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Gross and histopathological lesions associated with tuberculosis in two sloth bears (*Melursus ursinus*) in India

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Abstract

Post-mortem examination of two sloth bears which died in Bannerghatta Bear Rescue Centre, Bengaluru, Karnataka, were performed. Both the animals were anorectic and had considerable weight loss before death. Representative lung tissue samples were subjected to histopathology and staining. The lung tissues of the animals had diffuse congestion and subpleural petechial hemorrhages. In addition, small nodules of varied diameters were seen scattered on the lung lobes of both animals. On histopathological examination, the lung tissue of one of the animals showed extensive proliferation of blood vessels. Congestion and subpleural hemorrhages were seen in both cases. Few macrophages and epithelioid cells were seen scattered adjacent to a bronchiole. Kinyoun's acid fast staining of the histological sections revealed numerous acid fast bacilli indicative of tuberculosis.

Key words: Tuberculosis, sloth bear, post-mortem

Sloth bear (*Melursus ursinus*) is one of the four species of bears which are found in India. They are classified as vulnerable species according to the International Union for Conservation of Nature and included in schedule I of the Indian Wildlife Protection Act, 1972. Tuberculosis (TB), caused by *Mycobacterium tuberculosis* is a significant cause of morbidity and mortality in both wild

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and domestic animals worldwide (Lecu and Ball, 2011). Emergence of tuberculosis in sloth bears could be due to the spillover of *Mycobacterium tuberculosis* into this species because of their prolonged contact with humans. A significant number of sloth bears were poached by a community of people known as the Kalandars, and trained for street shows. There is inadequacy of diagnostic assays for detecting tuberculosis in these animals. Moreover, the affected bears appear apparently healthy and exhibit only mild or vague clinical signs in the terminal stages, which make diagnosis and treatment difficult. Hence diagnosis of tuberculosis in this species during post-mortem examination is important for considering preventive measures in live animals against TB.

Materials and methods

Two sloth bears, which died naturally at Bannerghatta Bear Rescue Centre, formed the sample of the study. Both the bears exhibited anorexia, isolation from the group and considerable weight loss before death. A thorough post-mortem examination of the animals was carried out and representative samples from lungs were collected in 10 per cent neutral buffered formalin for histopathology.

The formalin fixed samples were washed overnight, processed, cut at five micron thickness and stained with haematoxylin and eosin as per Suvarna *et al.*, 2012. Special staining technique, Kinyoun's acid fast staining, was done to demonstrate the presence of the acid fast bacteria. The staining procedure is briefed below.

The tissue sections were deparaffinized and hydrated to distilled water after which it was immersed in Kinyoun's carbol fuchsin and incubated at 50°C for one hour. The slides were differentiated in two changes of one per cent acid alcohol after washing in running tap water. Counterstaining was done using methylene blue solution and the slides were dehydrated in 95 and 100 per cent alcohol sequentially and cleared in two changes of xylene. The stained sections, after drying completely, were mounted in DPX and viewed under oil immersion objective (100x) in a compound microscope.

Fluorescent staining technique using auramine was also performed to further confirm the presence of acid fast bacilli. Staining technique was done as per Suvarna *et al.,* 2012 with slight modifications. The tissue sections were deparaffinized and hydrated to distilled water after which the slides were flooded with phenolic auramine – O and placed at 60°C for 10 minutes. It was then rinsed off with distilled water and decolourised using one per cent acid alcohol for 2 minutes. Counterstaining was done using Potassium permanganate solution for five minutes. The slides were then dehydrated in 95 and 100 per cent alcohol sequentially and cleared in two changes of xylene. The stained sections, after drying completely, were mounted in DPX and viewed under oil immersion objective (100X) of fluorescent microscope (Carl Ziess®).

Results and discussion

On gross examination, the carcasses of both the animals appeared emaciated. Diffuse congestion along with subpleural petechial hemorrhages were evident in all lung lobes of the first animal (Fig. 1). This finding is in agreement with Pereira (2016) who also reported presence of areas of congestion and hemorrhage as post-mortem findings in TB affected sloth bears. Small nodules of two centimeter diameter were seen scattered on both lung lobes. The right cranial lobe showed two distinct dark red colored nodules of three to four centimeter diameter. This is in agreement with Hedau and Kamdi (2016) who reported the same in a sloth bear carcass suspected to have died of TB.

The second animal showed multiple nodules of two to three centimeter diameter scattered over the parenchyma of both left and right lung lobes (Fig. 2). In addition, left lung lobes had diffuse areas of congestion and mild edema. The present findings are in agreement with Hunter (2011) who reported that granulomas were characteristic lesions of primary TB in immune-competent individuals.

Microscopically, extensive proliferation of capillaries along with severe congestion and hemorrhages were found in the lung tissue sample of the first animal, suggestive

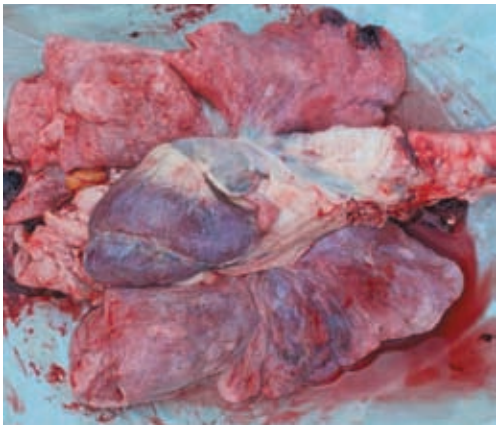

Fig. 1. Gross picture showing diffuse congestion and subpleural petechial haemorrhages in lung tissue

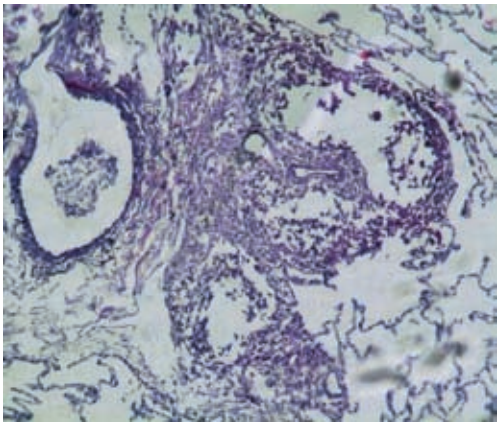

Fig. 4. Lung section with macrophages and epithelioid cells scattered adjacent to bronchiole, H and E (100x)

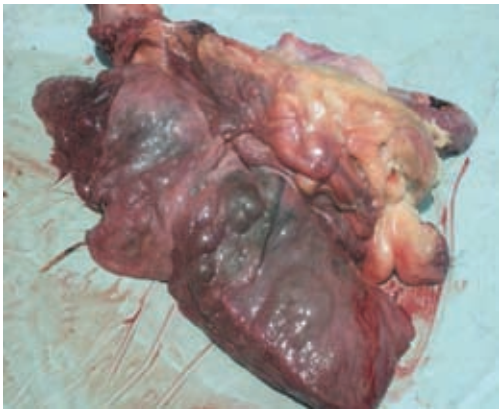

Fig. 2. Gross picture showing multiple nodular lesions in lung

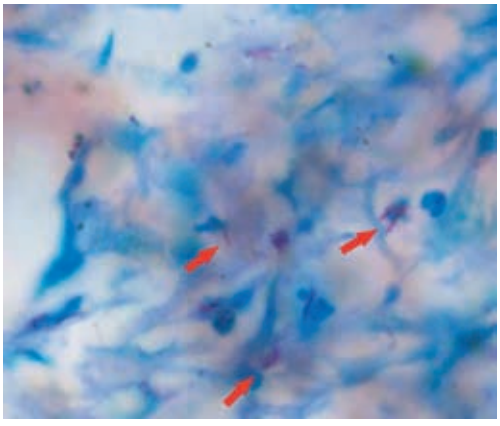

Fig. 5. Lung tissue with acid fast bacilli (arrow), Kinyoun's acid fast staining (1000x)

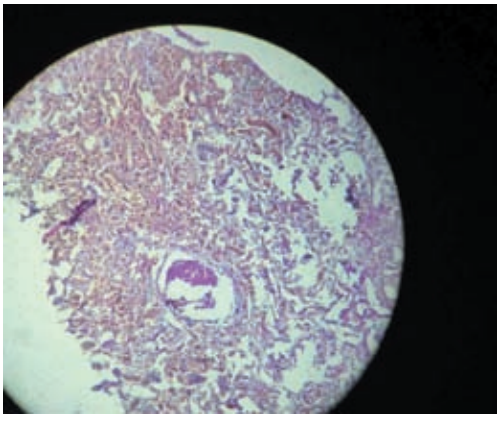

Fig. 3. Capillary proliferation with congestion and haemorrhage, H and E (100x)

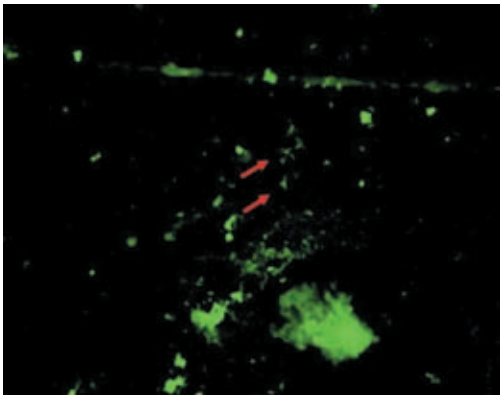

Fig. 6. Lung tissue with acid fast bacilli (arrow), Fluorescent staining (1000x)

of haemangioma (Fig. 3), whereas the lung tissue sample from second animal showed focal areas of macrophages and epithelioid cell proliferation adjacent to a bronchiole (Fig. 4). The typical tubercular granuloma with central caseous necrosis and surrounding inflammatory cells as described by Fefar *et al.* (2012) was not present in both the samples. However, Hernandez-Pando *et al.* (2000) had reported the presence of *M. tuberculosis* in lungs without typical histopathologic lesions of TB in case of human beings.

Both the tissue sections showed the presence of numerous acid fast bacilli when stained using Kinyoun's acid fast staining technique (Fig. 5). This is in agreement with Stephenson and Byard (2020) who reported that TB could be confirmed histologically by acid fast staining of suspected tissue sections. Rishikesavan *et al.* (2010) also confirmed TB in a captive male leopard by the presence of numerous acid fast bacilli in stained tissue impression smears.

Fluorescent staining for mycobacteria was also carried out in the tissue sections and both tissue samples showed the presence of numerous fluorescent acid fast bacilli when viewed under oil immersion objective (100X) of fluorescent microscope (Fig. 6). This is in agreement with Bodal *et al.* (2015) who reported that fluorescent staining was highly sensitive and can be used to confirm TB.

In the present study, both acid fast and fluorescent staining of histological sections showed identical results, which indicate that both these techniques are equally effective in confirming TB in post mortem samples. However, Kommareddi *et al.* (1984) reported that fluorescent staining using Auramine- O was simpler and had more sensitivity compared to Ziehl Neelsen (ZN) technique, at the same time, less specific than ZN technique.

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