

Pathomorphological Study of Bovine Tuberculosis in a Cow – A Case Report

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Abstract: The paper reports a case of bovine tuberculosis in a six-year-old dairy farm cow. Prior to death, the cow showed convulsion and froth from the mouth. At necropsy, lesions mostly observed in the lungs, lymph nodes, liver and kidney which were characterized by the circumscribed yellowish white lesions of various sizes and numbers. Histopathologically these lesions were characterized by tubercular granuloma displaying central necrosis with or without mineralization surrounded by macrophages, Langhan's giant cells, epithelioid cells, lymphocytes, plasma cells, neutrophils. Special stain Ziehl–Neelsen confirmed the acid fast bacteria in the tissue section of the lesions. Bacterial isolation was performed in the specialized laboratory for tuberculosis at IVRI from the samples, finally confirming the *Mycobacterium bovis* species.

Keywords: Pulmonary tuberculosis, Military dairy, Granuloma, Cow, histopathology

INTRODUCTION

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* is a chronic, infectious and progressive disease of cattle, other domesticated animals and certain free or captive wildlife species. It is characterized by the formation of non-vascular granulomas known as tubercles which occur most frequently in lungs, lymph nodes, liver, intestine, and kidney. The bovine TB is a significant veterinary disease that can spread to humans [1] via ingestion of contaminated milk and contact with infected animals. Bovine tuberculosis is still a major problem in developing countries for wildlife, public health, food safety, and the economy of livestock industries. Animals infected with BTB loose 10-25% of their productive efficiency in terms of milk production, weight gain, infertility and condemnation of meat [2]. Eradication of BTB is not easy in developing countries due to slam interaction between human and animal and lack of effective control measures. The disease in wildlife still poses a risk to livestock, tourism economy, and wildlife conservation even BTB has been mostly eradicated in the livestock industry of some developed countries through culling of infected animals and milk pasteurization [3]. Both officially bovine TB-free (OTF) and non-OTF countries reported an augment in the proportion of bovine TB positive cattle herds. Incidence and prevalence of BTB are escalating as per report[4]. Proper epidemiological status of bovine

tuberculosis in India is lacking but needs to be explored out in order to combat existing BTB situation.

The OIE recommends antemortem TB diagnostic method is the Intradermal Tuberculin Skin test (TST) which has been used in the eradication of bovine tuberculosis in the developing countries [4]. However, TST may give false-positive reactions due to exposure of some animals to environmental mycobacteria and may also give false-negative reactions due to immunosuppression, desensitization towards tuberculin, sub-potent use of tuberculin, and lengthy exposure to a field strain [5]. This test is labor intensive due to revisiting the animal for consecutive three days after tuberculin inoculation and usually impractical for free-ranging wildlife. TST cannot be repeated for at least 60 days because of systemic desensitization caused by the injection of tuberculin. The use of Comparative Intradermal Tuberculin Test (CITT) as a diagnostic test to overcome the problem of false positive reactions of the Single Intradermal test in India is restricted due to unavailability of avian tuberculin. *In vitro* blood IFN- γ assay measures the cell-mediated immune response of *M. bovis* infected animals[6] with the limitation of non-specific responses in a young animal, high logistic demand (culture start is required within 24 hours after blood collection), the requirement of well-trained personnel [5]. A test based on detection of humoral immune

response (i.e., antibody production) together with the test based on CMI increase level of pathogen detection can help to control bovine tuberculosis with some limitations [2].

At present, post-mortem diagnosis based on examination of gross lesions, followed by histopathology and culture, is widely used for surveillance of bovine tuberculosis in animals [5]. Bacteriological culture of clinical samples (i.e., milk, blood, nasal swab, and cattle tissues) is considered as gold standard for bovine tuberculosis diagnosis. But it requires a minimum of 3-6 weeks and presence of viable bacteria [5].

CASE HISTORY AND PATHOLOGICAL EXAMINATION

A 6-year-old cow, from Military dairy farm Bareilly was submitted to the necropsy with a progressive history of death. The cow showed convulsion and frothing from mouth prior to death. The animal was referred to Division of Pathology, IVRI, Izzatnagar, Bareilly (U.P) for necropsy. At necropsy, the most common lesions observed in the affected organs lungs, lymph nodes, liver and kidney which was characterized by the presence of circumscribed yellowish white lesions of various sizes and numbers (Fig.1). The lesions were observed in the lung and liver of the necropsied animal. Large encapsulated nodules containing yellowish white exudates were observed in some of the affected organs. There were also active

lesions with reddish to black demarcated necrotized areas, particularly on the lymph nodes of the affected animal, after cutting tubercle a gritty sensation that indicated the majority of the lesions collected were calcified. The tissues were collected in 10% neutral buffer formalin, were processed routinely and embedded in paraffin blocks. Then 4 to 5µm tissue sections were cut and stained with hematoxylin and eosin.

HISTOPATHOLOGICAL LESIONS

Histopathologically, the lesions were consisted of central areas of caseous necrosis, round, oval or irregular and often coalescing, eosinophilic, homogenous, with scant chromatin debris, few neutrophils and usually with moderate central mineralization. Often there was a surrounding moderate layer of epithelioid cells, Langhans-type multinucleated giant cells (Fig. 3) (round to oval, with less than 30 nuclei visible) collar of lymphocyte and plasma cells. Collagenous collective tissue was partially or completely surrounded most lesions, but with focal penetration of the capsule by the granulomatous process, extending the lesion into the surrounding parenchyma (Fig. 2 & 3). At the interface between the caseo-necrotic center and the surrounding inflammatory cell mantle, there were scattered foci of neutrophils and degenerate leucocytes observed. Acid-fast bacilli were observed in a tissue section by Ziehl-Neelsen stain that was found scattered randomly in both the central caseo-necrotic mass and granulomatous tissue (Fig.4).



Fig-1: Lung: grey and yellow nodular lesions were (arrows) seen with caseous-necrotic with center dry area

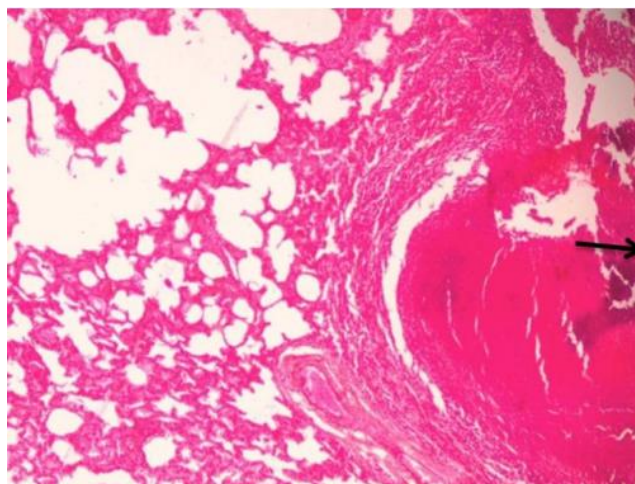


Fig-2: Typical granuloma in lung tissue section H&E x100

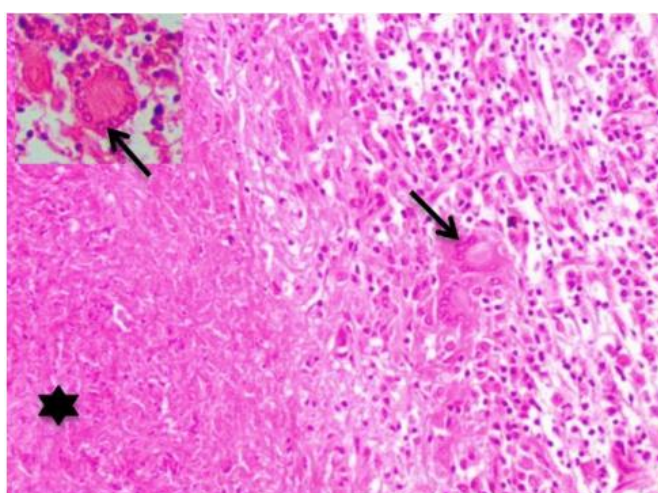


Fig-3: Mediastinal lymph node: the granuloma was consists of a central area of caseous necrosis (asterisk) with variable calcification, surrounded by epithelioid cells, Langhans' giant cells (arrows), lymphocytes, with a fibrous capsule infiltrated by lymphocytes. H&E x200

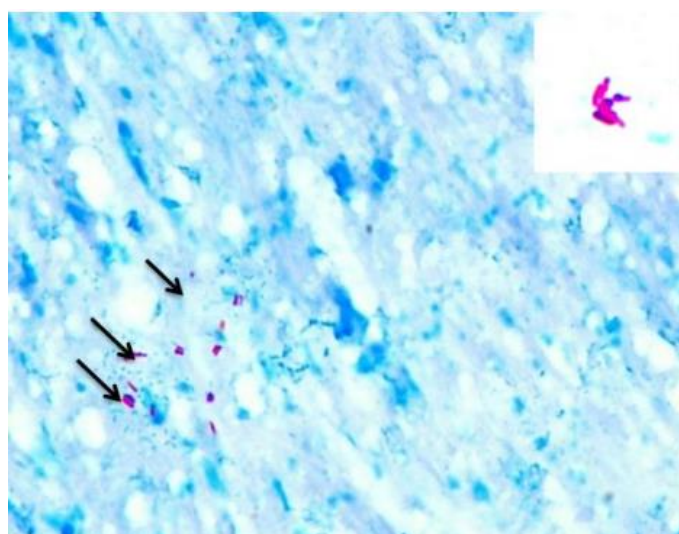


Fig-4: Tissue section stain: acid fast bacilli stained red rods by Ziehl–Neelsen stain (inset, arrows).

DISCUSSION

Bovine tuberculosis, caused by *M bovis*, has been on the increase in developed countries and

continues to occur in developing countries due to overcrowding and unhygienic management practice caused rapid spread of infection. Infectious diseases

study Subjects are one major reason for the economic losses in the dairy sectors. Bovine tuberculosis causes heavy economic losses and poses an enormous public health State [4].

In present case study, most common lesions observed in vital organs which were characterized by the presence of circumscribed yellowish white lesions of various sizes and numbers. The large encapsulated nodules containing yellowish white exudates were observed in some of the affected organs. Ameni *et al.* [10] reported the frequency and severity of the lesions are higher in the mesenteric lymph nodes than the thoracic lymph nodes and 90% of respiratory system infection. In present case study, similar lesion in mesenteric lymph node was also noted; the active lesion with reddish to black demarcated necrotized areas, after cutting a gritty sensation that indicated the lesion was calcified.

In the present study histopathological lesion in lung, liver, kidney and lymph node were characterized by the formation granuloma, from center to periphery necrosis with or without mineralization surrounded by macrophages, Langhan's giant cells, epithelioid cells, lymphocytes, plasma cells and neutrophils. Some workers recorded typical histopathological lesions previously [8, 9]. In present case acid-fast bacilli were observed in tissue section of lung, liver, kidney and lymph node by Ziehl-Neelsen stain that was found scattered randomly in both the central caseo-necrotic mass and granulomatous tissue. These finding agreement with previous studies [1,2,9]. One of essential systems applied to the eradication of bovine tuberculosis by *M. bovis* is the epidemiological surveillance of animals various dairy farms. This surveillance is conducted by means of inspection and taking samples, confirming the existence of the disease through the culture and molecular detection, which takes weeks before a result can be obtained [7].

CONCLUSIONS

A tentative diagnosis of bovine tuberculosis can be made by gross lesions on necropsy as the lesions of bovine tuberculosis are typical. Histopathological examination and special stain of the lesions confirm the diagnosis but bacteriological isolation from the lesion is the only way to make a definitive diagnosis of *Mycobacterium bovis* species. This disease is a significant zoonosis that can spread to humans, typically by the inhalation of aerosols or the ingestion of unpasteurized milk. Active animal tuberculosis outbreaks represent possible sources of infection to both animal and human populations.

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REFERENCES

1. Shah DH, Verma R, Bakshi CS, Singh RK. A multiplex-PCR for the differentiation of *Mycobacterium bovis* and *Mycobacterium tuberculosis*. *FEMS microbiology letters*. 2002 Aug 1; 214(1):39-43.
2. Radostits OM, Gay C, Hinchcliff KW, Constable PD. A textbook of the diseases of cattle, sheep, goats, pigs and horses. *Veterinary Medicine* 10th edition Bailliere, Tindall, London, UK. 2007:1576-80.
3. Michel AL, Müller B, van Helden PD. *Mycobacterium bovis* at the animal-human interface: A problem, or not. *Veterinary microbiology*. 2010 Jan 27; 140(3):371-81.
4. International Office of Epizootics. Biological Standards Commission, International Office of Epizootics. International Committee. Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds and bees. Office international des épizooties; 2008.
5. De la Rúa-Domenech R, Goodchild AT, Vordermeier HM, Hewinson RG, Christiansen KH, Clifton-Hadley RS. Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, γ -interferon assay and other ancillary diagnostic techniques. *Research in veterinary science*. 2006 Oct 31;81(2):190-210.
6. Wood PR, Jones SL. BOVIGAMTM: an in vitro cellular diagnostic test for bovine tuberculosis. *Tuberculosis*. 2001 Feb 1;81(1-2):147-55.
7. Parra A, García N, García A, Lacombe A, Moreno F, Freire F, Moran J, De Mendoza JH. Development of a molecular diagnostic test applied to experimental abattoir surveillance on bovine tuberculosis. *Veterinary microbiology*. 2008 Mar 18; 127(3):315-24.
8. Cassidy JP, Bryson DG, Pollock JM, Evans RT, Forster F, Neill SD. Lesions in cattle exposed to *Mycobacterium bovis*-inoculated calves. *Journal of comparative pathology*. 1999 Nov 1; 121(4):321-37.
9. Corner LA. Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. *Veterinary microbiology*. 1994 May 1; 40(1-2):53-63.
10. Ameni G, Aseffa A, Engers H, Young D, Gordon S, Hewinson G, Vordermeier M. High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to zebu breeds under field cattle husbandry in central Ethiopia. *Clinical and Vaccine Immunology*. 2007 Oct 1;14(10):1356-61.