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Letter to the editor

Molecular detection of *Mycobacterium leprae* by Polymerase Chain Reaction in captive and free-ranging wild animals

Dear Editor:

Leprosy, a disease neglected in many countries, is endemic in Brazil. With a wide diversity of fauna distributed in three biomes (Amazon Forest, Cerrado and Pantanal), the state of Mato Grosso (MT) in the Central-West Region has the highest prevalence of human cases: 7.75 per 10,000 inhabitants.[1] Despite the scarcity of data in the literature on wild animals naturally infected with *Mycobacterium leprae*, the possibility of transmission to humans cannot be ruled out. Armadillos, red squirrels, and non-human primates are important natural reservoirs of *M. leprae* reported in the literature, becoming possible sources of bacillary dissemination making it difficult to interrupt the leprosy transmission chain.[2] As data on natural infections are scarce, it is difficult to understand the role of wild animals in transmission of the disease. Therefore, we used PCR to detect the genetic material of *M. leprae* in nasal swabs of wild animals.

Nasal swabs were collected from 69 captive and free wild animals from the MT and Pantanal regions of Brazil, independent of clinical signs, and sent to the Laboratory of Microbiology and Molecular Biology, according to "Sistema de Autorização e Informação em Biodiversidade" (SISBIO), an authorization and information system for biodiversity (nos. 40617-1 and 42303). The samples were submitted for extraction of genetic material according to the phenol/chloroform method. PCR was performed according to Woods and Cole.[3] The PCR product was purified using a GFX^{TM} PCR DNA and Gel Band Purification kit (GE Healthcare, Piscataway, NJ, USA) and sequenced using an ABI-PRISM 3500 Genetic Analyzer (Life Technologies Corporation, USA). The sequences were deposited in GenBank and compared using the BLAST program (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Of the 69 samples (Table 1), six (8.69%) wild-type free and captive animals tested positive for *M. leprae* by PCR, including one margay (*Leopardus wiedii*), two lowland tapirs (*Tapirus terrestris*), two capuchin monkeys (*Sapajus apella*), and one owl monkey (*Aotus trivirgatus*). The detection in four different species of wild animals shows the ability of this bacillus to be carried in different hosts. In addition, two animals were from the zoo, that could have acquired *M. leprae* due to close contact to humans or environmental contamination. However, in literature the mechanism of transmission is not yet fully understood.[4]

Table 1 – Epidemiological data and *M. leprae* PCR test of the animals identified in Mato Grosso – Brazil.

ID	Free-ranging	City	Species	Scientific name	PCR
m962/16	Yes	Jangada	Jaguarundi	*Puma yagouaroundi*	Negative
m1016/16	Yes	Marcelândia	Jaguar	*Panthera onca*	Negative
m1102/16	No	Zoo[a]	Cougar	*Puma concolor*	Negative
m1122/16	No	Zoo	Coati	*Nasua nasua*	Negative
m1162/16	Yes	Cuiabá	Guinea pig	*Cavia porcellus*	Negative
m1226/16	Yes	Barra do Bugres	Ocelot	*Leopardus pardalis*	Negative
m1285/16	Yes	NA[b]	Jaguarundi	*Puma yagouaroundi*	Negative
m1294/16	Yes	Várzea Grande	Capybara	*Hydrochoerus hydrochaeris*	Negative
m1335/16	No	Zoo	Giant anteater	*Myrmecophaga tridactyla*	Negative
m1336/16	Yes	Rosário Oeste	Giant anteater	*Myrmecophaga tridactyla*	Negative
m1364/16	Yes	Cuiabá	Collared anteaters	*Myrmecophaga tetradactyla*	Negative
m1485/16	Yes	Santo Antônio do Leverger	Otter	*Lontra longicaudis*	Negative
m1491/16	Yes	NA	White-eared opossum	*Didelphis albiventris*	Negative

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– Table 1 (Continued)

ID	Free-ranging	City	Species	Scientific name	PCR
m1529/16	No	Zoo	Agouti	*Dasyprocta Aguti*	Negative
m1787/17	Yes	NA	Owl monkey	*Aoutus trivirgatus*	Positive Genbank MF975704
m1790/16	Yes	Tangará da Serra	Giant anteater	*Myrmecophaga tridactyla*	Negative
m1795/16	No	Zoo	Coati	*Nasua nasua*	Negative
m1796/16	No	Zoo	Agouti	*Dasyprocta aguti*	Negative
m1862/16	Yes	Várzea Grande	Collared anteaters	*Myrmecophaga tetradactyla*	Negative
m11/17	Yes	Cuiabá	Capuchin monkey	*Sapajus apella*	Positive Genbank MF975703
m74/17	No	Zoo	Cougar	*Puma concolor*	Negative
m153/17	Yes	Cuiabá	White-eared opossum	*Didelphis albiventris*	Negative
m234/17	No	Zoo	Cougar	*Puma concolor*	Negative
m235/17	Yes	Cuiabá	Sagui	*Callithrix* sp.	Negative
m248/17	No	Zoo	Maned wolf	*Chrysocyon brachyurus*	Negative
m261/17	Yes	NA	Black owler monkey	*Alouatta caraya*	Negative
m305/17	No	Zoo	Lowland tapirs	*Tapirus terrestris*	Positive Genbank MF975707
m345/17	No	Zoo	Cougar	*Puma concolor*	Negative
m379/17	Yes	NA	Black-tufted marmoset	*Callithrix penicillata*	Negative
m514/17	No	Zoo	White-cheeked spider monkey	*Ateles marginatus*	Negative
m520/17	No	Zoo	White-cheeked spider monkey	*Ateles marginatus*	Negative
m530/17	Yes	NA	Capuchin monkey	*Sapajus apella*	Positive Genbank MF818035
m539/17	Yes	NA	Monkey	NA	Negative
m542/17	Yes	Santo Antônio do Leverger	Owl monkey	*Aotus* sp.	Negative
m543/17	Yes	Santo Antônio do Leverger	Owl monkey	*Aotus* sp.	Negative
m705/17	Yes	Cuiabá	Monkey	NA	Negative
m709/17	Yes	Poconé	Giant anteater	*Myrmecophaga tridactyla*	Negative
m743/17	No	Zoo	Coati	*Nasua nasua*	Negative
m748/17	Yes	Poconé	Crab-eating fox	*Cerdocyon thous*	Negative
m765/17	No	Zoo	Ocelot	*Leopardus pardalis*	Negative
m787/17	No	Zoo	Margay	*Leopardus weidii*	Positive Genbank MF975706
m809/17	Yes	Rondonópolis	Lowland tapirs	*Tapirus terrestris*	Positive Genbank MF975705
m874/17	No	Zoo	Coati	*Nasua nasua*	Negative
m878/17	Yes	Campo Verde	Howler monkey	*Alouatta* sp.	Negative
m879/17	No	Zoo	Crab-eating fox	*Cerdocyon thous*	Negative
m742/17	No	Zoo	Coati	*Nasua nasua*	Negative
m721/17	No	Zoo	Coati	*Nasua nasua*	Negative
m871/17	Yes	Cuiabá	Owl monkey	*Aoutus azare*	Negative
m897/17	No	Zoo	Crab-eating fox	*Cerdocyon thous*	Negative
m881/17	No	Zoo	Crab-eating fox	*Cerdocyon thous*	Negative
m1055/17	Yes	NA	Capuchin monkey	*Sapajus apella*	Negative
m1070/17	Yes	NA	Capybara	*Hydrochoerus hydrochaeris*	Negative
m1126/17	Yes	Tangará da Serra	Cougar	*Puma concolor*	Negative
m1153/17	No	Zoo	Crab-eating fox	*Cerdocyon thous*	Negative
m1247/17	Yes	NA	Capuchin monkey	*Sapajus apella*	Negative
m1248/17	Yes	NA	Capuchin monkey	*Sapajus apella*	Negative
m1249/17	Yes	NA	Capuchin monkey	*Sapajus apella*	Negative
m1267/17	Yes	Poconé	Capybara	*Hydrochoerus hydrochaeris*	Negative
m1268/17	Yes	Poconé	Capybara	*Hydrochoerus hydrochaeris*	Negative
m1269/17	Yes	Poconé	Capybara	*Hydrochoerus hydrochaeris*	Negative
m1270/17	Yes	Poconé	Capybara	*Hydrochoerus hydrochaeris*	Negative
m1271/17	Yes	Poconé	Capybara	*Hydrochoerus hydrochaeris*	Negative
m1272/17	Yes	Poconé	Capybara	*Hydrochoerus hydrochaeris*	Negative
m1290/17	Yes	Cuiabá	Owl monkey	*Aoutus nigriceps*	Negative
m1309/17	Yes	Cáceres	Cougar	*Puma concolor*	Negative
m1313/17	Yes	Cuiabá	Capuchin monkey	*Sapajus apella*	Negative
m1327/17	Yes	NA	Black owler monkey	*Alouatta caraya*	Negative
m1338/17	Yes	Cuiabá	Capuchin monkey	*Sapajus apella*	Negative
m1339/17	Yes	Cuiabá	Sagui	*Callithrix* sp.	Negative

[a] Federal University of Mato Grosso-Cuiaba.
[b] NA, not available.

Knowledge of the environment surrounding the infected humans or animals, and route of infection and mode of transmission are necessary to understand endemics in certain regions.[4] Truman et al.[5] described that isolates from human and armadillos are identical genetically. Thus, we suggest that the possible contact of animals of this study, which may be possible carriers of the bacillus, with other animals or with humans can disseminate the disease, the bacillus was detected in nasal swabs. Thus, we observe that the detection in wild animals may be associated with high prevalence and endemicity in the state of MT, which makes them important sources of infection. In addition, these data contribute to a better understanding of the epidemiology of leprosy.

Disclaimers

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

Conflicts of interest

The authors declare no conflicts of interest.

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