

## Atypical subtype of sporadic bovine leukosis in an adult crossbred bull: A case report

Priyanka Syal, Nittin Dev Singh\*, Geeta Devi Leishangthem

Department of Veterinary Pathology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, India

**Address for Corresponding Author:**

Dr. Nittin Dev Singh, Department of Veterinary Pathology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, India; E-mail : [nitindevsingh@gadvasu.in](mailto:nitindevsingh@gadvasu.in)

Received: 31.10.25; Accepted: 27.11.25

### ABSTRACT

In the present study, a bull aged nine years with a history of fever and enlarged lymph nodes (LNs) was presented to the post mortem facility of Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences, University thorough necropsy examination revealed enlargement of prescapular, bronchial and mesenteric lymph nodes with loss of cortico-medullary differentiation and yellow creamish surface with haemorrhages. An interesting solitary hard mass was found near the right kidney. Cytological examination of LNs and hard mass revealed anaplastic changes in lymphoid cells. Histopathological examination of affected LNs and hard mass revealed medium to large sized lymphocytes with few mitotic figures that replaced the follicles and sinuses. Moreover, these neoplastic cells of LNs and hard mass were found to be immunopositive with CD3 antibody only and immunonegative with PAX-5 and BLV p24 (capsid protein) antibodies suggesting a case of sporadic bovine leukosis. However, the intriguing aspect of present case was that, neither the thymus nor the skin was affected along with the presence of atypical hard mass, indicating that it does not fall under either of the categorization of sporadic bovine leukosis. Hence, the present case based on age, gross, cytological, histological and immunohistochemical findings, was classified as an atypical form of Sporadic Bovine Leukosis.

**Keywords:** Atypical, bovine leukosis, BLV p24, CD3, Lymphoma, Pax-5, Sporadic

Lymphosarcoma is a round cell tumor which has hematomorphoid origin<sup>1</sup>. It is the second most common tumor in cattle after squamous cell carcinoma. Bovine lymphosarcoma has been categorized into two forms: Sporadic Bovine Leukosis (SBL), which has an unknown etiology and Enzootic Bovine Leukosis (EBL), which is caused by infection with BLV (Bovine Leukemia Virus)<sup>2</sup>. Further, SBL is commonly divided into three types: juvenile/ calf, thymic and cutaneous which mainly affects the bovine population under 3 years old while EBL is mostly found in dairy cows and is contagious with a peak incidence in mature animals above 3 years of age<sup>3</sup>.

Juvenile form mainly occurs in calves up to 6 months of age and characterized by multicentric lymphadenopathy<sup>4</sup>. The thymic form of SBL mainly affects the animals of 6-24 months and its clinical symptoms vary according to the tumour's location and size which is characterised by thymus involvement, cervical enlargement, bloating, dyspnoea, tachycardia, jugular distention, fever and forequarters edema<sup>5</sup>. In skin or cutaneous form, dermal plaques of diameter 1-5cm can be observed on the neck, rump, back and thigh which can be found in animals up to 30 months of age<sup>3</sup>. The cases which do not fit into these forms are classified as atypical subtype of sporadic bovine leukosis<sup>6</sup>.

The present communication case report described the pathomorphological diagnosis of atypical subtype of sporadic bovine leukosis in an adult crossbred bull, which was not fit in any category of sporadic bovine leukosis based on presence of mass, criteria of age, gross, cytological, histopathological and immunohistochemical findings.

Carcass of a crossbred bull aged nine years old was presented to the post mortem facility of Department of Veterinary Pathology, Guru Angad Dev

**How to cite this article:** Syal, P., Singh, N.D., Leishangthemlesha G.D. Atypical subtype of sporadic bovine leukosis in an adult crossbred bull: A case report. Indian J. Vet. Pathol., 50(1) : 75-79.

Veterinary and Animal Sciences University, Ludhiana with a history of fever and enlarged lymph nodes. A blood sample was collected from the animal at the time of its presentation to the Outpatient Department of the Large Animal Clinics, GADVASU, prior to its death. Total leukocyte count (TLC) and hemoglobin (Hb) were done by automatic haematology analyser machine (Orphee Mythic 18-VET) and differential leukocyte count was done manually after staining blood smear with Leishman stain<sup>7</sup>. A thorough necropsy was conducted and various

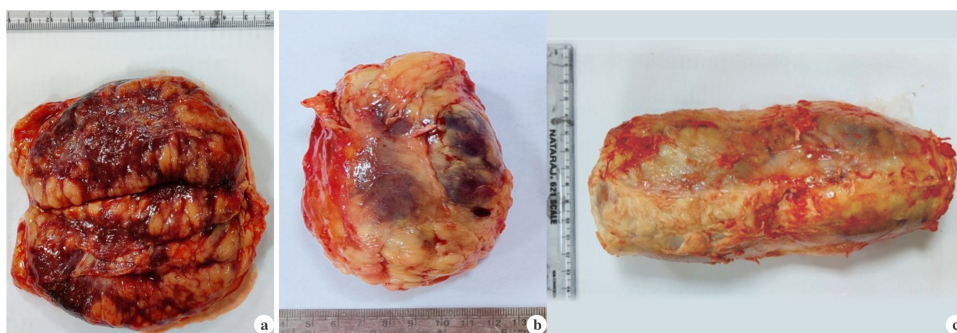


Fig. 1. Enlarged Prescapular (A), Bronchial (B) and Mesenteric (C) Lymph nodes

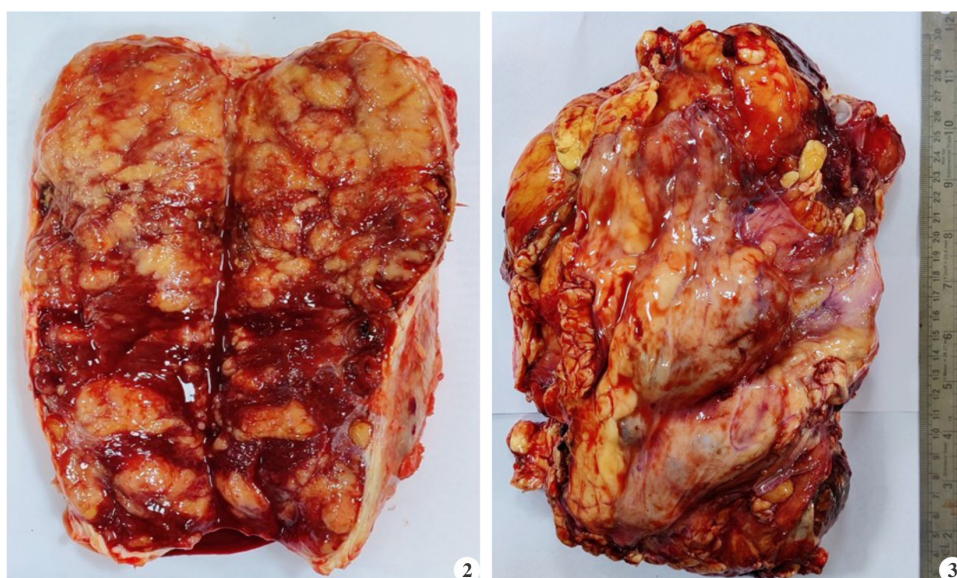


Fig. 2. Cutting of lymph node showed loss of corticomedullary differentiation and pale yellow surface with haemorrhages; Fig 3. Solitary hard mass with 27cm length and 17cm width near kidneys.

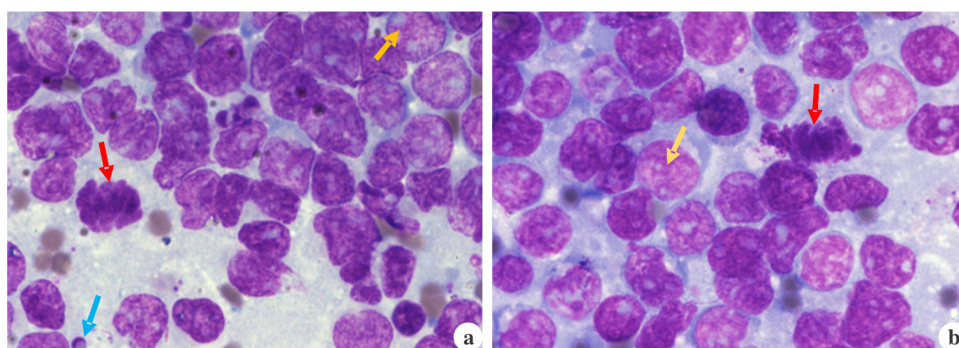
gross findings were recorded. Impression smears were made by taking cytological samples from enlarged lymph nodes (prescapular, bronchial and mesenteric lymph nodes) and hard mass found in the abdominal cavity near the right kidney on a clean glass slide. All impression smear were stained with Leishman stain. Representative tissue samples were collected *viz.*, lymph nodes, liver, spleen, lungs and hard mass in 10% neutral buffered formalin for histopathological analysis. The tissue sections of 5 $\mu$ m thickness were cut with a rotary microtome (Leica) and stained with the standard Haematoxylin and Eosin method<sup>8</sup>. For confirmatory diagnosis immunohistochemistry was performed by using CD3 antibody (Pathnsitu, Polyclonal antibody, Ready to use), PAX-5 antibody (Invitrogen, Monoclonal antibody and Dilution 1:250) and monoclonal antibodies against the p24 protein of the bovine leukemia virus capsid with a dilution of 1:500 (VMRD, Pullman, WA, USA)<sup>9</sup>. Antigen labelling was performed by using DAB (3, 3-diaminobenzidine) solution (Vector, ImmPACT peroxidase substrate kit, USA) for visualizing the antigen-antibody-peroxidase reaction. All the images were taken by using an Olympus Microscope (BX51).

On clinical examination, an animal was found to be suffering from fever and enlarged lymph nodes. Haematological examination revealed 9.5 g/dl of Hb and 14900/ $\mu$ l TLC with a differential count of 68% lymphocytes and 32% neutrophils. Resultant absolute count of lymphocytes and neutrophils were 10132/ $\mu$ l and 4768/

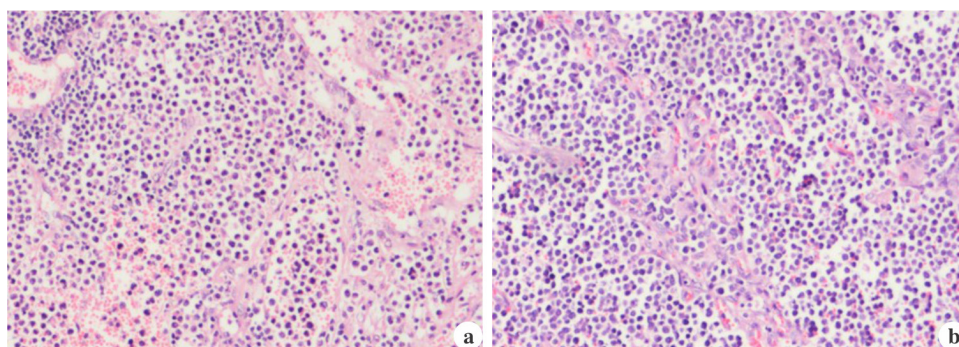
$\mu$ l respectively. Therefore, hematology revealed leukocytosis with absolute lymphocytosis.

Grossly, prescapular (11cm length and 8.5cm width), bronchial (9cm length and 6.9cm width) and mesenteric (20.1cm length and 7.6cm width) lymph nodes were enlarged (Fig. 1A, 1B, 1C). On cutting the lymph nodes, the gross corticomedullary differentiation was found to be lost with pale yellow to reddish discoloration (Fig. 2). The kidneys were slightly congested and one interesting solitary hard mass of 27cm length and 17cm width was seen near right kidney (Fig. 3). The mass had yellowish discoloration with haemorrhages on their cut surfaces. Liver was enlarged with round edges and multiple sterile cysts were observed on lungs, liver and spleen. Moreover, the spleen revealed petechiae on its surface and lungs showed emphysema as crepitating sounds were evident by tactile sensation.

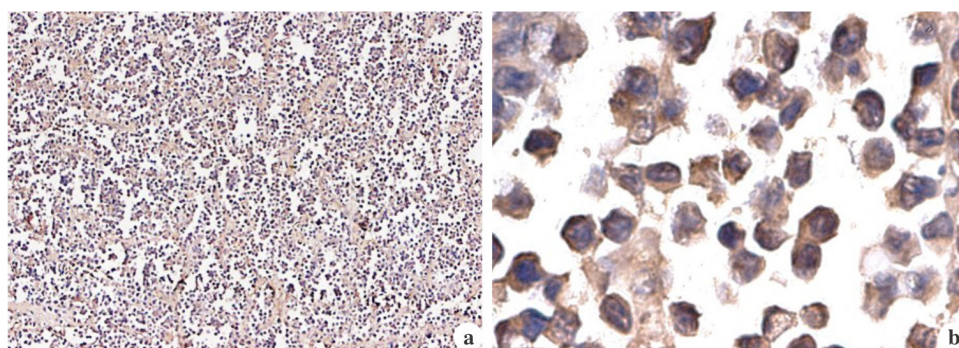
Cytological examination of the impression smear from lymph node revealed medium to large sized population of lymphocytes (89.29 $\mu$ m to 160.36 $\mu$ m in size) having large nucleus varying from 82.85 $\mu$ m to 157.08 $\mu$ m with prominent nucleoli of varying sizes (15.85 $\mu$ m to 24.26 $\mu$ m), coarse aggregated chromatin and less cytoplasm with basophilic intensity suggesting lymphoma. Mean  $\pm$  S.E of cell size, nucleus size and nucleolus size were 108.83  $\pm$  7.16, 107.66  $\pm$  5.90 and 19.57  $\pm$  1, respectively. In some areas, two to four



**Fig. 4.** Cytological examination of impression smear of mesenteric lymph node (A) and solitary mass (B) near kidney revealed uniform population of lymphoblasts having large nucleus with prominent multiple nucleoli (yellow arrow) and small basophilic cytoplasm, mitotic figures (red arrow) and lymphoglandular bodies (blue arrow) suggesting lymphoma (Leishman, Bar=10 $\mu$ m)



**Fig 5.** Lymph node (5A) and Solitary mass (5B) revealed small to medium sized neoplastic cells and lymphoblasts (H&E, Bar=100 $\mu$ m).



**Fig 6.** Immunoreactivity with CD3 by neoplastic cells in lymph node (Bar=100 $\mu$ m) (A) and solitary mass (B) (Bar=10 $\mu$ m).

mitotic figures were also observed (Fig. 4A). Further, impression smear from the solitary hard mass revealed similar changes such as medium to large sized lymphoblasts, with large nucleus, prominent nucleoli and coarsely aggregated chromatin (Fig. 4B).

Histopathological examination of enlarged lymph nodes revealed small to medium sized lymphocytes and lymphoblasts with neoplastic characteristics *viz.*, pleomorphic nucleus, karyomegaly, prominent nucleoli and coarse chromatin with few mitotic figures. These cells totally replaced the normal lymphoid follicles and sinuses of the lymph nodes (Fig. 5A). The neoplastic cells were separated by thin connective tissue and nucleus was surrounded by a thin margin of pale eosinophilic cytoplasm. A widespread sclerotic area was detected with marked proliferation of

fibrous tissue in a lymph node suggesting desmoplasia. Moreover, the tissue section of solitary mass seen in the abdominal cavity near right kidney also revealed similar neoplastic changes (Fig. 5B), which led to the conclusion that this mass also represented lymphosarcoma. The histology of this mass was fully altered and only neoplastic cells were observed. Liver, spleen and lungs revealed sterile cyst showing a thin, fibrous capsule on the outside and a single layer of cuboidal tissue inside. There was lymphoid depletion and hemosiderosis in spleen. Lungs revealed severe dilatation of alveoli suggesting emphysema. The thymus was not located as due to the animal's age, it might have involuted. Further, Immunohistochemical staining of the lymph nodes and solitary hard mass showed immunopositivity with CD3 antibody only (Fig. 6A, 6B). No immunoreactivity was found with PAX-5 antibody (Fig. 7A, 7B) and BLV viral capsid protein p24 (Fig. 8A, 8B).

In the present case, cytological and histopathological examination of lymph node and hard mass discovered that the animal was suffering from lymphosarcoma or malignant lymphoma<sup>10</sup>. Further, the confirmatory diagnosis was done by immunohistochemical studies that revealed immunoreactivity of neoplastic cells with CD3 only which is a membrane T-cell marker suggesting T-cell origin of neoplastic cells<sup>11</sup> and no immunoreactivity was found with PAX-5 antibodies

which is expressed by B-lymphocytes<sup>12</sup>.

Moreover, no viral isolate was found by using BLV capsid protein p24 antibody that revealed lymphoma in aged bull was not induced by bovine leukemia virus *i.e.*, EBL form<sup>13</sup>. Therefore, it was confirmed that the animal was suffering from sporadic bovine leukosis.

According to previous reports, T-cells are primarily involved two forms of sporadic bovine leukosis *viz.*, thymic and cutaneous form which primarily affects young animals *i.e.*, up to 30 months of age<sup>14</sup>. However, the intriguing aspect of present case was that, neither the thymus nor the skin was affected, indicating that it does not fall under either of these forms of sporadic bovine leukosis. The third category of SBL is juvenile form that is seen up to six months of age with multicentric lymphadenopathy and B-cell involvement<sup>3</sup>. Although multicentric lymphadenopathy with T-cell involvement was evident in the present case, but, the curious turning point was the bull's age which was nine years that precluded it from being classified as a juvenile form.

Therefore, the present case deviated from the classification of SBL and classified as atypical subtype of SBL. Moreover, along with the age factor, a solitary hard mass of unknown origin due to its altered histology found in the abdominal cavity showing features of lymphosarcoma also representing an atypical form of sporadic bovine leukosis. The comparable results of hard mass with the lymph nodes might represent it as an enlarged mesenteric lymph node but it was not confirmed. Additionally, the lymphocytic leucocytosis of present case also corroborated the finding that the cancer was limited to lymph nodes and had not transformed into leukemic form as no bizarre lymphocytes were observed in the blood smear.

Most of the cases of atypical SBL were reported in young animals<sup>15,16,17,18</sup>. Only one case of atypical SBL was reported in an adult cattle<sup>6</sup>. SBL is rarely detected and is estimated to affect one animal out of every 100,000 cattle<sup>19</sup>. It is nontransmissible and noncontagious, and it normally exclusively affects young cattle typically those under a year old in a herd.

Moreover, the fever in bull might be a paraneoplastic syndrome as neoplasia leads to release of various cytokines like IL-1, IL-6, IL-8 and INF- $\gamma$  which are mainly responsible for affecting the thermoregulatory centre<sup>20</sup> and concomitant secondary infections that was evident from increased total leukocyte count of present case. Bovine tuberculosis was ruled out as a differential diagnosis based on enlarged lymph nodes. However, the pathological examination revealed no evidence of tuberculosis and acid-fast staining came negative on impression smear taken from lymph nodes and their tissue sections. Cysts on liver, lung and spleen of present

case was incidental finding that was investigated on histopathological examination for scolex of tapeworm *Echinococcus granulosus* but it was not found anywhere. Therefore, these cysts could be suggested as sterile cysts. Sporadic bovine leukosis remains a clinical and pathologic mystery despite recent attempts to gain a better understanding of the condition. In comparison to enzootic bovine leukosis, it is far less common.

In the present study, an aged adult bull presented with fever and enlarged lymph node was found to be suffering from lymphosarcoma based on cytological and histopathological findings. Further, to confirm the form of lymphoma *i.e.*, sporadic bovine leukosis or enzootic bovine leukosis, immunohistochemical analysis was done that revealed immunoreactivity with T-lymphocytes only describing the present case was of sporadic bovine leukosis. Nevertheless, the criteria of age and atypical hard mass with altered histology deviated the animal to not fall under any described categories of sporadic bovine leukosis. Hence, the present case based on age, gross, cytological, histological and immunohistochemical findings, was classified as an atypical form of Sporadic Bovine Leukosis. This was the first instance of atypical sporadic bovine leukosis in an adult cattle reported in India.

## ACKNOWLEDGMENT

Authors are thankful to the Head of Department of Veterinary Pathology, College of Veterinary science, Guru Angad Veterinary and Animal Sciences University, Ludhiana for providing all facilities in the Department.

**Financial support & sponsorship:** None.

**Conflict of Interest:** There exists no conflict of interest among authors

**Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation:** The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

## REFERENCES

1. Sharma S, Kamala R, Nair D, Mhatre S, Sabharwal R, Choudhury B, Rana V. 2017. Round cell tumors: classification and immunohistochemistry. *Indian J Med Paediatr Oncol* 38:349-353.
2. Angelos JA, Thurmond MC. 2015. Bovine lymphoma. In: Smith BP, ed. *Large Animal Internal Medicine*. Elsevier, St Louis. 1070-1073.
3. Thompson BS, Goodrick ER. 2018. Miscellaneous infectious diseases. In: Peek SF, Divers TJ, eds. *Rebhun's Diseases of Dairy Cattle*. Elsevier, St. Louis. 737-783.
4. Diehl BN, Alejandro KG, de Oliveira HH, Darwin L, Craft W, Cabrera C. 2023. Peripheral lymphadenopathy in twin male Brahman calves. *J Am Vet Med Assoc* 261:1-3.
5. Hishamnuri WN, Nakagun S, Maezawa M, Sakaguchi K,

- Akiyama N, Watanabe KI, Horiuchi N, Kobayashi Y, Inokuma H. 2019. Disseminated thymic B-cell lymphoma in a Holstein heifer. *J Vet Diagn Invest* **31**:852-855.
6. Grünberg W, Eisenberg SW. 2013. Atypical form of sporadic bovine leukosis (SBL) in the Netherlands. *Vet Rec* **173**: 398.
  7. Gajendra S, Jha B, Goel S, Sahni T, Sharma R, Shariq M, Jaiswal S, Sachdev R. 2015. Leishman and Giemsa stain: a new reliable staining technique for blood/bone marrow smears. *Int J Lab Hematol* **37**:774-782.
  8. Luna LG. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 2nd edn. Blakiston Division, McGraw-Hill.
  9. Kim SW, Roh J, Park CS. 2016. Immunohistochemistry for pathologists: protocols, pitfalls, and tips. *J Pathol Transl Med* **50**:411-418.
  10. Rahman S, Agrawal R, Nashiruddullah N, Sood S, Pande N, Tikoo A. 2023. Juvenile lymphosarcoma in Calf: A Cytohistological Analysis. *Explor Anim Med Res* **13**:136-139.
  11. Frankel AE, Zuckero SL, Mankin AA, Grable M, Mitchell K, Lee YJ, Neville DM, Woo JH. 2009. Anti-CD3 recombinant diphtheria immunotoxin therapy of cutaneous T cell lymphoma. *Curr Drug Targets* **10**:104-109.
  12. Adams B, Dörfler P, Aguzzi A, Kozmik Z, Urbanek P, Maurer-Fogy I, Busslinger M. 1992. Pax-5 encodes the transcription factor BSAP and is expressed in B lymphocytes, the developing CNS, and adult testis. *Genes Dev* **6**:1589-1607.
  13. De Oliveira CH, Barbosa JD, Damasceno KA, Cassali GD, Oliveira C. 2016. Multicentric lymphoma in buffaloes in the Amazon region, Brazil. *BMC Vet Res* **12**: 1-10.
  14. Bundza A, Greig AS, Chander S, Dukes TW. 1980. Sporadic bovine leukosis: a description of eight calves received at Animal Diseases Research Institute from 1974-1980. *Can J Vet Res* **21**(10):280.
  15. Lyons N, Browne E, Aldridge B. 2010. An atypical presentation of sporadic bovine leukosis in a steer. *UK Vet Liv* **15**:26-29.
  16. Matsuyama Y, Murakami T, Ohbayashi T, Kobayashi Y, Matsumoto K, Matsui T, Inokuma H. 2010. Atypical sporadic bovine leukosis with astasia in a Holstein heifer. *J Jpn Vet Med Assoc* **63**:355-358.
  17. Dalgleish R, Callanan JJ, McNeil PE. 1991. An atypical case of lymphosarcoma (sporadic bovine leukosis) in a heifer. *Vet Rec* **129**:308-310.
  18. Hendrick SH. 2002. Atypical sporadic bovine leukosis in a beef feedlot heifer. *Can Vet J* **43**(8):617.
  19. Oliver-Espinosa O, Physick-Sheard PW, Wollenberg GK, Taylor J. 1994. Sporadic bovine leukosis associated with ataxia and tibiotarsal joint swelling: A case report. *Can Vet J* **35**: 777-779.
  20. McGavin MD, Zachary JF. 2006. Pathologic basis of veterinary disease. Elsevier Health Sciences; 2006.