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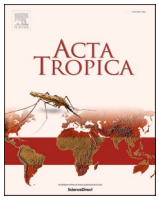

Prevalence of *Mycobacterium leprae* and *Mycobacterium lepromatosis* in roadkill armadillos in Brazil

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ABSTRACT

To evaluate the prevalence of *Mycobacterium leprae* and *Mycobacterium lepromatosis* in road killed armadillos identified along Brazilian regions, samples of liver, spleen, muscle, ear, nose and tail were collected on highways from 78 animals. The armadillos were of four different species, *Cabassous tatouay, Dasypus novemcinctus, Dasypus septemcinctus* and *Euphractus sexcinctus*. After DNA extraction from two tissues, specific primers were used for the detection of each pathogen using SYBR green qualitative Real-Time PCR, and amplicons were sequenced. The species with the highest prevalence was *D. novemcinctus*, mainly in the Central-West, South, and Southeast regions of Brazil. We detected *M. leprae* DNA in 32 (41 %) of the 78 individuals and *M. lepromatosis* DNA was not identified in any of the examined samples. The zoonotic component of leprosy may play a role in the transmission of the disease in endemic areas in which environmental conditions and contact with reservoirs must be investigated.

1. Introduction

Leprosy is among the neglected diseases listed by the World Health Organization (WHO) for elimination by 2030 (World Health Organization, 2023). It is a chronic infectious disease that persists in tropical countries, in socially vulnerable populations with precarious living and health conditions (Nery et al., 2019).

Leprosy is reported in 119 countries with 80 % of cases occurring in India, Brazil, and Indonesia (World Health Organization, 2023). In Brazil, 269,086 new cases of leprosy were diagnosed between 2012 and 2021, although there was a 50 % reduction in the detection rate in the last decade (2012–2021). Nevertheless, identifying cases in children (<15 years old) persists, meaning active bacteria circulation. Furthermore, there is a high percentage of individuals diagnosed with leprosy and physical disability, which translates into late diagnosis (Brasil, 2023).

The most important etiological agent of leprosy is *Mycobacterium leprae*, an obligate non-culturable intracellular bacteria (Han et al., 2008). In 2008, *Mycobacterium lepromatosis* was identified by molecular techniques and sequencing in two Mexican patients who had a diffuse multibacillary clinical form of leprosy (Han et al., 2008).

Mycobacterium leprae and *M. lepromatosis* have a similar genome size (~3.27 MB) and the genes that encode proteins share 93 % of the nucleotide sequence. Phylogenetic studies have indicated a divergence from its most recent common ancestor of 10 to 14 million years. Despite this separation, the two bacilli retain their pathogenic ability. Genetic and phylogenetic studies do not provide evidence of differences in transmissibility or virulence between the two species while causing human leprosy (Silva et al., 2022; Singh et al., 2015).

More than 154 cases of *M. lepromatosis* leprosy have been reported in 11 Asian and American countries, the majority in Mexico (Romero-Navarrete et al., 2022; Deps and Collin, 2021; Fernández et al., 2022; Han et al., 2012). In Brazil, *M. lepromatosis* was identified in 10 of 80 patients, of whom three had co-infection with *M. leprae* (Deps and

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Collin, 2021).

Hypothetically, leprosy transmission occurs through aerosols, through direct and prolonged contact, mainly from an untreated infected person to another, however, there is evidence that not only humans are reservoirs of *M. leprae* and *M. lepromatosis,* supporting the occurrence of an environmental and zoonotic transmission cycle (Deps and Collin, 2021). *D. novemcinctus* stands out as the most important wildlife reservoir of *M. leprae* (Vera-Cabrera et al., 2022; Ploemacher et al., 2020; Sharma et al., 2015; Truman et al., 2011; Avanzi et al., 2016; Hockings et al., 2021). At the same time, other species of wild animals such as the red squirrels (*Sciurus vulgaris*) from the British Isles are also naturally infected (Avanzi et al., 2016). Other mammals require further investigation for their role as potential wildlife reservoirs (i.e., non-human primates) (Avanzi et al., 2016).

Natural infection of *M. leprae* in armadillos was first reported in 1974 in Louisiana, United States (US) in the species *D. novemcinctus*, known as the nine-banded armadillo (Ploemacher et al., 2020; Avanzi et al., 2016; Frota et al., 2012; Walsh et al., 1986). In the US, this species was declared a natural reservoir of *M. leprae* when the same strains were found in samples from armadillos and human patients (Vera-Cabrera et al., 2022; Ploemacher et al., 2020; Sharma et al., 2015; Truman et al., 2011). In Brazil, the first report of natural infection of *M. leprae* in nine-banded armadillos was in 2002 in the state of Espírito Santo (Deps et al., 2002). Also, in Ceará state, the positivity of *M. leprae* was recorded in individuals of the species *D. novemcinctus* and *Euphractus sexcinctus* hunted by residents (Kerr et al., 2015). Zoonotic infection in Brazil has been associated with various forms of contact between human populations and the wild reservoir, including hunting, use of carapace, and rearing in peridomicile, common practices in some regions of Brazil (Kerr et al., 2015; Stefani et al., 2019; Silva et al., 2018; Deps et al., 2020; Kerr-Pontes et al., 2006). *M. lepromatosis* has not been investigated in Brazilian armadillos.

The present study aimed to evaluate the presence of *M. leprae* and *M. lepromatosis* in armadillos roadkill on Brazilian roads to contribute to a better understanding of the epidemiological scenario of leprosy transmission in the country and the potential zoonotic component of its transmission.

2. Material and methods

This is a prevalence study of *M. leprae* and *M. lepromatosis* armadillo infection via molecular biology tools. Samples of differents organs such as the liver, spleen, muscle, ear, tail, and nose were collected from armadillos roadkill on Brazilian roads in the Central-West, Southeast, South, and southern parts of the Northeast and North regions.

2.1. Ethics approval

This research was approved by Universidade Estadual de Campinas UNICAMP's Animal Ethics Committee and authorized by Chico Mendes Institute for Biodiversity Conservation, 8-30-2021, ICMBio SISBIO 78,721–1.

2.2. Collection of tissue samples in the field

Tissue samples from roadkill armadillos were collected on different roads in Brazil from September 2021 to May 2022, traversing 41,728 km on roads in 371 different cities, which corresponds to 6.7 % of the 5,570 Brazilian municipalities. Itinerary planning followed the mapping of the most likely places to find roadkilled animals (Cirino et al., 2022) from the Dasypodidae family. Geographic coordinates (i.e., latitude and longitude) were obtained from the location where animals were collected.

After necropsies at the site of collection, samples of tissues with unknown time of death were stored in two mL microtubes with 1000 μL of 70 % ethanol. The samples were subsequently transported to the Laboratory of Applied Research in Dermatology and Bartonella Infections (PADIB), Campinas, São Paulo, Brazil, for storage in a −20º C freezer until processing. In our lab, we rule out contamination due to a strict workflow with a room for DNA extraction, another for manipulating the DNA, and another for post-PCR procedures as described in detail by Drummond et al. (2023).

2.3. DNA extraction and molecular analysis

Tissues from the liver, spleen, and muscle were prioritized. DNA extraction from tissue samples was performed using the Invitrogen™ PureLink™ Genomic DNA Mini Kit by Thermo Fisher Scientific following the manufacturer's instructions. The extracted DNA was eluted in 120 μL buffer and stored at −20º C. To verify the quality of the extracted DNA, a conventional Polymerase Chain Reaction (PCR) reaction for constitutive gene amplification (i.e., *GAPDH*) was performed (Birkenheuer et al., 2003). Measurement of the nucleic acid concentration and purity of the samples was performed with a Thermo Scientific NanoDrop® 2000 Spectrophotometer.

2.4. PCR reaction

In this study, we used qualitative Real-Time PCR results. The qualitative SYBR green PCR Real-Time (ThermoFisher Scientific) detects and measures small amounts of nucleic acids, correlating PCR products with fluorescence intensity, showing in which PCR cycle the amplification of interest is detected.

Specific primers were used for the detection of *M. leprae*: LP1 (RLEP) (F) TGCATGTCATGGCCTTGAGG and LP2 (RLEP) (R) CACCGATACCAGCGGCAGAA, amplifying fragments of 129 base pairs (Romero-Alvarez et al., 2023). The sensitivity of these primers is high because this region is repeated several times throughout the genome. For the detection of *M. lepromatosis*, primers RLPM (F) TGGTGATCGGGGTCGGCTGGA and RLPM (R) CCCCACCGGACACCACCAACC, amplifying a fragment of 100 base pairs (Romero-Alvarez et al., 2023).

The conditions for the SYBR Real-Time PCR were: 3 μL of water, 10 μL of SYBR, 1μL of each primer, and 5μL of DNA for a total volume of 20μL per reaction. The cycling was established as 40 cycles, holding stage: 95 °C for 20 s, cycling stage: denaturation 95 °C for 3 s, annealing 60 °C for 30 s, and Melt curve: 95 °C for 15 s, 60 °C for 1 min and 85 °C for 15 s. DNA extracted from previously sequenced *M. leprae* and *M. lepromatosis* was used as a positive control for each reaction. We also performed electrophoresis, and the positive samples were confirmed by the presence of a band in the 1.5 % agarose gel stained with GelRed.

2.5. Sequencing

After identifying *M. leprae* DNA, samples were sent for Sanger sequencing at the Central Laboratory of High-Performance Technologies in Life Sciences (LaCTAD) at the UNICAMP. The results were analyzed using Chromas 2.6.6 software and compared to the GenBank database using the BLAST tool from the National Center for Biotechnology Information (NCBI).

2.6. Statistical analysis

The prevalence of positive animals tested by SYBR green Real-Time PCR was calculated. The proportion of positivity according to species, collection region, and type of tissue analyzed was compared using Fisher's exact test, considering a statistical significance level of 0.05. Regions and species with less than two individuals were not included in the statistical test. We performed all analyses in the R programming language (R Core Team, 2021).

3. Results

Tissues of 78 individuals belonging to the following species were collected: *D. novemcinctus* $n = 50$ (64.1 %), *Euphractus sexcinctus* $n = 25$ (32.1 %), *Cabassous tatouay* $n = 2$ (2.6 %), and *D. septemcinctus* $n = 1$ (1.3 %). The most common species was *D. novemcinctus*. The species studied were found in the South region (44.9 %) in the states of Rio Grande do Sul and Paraná, followed by the Central-West region (30.8 %) in the states of Mato Grosso do Sul and Mato Grosso (Table 1, Fig. 1).

The GAPDH gene was amplified in all samples, demonstrating the presence of amplifiable DNA. After performing the SYBR green Real-Time PCR, considering the Ct cutoff point 40, DNA of *M. leprae* was detected in 41 % (32/78) armadillos, from 155 tissues. There was no detection of *M. lepromatosis* DNA. The Southeast region had the highest prevalence of infection among the armadillos collected (52.9 %), followed by the South with 40 %, and the Central-West region with prevalence of 33.3 % (Table 1).

There was no statistical difference between the positivity for *M. leprae* DNA detection of animals according to the region of collection Central-West, Southeast, and South ($p = 0.4491$). Northeast, with less than two individuals each, was not included in the Fisher test.

Animals collected in ten states showed positivity for *M. leprae* in molecular tests: Bahia (1/1), Goiás (5/6), Minas Gerais (2/4), Mato Grosso do Sul (3/10), Paraná (10/17), Rio Grande do Sul (3/16), Santa Catarina (1/2), São Paulo (7/13). No positivity was observed in Mato Grosso state (0/8) and Roraima state (0/1) (Table 1).

Forty-four of all tissues examined ($n = 155$) showed positivity for *M. leprae*, especially the spleen (40 %) and liver (29.7 %) (Table 2). From the 32 positive armadillos, 11 (34.4 %) had two tissues that tested positive.

All sequenced samples were submitted do BLAST (National Institutes of Health) and showed 99 % to 100 % similarity with another Brazilian sequence of *Mycobacterium leprae* (GenBank® accession FM211192).

4. Discussion

We study four different species of armadillos covering all five regions of Brazil: South, Southeast, Central-West, and in the southern part of the Northeast and North regions. Armadillos are widely distributed in the country, found in all Brazilian biomes such as the Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal (Wetland) (Rodrigues et al., 2020). The armadillo species most frequently found in the study area was *D. novemcinctus* (64.1 %).

Using the qualitative SYBR green Real-Time PCR, we detected *M. leprae* DNA in 41 % of the animals and no positive samples for *M. lepromatosis* in the armadillos evaluated. The species with the highest prevalence of infection was *D. novemcinctus* with 56 %. Positive samples were more prevalent in the Southeast region (52.9 %) of Brazil followed by the South region.

Our results reinforce the role of *D. novemcinctus* as the most common armadillo species in the country with the highest prevalence of infection. This armadillo is the predominant Dasypodidae species in the Americas due to its great ability to adapt to land devastated by monocultures (Rodrigues et al., 2020). The proximity between wild and domestic animals increases the risk of pathogen circulation and the occurrence of zoonoses with an impact on animal and human health. (Fagre et al., 2022).

In Brazil, the *M. leprae* DNA detection in *D. novemcinctus* ranged from 0 to 56 %. *D. novemcinctus* was the most examined species in the state of Espírito Santo (Deps et al., 2020), in Ceará (Kerr et al., 2015); in Pará (Stefani et al., 2019); and Amazonas (Silva et al., 2018). Infection with *M. leprae* in *D. novemcinctus* has already been notified in Brazil through serological (ELISA) and molecular tests in animals captured by hunters in the state of Espírito Santo, with prevalences of 11 % (5/47) and 53 % (19/36), respectively (Deps et al., 2020). Moreover, *M. leprae* has been reported in *E. sexcinctus* in the state of Rio Grande do Norte with a 100 % prevalence (20/20) (Deps et al., 2020). In this study, the presence of *M. leprae* was detected for the first time in a *C. tatouay* individual. This is an important finding since other studies conducted with this same armadillo species did not report the presence of *M. leprae* DNA detection

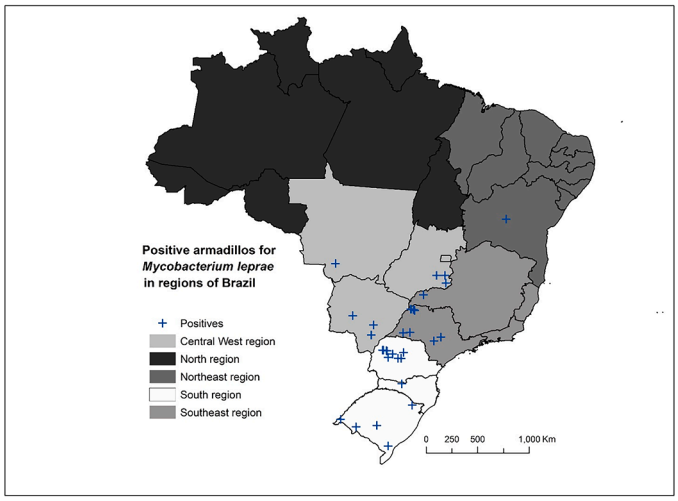

Fig. 1. Positives roadkill armadillos for *Mycobacterium leprae* in regions of Brazil.

Table 1
Characteristics of armadillos roadkill on Brazilian roads tested for *Mycobacterium leprae*.

Category	No. (%) animals	No. (%) positive for *M. leprae*
Species		
Dasypus novemcinctus	50 (64.1)	28 (56.0)
Euphractus sexcinctus	25 (32.1)	3 (12.0)
Cabassous tatouay	2 (2.6)	1 (50.0)
Dasupus septemcinctus	1 (1.3)	0
Total	78 (100)	32 (41)
Region		
South	35 (44.9)	14 (40.0)
Central-West	24 (30.8)	8 (33.3)
Southeast	17 (21.8)	9 (52.9)
North	1 (1.3)	0
Northeast	1 (1.3)	1 (100)
Total	78 (100)	32 (41)
State		
Paraná	17 (21.8)	10 (58.8)
Rio Grande do Sul	16 (20.5)	3 (18.8)
São Paulo	13 (16.7)	7 (53.8)
Mato Grosso do Sul	10 (12.8)	3 (30.0)
Mato Grosso	8 (10.3)	0
Goiás	6 (7.7)	5 (83.3)
Minas Gerais	4 (5.1)	2 (50.0)
Santa Catarina	2 (2.6)	1 (50.0)
Roraima	1 (1.3)	0
Bahia	1 (1.3)	1 (100)
Total	78 (100)	32 (41)

Table 2
Positivity for *Mycobacterium leprae* in tissue samples from armadillos ($n = 155$) roadkill on Brazilian highways.

Tested tissues	No (%) tissue samples	No (%) positive tested tissues
Ear	40 (25.8)	10 (25)
Muscle	39 (25.2)	8 (20.5)
Liver	37 (23.9)	11 (29.7)
Spleen	25 (16.1)	10 (40.0)
Tail	11 (7.1)	3 (27.3)
Nose	2 (1.3)	2 (100)
Mix	1 (0.6)	0 (0)
Total	155 (100)	44 (28.4)

(Pedrini et al., 2010; Ploemacher et al., 2020). Brazil is a continental size country. Deps et al. (2020) showed in their meta-analysis of the Brazilian states where armadillos were studied (seven from 27 Federative Units) (Deps et al., 2020). We got armadillo samples from 10 Brazilian states. In seven of them, the occurrence of *M. leprae* DNA detection in these animals had not yet been recorded and/or studied.

Liver and spleen were the tissues that showed the higher *M. leprae* counts followed by ears and tails and lastly muscles. It is different from the data obtained by Sharma et al. (2020). Despite using tissues from roadkill armadillos, good quality DNA was obtained and downstream applications such as sequencing were conducted that facilitated the estimate of *M. leprae* prevalence in roadkill armadillos in Brazil. The positive result of the tissue may be related to the phase of the disease in each armadillo, which may be decisive for the number of bacilli present in certain organs at the time of death (Deps et al., 2020). We used the qualitative SYBR green Real-Time PCR that was able to amplify *M. leprae* DNA in extracted from different tissues. We chose to collect roadkill armadillos and not capture and euthanize them, because these animals play an important role in ecosystems, dispersing seeds and controlling pests through invertebrate predation (Rodrigues et al., 2020). Furthermore, considering all Brazilian paved roads, the total number of medium and large mammals roadkill can reach almost nine million annually (Pinto et al., 2022). Therefore, using roadkill animals to identify the prevalence of specific pathogens, in different regions of the country, is also a way of giving a destination to these dead animals that until then would not have been used for this scientific purpose (Molbert et al., 2023).

As in other studies, *M. lepromatosis* was not detected in wild animals tested tissues, however, the challenge remains in identifying *M. lepromatosis* in armadillos in Brazil and in other species that are potential hosts or reservoirs of the bacillus (Deps and Collin, 2021; Fernández et al., 2022; Han et al., 2012; Vera-Cabrera et al., 2022; Ploemacher et al., 2020; Hockings et al., 2021).

There was no statistical difference between the prevalence of *M. leprae* DNA detection in armadillos in different regions of the country where we got more specimens. Nevertheless, Central-West, where we found a lower *M. leprae* detection percentage (33.3 %), presents the highest leprosy detection coefficient in the country, while the Southeast and South regions have the lowest. The latter are areas with better social and health indicators (Brasil, 2023), more similar to areas where leprosy is considered zoonotic, and we got the highest *M. leprae* DNA detection percentage (52.9 % and 40.0 %, respectively). We need greater sample sizes to analyze these data.

The transmission of *M. leprae* is multifactorial, involving humans, the environment, and reservoirs that may maintain the circulation of the pathogen and transmission to humans in a zoonotic cycle in specific areas (Ploemacher et al., 2020). Given the high prevalence of *M. leprae* detection in armadillos, it is necessary to expand surveillance actions and prevention also focusing on the potential zoonotic transmission of the disease. To fulfill the commitment to eliminate the disease by 2030 (World Health Organization, 2023), recommendations for changing habits and behaviors, like hunting and eating meat from animals that act not only as reservoirs, but sources of infection are strategic. Moreover, structured and sensitive epidemiological surveillance, early diagnosis, timely and complete treatment, and the active search for human cases with person-to-person transmission should be reinforced.

5. Conclusion

We found *M. leprae* DNA in two out five armadillos in Brasil. The high prevalence of *M. leprae* in roadkill armadillos reinforces the hypothesis of a potential zoonotic component of leprosy in the maintenance of *M. leprae* transmission in leprosy endemic areas, as occurrs in Brazil. The biological and epidemiological relevance of *M. lepromatosis* among vertebrate reservoirs remains an issue to be analyzed in additional studies.

Author statement

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CRediT authorship contribution statement

J Monsalve-Lara: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **M Drummond:** Visualization, Validation, Methodology, Investigation, Data curation. **D Romero-Alvarez:** Writing – review & editing, Visualization, Methodology, Conceptualization. **PENF Velho:** Writing – review & editing, Validation, Methodology, Formal analysis. **D Jiménez-García:** Visualization, Investigation, Conceptualization. **R Marques:** Visualization, Investigation, Conceptualization. **AT Peterson:** Writing – review & editing, Visualization, Conceptualization. **RN Angerami:** Writing – review & editing, Visualization. **DP Silva:** Investigation. **MR Donalisio:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for *[ActaTropica]* and was not involved in the editorial review or the decision to publish this article.

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Data availability

Data will be made available on request.

References

Avanzi, C., Del-Pozo, J., Benjak, A., Stevenson, K., Simpson, V.R., Busso, P., et al., 2016. Red squirrels in the British Isles are infected with leprosy bacilli. J. Sci. 354, 744–747. https://doi.org/10.1126/science.aah3783.

Birkenheuer, A.J., Levy, M.G., Breitschwerdt, E.B., 2003. Development and evaluation of a seminested PCR for detection and dif-27ferentiation of *Babesia gibsoni* (Asian genotype) and *B. canis* DNA in canine blood samples. J. Clin. Microbiol. 41, 4172–4177. https://doi.org/10.1128/JCM.41.9.4172-4177.2003.

Brasil 2023. Boletim Epidemiológico de Hanseníase. Ministério da Saúde, Secretária de Vigilância em Saúde; 2023 [cited 2023 Dic 5]. http://antigo.aids.gov.br/pt-br/pub/2023/boletim-epidemiologico-de-hanseniase-2022.

Cirino, D.W., Lupinetti-Cunha, A., Freitas, C.H., de Freitas, S.R., 2022. Do the roadkills of different mammal species respond the same way to habitat and matrix? Nat. Conserv. 47, 65–85. https://doi.org/10.3897/natureconservation.47.73010.

Deps, P., Collin, S.M., 2021. *Mycobacterium lepromatosis* as a Second Agent of Hansen's Disease. Front. Microbiol. 12, 698588 https://doi.org/10.3389/fmicb.2021.698588.

Deps, P.D., Santos, A.R., Yamashita-Tomimori, J., 2002. Detection of *Mycobacterium leprae* DNA by PCR in blood sample from nine-banded armadillo: preliminary results. Int. J. Lepr. Other Mycobact. Dis. 70, 34–35.

Deps, P., Antunes, J.M., Santos, A.R., Collin, S.M., 2020. Prevalence of *Mycobacterium leprae* in armadillos in Brazil: a systematic review and meta-analysis. PLoS. Negl. Trop. Dis. 14, e0008127 https://doi.org/10.1371/journal.pntd.0008127.

Drummond, M.R., Dos Santos, L.S., de Almeida, A.R., Lins, K.A., Barjas-Castro, M.L., Diniz, P.P.V.P., Velho, P.E.N.F, 2023. Comparison of molecular methods for Bartonella henselae detection in blood donors. PLoS. Negl. Trop. Dis. 17 (6), e0011336 https://doi.org/10.1371/journal.pntd.0011336. PMID: 37262044; PMCID: PMC10234562.

Fagre, A.C., Cohen, L.E., Eskew, E.A., Farrell, M., Glennon, E., Joseph, M.B., et al., 2022. Assessing the risk of human-to-wildlife pathogen transmission for conservation and public health. Ecol. Lett. 25 (6), 1534–1549. https://doi.org/10.1111/ele.14003.

Fernández, J.D.P., Pou-Soarez, V.E., Arenas, R., Juárez-Duran, E.R., Luna-Rojas, S.L., Xicohtencatl-Cortes, J., et al., 2022. *Mycobacterium leprae* and *Mycobacterium lepromatosis* Infection: a Report of Six Multibacillary Cases of Leprosy in the Dominican Republic. Jpn. J. Infect. Dis. 75, 427–430. https://doi.org/10.7883/yoken.JJID.2021.709.

Frota, C.C., Lima, L.N.C., Rocha A da, S., Suffys, P.N., Rolim, B.N., Rodrigues, L.C., et al., 2012. *Mycobacterium leprae* in six-banded (*Euphractus sexcinctus*) and nine-banded armadillos (*Dasypus novemcinctus*) in Northeast Brazil. Mem. Inst. Oswaldo Cruz. 107, 209–213. https://doi.org/10.1590/S0074-02762012000900029.

Han, X.Y., Seo, Y.-H., Sizer, K.C., Schoberle, T., May, G.S., Spencer, J.S., et al., 2008. A new Mycobacterium species causing diffuse lepromatous leprosy. Am. J. Clin. Pathol. 130, 856–864. https://doi.org/10.1309/AJCPP72FJZZRRVMM.

Han, X.Y., Sizer, K.C., Velarde-Félix, J.S., Frias-Castro, L.O., Vargas-Ocampo, F., 2012. The Leprosy Agents *Mycobacterium lepromatosis* and *Mycobacterium leprae* in Mexico. Int. J. Dermatol. 51, 952–959. https://doi.org/10.1111/j.1365-4632.2011.05414.x.

Hockings, K.J., Mubemba, B., Avanzi, C., Pleh, K., Düx, A., Bersacola, E., et al., 2021. Leprosy in wild chimpanzees. Nature 598 (7882), 652–656. https://doi.org/10.1038/s41586-021-03968-4.

Kerr, L., Kendall, C., de Sousa, C.A., Frota, C., Graham, J., Rodrigues, L., et al., 2015. Human-armadillo interaction in Ceará, Brazil: potential for transmission of *M. leprae*. Acta Trop. 152, 74–79. https://doi.org/10.1016/j.actatropica.2015.07.023.

Kerr-Pontes, L.R., Barreto, M.L., Evangelista, C.M., Rodrigues, L.C., Heukelbach, J., Feldmeier, H., 2006. Socioeconomic, environmental, and behavioral risk factors for leprosy in North-east Brazil: results of a case–control study. Int. J. Epidemio 35, 994–1000. https://doi.org/10.1093/ije/dyl072.

Molbert, N., Ghanavi, H.R., Johansson, T., Mostadius, M., Hansson, M.C., 2023. An evaluation of DNA extraction methods on historical and roadkill mammalian specimen. Sci. Rep. 13 (1), 13080. https://doi.org/10.1038/s41598-023-39465-z.

Nery, J.S., Ramond, A., Pescarini, J.M., Alves, A., Strina, A., Ichihara, M.Y., et al., 2019. Socioeconomic determinants of leprosy new case detection in the 100 Million Brazilian Cohort: a population-based linkage study. Lancet 7, e1226–e1236. https://doi.org/10.1016/S2214-109X(19)30260-8.

Pedrini, S., Rosa, P.S., Medri, Í., Mourão, G., Bagagli, E., de Magalhães Lopes, C.A., 2010. Search for *Mycobacterium leprae* in wild mammals. BJID 14 (1), 47–53.

Pinto, F.A.S., Cirino, D.W., Cerqueira, R.C., Rosa, C., Freitas, S.R., 2022. How many mammals are killed on Brazilian roads? Assessing impacts and conservation implications. J. Divers. 14 (10), 835. https://doi.org/10.3390/d14100835.

Ploemacher, T., Faber, W.R., Menke, H., Rutten, V., Pieters, T., 2020a. Reservoirs and transmission routes of leprosy; A systematic review. PLoS. Negl. Trop. Dis. 14, e0008276 https://doi.org/10.1371/journal.pntd.0008276.

Ploemacher, T., Faber, W.R., Menke, H., Rutten, V., Pieters, T., 2020b. Reservoirs and transmission routes of leprosy; A systematic review. PLoS Negl Trop. Dis. 14 (4) https://doi.org/10.1371/journal.pntd.0008276.

R Core Team, 2021. R: A language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Rodrigues, T.F., Mantellatto, A.M.B., Superina, M., Chiarello, A.G., 2020. Ecosystem services provided by armadillos. Biol. Rev. 95, 1–21. https://doi.org/10.1111/brv.12551.

Romero-Alvarez, D., Garzon-Chavez, D., Jackson, M., Avanzi, C., Peterson, A.T, 2023. *Mycobacterium leprae* in Armadillo Tissues from Museum Collections, United States. Emerg. Infect. Dis. 29, 622–626. https://doi.org/10.3201/eid2903.221636.

Romero-Navarrete, M., Arenas, R., Han, X.Y., Vega-Memije, M.E., Castillo-Solana, A.D., 2022. Leprosy Caused by *Mycobacterium lepromatosis*: literature Review and Report of a Family in Acapulco, Mexico. Am. J. Clin. Pathol. 158, 678–686. https://doi.org/10.1093/ajcp/aqac110.

Sharma, R., Singh, P., Loughry, W.J., Lockhart, J.M., Inman, W.B., Duthie, M.S., Pena, M. T., Marcos, L.A., Scollard, D.M., Cole, S.T., Truman, R.W, 2015. Zoonotic Leprosy in the Southeastern United States. Emerg. Infect. Dis. 21 (12), 2127–2134. https://doi.org/10.3201/eid2112.150501.

Sharma, R., Singh, P., McCoy, R.C., Lenz, S.M., Donovan, K., Ochoa, M.T., et al., 2020. Isolation of mycobacterium lepromatosis and development of molecular diagnostic assays to distinguish mycobacterium leprae and M. lepromatosis. Clin. Infect. Dis. 71, e262–e269. https://doi.org/10.1093/cid/ciz1121.

Silva, MB da, Portela, J.M., Li, W., Jackson, M., Gonzalez-Juarrero, M., Hidalgo, A.S., et al., 2018. Evidence of zoonotic leprosy in Pará, Brazilian Amazon, and risks associated with human contact or consumption of armadillos. PLoS. Negl. Trop. Dis. 12, e0006532 https://doi.org/10.1371/journal.pntd.0006532.

Silva, F.J., Santos-Garcia, D., Zheng, X., et al., 2022. Construction and analysis of the complete genome sequence of leprosy agent *Mycobacterium lepromatosis*. Microbiol. Spectr. 25, e0169221 https://doi.org/10.1128/spectrum.01692-21.

Singh, P., Benjak, A., Schuenemann, V.J., Herbig, A., Avanzi, C., Busso, P., et al., 2015. Insight into the evolution and origin of leprosy bacilli from the genome sequence of *Mycobacterium lepromatosis*. Proc. Natl. Acad. Sci. USA. 112, 4459–4464. https://doi.org/10.1073/pnas.1421504112.

Stefani, M.M.A., Rosa, P.S., Costa, M.B., Schetinni, A.P.M., Manhães, I., Pontes, M.A.A., et al., 2019. Leprosy survey among rural communities and wild armadillos from Amazonas state, Northern Brazil. PLoS. One 14, e0209491. https://doi.org/10.1371/journal.pone.0209491.

Truman, R.W., Singh, P., Sharma, R., et al., 2011. Probable zoonotic leprosy in the southern United States. N. Engl. J. Med. 364, 1626–1633.

Vera-Cabrera, L., Ramos-Cavazos, C.J., Youssef, N.A., Pearce, C.M., Molina-Torres, C.A., Avalos-Ramirez, R., et al., 2022. *Mycobacterium leprae* Infection in a Wild Nine-Banded Armadillo, Nuevo León, Mexico. Emerg. Infect. Dis. 28, 747–749. https://doi.org/10.3201/eid2803.211295.

Walsh, G.P., Meyers, W.M., Binford, C.H., 1986. Naturally Acquired Leprosy in the Nine-Banded Armadillo: a Decade of Experience 1975–1985. J. Leukoc. Biol. 40, 645–656. https://doi.org/10.1002/jlb.40.5.645.

World Health Organization. Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030 [cited 2023. https://www.who.int/publications/i/item/9789240010352.