

***Mycobacterium tuberculosis* complex infection in Barbary sheep (*Ammotragus lervia*) at the Curitiba Zoo, southern Brazil: case report**

Infecção pelo complexo *Mycobacterium tuberculosis* em carneiro da Barbária (*Ammotragus lervia*) no Zoológico de Curitiba, sul do Brasil: relato de caso

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Abstract

Tuberculosis is one of the most important mandatory notification diseases in the world caused by bacteria of the *Mycobacterium tuberculosis* complex, infecting both humans and animals. A sudden death of a Barbary sheep in Curitiba Zoo, and presence of multifocal nodules in lungs at necropsy raised suspicion of tuberculosis. Quantitative polymerase chain reaction (qPCR) from organs and fluid was performed and detected *M. tuberculosis* complex in a lung sample. This research reports the *M. tuberculosis* complex infection in Barbary sheep, a zoonosis of great relevance to public health and emphasizes the need to implement prevention measures. Furthermore, the research may provide a better understanding for species conservation, occurrence and transmission of diseases in captivity, reservoir potential and public health impact to zoo personnel and visitors.

Keywords: *Ammotragus lervia*. *Mycobacterium tuberculosis* complex. Zoos.

Resumo

A tuberculose é uma das doenças mundiais de notificação obrigatória mais importantes causada pelo complexo *Mycobacterium tuberculosis* que pode infectar pessoas e animais. A morte repentina de um carneiro da Barbária no Zoológico de Curitiba, que apresentou nódulos multifocais no pulmão à necropsia, levantou a suspeita de tuberculose. Foi realizada a Reação em Cadeia da Polimerase Quantitativa (qPCR) de fragmentos de órgãos e fluido. A qPCR detectou a presença do complexo *M. tuberculosis* nas amostras de pulmão. Este estudo relata a infecção pelo complexo *M. tuberculosis* no carneiro da Barbária, uma zoonose de grande relevância para a saúde pública, ressaltando-se a necessidade da implementação de medidas de prevenção. Além disso, pode prover um melhor entendimento sobre conservação de espécies, ocorrência e transmissão de doenças em cativeiro, potencial reservatório e impacto na saúde pública para visitantes e funcionários dos zoológicos.

Palavras-chave: *Ammotragus lervia*. Complexo *Mycobacterium tuberculosis*. Zoológicos.

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The introduction of exotic species, considered as a common practice in zoos, may characterize significant risks to the resident population, including animals and humans, since new pathogens can be introduced to a controlled environment (YESILBAG et al., 2011). Wildlife may represent a massive and unknown

reservoir system that can potentially become a source to emerging or reemerging diseases, particularly zoonoses, such as tuberculosis (CHOMEL et al., 2007). Tuberculosis is one of the most important mandatory notification diseases in the world and annually more than nine million human cases are reported (WHO, 2015). The disease is caused by bacteria of the *Mycobacterium* complex, including *Mycobacterium bovis*, infecting mostly animals, and *Mycobacterium tuberculosis*, which is considered primarily a human pathogen despite been reported in domestic and wildlife species living in close contact with humans (MICHALAK et al., 1998). This disease has been reported in several mammals, including wild and captive animals, and a common finding in these cases is the exposure to infected animals or humans (KANEENE et al., 2010), highlighting the importance to public health. Active surveillance of animals and humans is an important preventive measure and it will allow a rapid diagnostic and proper management in order to avoid spread of the pathogen to other species, including humans.

Some species of wild animals, such as the Barbary sheep (*Ammotragus lervia*), have the potential to act as reservoirs for important infectious diseases (CANDELA et al., 2009). The Barbary sheep is an African species with some unique primitive features, grouped into suborder Ruminantia, subfamily Caprinae, which includes the domesticated genera *Ovis* and *Capra*. A closer phylogenetic connection between the genera *Ammotragus* and *Capra* was recently observed (MEREU et al., 2008). The role of Barbary sheep as a pathogenic reservoir to humans remains to be fully established, but a previous study described co-infections with *M. tuberculosis* complex, showing high prevalence of antibodies against *M. bovis* and *M. avium* ssp. *paratuberculosis*, suggesting that these animals can act as hosts or reservoirs and help in the dissemination of *Mycobacterium* spp. (CANDELA et al., 2009). The active surveillance of infectious diseases in zoo animals can be an indicative of the health status among these species, and also can allow the settlement of early

intervention protocols, in order to reduce the risk of infection to the workers, visitors and to other captive animals. The present study documents an infection of *M. tuberculosis* complex in Barbary sheep at Curitiba Zoo, southern Brazil.

Curitiba Zoo, established in 1982, is among the largest Brazilian zoos with approximately 2,300 animals of 300 species, several of which were born in captivity, distributed across an area of 530 square meters (JAVOROUSKI; BISCAIA, 2012). In April 2013 an apparently healthy eleven-year-old male captive-born Barbary sheep died without any specific clinical sign. The animal was kept in an isolation area with another four healthy males.

Post-mortem examination revealed multifocal yellowish-white nodules in its lungs and enlarged mediastinal lymph nodes with multiple nodules. A significant volume of free sero-sanguineous fluid was also found in the thoracic cavity. Multiple organs fragments were fixed in formaldehyde 10% and sent to histological exam. Pronounced diffuse neutrophilic inflammatory infiltrate was observed within alveoli and bronchioles, associated with multifocal necrosis areas and marked autolysis. Morphological diagnosis was suppurative bronchopneumonia associated with necrosis areas and it was not possible to completely rule out the development of an infectious process, such as tuberculosis.

Lungs, lymph nodes, nodules, liver fragments and fluid were sent to the Central Laboratory of the Parana State. DNA extraction and purification were performed using the automated platform Nuclisens easyMAG (bioMérieux, Boxtel, Netherlands). During the extraction procedure, 20µl of plasmid is added to each specimen in order to provide the target for the inhibition control system. Using molecular techniques, quantitative polymerase chain reaction (qPCR) (MTB Q - PCR Alert Kit, Nanogen Advanced Diagnostics®), *M. tuberculosis* complex was detected in the lung tissue sample. qPCR was used to detect the IS6110 region, which is specific for *M. tuberculosis* complex (EISENACH et al., 1990). The ABI Prism 7500

(Applied Biosystems, Foster City, CA, USA) was used as defined by the manufacturer's instructions. The animal tested positive for *M. tuberculosis* in the lung sample and the Sanitary Defense Agency of Parana State was notified. The other Barbary sheep housed in the isolation area were submitted to a comparative tuberculin test. The standard interpretation for cattle was used as recommended by the Brazilian Ministry of Agriculture, Livestock and Supply. Bovine and avian tuberculin PPD, property of the Parana Institute of Technology, were intradermally administered. After 72 hours, the increase in skinfold thickness is measured and a positive reaction is usually considered if there is an increase equal or more than four mm in the skinfold thickness (BRASIL, 2006). All of the animals tested were negative and monitored every six months thereafter. All keepers and veterinary staff were submitted to bacilloscopy and radiographic examinations. There is no report of anyone positive for tuberculosis.

The importance of this pathogen occurrence in zoos or animal parks is growing and emphasized by the difficulty of replacing some rare and endangered species, as well as economic losses and risks to public health (THOEN et al., 2009). Furthermore, there is some reluctance to publicize findings due to public relations impact and loss of revenue (KANEENE et al., 2010). The present study revealed the occurrence of *M. tuberculosis* complex in Barbary sheep, an exotic species; however, the same Brazilian zoo described in 2006 the first report of RD^{Rio} *M. tuberculosis* infection in tapirs (MURAKAMI et al., 2012). Not surprisingly, wild animal species are thought to be reservoirs for more than 70% of all emerging infections (KUIKEN et al., 2005; CHOMEL et al., 2007), despite efforts to control such infections in zoo facilities through the adoption of preventive measures like restricted access to employees, use of personnel protective equipment, exclusive material for each enclosure and quarantine.

Reports of zoo wildlife species that could harbor *M.*

tuberculosis have greatly increased. Some of these species include chimpanzees, Asian elephants (CHARLESWORTH et al., 2013) Brazilian tapir (MICHEL et al., 2003), antelopes, capuchin and vervet monkeys (MICHEL et al., 2013). It is important to emphasize that clinical signs are only rarely apparent in wild animals (KANEENE et al., 2010). Furthermore, treatment of infected wildlife has been limited to animals in captivity and is not recommended for animals that cannot be monitored.

Since 2006, the Curitiba Zoo adopted a protocol of strict standards of hygiene, including restricting animal access to specific staff members, use of personnel protective clothing (including masks, gloves), and not removing utensils from the housings (MURAKAMI et al., 2012). Considering that the infected captive-born Barbary sheep were kept in a non-visiting and isolation area since birth, the probable infection source and zoonotic transmission may have been the animal keepers and veterinary staff, acting as carriers of *M. tuberculosis* complex from other zoo areas. However, none of the staff presented symptoms of active disease to date. Due to it being a zoonosis of great relevance to the public health, all keepers and veterinary staff are periodically submitted to occupational periodic exams, which include blood tests, bacilloscopy and radiographic examinations.

In conclusion, the present study has shown that Barbary sheep in the Curitiba Zoo, Parana, southern Brazil, have been infected by *M. tuberculosis* complex. This occurrence may provide a better understanding for species conservation, occurrence and transmission of diseases in captivity, reservoir potential and public health impact to zoo personnel and visitors.

Conflict of interest

The authors declare that there is no conflict of interest.

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