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# Histomorphological features of pulmonary tuberculosis in Asian elephants (*Elephas maximus*)

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Tuberculosis in Asian elephants usually shows involvement of lungs and associated lymph nodes. Grossly, the affected lobes of lungs reveal circumscribed lesions of varying sizes with central areas of caseation and necrosis. This report documents histomorphological features of pulmonary tuberculosis in six Asian elephants. The important cell types present were lymphocytes, foamy macrophages, epithelioid macrophages and Langhan's type of giant cells. In addition, caseation necrosis, fibrotic capsule, and mineralisation (calcification) were also observed. Characteristic features of tuberculoid granulomas observed in the six cases showed similarities with other species. The six cases showed varying proportions of the seven defined features but all features were present together only in one case.

## Keywords: Granuloma, histopathology

Pulmonary tuberculosis caused by the *Mycobacterium tuberculosis* complex (MTC) pathogens in mammals is characterised by granulomatous reaction consisting of different types of cells like foamy macrophages, epithelioid macrophages, and Langhan's giant cells with a peripheral rim of lymphocytes and a fibrous capsule (Sakamoto, 2012). A few granulocytes and plasma cells may also be present in these granulomas with a central core of necrosis and mineralisation. Minor variations in the proportion of these components may be seen in the lesions caused by various species of MTC pathogens in different mammalian hosts (Palmer *et al.*, 2022). The nature of the granulomas may also vary with age of the lesion and the stage of the disease (Dartois, 2011). Few reports have studied histomorphological features of pulmonary tuberculosis in Asian elephants, but have lacked descriptions of histomorphology (Landolfi *et al.*, 2015). This report describes the important types of cells and other lesions observed in pulmonary tuberculosis granuloma of Asian elephants.

Antemortem serological testing of six adult male captive Asian elephants (*Elephas maximus*) gave positive results on the DPP<sup>®</sup> Assay (Dual Path Platform Vet-TB Elephant assay kits, Chembio Diagnostic Systems Inc., USA). DPP is a rapid immunochromatographic test that was licenced by the United States Department of Agriculture for tuberculosis

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Fig.1: Acid-fast bacilli observed in a granuloma.



Fig. 2: Low magnification photomicrograph depicting multiple coalescing (CG) and non-coalescing (NCG) granulomas.



**Fig. 3:** Granuloma with a central core of caseation necrosis (CN). The granuloma depicted here lacked a fibrotic capsule.

screening in Asian and African elephants (Lyashchenko *et al.*, 2012). Over an extended period, the six animals died due to various reasons, which were mostly accidents associated with the annual episodes of musth. During postmortem examinations of these elephants, gross lesions suggestive of pulmonary tuberculosis were observed in the lung parenchyma of all elephants. Representative tissue



Fig. 4: Macrophage reaction in a granuloma, composed of many foamy macrophages with vacuolated cytoplasm (black arrow) and epithelioid macrophages (white arrow).



**Fig. 5:** Multinucleated giant cell reaction in a granuloma. Typical Langhan's cells (black arrow) with several nuclei arranged along the periphery of the cell in a horseshoe pattern. Some of them had only fewer nuclei (white arrow, in these cases binucleated cells). Scattered lymphocytes with scanty cytoplasm around the zone of giant cell reaction.

samples were collected and fixed in 10 per cent neutral buffered formalin, embedded in paraffin blocks (Suvarna *et al.*, 2018).

Zeel Neilsen staining of tissue sections was done to screen for the presence of acid-fast bacilli. Haematoxylin and eosin-stained sections were then evaluated for the presence of tuberculoid granulomas. Seven features of a typical tuberculoid granuloma that were evaluated included presence of lymphocytes, foamy macrophages, epithelioid macrophages and Langhan's type of giant cells along with lesions indicative of caseation necrosis, fibrotic capsule, and mineralisation (calcification). Representative photomicrographs were taken with an Olympus BX51 microscope loaded with DP71 camera.

Zeel Neilsen staining of tissue sections revealed the presence of acid-fast bacilli in all cases (Fig. 1). Typical coalescing and non-coalescing granulomas were observed in the six cases (Fig. 2). All the seven defined



Fig. 6.: Trinucleated cell (arrow) in a granuloma with a prominent fibrous capsule (star). Few lymphocytes are present along the rim of the caseating mass, inside the capsule.



Fig. 7: Mineralization (calcification) in a granuloma (white star) and prominent fibrous tissue capsule (black star).

features were observed together only in one case. Remaining five cases showed variations in presence of the seven features. Representative photomicrographs of each of the four defined cell types, namely, lymphocytes, foamy macrophages, epithelioid macrophages, Langhan's type of giant cells and the three typical lesions like caseation necrosis, fibrotic capsule, and mineralisation (calcification) identified in the six cases are shown in Fig. 3 to 7.

The major cell types in the histopathological lesions of tuberculoid granulomas described in humans and other animals were observed in the pulmonary tuberculosis granulomas of the Asian elephant. Variations in the nature and type of cells present need to be evaluated further with a larger sample size, with respect to the stage of disease in the host and the age and size of individual granulomas (Dartois, 2011). The failure to appreciate all features in the five cases can also be due to inadequate sampling. Such qualitative evaluations provide better insights on the pathogenesis and progression of the disease. Adjunct diagnostic procedures like culture and nucleic acid amplification techniques are required to confirm the different species of MTC organisms in these lesions.

### Summary

Histomorphological features of tuberculoid granulomas in Asian elephants show similarities to the lesions of pulmonary tuberculosis observed in other mammalian species.

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#### Conflict of interest

The authors declare that they have no conflict of interest.

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