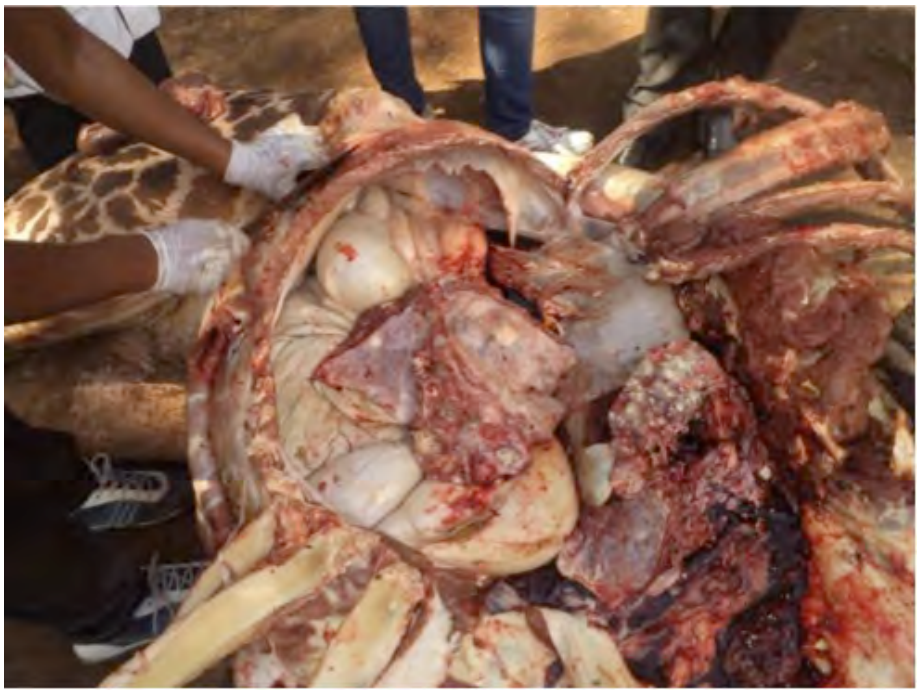
Generalized tuberculosis in captive Giraffe *Giraffa camelopardalis* - a case report



IUCN Red List:

Global — Vulnerable A2acd (Muller et al. 2016)

Post mortem inspection (thoracic cavity) of Giraffe

Mammalia
[Class of Mammals]

Cetartiodactyla
[Order of Even-toed ungulate]

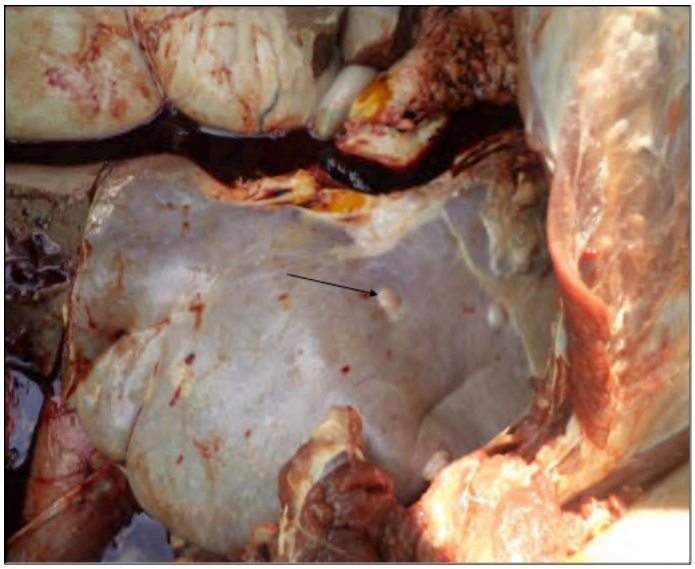
Giraffidae
[Family of ruminant artiodactyl mammals]

Giraffa camelopardalis
[Giraffe]

Species described by Linnaeus in 1758

Tuberculosis caused by *Mycobacterium tuberculosis* Complex, is one of the most important zoonotic diseases which infect animal as well as human. It is a chronic devastating disease which occurs in diverse group of animal (domestic, certain free and captive wild species) may allow the host to survive for months, even years, without any clinical symptoms (Van Soolingen et al. 1997; Behr et al. 1999). The concurrent infections and other stress factors may result into the clinical form of disease (Gupta et al. 2009). Zoo animals are also infected with tuberculosis due to more interaction between human, animals and environment. Tuberculosis has been reported from different captive herbivore and non-human primates (Rajkonwar 2003; Singh et al. 2006; Nath et al. 2012) of Assam. In this study, a case of tuberculosis has been reported in a giraffe of Assam State Zoo, Guwahati, Assam, India.

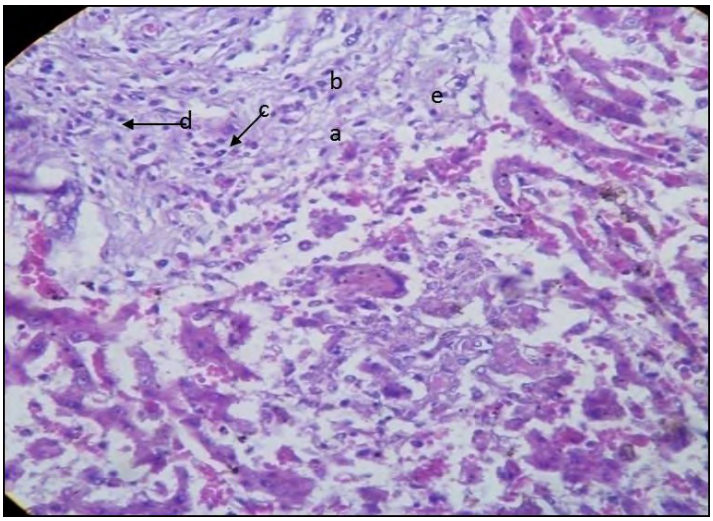
Post-mortem examination was done and the gross changes were recorded. Tissue samples were collected for isolation of the organisms as well as for histopathological examination in 10% formalin solution. Isolation of *Mycobacterium* spp. was done on Lowenstein-Jensen (L-J) media slants with or without pyruvate and incubated at 37^{0}C for 8 weeks. For histopathological examination representative tissue samples showing typical lesions of tuberculosis were collected from different organs *viz*. lung, liver, lymphnodes. Tissues were processed and stained with routine Haematoxylin and Eosin (H&E) method of staining (Culling 1974). DNA was extracted from the isolated *Mycobacterium* spp. with the help of DNA Sure Tissue Mini Kit (Genetics Brand) following manufacture's instruction. PCR was performed for each culture of *Mycobacterium* spp. following the method described by De los Monteros et al. (1998) to detect *pncA* and *oxyR* genes of *Mycobacterium* species using oligonucleotide primers. Biochemical tests *viz.* Nitrate Reduction test, Pyrazinamidase test, Niacin Detection test was done for species identification.



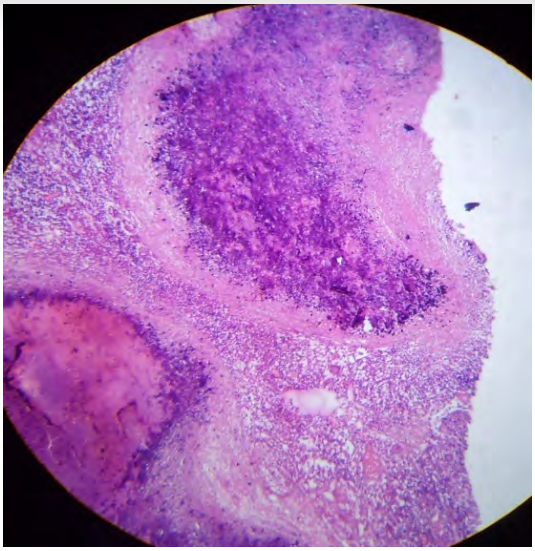
Liver showing solitary tubercles

Global Distribution:
Angola (Angola), Botswana, Cameroon, Central African Republic, Chad, Congo, The Democratic Republic of the, Ethiopia, Kenya, Mozambique, Namibia, Niger, Somalia, South Africa, South Sudan, Tanzania, United Republic of, Uganda, Zambia, Zimbabwe

The post-mortem examination displayed dry and calcified areas on incision from the lungs. Jones et al. (1997) and Vural & Alcigir (2010) have reported similar findings. Nodules were filled with yellowish green purulent exudates. It might be due to exudative type of lesions which is generally seen in more acute cases. Cut section of the enlarged lymphnodes displayed widespread yellowish-white caseo-necrotic foci in cortical region with

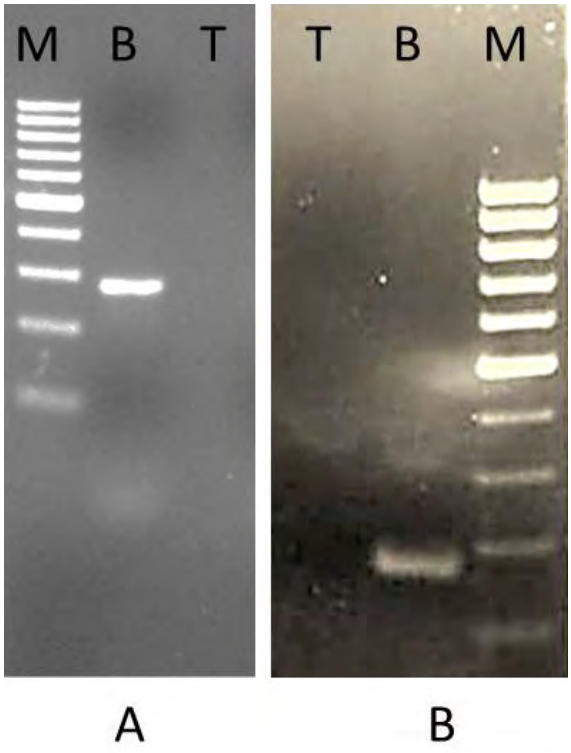


Photomicrograph of liver showing (a) fibrosis (b) necrosis with (c) infiltration of epithelioid cell (d) macrophage and (e) formation of giant cell. (H&E at 400X)



Photomicrograph of lymph node showing granulomatus lesions with calcification. (H&E at 100X)

or without central calcification. Similar findings were also observed by Vural & Alcigir (2010). *Mycobacterium* was isolated from the tissue samples (lungs, liver, lymph nodes, pleura, peritoneum and spleen) of necropsied animals died of tuberculosis by Muller et al. (2008) and Thakur et al. (2010). In H&E stain, liver showed fibrosis, necrosis with infiltration of epithelioid cell and macrophage. Microscopically, necrotic cores of lungs and lymph node contained dystrophic mineralization. Epithelioid macrophages and multinucleated giant cells surrounded necrotic areas and there were often moderate-to-marked infiltration of lymphocytes. Goswami et al. (2014) and Youssef & Ahmed (2014) also reported the same findings. Depletion of lymphocytes from the lymphoid follicles that was found in the present study might be associated with degeneration and necrosis of lymphocytes.



(A) Amplification of *M. bovis* specific *oxy*R with 280bp (lane B) but not with *M. tuberculosis* (lane T). (B) Amplification *M. bovis* specific *pncA* with 185bp (lane B) but negative for *M. tuberculosis* (lane T). Lane M indicates 100bp DNA marker.

Mycobacterium isolated from lung, liver and lymph nodes generated products of *Mycobacterium bovis* specific *pncA* and *oxyR* at 185bp and 280bp, respectively, while no products in *M. tuberculosis* specific *pncA* and *oxyR* gene was observed. Vathsala et al. (2007) also diagnosed *M. bovis* in wild animals with PCR by using primer sequence of *pncA* specific for *M. bovis*. All the isolates found to be negative for all biochemical tests which indicates positive for *M. bovis*.

Tuberculosis can be easily spread in the environment. So there is every possibility that the nearby captive animals in the zoo can get infected. Specification of organism has been necessary for proper curative treatment as *M. bovis* is resistant to pyrazinamide. Thus, the present study confirms the utility of *pncA* and *oxyR* gene based PCR to differentiate two closely

related species i.e. *Mycobacterium bovis* and *Mycobacterium tuberculosis* with high specificity and sensitivity.

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Acknowledgements

Authors are thankful to ICAR, New Delhi for funding the "Outreach Project on Zoonotic diseases" and Director of Research (Veterinary) for providing necessary facilities to carry out research work. Due acknowledgement is also extended to the Zoo animal attendants and workers who helped during the process.

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Citation: Konch, P., A.G. Barua, P. Sarma, H. Raj, B. Dutta & D. Sonowal (2017). Generalized tuberculosis in captive Giraffe *Giraffa camelopardalis* - a case report. Vet Brief #4. In: *Zoo's Print* 32(7): 23:26