Potential diagnostic biomarkers for pulmonary tuberculosis in humans are not elevated in *Mycobacterium tuberculosis* culture positive Asian elephants (*Elephas maximus*)

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Asian elephant (Photo Credit: Oregon Zoo)

Background:

Mycobacterium tuberculosis (M.tb) infection is a threat to elephant health and conservation and human public health worldwide. Cases of tuberculosis (TB), the disease due to *M.tb* in those susceptible, have occurred on multiple continents and affected both wild and captive African and Asian elephants. Humans are the natural host for *M.tb* and main source of transmission to elephants. Infection can then spread from elephants to other elephants, to other mammals, and possibly back to humans. The ability to quickly and accurately diagnose *M.tb* infection in elephants is highly important. However, diagnosing these infections is complicated by the fact that much remains unknown about elephants' immune response to *M.tb* infection and elephants often show no clinical signs until the disease has significantly progressed. Identification of serum biomarkers of *M.tb* infection in elephants may improve diagnostic testing capabilities, allowing for earlier detection of *M.tb* positive elephants and reduced risk of spread to other species.

Specific Aims and Hypotheses:

1. To determine if CXCL1, MMP8, IL-10, IFN-γ, and TNF-α can be detected in serum from Asian elephants
2. To determine if concentrations of these biomarkers are significantly elevated in serum from *M.tb* culture positive elephants compared to *M.tb* culture negative elephants

CXCL1, MMP8, IL-10, IFN-γ, and TNF-α were recently identified as potential diagnostic biomarkers for *M.tb* infection in experimental studies in animals and humans. Therefore, we hypothesized that these biomarkers would be detectable and significantly elevated in *M.tb* culture positive elephants compared to *M.tb* culture negative elephants.

Materials and Methods:

Elephant Serum Samples:
We obtained banked Asian elephant serum samples from Oregon Zoo and a second U.S. zoo that requested to remain anonymous. Samples from *M.tb* culture positive elephants included ten samples from five elephants (two samples per elephant) that had tested positive for *M.tb* infection via trunk wash culture. None of the *M.tb* culture positive elephants had clinical signs of disease. Samples from *M.tb* culture negative elephants included 91 samples from six elephants (range of 8–39 samples per elephant) that tested negative for *M.tb* infection via trunk wash culture during routine screening. Zoo research committees at collaborating zoos reviewed and approved the study.

Biomarker Immunoassays:
We used commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits to determine the concentrations of CXCL1, MMP8, IL-10, IFN-γ, and TNF-α in elephant serum samples. Prior to testing all samples, ELISAs were optimized for laboratory conditions, and a pilot study was performed on sera from a few *M.tb* culture positive and *M.tb* culture negative elephants to determine the optimal dilution series that would allow us to calculate the concentration of each biomarker on our standard curve. We used Gen5 Software, Excel, and GraphPad Prism to analyze the results of the assays. We calculated the limit of detection for each assay by adding two standard deviations to the optical density (OD) of the blank (i.e., controls with no sample or standard added), and interpolating the concentration at this OD value from the standard curve.

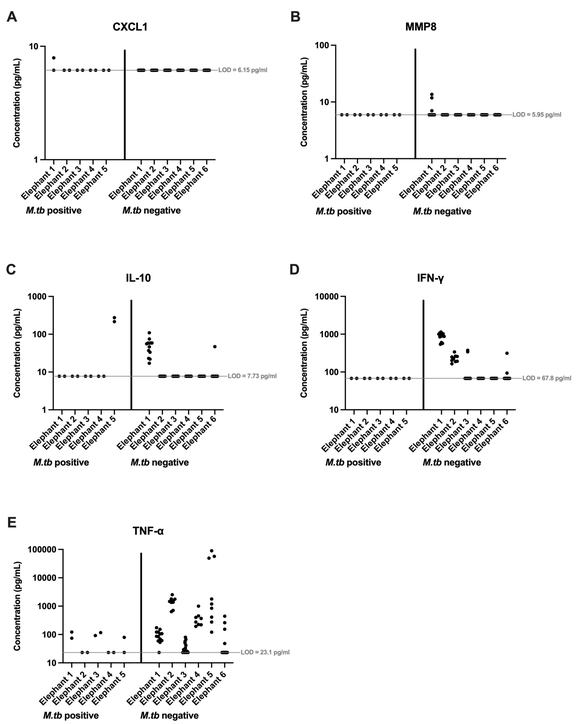

Figure 1. Concentrations of (A) CXCL1, (B) MMP8, (C) IL-10, (D) IFN-γ, and (E) TNF-α in samples from *Mycobacterium tuberculosis* (*M.tb)* positive and *M.tb* negative elephants. Symbols represent the average concentration of the specified biomarker in one sample, as calculated by averaging the measured concentrations in three replicates. The calculated limit of detection (LOD) for each biomarker assay is shown as a grey line.

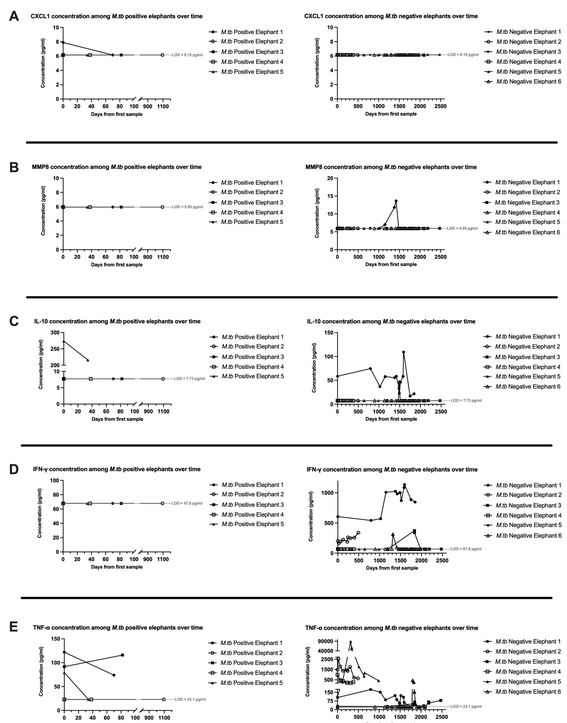

Figure 2. Concentrations of (A) CXCL1, (B) MMP8, (C) IL-10, (D) IFN-γ, and (E) TNF-α in samples from *Mycobacterium tuberculosis* (*M.tb)* positive and *M.tb* negative elephants, shown as a function of time from the date of the first sample received from each elephant (Day 0). Symbols represent the average concentration of the specified biomarker in one sample, as calculated by averaging the measured concentrations in three replicates. The calculated limit of detection (LOD) for each biomarker assay is shown as a grey line.

Results:

Biomarker concentrations were below the limit of detection for the assay in 100/101 (99%) samples for CXCL1, 98/101 (97%) samples for MMP8, 85/101 (84%) samples for IL-10, 75/101 (74%) samples for IFN-γ, and 45/101 (45%) samples for TNF-α. Except for TNF-α, most samples were below the limit of detection regardless of *M.tb* status. Two of the five *M.tb* culture positive elephants (*M.tb* positive elephants 2 and 4 in Figures 1 and 2) did not have detectable levels of any of the five biomarkers.

Conclusions:

Concentrations of CXCL1, MMP8, IL-10, IFN-γ, and TNF-α were below the limit of detection for our assays in most samples. However, a concentration above the limit of detection was present in at least one sample for each of the five biomarkers, confirming that these biomarkers can be detected in the serum of Asian elephants. Biomarkers were not substantially elevated in *M.tb* culture positive elephants compared to *M.tb* culture negative elephants. However, this may differ in elephants in later stages of disease that are demonstrating active clinical signs. More sensitive assays are needed to obtain a more accurate understanding of the presence of these biomarkers, and concentrations if present, in Asian elephant serum and better evaluate their utility in detecting *M.tb* infection in Asian elephants.

Acknowledgements:

Funding for this project was provided by the Tufts Elephant Conservation Alliance (TECA) Elephant Conservation Fellowship and Cummings School of Veterinary Medicine at Tufts University. We thank these funders, as well as the zoos that contributed banked elephant serum samples to make this project possible.

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