

GROSS PATHOLOGY AND HISTOPATHOLOGY OF TUBERCULOSIS

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Abstract: *The current research work was carried out with the objective of evaluating gross and histopathological changes in different organs due to tuberculosis in 15 tuberculin test positive animals. Out of fifteen cases 12 animals revealed pulmonary form of tuberculosis, 01 with generalized form and 2 animals showed no observable lesions. The frequency of caseative lesions in affected animals were 73.73% (11/15) in bronchial lymphnode, 66.67% (10/15) in lungs, 40.0% (06/15) in mediastinal lymphnode. The remaining organs such as prescapular, retropharyngeal, mesenteric lymphnode, intestine and liver showed lesion at 6.67% (1/15). In histopathological study, granulomatous inflammation was observed in all affected organ characterized by focal or multifocal areas of central caseation with or without calcification, surrounded by a zone of inflammatory cells consisting of lymphoid cells, epitheloid cells and Langhan's type of giant cells.*

Key words: Bovine Tuberculosis, Mycobacterium, Histopathology

INTRODUCTION

Bovine tuberculosis (BTB) is a zoonotic disease that causes respiratory disorder in both cattle and humans. Active animal tuberculosis outbreaks represent possible sources of infection to both animal and human populations [1,2]. The Office International des Epizooties classifies bovine tuberculosis as a List B disease, a disease which is considered to be of socio-economic or public health importance and of great significance to the international trade of animals and animal products [3,4]. *Mycobacterium bovis* is a zoonotic bacteria and is the major cause of human infection in underdeveloped countries [5]. The infection in bovines occurs due to many reasons, such as colostrum/milk to calves [6], ingestion of infected flies [7], bird droppings [8], aerosol [9], contact with each other and with other wildlife [10], and excreta of flies [7]. It may be further mentioned that *tuberculosis bacilli* remain viable in the soil for up to 2 years [11]. The pure exotic breeds show higher susceptibility compared to the indigenous breeds [12].

Bovine tuberculosis is characterized by the formation of granulomas (tubercles) where bacteria are habitating. These granulomas are usually yellowish and either caseous, caseo-calcareous or calcified. They are often encapsulated and mostly restricted to thoracic region [13]. The involvement of various organs order wise include lungs, pleura and peritoneum, liver, udder, kidney, intestine, heart and adrenal glands. Further involvement of various lymphnodes were in the order of bronchial/mediastinal, prescapular, mesenteric, sternal, supramammary cervical, mandibular, retropharyngeal, parotid and tonsillar lymphnode [12]. As evident from the preceding discussion, the prevalence of the disease varies from region to region and also from one farm to another in same locality. The present study was designed to investigate gross pathological and histopathological lesions in cattle affected with tuberculosis.

MATERIALS AND METHODS

The study was carried out on the tuberculin test

positive animals (n=15) which were culled and a detailed autopsy was conducted on all the carcasses with recording gross pathological lesions. Representative tissue samples consisting of lungs, lymphnodes (bronchial, mediastinal, prescapular, retropharyngeal and mesentric), mammary gland, intestine, liver, kidneys, heart, spleen, pituitary gland, adrenal glands, thyroid gland, brain and pancreas were collected and preserved in neutral buffered formalin. Impression smears from the lesions were prepared for detecting the presence of acid-fast bacilli by Ziehl-Neelson's method of staining and oramine stain for culture smears. The formalin fixed materials were processed by routine paraffin embedding method, sections of 4-5 microns thickness were cut and stained with Haematoxylin and Eosin and Ziehl - Neelson methods [14] to study histopathology and to demonstrate acid fast organisms respectively. Von Kossa's stain was used to demonstrate calcium. Samples were also collected for culture purpose [14].

RESULTS

The tubercular lesions(gross or microscopic) involving different organs and systems have been studied in detail and classified (Table no 1 and Graph 1).Of the 15 tuberculin positive cases taken up for the study 12 animals revealed pulmonary tuberculosis, one with generalized tuberculosis and other two animals did not reveal any observable lesions.

Lungs: It revealed multiple nodular lesions measuring about 0.5-2.0 cms in diameter. The lungs were moderately emphysematous, moderate to severely congested and areas of consolidation (Fig.1a) with occasional focal areas of hemorrhages. Caseative nodules measuring about 0.5 to 5 cm in diameter were observed

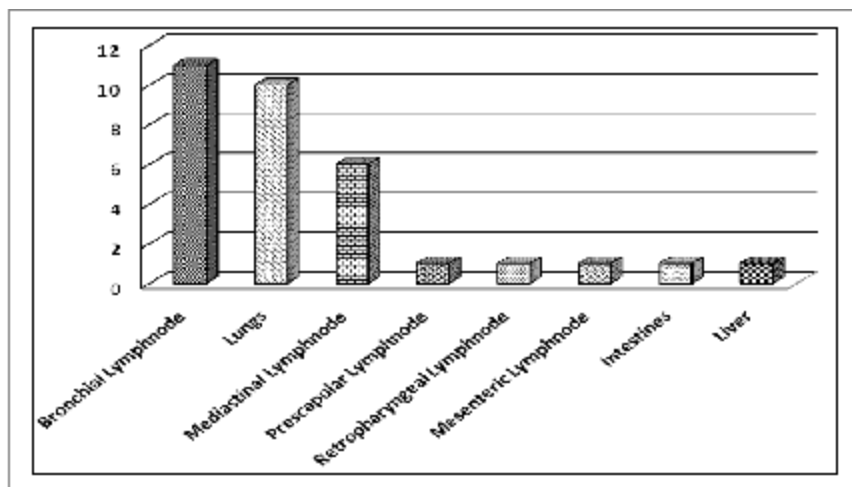
Table 1. Involvement of Individual Organs with Tuberculosis

No.	Organ / Lymphnode	(n)	per cent
1.	Bronchial Lymphnode	11	73.73
2.	Lungs	10	66.67
3.	Mediastinal Lymphnode	06	40.00
4.	Prescapular Lymphnode	01	6.67
5.	Retropharyngeal Lymphnode	01	6.67
6.	Mesenteric Lymphnode	01	6.67
7.	Intestines	01	6.67
8.	Liver	01	6.67

in different lobes of the lungs in ten cases and some of these nodules were also calcified (Fig 1.b).

Microscopically the parenchyma of lungs revealed mild to moderate degree of congestion of vessels and capillaries, multifocal areas of hemorrhage, oedematous exudate in a few alveoli along with cellular debris and alveolar emphysema. Granulomatous inflammation in the lungs was observed in ten cases and it was characterized by focal or multifocal areas of central caseation with or without calcification, surrounded by a zone of inflammatory cells consisting of lymphoid cells, epitheloid cells and Langhan's type of giant cells (Fig1.c). In eight cases the granuloma showed fibrous tissue encapsulation. Acid fast bacilli were demonstrated in the lung sections using Ziehl-Neelson's staining (Fig 1.d).

Lymphnodes: The bronchial and mediastinal lymph nodes showed tubercular lymphadenopathy in ten and six cases respectively. These lymph nodes were enlarged and oedematous and contained circumscribed nodular lesions. The cut surfaces revealed yellowish to white caseo-calcified material (Figs. 1e,f). Similar lesions were also seen in prescapular, retropharyngeal and mesenteric lymphnodes (Fig 2.a) in one case each. Smears from the granulomatous lesion revealed presence of acid fast bacilli with



Graph 1. Involvement of individual organs with tuberculosis



Fig. 1a: Lung -Emphysema - Consolidation – Congestion

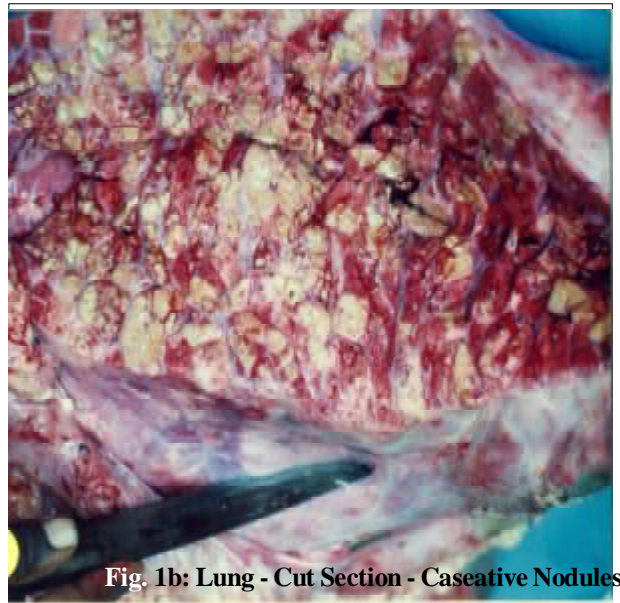


Fig. 1b: Lung - Cut Section - Caseative Nodules

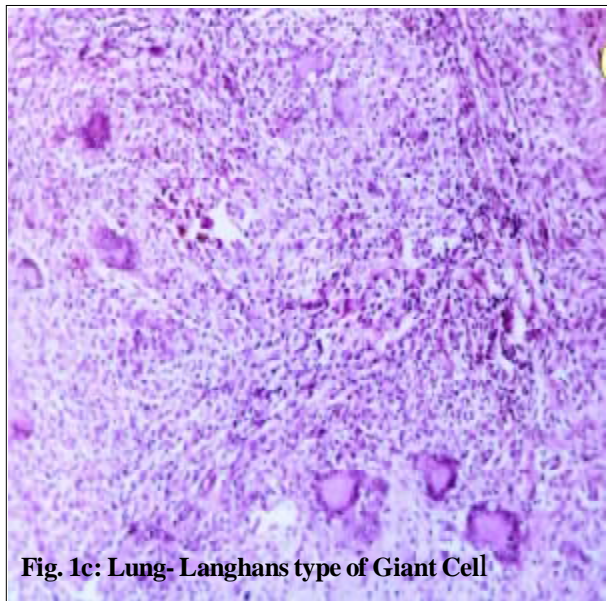


Fig. 1c: Lung- Langhans type of Giant Cell

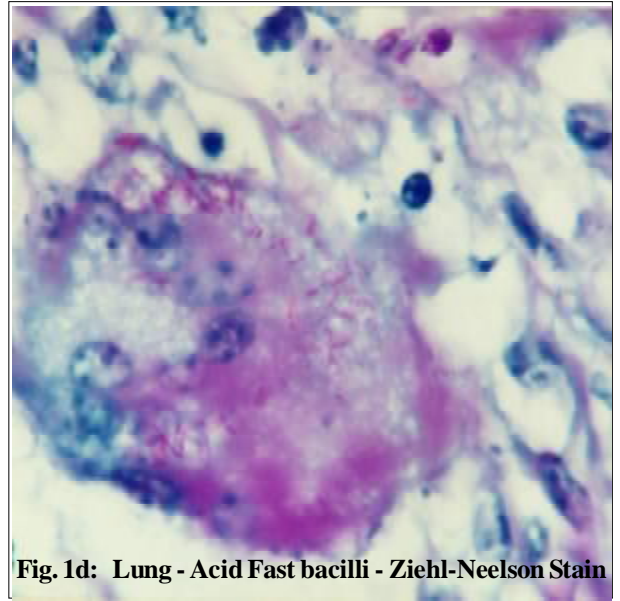


Fig. 1d: Lung - Acid Fast bacilli - Ziehl-Neelson Stain



Fig. 1 e: Bronchial Lymphnode - Greyish white - Caseative Nodule



Fig. 1 f: Mediastinal Lymph node - Caseative tubercles

Ziehl-Neelson's staining and cultures also revealed the presence of bacilli by oramine staining (Fig 2.b).

Microscopically the lesions in all the lymph nodes consisted of focal to multi-focal areas of granulomatous inflammation characterized by central areas of caseation with (Fig 2c) or without calcification surrounded by epithelioid cells, giant cells (different stages of formation) and lymphoid cells (Fig. 2d). Moderate degree of lymphoid (CT) hyperplasia with connective tissue proliferation was observed in seven cases. The capsule was thickened either due to subcapsular oedema or due to capsular connective tissue proliferation along with eosinophil infiltration in two cases. In three instances the involvement granulomatous inflammation in bronchial lymph nodes was extensive, leading to coalescence of the tubercular lesions with resultant loss of lymphoid architecture. In these cases additional features included massive CT proliferation mimicking sarcomatous picture. Other microscopic changes were congestion of blood vessels with areas of haemorrhage involving the medullary region. Anthracosis of mild to moderate degree was also seen.

Mammary Gland: Grossly mammary glands did not reveal any lesions. Microscopically in most of the acini were filled with eosinophilic exudate along with cellular debris and infiltration of large number of neutrophils also some of the cases showed vacuolated acini's with calcium concretions. Slight thickening and oedema of inter acinar connective tissue was observed.

Intestines: Grossly the mucosa of the small intestines showed mild to moderate degree of congestion. Histologically, well circumscribed caseo-calcareous tubercular lesion encapsulated by fibrous tissue (Fig 2.e). Other changes in the intestines included, multifocal areas of haemorrhages, massive infiltration of mononuclear cells and eosinophils in the lamina propria were evident. In some of the cases occasionally, villi showed desquamation of the epithelium and goblet cell hyperplasia. Tunica serosa and muscularis showed stray infiltration of lymphoid cells with mild degree of edema in seven cases.

Liver: Grossly in 12 cases the liver was dark red in color, surface was smooth and the borders were slightly rounded. Microscopically a single case revealed foci of early granulomatous lesion characterized by infiltration of lymphocytes and epithelioid cells (Fig. 2f).

Spleen: Grossly in all the cases spleen appeared congested with occasional haemorrhagic areas. Sections of spleen revealed massive degree of haemorrhages and hemosiderosis. Lymphocytolytic activity was evident in some of the cases with hyperplasia of splenic corpuscles.

Kidney: Grossly in almost all the carcasses both the kidneys were haemorrhagic and congested. Microscopically kidneys showed moderate degree of congestion of vessels and glomeruli, sclerosis of vessels, Lymphoid cell infiltration, segmental thickening of Bowman's capsule and periglomerulitis in a single case. Other changes included granular cell swelling of the tubular epithelium along with desquamation and degenerative changes with presence of proteinaceous material in the tubular lumen and mineralization. Brain and Pancreas did not reveal any changes.

Thyroid Gland: Grossly thyroid glands were slightly congested. Microscopically capillaries and blood vessels were congested, along with focal areas of haemorrhages. In most of cases there was evidence of colloid goitre characterised by highly distended follicles filled with colloid in addition to rupture of occasional follicles and formation of satellite and secondary follicles.

Pituitary did not reveal any changes grossly. Microscopically severe congestion of the blood vessels and occasional areas of haemorrhage were noticed in three cases. Numerous cysts of varying size lined by low cuboidal to flat epithelial cells filled with faintly eosinophilic material were observed in pars distalis.

Adrenals: Grossly adrenals appeared slightly enlarged in size and it did not reveal any visible lesions on incision. Microscopically, moderate degree of cortical hyperplasia and medullary hyperplasia were observed.

DISCUSSION

Pulmonary form of tuberculosis was the feature observed in the present study. Similar observations were made by [15-21]. It is a well established fact that pulmonary tuberculosis is the most common form of tuberculosis in cattle. In the present study, the frequency of tubercular lesions encountered in bronchial lymph node was 73.37 per cent and 40.0 percent in mediastinal lymph node. Whereas [18]



Fig. 2a: Mesenteric Lymph node - Caseative Lesions

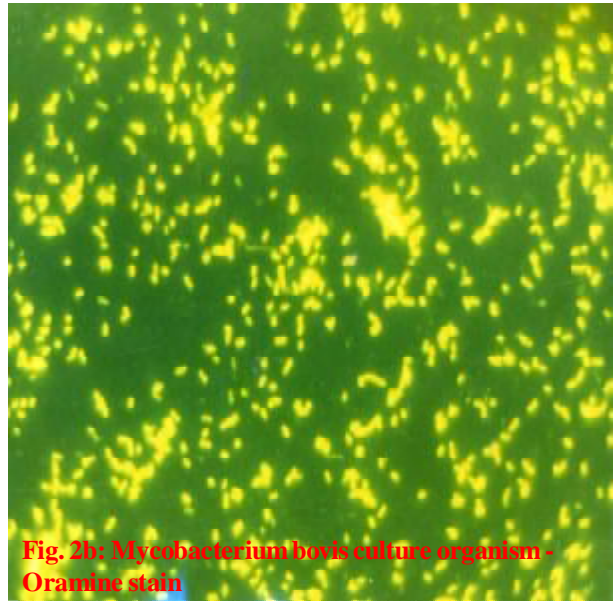


Fig. 2b: Mycobacterium bovis culture organism - Oramine stain



Fig. 2c: Lungs -Dystrophic calcium - Von Kossa's stain

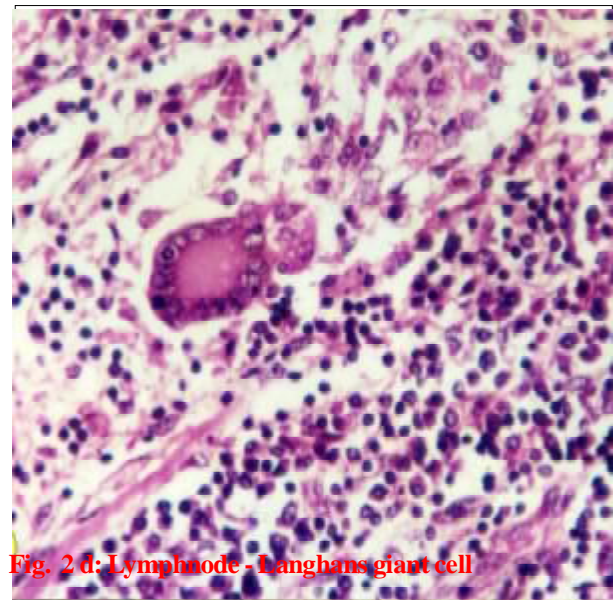


Fig. 2 d: Lymphnode - Langhans giant cell

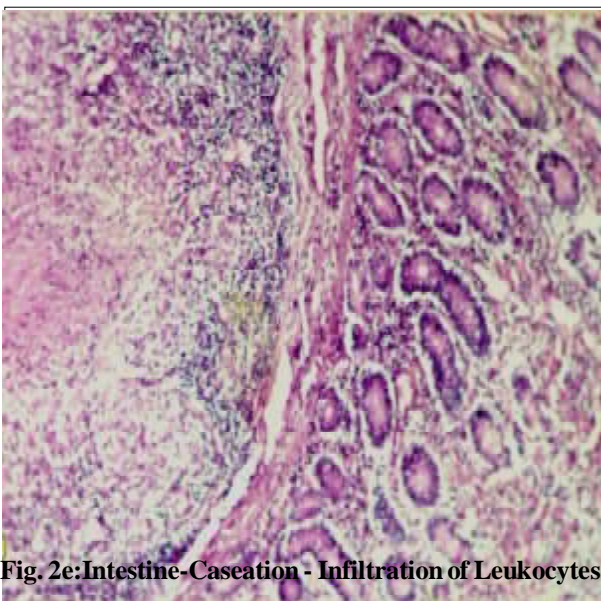


Fig. 2e: Intestine-Caseation - Infiltration of Leukocytes

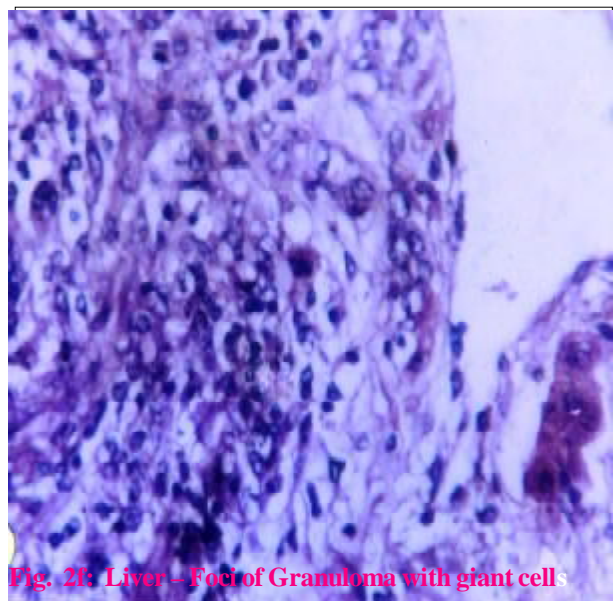


Fig. 2f: Liver - Foci of Granuloma with giant cell

recorded 100 per cent involvement of lungs and lymph nodes and [12] recorded 82.8 per cent in lungs and 92.4 per cent involving lymph nodes.

Tubercular lesions in intestines are relatively uncommon and they occur more frequently in calves than in adults [22]. However in the present study a solitary case of intestinal tuberculosis was noticed along with lesions in the mesenteric lymph nodes. Similar report of an incidence of 4.68 per cent of intestinal tuberculosis [18] and 1.8 per cent were observed [12]. Lymphoid hyperplasia of different degrees was observed in the lymph nodes with or without tubercular lesions. This hyperplasia may be due to the exposure of lymph nodes to an antigen which reacts with immunocompetent antibody producing cells, bringing about paracortical and follicular hyperplasia [23]. A cystic space in pituitary was observed in the current study which could be due to hyperestrogenism and stress due to disease process [22]. Mild to moderate degree of colloid goitre was observed in nine cases which may be due to deficiency of iodine in soil and water [24-26]. Cortical and medullary hyperplasia and haemorrhages of adrenal gland was observed, which was in accordance with the observation of other researchers [22,26]. These changes could be attributed to chronic infection and stress [27,28]. Bovine tuberculosis poses a significant risk to human and animal health. The only way to be protected from the disease is through prevention. It is important to limit the exposure of the herd to other infected cattle. Testing and eradication of the infected animals is the current method of control, though additional research is currently being explored in the areas of vaccinations and other possible preventative measures. It can be concluded from the present study that tuberculosis primarily affects the lungs and the sentinel lymph nodes characterized by the formation of tubercles with caseative necrosis at the centre and microscopically, they are characterized by the presence of epithelioid cells, lymphocytes, giant cells and fibrous tissue encapsulation.

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