
NONTUBERCULOUS MYCOBACTERIAL LUNG INFECTION IN AN AFRICAN ELEPHANT (*Loxodonta africana*) AND A GREATER ONE-HORNED RHINOCEROS (*Rhinoceros unicornis*) CAUSED BY *Mycobacterium avium* ssp. *hominissuis* AND *Mycobacterium nebraskense* AND THE REACTION TO ANTE- AND POSTMORTEM TESTS

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Abstract

A 33-year-old captive female greater one-horned rhinoceros (*Rhinoceros unicornis*) and a 47-year-old captive female trunk-wash negative African elephant (*Loxodonta africana*) showed chronic-intermittent weight loss and weakness (both), a chronic sole ulcer of the left hind limb and a chronic abscess on the left shoulder (rhino), and sudden falls with final recumbency (elephant). Both animals were euthanized and necropsy revealed a multifocal chronic granulomatous pneumonia and lymphadenitis of lung-associated lymph nodes. Opportunistic ante- and postmortem laboratory tests were performed (Tables 1 and 2). Acid-fast bacteria were microscopically detected in lung granulomas and culture of the granulomas for mycobacteria was positive in both animals. However, specific real-time PCR and culture for *Mycobacterium tuberculosis*-complex (MTB) bacteria tested negative. Sequencing of the cultured nontuberculous mycobacteria (NTM) resulted in highest homology to *Mycobacterium nebraskense* for rhino samples and *Mycobacterium avium* ssp. *hominissuis* for elephant samples. Two independent microbiological laboratories reported similar results for the elephant tissue samples. Based on these findings a diagnosis of pulmonary infection with NTM was made for both animals.

Granulomatous pulmonary disease caused by NTM is rarely reported in elephants^{1,2} and has not been reported in rhinos. However, NTM continue to emerge as a major cause of opportunistic infection and the differentiation from MTB-complex infection is challenging. Opportunistic indirect antemortem screening TB-tests were all negative or non-reactive with the exception of one immunoassay on elephant serum (TB-ST+2 Tuberculosis Rapid Test, Lionex GmbH, D-38126 Braunschweig). Dual path platform technology (DPP® VetTB Assay, Chembio Diagnostic Systems Inc., Medford, New York 11763, USA) tests remained non-reactive over time including samples from the day of euthanasia. The present results highlight the importance of choosing appropriate diagnostic tools for the ante- and postmortem detection and differentiation of MTB complex and NTM infection in zoo animals.

Key words: African elephant, greater one-horned rhinoceros, *Loxodonta africana*, *Mycobacterium avium* ssp. *hominissuis*, *Mycobacterium nebraskense*, *Rhinoceros unicornis*

Table 1. Antemortem tb testing of an African elephant and a greater one-horned rhinoceros with a pulmonary infection caused by nontuberculous mycobacteria (NTM).

| Animal | Days before euthanasia | Test | Result |
|----------|------------------------|--------------------------------------|--------------|
| Elephant | 0 | DPP® ^a | non-reactive |
| Elephant | 303 | 3-day trunk wash MTB PCR and culture | negative |
| Elephant | 800 | DPP® ^a | non-reactive |
| Elephant | 800 | TB-ST+2 ^b | reactive |
| Elephant | 943 | DPP® ^a | non-reactive |
| Elephant | 1744 | DPP® ^a | non-reactive |
| Rhino | 0 | DPP® ^a | non-reactive |
| Rhino | 185 | DPP® ^a | non-reactive |

^aDPP® VetTB Assay for Elephants (Chembio Diagnostic Systems Inc.).

^bTB-ST+2 Tuberculosis Rapid Test (Lionex GmbH).

Table 2. Postmortem tb testing of granulomatous tissue samples from the lung of an African elephant and a greater one-horned rhinoceros with a pulmonary infection caused by nontuberculous mycobacteria (NTM).

| Animal | Test | Result |
|----------|--|--|
| Elephant | Ziehl-Neelsen stain, histology | negative |
| Elephant | Ziehl-Neelsen stain, bacteriology | positive (Lab 1) |
| | Auramin stain, bacteriology | positive (Lab 2) |
| Elephant | Real-time PCR MTB-complex | negative (Lab 1) |
| | | negative (Lab 2) |
| Elephant | Culture MTB-complex | negative |
| Elephant | Culture NTM | positive (Lab 1) |
| | | positive (Lab 2) |
| Elephant | Sequencing hsp65 gene of cultured NTM Identification | <i>Mycobacterium avium</i> ssp. <i>hominissuis</i> (Lab 1) <i>Mycobacterium avium</i> (Lab 2) |
| Rhino | Ziehl-Neelsen, histology | negative |
| Rhino | Auramin, bacteriology | positive |
| Rhino | Real-time PCR MTB-complex | negative |
| Rhino | Culture MTB-complex | negative |
| Rhino | Culture NTM | positive |
| Rhino | Sequencing 16S rDNA gene of cultured NTM | <i>Mycobacterium nebraskense</i> |

LITERATURE CITED

1. Lacasse C, Terio K, Kinsel MJ, Farina LL, Travis DA, Greenwald R, Lyashchenko KP, Miller M, Gamble KC. Two cases of atypical mycobacteriosis caused by *Mycobacterium szulgai* associated with mortality in captive African elephants (*Loxodonta africana*). J Zoo Wildl Med. 2007;38(1):101-107.
2. Yong H., Choi GE, Byung SL, Lee BS, Whang J, Shin SJ. Disseminated infection due to *Mycobacterium avium* subsp. *avium* in an Asian elephant (*Elephas maximus*). J Zoo Wildl Med. 2011;42(2):743-746.