Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(2): 1130-1133

Received: 01-01-2020
Accepted: 03-02-2020

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Histopathological alterations in lymph node of *Theileriosis* affected crossbred cattle: A case report

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Abstract
A nine (9) year old Jersey crossbred cow carcass was presented for necropsy to local veterinary dispensary with a history of inappetance, pyrexia (106^0F), lacrimation, decreased milk production, lethargy, dark brown coloured urine and prolonged lateral recumbency. On necropsy, petechial conjunctiva, pallor visible mucosa and enlarged prescapular lymph node were observed. Smears (blood and lymph node aspirate) and lymph node tissue sections were stained with wright-giemsa and haematoxylin-eosin stain respectively at Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad. Cytologically, smears showed presence of pleomorphic piroplasms in erythrocytes and schizonts in mononuclear cells. Histopathologically, lymph node sections revealed depleted lymphocytes in cortical lymphoid follicles, lymphocyte proliferation and degenerated high endothelial venules in paracortex. Medulla showed distended sinuses with endothelial degeneration and distorted cords with decreased cell density. In conclusion, the findings noticed in the present paper revealed that *Theileriosis* caused by *T. annulata* is associated with lymphoid tissue alterations.

Keywords: *Theileria annulata*, Bovine, pleomorphic piroplasms, schizont, lymph node, histopathology

1. Introduction
Theileriosis is a group of disease caused by various species of *Theileria* which is an apicomplexan obligatory intracellular protozoan parasite and has a complex life cycle involving both vertebrate and invertebrate host [1, 2]. Six *Theileria* species infest Bovidae among which most pathogenic species are *T. annulata* and *T. parva* which mainly affects lymphoid tissue such as Lymph nodes [3]. Based on the study conducted by All India Coordinated Research project of Indian Council of Agriculture Research during 1989 it was revealed that *T. annulata* causing Bovine Tropical *Theileriosis* was predominant across Indian subcontinent with an estimated annual losses of about US$ 384.3 million due to high mortality, decreased production, and reproductive problems [4, 5]. The endemic disease of the country was transmitted by tick *Hyalomma anatolicum anatolicum* and mainly recorded in exotic and crossbreed cattle of all age groups with highest prevalence in young calves of less than one (1) month age [2, 6].
Clinical signs shown by the affected animal include high fever (107^0F), anorexia, weight loss, debility, long lasting anaemia, jaundice, tachypnea, cough, salivation, lacrimation, petechiae on conjunctiva, enlarged lymph nodes and abortions or stillbirths [3, 7-12]. Major post-mortem lesions observed *viz.*, enlarged, edematous and haemorrhagic lymph nodes (prescapular and sub maxillary), haemorrhagic spots on subcutis, pleurae, peritoneum, omentum, serous surface of gastrointestinal tract (GIT), mucous membrane of lips, skeletal muscles, pericardium, myocardium, endocardium and tongue, splenomegaly, punched out ulcers having necrotic centers with haemorrhagic borders on mucosal surface of abomasum and haemorrhagic spots on liver and kidney parenchyma [13, 14]. Diagnosis of the disease is based on severe clinical signs and confirmation is by microscopic examination of Giemsa stained thin blood or lymph node smears for presence of piroplasms in erythrocytes and Koch's blue bodies in lymphocytes [15]. Present communication describes the pathogenic effect of *T. annulata* on blood cells and lymphoid tissues like lymph node.

2. Materials and methods
A nine (9) year old Jersey crossbred cow carcass from Gundur village in Nagarkurnool district of Telangana state was presented for necropsy to local veterinary dispensary (VD) immediately after death. According to history collected from owner, ailing animal exhibited

clinical signs *viz.*, inappetance, severe pyrexia (106^0F), lacrimation, decreased milk production, lethargy, dark brown coloured urine and prolonged lateral recumbency with open mouth breathing. All animals in the herd were injected with Hitek (Ivermectin) at the rate of 1 mL/ 50 Kg b.wt *via* subcutaneous route one (1) week prior to animal death and hence, no ticks were recovered from carcass. Thin smears prepared from lymph node aspirate and peripheral blood were air dried, fixed in methanol and then wrapped in tissue paper with suitable labelling. After necropsy, organs like lymph nodes were collected in ten (10) percent neutral buffered formalin (NBF) for histopathology and then sent to Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad along with the smears. For cytology, smears were stained with 10 percent wright-geimsa stain and examined under oil immersion lens of microscope. After seventy-two (72) hours of fixation in 10 percent NBF, tissue slices were washed under running tap water for overnight and further dehydrated in alcohol, embedded in paraffin and 5 microns thick sections were cut with the microtome. Then sections were stained with Hematoxylin and Eosin (H&E) and examined under microscope [16].

3. Results and Discussion

The present paper depicts a case report of bovine tropical theileriosis in cross breed cattle based on necropsy findings, cytology and histopathology.

External examination showed that the carcass was in rigor mortis and other abnormalities recorded include petechiation of conjunctiva, pallor visible mucosa and a palpable swollen area at the point of shoulder (Fig 1). On necropsy, it was revealed that the swollen area was due to enlarged prescapular lymph node (8.5 cm × 5 cm) with hard consistency (Fig 2). In the present case, lymph node enlargement could be due to focal areas of lymphocyte proliferation in deeper cortex and this opinion has been supported by authors [17]. Inappetance and lethargy were attributed to pyrexia which could due to increased thermoregulatory set point in the hypothalamus by endogenous pyrogens released from parasite mediated host cell lysis. The opinion was agreed with statement given by various researchers [18, 19]. Dark brown coloured urine and pallor mucosa in the current report could be due to hemolysis resulted from erythrophagocytosis of piroplasmic erythrocytes by macrophages of reticuloendothelial system [20]. In the present case report, clinical signs and necropsy lesions observed were in accordance with the earlier findings of authors [20-22].

Fig 1: Cattle carcass showing enlarged prescapular lymph node (arrow).

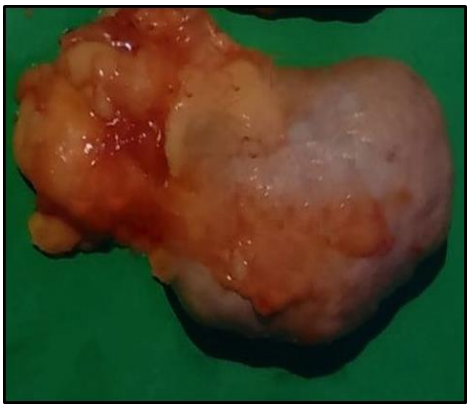

Fig 2: Enlarged prescapular lymph node (arrow).

Cytological examination of wright-giemsa stained smears showed presence of both pleomorphic piroplasms (annular and comma) in erythrocytes (Fig 3) and schizonts in mononuclear cells (lymphocytes) (Fig 4). The findings were similar to the observations of authors [11, 17, 23].

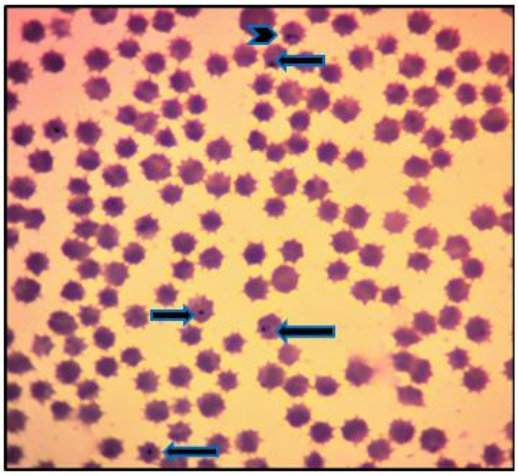

Fig 3: Peripheral blood smear showing annular (arrow) and comma (arrow head) shaped piroplasms of *T. annulata* inside erythrocytes (Wright-giemsa stain x 100X).

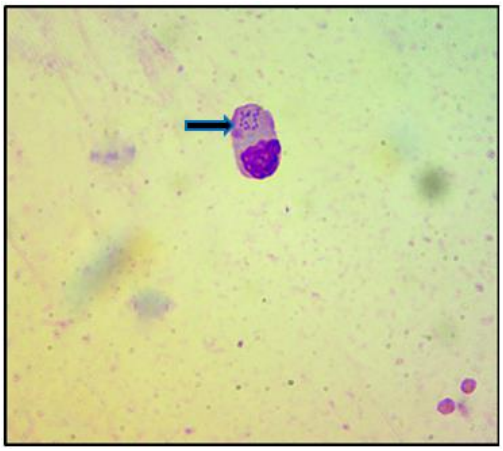

Fig 4: Lymph node aspirate smear showing multi nucleated schizont (arrow) in lymphocytic cytoplasm (Wright-giemsa stain x 100X).

Histopathologically, sections of lymph node cortex revealed decreased lymphocyte density in germinal center (GC) of secondary lymphoid follicle (Fig 5) whereas paracortex showed degenerated high endothelial venules (HEV) and focal areas of lymphocyte proliferation with prominent demarcation of cortico-medullary junction (Fig 6). Sections of

medullary region showed distorted medullary cords with decreased cell density, few cords with ruptured capillaries and distension of medullary sinus with endothelial degeneration (Fig 7). The pathological changes were in agreement with the previous studies of a number authors [24-26]. Contrary to this, hyperplasia of lymphoid follicles was observed by different authors [13, 27].

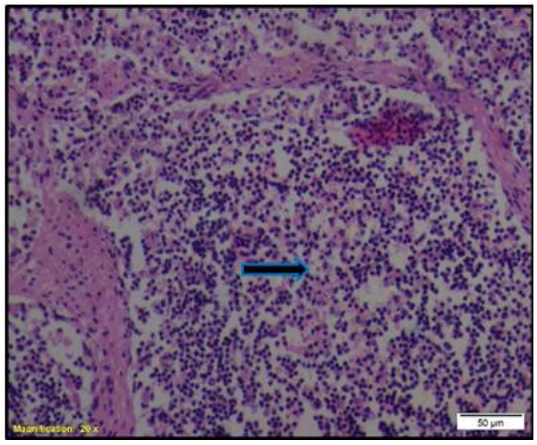

Fig 5: Photomicropgraph of lymph node cortex showing depleted lymphocytic population in germinal center of secondary lymphoid follicle (arrow): H&E 50µm.

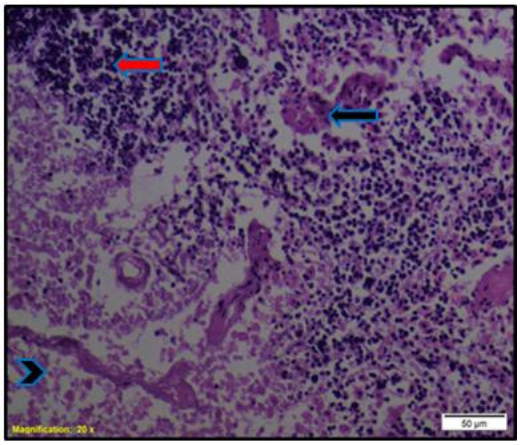

Fig 6: Photomicrograph of lymph node paracortex showing lymphocyte proliferation (red arrow), degenerated high endothelial venules (black arrow) and reticular frame work degeneration (arrow head): H&E 50µm.

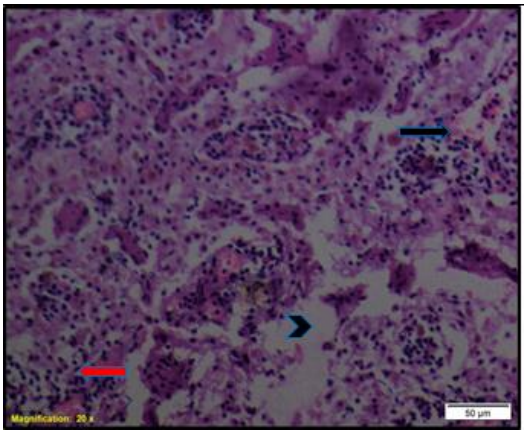

Fig 7: Photomicrograph of lymph node medulla showing distorted medullary cords with decreased cell density (red arrow), few cords with ruptured capillaries (black arrow) and distension of medullary sinuses with endothelial degeneration (arrow head): H&E 50µm.

4. Conclusion

In conclusion, the current paper emphasizes the pathogenic effect of *Theileria* on blood cells and lymphoid tissues such as lymph node which was diagnosed on the basis of necropsy findings, cytology and histopathology. In the present case report, based on the available disease epidemiological data of *Theileria* in Telangana state, it was assumed that the infection could be due to *T. annulata*. Further there is a scope for species identification which can be done through gene expression techniques.

5. Acknowledgements

The authors are extremely thankful to P V Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad for providing support and necessary facilities to carry out the work.

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