

**Molecular determination of *Mycobacterium leprae* viability in samples of *Dasypus novemcinctus* armadillo tissue in a region endemic to leprosy.**

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*Mycobacterium leprae* (*M. leprae*), the causative agent of leprosy, is not cultivated in vitro and this mycobacteria can only be maintained in alive in passages into mouse foodpads and nine banded armadillos. According to the literature, leprosy is believed to be spread from person to person through the upper respiratory tract droplets. For the last three decades, it has been considered that the existence of naturally infected animals is through *M. leprae*. Most of the studies have shown the presence of *M. leprae* DNA using different target genes. Also, the similar phylogenetic profile of mycobacteria in naturally infected armadillos and leprosy patients have suggested the zoonotic transmission. The objective of the present study was to identify the presence and viability of *M. leprae* in armadillos hunting for consumption in a hyperendemic region in Southern Amazonia. Samples of 11 ears and 8 samples of livers and spleens respectively were collected in RNeasy <sup>TM</sup> stabilization solution and maintained at -20°C until processing. RNA and DNA were extracted using the FastPrep<sup>®</sup> ProBlue kit (MP-Biomedicals) according to the manufacturer's recommendations. RNA is transcribed into cDNA. The levels of 16S rRNA in *M. leprae* from armadillo tissues were determined using real-time RT-PCR. These levels were normalized for bacterial numbers using a previously characterized, DNA-based, real-time PCR assay for RLEP. Results were evaluated based on the Ct. Positive sample were those with Ct below 37. The RLEP assay was shown to be the most sensitive (100%) in all armadillo tissue samples (Ct ranging 25 a 35). Conversely, the 16S rRNA assay was considered positive in 82% of the ears and 12.5% of the spleen samples. No amplification was obtained in the liver samples. Ct ranged from 31 to 35 in the ear samples and in a single spleen sample a Ct 35 was obtained. The 16S rRNA/RLEP assay consistently identified the presence of *M. leprae* in eight of the ears. *D. novemcinctus* armadillo's positive for the 16S rRNA / RLEP assay may represent an active and recent dispersion dynamics. Based on the longevity of armadillos, ranging from 12 to 15 years and to the period for development of the disease in humans varying between two and seven years after infection, this animal may have an important role on dissemination of *M. leprae*.

Keywords: *Mycobacterium leprae*, 16S rRNA, RLEP, *Dasypus novemcinctus*; zoonotic transmission