Original Research

Characterization of Two Natural Cases of Lymphomas in Bovines

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Abstract

Lymph nodes are frequently affected in lymphomas in animals and people. The present research was undertaken to characterize two cases of lymphomas causing lymphadenopathy in bovines. Impression smears from affected lymph nodes and visceral organs were stained with Wright's stain for cytology. Tissue samples were collected in 10% neutral buffered formalin and processed for histopathology and immunohistochemistry. Grossly there was enlargement of various peripheral and visceral lymph nodes in first case whereas second case revealed enlargement of mediastinal lymph node with involvement of lung. Histopathological examination showed presence of pleomorphic small neoplastic lymphocytes in lymph nodes, lungs and heart in first case. However, there was diffuse monomorphic cell infiltration of small lymphocytes in mediastinal lymph node in the second case. Based on the positive immunoreactivity for CD3, CD4, CD8, and focal positive reactivity for p-27, the first case was diagnosed as mixed T and B-cell lymphoma. Whereas, the second case was diagnosed as T-cell lymphoma based on positive reactivity for CD3 and CD4. The study concluded that immunohistochemistry was helpful tool in further characterization lymphoma into B- and T-cell lineage.

Key words: Bovine, Immunohistochemistry, Lymphadenopathies, Lymphoma

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Introduction

Lymphoma is a neoplastic disease caused by bovine leukemia virus (BLV), a B-lymphotropic oncogenic virus belonging to retroviridae family that infects cattle worldwide and is the causative agent of enzootic bovine leukosis (EBL), a neoplastic proliferation of B cells (Burny *et al*., 1988; Kettmann *et al*., 1982). BLV infection is characterized by a long period of viral latency and by the absence of viremia. Four epidemiologic and anatomic forms of bovine leukosis have been described: adult multicentric form, cutaneous form, adolescent thymic form (TBL) and the multicentric calf (juvenile) form (CBL). Only the

adult multicentric form is enzootic (Enzootic bovine leukosis, EBL), and is associated with BLV infection. It occurs predominantly in adult cattle with a peak incidence in 5 to 8-year old age group (Vernau *et al.*, 1992). The other three forms are sporadic (Sporadic bovine leukosis, SBL). The calf form mostly exists in cattle around 6 months of age and results in lymphadenopathy. Whereas, the thymic and skin form seems to occur in between 7-24 years of age (Asahina *et al*., 1995). Tumor cells in EBL predominantly consist of B-cell lineage (Takashima *et al*., 1977; Onuma *et al*., 1982) and in SBL the tumor cells particularly comprise of T-cell lineage (Koyama *et al*., 1983; Ishiguro *et al*., 1994). Three different forms of BLV infection have been reported; BLV infection alone without any clinical expression; increase of the absolute number of peripheral blood lymphocytes and the lymphoma; common form recorded in adults (Burny *et al.*, 1988; Radostitis *et al.*, 1994). Bovine lymphoma is generally classified according to age and epidemiologic association with BLV infection. Lymph nodes are involved in bovine lymphoma and thus examination of lymph nodes helps in the diagnosis of lymphoma. So, the present study was undertaken to characterize two cases of lymphomas associated with lymphadenopathy in bovines.

Materials and Methods

The present study was conducted on two suspected cases of bovine lymphomas on the basis of peripheral blood smear examination and gross lesions in lymph nodes. Both the animals were presented for necropsy examination to the Department of Veterinary Pathology, GADVASU, Ludhiana. Tissue samples of affected lymph nodes and associated visceral organs were collected and fixed in 10% neutral buffered formalin for routine histopathological examination and immunohistochemical analysis. Formalin fixed tissues were processed for paraffin wax embedding, sectioned at 4–5 µm and stained with haematoxylin and eosin (H&E) for routine histopathology (Luna, 1968). In addition, impression smears were prepared from affected lymph nodes and visceral organs and stained with Wright's method for cytology.

For immunohistochemical analysis, 3µ thick paraffin tissue sections were spread on positively charged microscopic slides (Fisher Scientific, USA). Antigen retrieval was done in EZ antigen retrieval solutions using EZ-Retriever System (BioGenex Laboratories Inc., California). After endogenous peroxidase and nonspecific protein blocking, the sections were incubated with a panel of primary antibodies including CD20, CD79, p-27, CD3, CD4 and CD8 (Table 1) in a humidified chamber at 4^0C overnight. Secondary antibody conjugated with HRP (Vector Laboratories, USA) was added and incubated for 30 min at room temperature. Antigen-antibody complex was visualized using ImmPACT™ DAB Peroxidase Substrate Kit (Vector Laboratories, USA) followed by counterstaining with hematoxylin. The stained slides were evaluated for immunohistochemical reaction using microscope under oil immersion.

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Table 1: Panel of antibodies used for immunohistochemical characterization of lymphoma

S. No.	Antibody (Catalog No.)	Company	Clonity	Dilution used
1	CD3	Sigma- Aldrich Steinheim	Monoclonal	0.180555556
2	CD4 (ILA11)	VMRD, Pullman, WA	Monoclonal	1:20
3	CD8 (BAQ111A)	VMRD, Pullman, WA	Monoclonal	1:20
4	CD79	AbD Serotec	Monoclonal	0.111111111
5	p-27	BioGenex, USA	Monoclonal	1:30
6	CD20	BioGenex, USA	Monoclonal	RTU

Results

In the present study, two cases of bovine lymphomas were suspected on the basis of gross pathological lesions in lymph nodes and associated organs. First case was presented to the Large Animal Clinics of the University. Hematological examination revealed high leukocyte count (3, 65,400/µl) with almost 100% lymphocytes and it was diagnosed as lymphocytic leukemia (Fig.1).

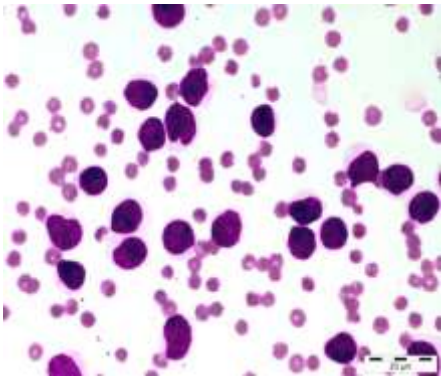

Fig. 1: Blood smear showing pleomorphic nuclei in lymphocytes. Giemsa stain, Bar=20µm

Grossly there was enlargement of various peripheral and visceral lymph nodes including pre-scapular and pre-femoral lymph nodes (Fig.2A & B).

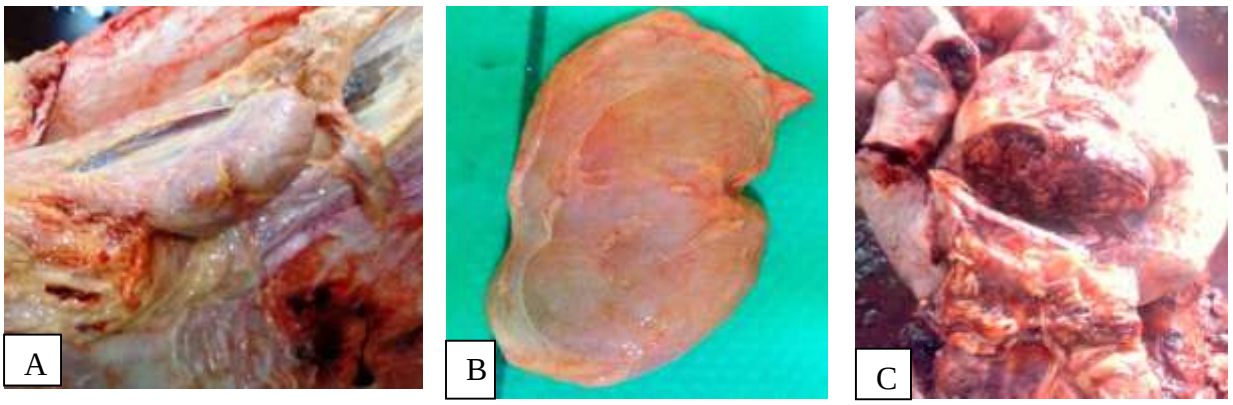

Fig. 2: Enlarged prescapular lymph node (A), prefemoral lymph node (B) and mediastinal lymph node (C) in cases of lymphoma

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Histopathological examination of the first case showed presence of pleomorphic small neoplastic lymphocytes in lymph nodes, lungs and heart (Fig.3). The second case was presented for post mortem examination which revealed enlargement of mediastinal lymph node along with involvement of lungs (Fig.2C).

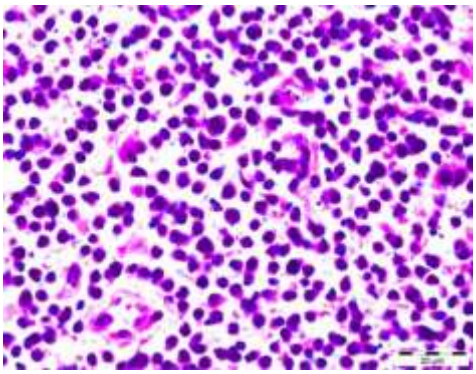

Fig. 3: Prescapular Lymph node: Photomicrograph showing small to intermediate pleomorphic cells. H&E stain, Bar=20µm

Cytological smears prepared from affected lymph nodes and lungs showed pleomorphic lymphocytes with increase in nuclear to cytoplasmic ratio and prominent nucleoli. Histopathological examination of mediastinal lymph node showed diffuse monomorphic cell infiltration of small lymphocytes (Fig.4).

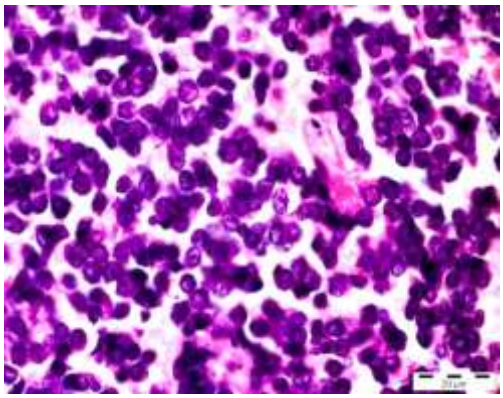

Fig. 4: Mediastinal Lymph node: Photomicrograph showing diffuse monomorphic lymphocytic lymphoma. H&E stain, Bar=20µm.

In lungs, there was infiltration of mixed inflammatory cells and proliferation of lymphoblasts leading to pseudo-lobulation with presence of edema and thickening of pleura. In addition,

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lymphoid cell infiltration along with necrosis was observed in liver, intestine and stomach, whereas, spleen showed presence of hemosiderin laden macrophages with chronic splenitis.

Immunohistochemical staining of the first case revealed positive reactivity of CD3 and CD8 along with mild reactivity for CD4. In addition, focal positive reactivity of p-27 was also observed in some areas. So on the basis of imunohistochemical staining the case was diagnosed as mixed T-cell and B-cell lymphoma (Fig.5-7).

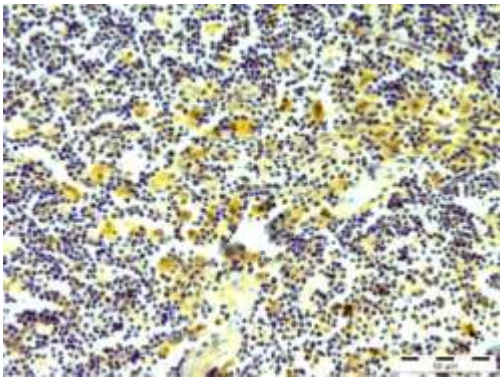

Fig. 5: Prefemoral lymph node showing positive immunoreactivity for CD8. One step polymer HRPO staining. Counterstain by hematoxylin. Bar=50µm.

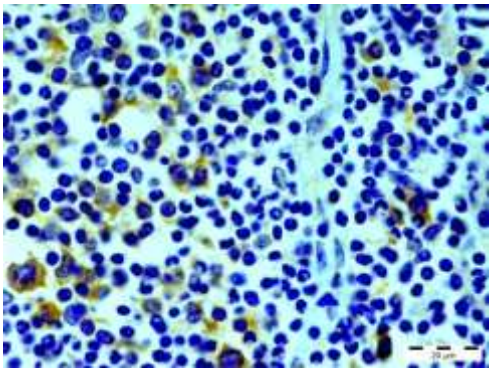

Fig. 6: Prefemoral lymph node showing positive immunoreactivity for CD3. One step polymer HRPO staining. Counterstain by hematoxylin. Bar=20µm.

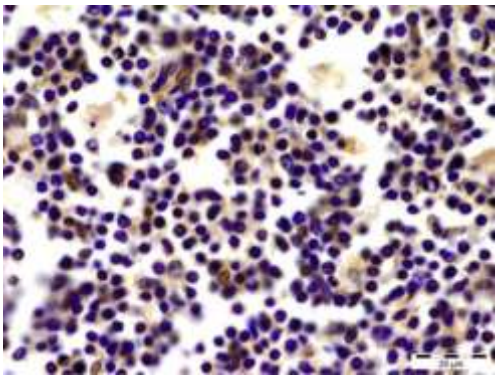

Fig. 7: Prefemoral lymph node showing mild positive immunoreactivity for p-27. One step polymer HRPO staining. Counterstain by hematoxylin. Bar=20µm.

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In the second case, marked positive reactivity of CD3 was observed along with very mild focal positive reactivity of CD8 in some areas. Therefore based on the immunohistochemical staining the second case was diagnosed as T-cell lymphoma (Fig. 8-9). No reactivity for CD20 and CD79 was observed in both the cases.

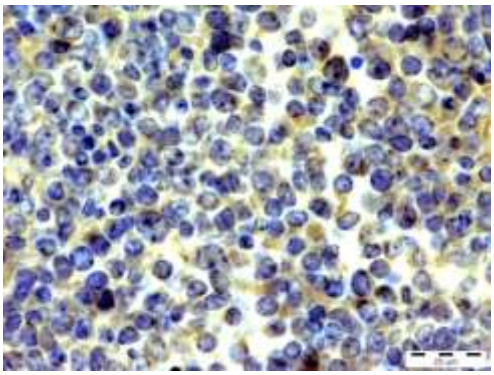

Fig. 8: Mediastinal lymph node showing positive immunoreactivity for CD3 in another case. One step polymer HRPO staining. Counterstain by hematoxylin. Bar=20µm.

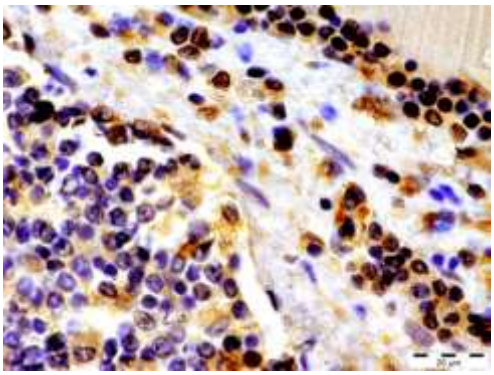

Fig. 9: Lymphoma: Lung showing mild positive immunoreactivity for p-27. One step polymer HRPO staining. Counterstain by hematoxylin. Bar=20µm.

Discussion

Lymphoma is second most common neoplasm in dairy cattle after squamous cell carcinoma, and its high prevalence may be attributed to BLV infection (Vernau *et al*., 1992). It is one of the most important cause of lymphadenopathy in bovines. In the present study, 32 cases of lymphadenopathies were recorded in bovines. Out of these two cases of bovine lymphoma were suspected on the basis of peripheral blood smear examination and gross lesions in lymph nodes and visceral organs. In the first case, besides lymphocytic leukemia, there was enlargement of peripheral and visceral lymph nodes including pre-scapular lymph node, pre-femoral lymph nodes and spleen. Whereas, in the second case there was enlargement of mediastinal lymph node with presence of lesions in lungs. Singh *et al*. (1979, 1980) described lymphosarcomatous involvement of both male and female reproductive organs and endocrine glands of Indian buffaloes with enlargement of superficial and visceral lymph nodes. Yoon *et al.* (2005) reported that lesions in bovine lymphomas were most frequently observed in lymph nodes, followed by intestine, heart and stomach. They were of the opinion that enlargement of lymph nodes and gross lesions in visceral organs can be used as criteria to detect BLV-associated lymphoma in bovines. Similarly, Kagawa *et al*. (2009) have also reported neoplastic involvement of lymph nodes, thymus, liver, spleen, kidneys, omasum, abomasum, intestines, pleura and peritoneum in cases of lymphoma in calves. Otrocka-Domagala *et al.* (2012) reported involvement of superficial and visceral lymph nodes and spleen in a case of cutaneous T-

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cell lymphoma in a cow. Cytological examination of affected lymph nodes in the present study showed varying sized, pleomorphic lymphocytes having increased nuclear to cytoplasmic ratio and prominent nucleoli. Similarly, pleomorphic nuclei with irregular contours and prominent nucleoli along with large atypical cells in cytological smears of lymphoma cases has been reported earlier by Kagawa *et al*. (2009). On the other hand, Sampaio *et al*., 2012, observed large number of neoplastic lymphocytes, lymphoblasts and marked atypia in cytogical smears in a case of thymic lymphoma in a cow.

In the present study, microscopic examination in the first case revealed proliferation of pleomorphic small to intermediate sized lymphocytes in the lymph nodes, heart and lungs. Whereas, in the second case monomorphic cell infiltration was observed in case of mediastinal lymph nodes and lungs. Microscopically, Sampaio *et al.* (2012) reported sheets of neoplastic lymphocytes showing pleomorphism, anisokaryosis, granular chromatin, hyperchromasia and apoptosis in pericardium, lungs and intercostal muscles of cow suffering from lymphoma. Similarly, infiltration of round to polygonal neoplastic lymphocytes having round nuclei, clumped chromatin, prominent nucleoli and eosinophilic cytoplasm has been reported earlier in skin and superficial lymph nodes in a case of cutaneous lymphoma in a cow. Major problem in the classification of lymphomas based on morphological grounds is extreme diversity in histologic appearance of neoplasm, so immunohistochemical staining is carried out to classify the lymphomas into B and T cell lineage. In the present study, on the basis of positive reactivity of CD8, CD3 and CD4 and focal positive reactivity of p-27 the first case was diagnosed as T-cell dominant B-cell lymphoma. Whereas, the second case was diagnosed as T-cell lymphoma on the basis of positive reactivity for CD3 and CD8. Immunohistochemical staining has been similarly used by earlier workers for classification of lymphomas (Stober, 1981; Suchi *et al.,* 1987; Schweizer *et al.,* 2003; Buczinski *et al.,* 2006; Yamamoto *et al.,* 2007; Kagawa *et al.,* 2009; Sampaio *et al*., 2012; Otrocka-Domagala *et al*., 2012).

Conclusion

In conclusion, it may be stated that lymphomas are found to be one of cause of lymphadenoapthy in bovines, and immunohistochemistry could serve as further diagnostic technique in classification of B- and T- cells lineage.

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