associate with the armadillos habitats. The main objective of this study was to investigate the possible correlation between the water source and *M. leprae* infection of wild armadillos, using the ELISA test, ArcGIS 9.0 program and statistical analysis. The majority of armadillos that were close to water source were negatives for the ELISA test. After statistical analysis, the study not found correlation with infected armadillos and proximity to water source.

Key words: *Mycobacterium leprae*, armadillos, water source, leprosy.

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DIAGNÓSTICO DE LA *Mycobacterium leprae* EN ARMADILLOS (*Dasypus novemcinctus*) Y SU CORRELATIÓN CON LA PROXIMIDAD DE LAS FUENTES DE ÁGUA EN EL CONDADO DE RIVE, ESTADO DE ESPÍRITO SANTO-BRASIL

RESUMEN

Armadillos (*Dasypus novemcinctus*) por mucho tiempo se consideraron reservatorios del *Mycobacterium leprae*. Los seres humanos son los principales reservatorios de la hanseníasis; el água y el suelo también se consideran reservatorios no humanos de la hanseníasis. Las fuentes de água se consideran en asociación con los hábitats de los armadillos. El objetivo de este estudio fue investigar la posible correlación entre fuentes de água e infección del *M. leprae* en armadillos, utilizando el test de ELISA, o el programa AcrGIS 9.0 y análisis estatística. La mayoria de los armadillos que se encontraban cerca de las fuentes de água fueran negativos para el test de ELISA. Después de la analisis estatísitica, el estudio no encontró correlatión entre los armadillos infectados y la proximidad con la fuente de água.

Palabras-clave: *Mycobacterium leprae*; armadillos; fuentes de água; hanseníasis.

INTRODUCTION

Humans are considered the main reservoir of *Mycobacterium leprae,* the etiologic agent of leprosy (1). However, natural infection among nine-banded armadillos (*Dasypus novemcinctus*) was first reported in 1975 (2), and a large zoonotic reservoir is recognized among armadillos in the South Eastern of United States was described (3, 4). Truman et al. (5) screened leprosy among 565 armadillos from Louisiana and Texas and found immunoglobulin class M (IgM) antibodies to the phenolic glycolipid-I antigens (PGL-1) of *M. leprae* in 16% of the animals. Job et al. (6) showed by polymerase chain reaction (PCR) technique, 16 of 30 (53,3%) wild nine-banded armadillos captured in Louisiana had evidence of *M. leprae* infection. In armadillos, the PCR has the highest sensitivity to detect the presence of *M. leprae*, followed by autopsy, IgM antibodies to PGL-I, inguinal lymph node biopsy and ear biopsy (6).

Globally, Brazil is second after India in the number of leprosy cases worldwide (7). While leprosy appears to be quite common among armadillos in the United States, the disease has received relatively little attention elsewhere and comparatively little information is available about leprosy among armadillos in other countries (1). Besides USA (2, 5), *M. leprae* infection in wild armadillos has been reported in Mexico (8), Argentina (9), and in Brazil (10-12). Controversy remains if armadillos are sources of *M. leprae* and contribute to leprosy transmission in Brazil (10-13).

Armadillos are present only in countries of the new world and they cannot be considered a universal reservoir of leprosy, however, other non-human reservoirs have been suggested such as soil and water (14, 15). In Brazil, Salem and Fonseca (16) described the presence of acid-fast organism (AFB) in water from the lake near the Amazon River. Armadillos occupy a diverse range of ecological habitats, but are usually found in close association with water supplies (5, 17). The degree to which leprosy infected armadillos contribute to human leprosy infections and the role these animals play in perpetuating leprosy in some high endemic countries is an important basic scientific question which may have considerable impact on the health of local citizens (1, 18). The armadillos have been diagnosed positives for leprosy near to water sources (5, 11, 18). The main objective of this

study was to investigate the possible correlation between the water source and *M. leprae* infection of wild armadillos.

MATERIALS AND METHODS

The natural infection with *M. leprae,* was analyzed in 20 wild nine-banded armadillos, *D. novemcinctus* species, from Espirito Santo state, localized in Southern region of Brazil. Armadillos were trapped from the wild by a biothechnician from Rive (20° 45' 29" S 41° 27' 32" W), a county of Alegre municipality (20° 45' 49" S 41° 31' 58" W), under license from Brazilian Institute of the Environment and the Natural Renewable Resources-IBAMA (02009.000553/00-45), and the Ethical Committee from São Paulo Federal University-UNIFESP (107/01), for studies with *M. leprae* in Espírito Santo State, Brazil. The collections were made from July 2004 to July 2006. During that time, 20 armadillos were captured and transported to the Experimental Leprosy Laboratory (LHE), Espirito Santo University, Vitória, Brazil. At LHE, the animals were anesthetized using Tiletamine/Zolazepan at 8.5mg/Kg which was found to be effective and safe agents for 30 to 40 minute immobilizations (19). After the anesthesia, the blood was collected from the subclavian vein and put into a tube containing Ethylene diamine tetracetic acid (EDTA). The blood was then centrifuged and the serum was separated and stored at -20ºC.

The serum samples were tested in an Enzyme-Linked Immunosorbent Assay (ELISA) for immunoglobulin M (IgM) class antibodies to phenolic-glycolipid-1 antigen (PGL-1) of *M. leprae* using the method described previously. The PGL-1 antigen was prepared by Dr Patrick Brennan (Colorado Sate University) and the resulting ELISA absorbance were judge for positive and negative using the earlier definitions (20). Values from 0 to 580 (OD X 10) were considered negative, from 580 to 720 were equivocal and 721 and above were positives. Specificity of the reactions was confirmed by absorbing presumed positives plasmas with whole *M. leprae* and other mycobacterial species. (20).

Data was entered into an Excel spreadsheet by accession number. The computer program ArcGIS 9.0 was used to map and to perform the analysis for the epidemiology of *M. leprae* infections in armadillos by ELISA test. Serological results were examined with Geographic Information System (GIS) methods to discern geographic or environmental trends. The study took into consideration that armadillos have a linear home range of 157 meters (17). Although, if any armadillo that has a water source within their home range, was considered to be close to a water source. If the water source was not within the armadillo home range, then they were considered not close to a water source.

Data analysis included calculation of some statistical rates. In this study, 20 armadillos were followed through the GIS to determine the distance between the armadillos and the water source. The hypothesis, that the animals could be infected due to water source was tested. The statistical analyses were made in 2X2 small table (frequency of distance of the animals from the water source X animals diagnosed positives for leprosy), and submitted to the analysis with Chi-Square, Mantel-Haenszel Chi-Square, and Fisher's Exact Test ($p>0.05$).

RESULTS AND DISCUSSION

The number of samples (armadillos, n=20), represent only one county of one municipality from Espírito Santo state, and admit that armadillo's population and edaphic-climatic and geographic conditions show the majority of the regions of Espírito Santo State. Of those, 3 with positive ELISA test result were close to a water source. On the other hand, there was one animal with positive result that is not close to a water source. The numbers of negative ELISA animals close to a water source were bigger than the number of positive

animals that were close to a water source. Of the negative animals, ten were close to a water source and six were not close to a water source.

Until the present date, there are reports about environmental sources of leprosy (14, 15, 21) and others reports where the researchers describe the close association of armadillos with water supplies (5, 17). However, the infection in armadillos appears to have evolved by natural means (1). In this study, did not notice association between infected armadillos and proximity of water source, through the statistical analysis (Chi-Square = 0.22, p=0.63, Mantel-Haenszel Chi-Square = 0.20, p=0.64). By the Fisher's Exact Test, it was observed, that 6 animals (negative by serology) were quite distanced from de water source, with 0.85 of probability (p=0.85), suggesting that the water supplies are not the source of infection.

The ELISA was considered positive when the values were identical to 721 and above in the absorbance lecture for PGL-1 antigen (Figure 1). IgM antibodies to PGL-1 were detected in four (20%) of the 20 animal. With the present cut-off values, the screening test for IgM antibodies to PGL-1 was not sensitive enough to detect very early diseases. Nevertheless, the test is highly specific and virtually always positive in lepromatous leprosy with high bacterial load. This finding is according with earlier reports (22, 23).

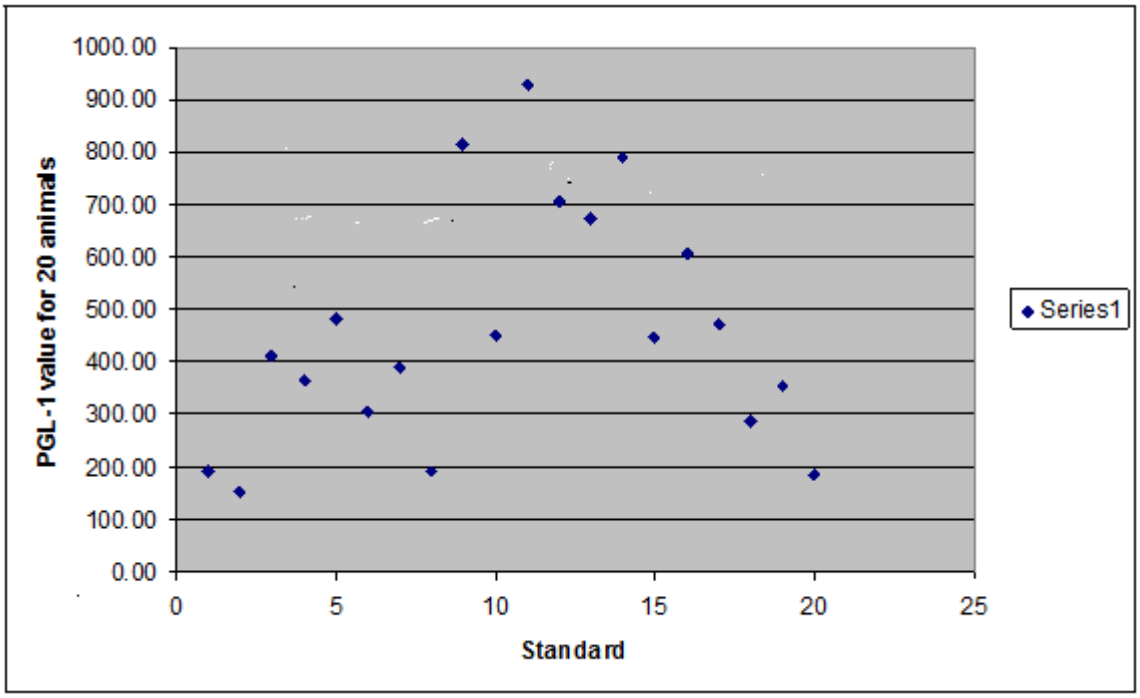

Figure 1. ELISA for PGL-1 specific antigen for *M. leprae.* Values from 0 to 580 (OD X 10) were considered negative, from 580 to 720 were equivocal and 721 and above were positives

Figure 2 shows the correlation between the presence of water source and ELISA results. The map was constructed using the computer program ArcGIS 9.0. Using this program it was demonstrated the main map of Espirito Santo State which points out the areas where the armadillos were captured (Figure 3). In the second map, was showed the image more closely to the exact point where the armadillos were captured. In these exact points, were constructed around them a kind of buffer (armadillo home range from water source) to know that the armadillos were close or were not close to water source (Figure 4).

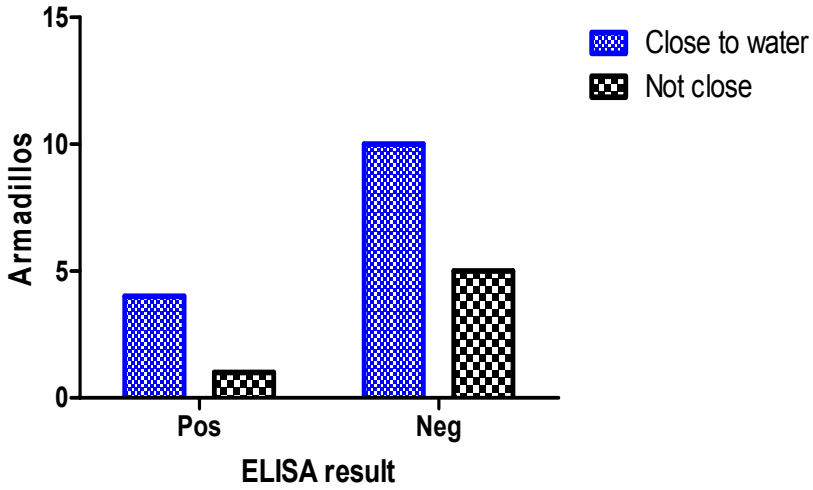

Figure 2. Correlation between presence of water source and ELISA results

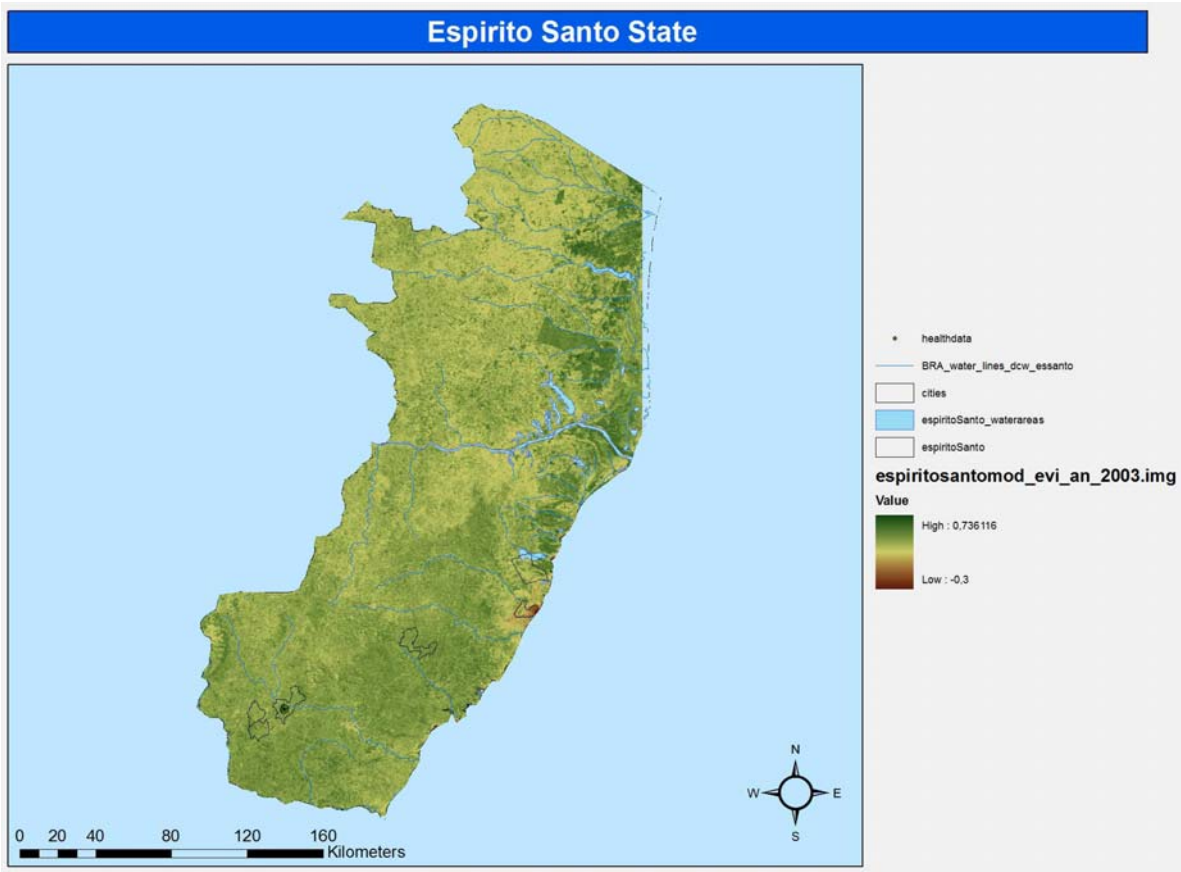

Figure 3. Main map of Espirito Santo State, Brazil, showing the areas where the armadillos were captured (red points). In this map the blue lines are water sources.

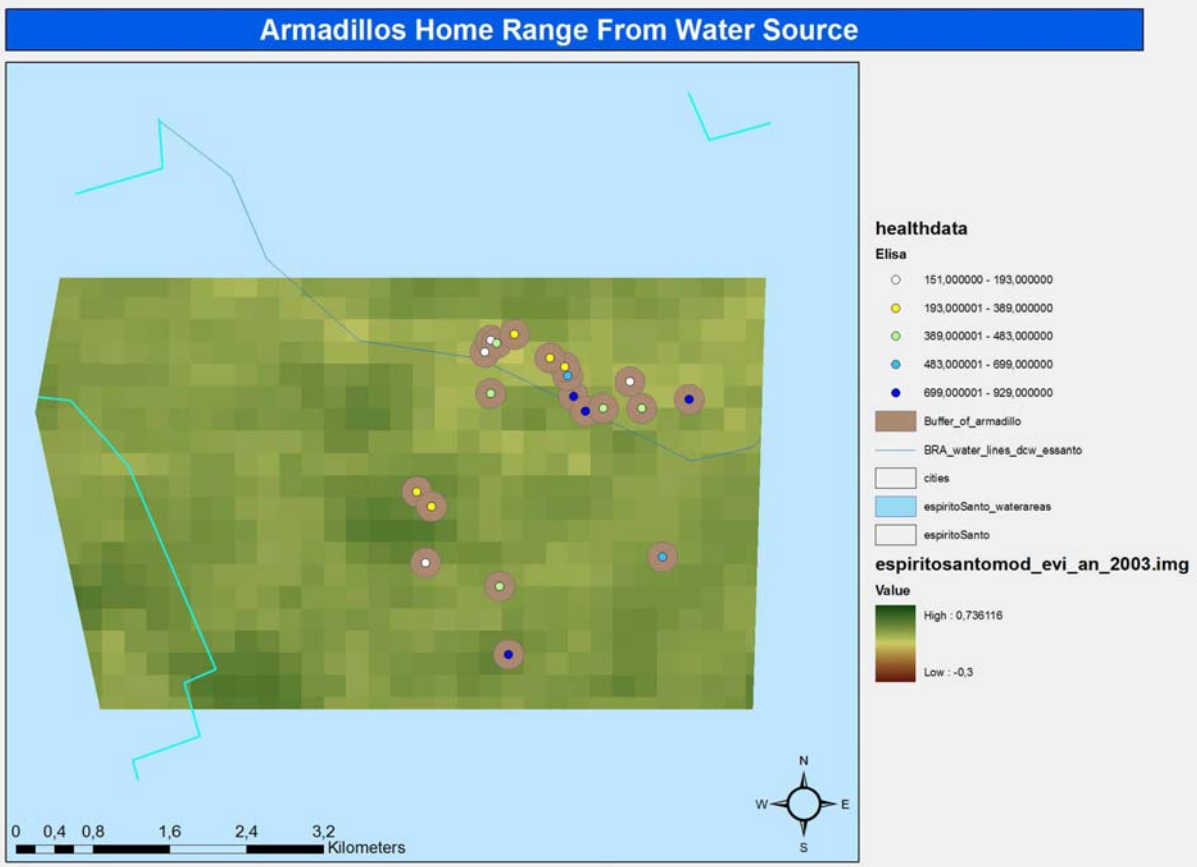

Figure 4. Zoom from the area where the armadillos were captured. A kind of buffer (armadillo home range from water source) was constructed around these points to know that the armadillos were close or were not close to water source. The ELISA positives animals are showed with blue points. In this map, the blue lines are water sources. If the buffer was inside or in contact with the blue lines, the armadillo was close to water source.

CONCLUSION

After statistical analysis, the study not found correlation with infected armadillos and proximity to water source. For this researched region, the water supplies are not a source of infection to armadillos.

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