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REVIEW ARTICLE
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Bovine Leukosis: Classification, Clinical Findings, Clinical Pathology, Diagnosis, Necropsy and Control Measures

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ABSTRACT

Bovine leukosis is divided into two types: enzootic bovine leukosis (EBL), which is caused by bovine leukemia virus (BLV) infection, and sporadic bovine leukosis (SBL) of unknown cause. EBL is a non-benign tumorous disease of the lympho-reticular system in cattle. It is manifested by inveterate low-level viremia and a considerably long passivity period. BLV is an oncogenic class of the Delta retroviruses, and it is exceedingly linked to the human T-cell leukemia virus. Worldwide, BLV hits cattle causing huge financial losses. Genetic tendency plays a vital role and is shown in the fact that few cattle are apparently affected. Infected animals are constant carriers of the virus; however, infected cows are asymptomatic, with few cattle showing passing viremia. Three to four months post-infection, BLV virus is difficult to be sequestered from these cows. Almost thirty percent of infected cows are sero-convert and may develop constant lymphocytosis within three months to some years post-infection. Only one to five percent of carrier cattle show the tumorous disease. SBL is typically classified into three forms according to onset age and lesion site: calf or juvenile form, thymic or adolescent form, and skin or cutaneous form. These forms are sporadic with no implicit infectious cause, like a virus, has been isolated for these forms. However, the popular form, enzootic or adult multi-centric form, is always linked with BLV infection. In the later form, affected cattle are usually four to eight years old, and neoplasms are usually located in the abomasum, uterus, cardiac right atrium, or superficial lymph nodes. Clinical manifestations, laboratory findings, diagnostic criteria, necropsy findings, and control measures are clarified. As a consequence of EBL, large financial losses occur due to the collapse of affected cattle, condemnation of diseased cases, loss of export benefits, and veterinary charges. This review was written to emphasize the categories, clinical findings, clinical pathology, diagnosis, necropsy, and control measures of bovine leukosis in cattle.

Keywords: Cattle; Bovine leukosis; Enzootic bovine leukosis; Lymphosarcoma; Pathology

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INTRODUCTION

Bovine leukosis is divided into two types: enzootic bovine leukosis (EBL), which is caused by bovine leukemia virus (BLV) infection, and sporadic bovine leukosis (SBL) of unknown cause. SBL is typically classified into three forms according to onset age and lesion site: calf or juvenile form, thymic or adolescent form, and skin or cutaneous form (Constable et al., 2017). The common, adult, enzootic, or multi-centric form, is always associated with infection with BLV virus. Cattle with the later form are usually four to eight years and neoplasms are commonly found in the

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abomasum, uterus, cardiac right atrium, or superficial lymph nodes. Huge financial losses occur due to collapse of diseased cattle, condemnation of affected cases, veterinary charges and loss of export benefits (Smith, 2015).

EBL is a malignant tumorous disease of the lympho-reticular system in cows. It is manifested by lasting low-level viremia and a long passivity period. Only one to five percent of affected cattle shows the tumorous disease (Maezawa et al., 2022; Lancheros-Buitrago et al., 2023). Both B lymphocytes and T lymphocytes may be affected with BLV; however, the resulting neoplasms are consisted mainly of B lymphocytes (Hirsh and Chung Zee 1999; Mohamed et al., 2011; Tharwat and Abd El-Rahim, 2012). Only one to five percent of carrier cattle show the tumorous disease (Smith, 2015; Constable et al., 2017).

EBL is caused by BLV, a tumorous class of the Delta retroviruses. This virus is linked mainly to human T cell leukemia virus. Worldwide, BLV affects cows, causing considerable financial losses (Polat et al., 2017; Konishi et al., 2019). EBL pathogenesis is very complicated. Genetic tendency plays a vital role and is shown in the fact that few cattle are apparently affected (Hopkins et al., 1991). Infected animals are constant carriers of the virus; however, infected cows are asymptomatic, with few cattle showing passing viremia. Three to four months post-infection, BLV virus is difficult to be isolated from these cows. Almost thirty percent of diseased cattle are sero-convert and may show constant lymphocytosis within three months to some years post-infection. This review was written to emphasize the categories, clinical findings, clinical pathology, diagnosis, necropsy and control measures of bovine leukosis in cattle.

Classification of Bovine Leukosis

Enzootic Bovine Leukosis

Clinical Findings

Cattle affected with the enzootic or adult type of lymphoma are usually over four years old but may also be affected as young as two years. Affected animals are often admitted with a complaint of decreased body condition, a sharp decrease in milk yield, enlarged superficial lymph nodes, protrusion of the third eyelid, inappetence or anorexia, and exophthalmos. Subclinical form of lymphoma may be detected in cows presented for routine gynecological examinations. Other symptoms may include ataxia, diarrhea, ketosis, paresis and infertility (Smith, 2015; Constable et al., 2017).

Clinical examination may be revealing an organ and/or system dysfunctions due to neoplasm involvement. Auscultation of the thoracic cavity may show tachycardia, cardiac dysrhythmia, hyperpnea and tachypnea. Common locations of neoplasms include heart especially in the right atrium, uterus, abomasum, spinal cord, kidney, rumen/reticulum and retrobulbar space. Enlarged superficial nodes especially are the femoral, prescapular, and mammary lymph nodes. Feces are passed pasty, scant or even watery, reflecting lesions presence in the gastrointestinal tract. Melena may also be found in cattle with abomasal ulceration and infiltration of neoplastic cells in the wall of the abomasum. Rectal examination can be beneficial in diseased cases without exophthalmos or enlargement of the peripheral nodes. Multiple neoplastic masses are usually detected in the abdomen and their sizes range from only slightly large nodes to severe lesions fifty centimeters in diameter (Fig. 1, 2, and 3).

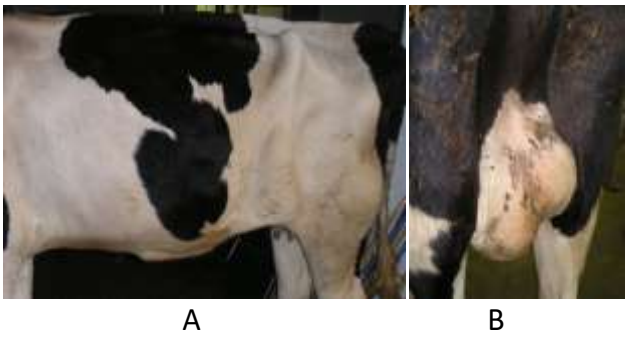

Fig. 1: A Holstein cow seropositive for bovine leukemia virus. Enlargement of the prefemoral (**A**) and supramammary lymph nodes (**B**) is seen.

Fig. 2: A Holstein cow seropositive for bovine leukemia virus. Loss of body condition and raising tail were presenting findings. Rectal examination revealed numerous pelvic masses

Fig. 3: Ocular lesions in cows seropositive for bovine leukemia virus. Bilateral exophthalmia is apparent in image **A**. Exophthalmia is evident in the right (**B**) and left (**C**) eyes. Protrusion of the third eyelid is clear in image **D**.

Clinical Pathology

Hemogram finings in cattle with EBL is often non-helpful. A microcytic, hypochromic anemia may be found in cases with gastrointestinal bleeding. Thirty percent of animals with BLV show constant life-long lymphocytosis, consisted mostly of B cells. Lymphocytotic cases have

elevated BLV proviral load (PVL) in their peripheral blood lymphocytes versus non-lymphocytotic cases, however some non-lymphocytotic cases may have elevated BLV PVL representing obstacles for the successful efforts done for disease eradication. In the plurality of calves infected experimentally with BLV, animals became polymerase chain reaction (PCR) positive faster than when they become seroconverted (Juliarena et al., 2007; Nagy et al., 2007; Smith, 2015; Constable et al., 2017). It was reported also that there are statistically significant differences of DNA damage between the peripheral blood mono-nuclear cells (PBMC) and polymorpho-nuclear neutrophils leukocytes (PMN) detected from infected cows with BLV versus PBMC and PMN collected from healthy cattle (Fig. 4). The latter methodology to detect DNA damage in PBMC and PMN is simple and fast and is used to detect apoptosis in blood as well as hepatic cells (Tharwat et al., 2012a,b,c; Tharwat and Oikawa, 2023).

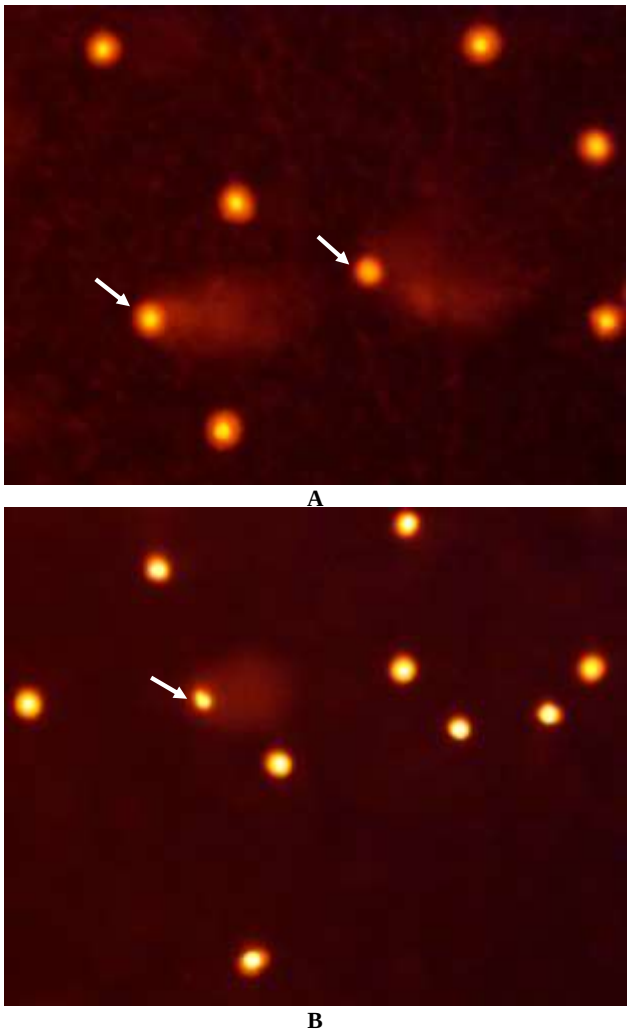

Fig. 4: Apoptotic changes of peripheral blood mononuclear cells (arrows) (**A**) and polymorphonuclear neutrophils leukocytes (arrow) (**B**) assessed by the Comet assay in cattle with enzootic bovine leukosis.

Diagnosis

Emaciation, protrusion of the eye, enlargement of the superficial lymph nodes, palpable neoplastic masses in the abdomen, increases in peripheral lymphocytes (12,000 cells/mm3), and/or presence of tumorous B cells in the peripheral blood are usually diagnostic (Konishi et al., 2019). Fine needle aspirate from the enlarged lymph nodes shows a monomorphic population of abnormal lymphocytes (Fig. 5). Interestingly, over fifty percent of cattle affected with EBL were discovered incidentally at slaughterhouses (Kobayashi et al., 2016).

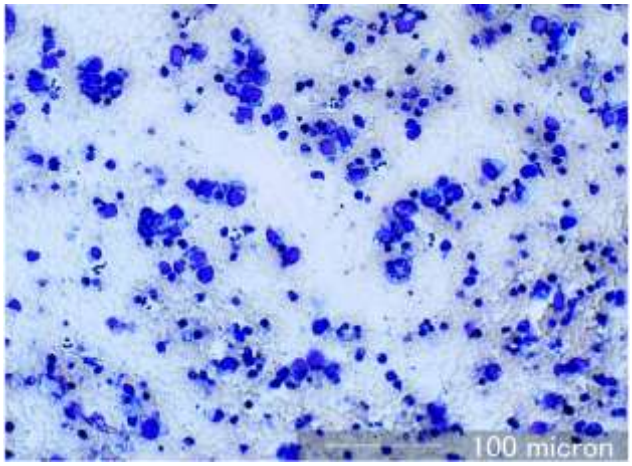

Fig. 5: Fine needle aspirates from a prefemoral lymph node in a cow with enzootic bovine leukosis showing a monomorphic population of abnormal lymphocytes.

Reports published by Konnai et al. (2005) and Ikeda et al. (2005) stressed the potentials of various gene expression patterns of tumor necrosis factor receptors between EBL cattle versus non-EBL cattle. The usefulness of the enzymatic activity of serum thymidine kinase (TK) in the diagnosis of EBL as well as the potency of using the PVL in lymph nodes for the detection of EBL has also been reported (Tawfeeq et al., 2013; Somura et al., 2014). Currently, enzyme-linked immunosorbent assay (ELISA) and PCR are also implemented to diagnose the seropositive cattle for BLV (Simard et al., 2000; Mohamed et al., 2011; Tharwat Abd El-Rahim, 2012; Nishimori et al., 2021).

A recent study conducted by Konishi et al., (2019) showed that TK and lactate dehydrogenase isozyme 2 had high sensitivity and specificity, and these enzymes could distinguish EBL cattle from cases with persistent lymphocytosis with have high reliability and are attainable and easy to perform. The TK and LDH isozymes could also help to detect EBL cases antemortem thus improving the effectiveness of diagnosing EBL cows on farms performing EBL-eradication plans (Konishi et al., 2019).

Postmortem Findings

A diagnosis of EBL is usually made at postmortem examination. Necropsy findings include a severely enlarged lymph nodes which is hemorrhagic on cut section, pelvic masses within the pelvic cavity which are also hemorrhagic on cut section, and enlarged mesenteric lymph nodes (Fig. 6, and 7). Neoplasms may be firm not hard and may be felt lobulated. Carcinomas tend to be of similar consistency but are seldom larger than 15 cm in diameter and are usually associated with intestinal tissue. Lymph nodes of cows with carcinoma are not usually

enlarged unless a secondary infection is present. Melanomas are generally less than 15 cm in diameter and hard, sometimes with protrusions 1 to 2 cm high along the surface, and not usually found associated with lymph nodes. Masses of fat necrosis tend to be firm and are usually associated with omental tissue. Internal abscesses are often single and associated with the uterus, as a consequence of uterine tear and infection during parturition, or with gluteal muscles of the pelvic ceiling, as a consequence of injection-related infections. Laparotomy may be signalized as a further diagnostic method in severe cases, especially in valuable animals (Smith, 2015; Constable et al., 2017).

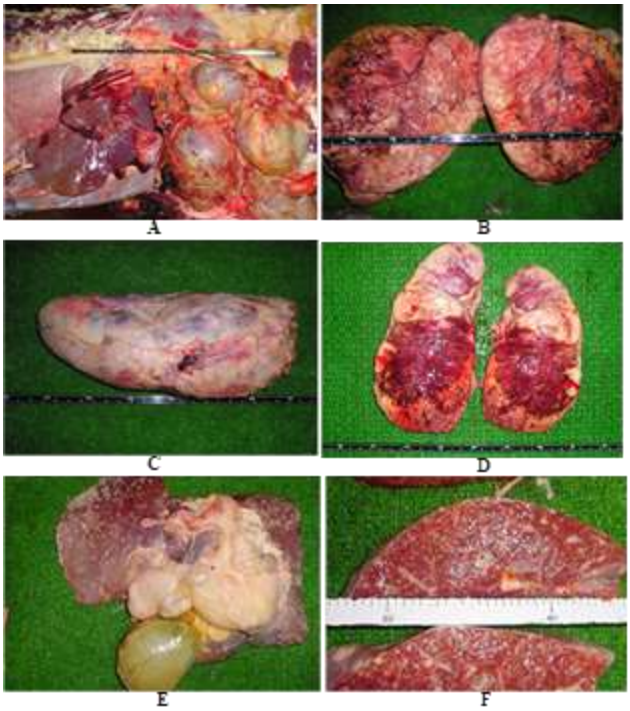

Fig. 6: Postmortem examination in a Holstein cow with enzootic bovine leukosis. Three large pelvic masses were found (**A**). Image **B** shows a cross section through the pelvic masses where the cut surface was hemorrhagic. Image **C** shows a severely enlarged prefemoral lymph node. Image **D** shows a cross section through the enlarged prefemoral lymph node where the cut surface was also hemorrhagic. Multiple nodular masses are found on the hepatic capsule (E) which are also present within the hepatic parenchyma (F).

Control Measures

No curative treatment for lymphoma exists. Supportive measurements may be prescribed to minimize discomfort and extend lifelong sufficient to collect valuable ova and/or embryos or offspring or to collect also semen (Smith, 2015; Constable et al., 2017). Initial control measures depend on culling cases with elevated lymphocyte count (Bendixen, 2006) but recently, eradication depends on removal of all an BLV-antibody response. However, the later control measure is often impossible economically for farms with high incidence rates. However, strict managemental interferences for example using hypodermic needles and gloves once have not often been valuable in lowering transmission (Ruggiero and Bartlett, 2019). Other control measures are by vaccination as well as selection of genetic host. Recent evolution of Quantitative PCR to estimate BLV PVL are promising in detecting infectious cases so they can be eradicated via culling or segregating. It was demonstrated in three herds that culling or segregation of cattle with elevated PVL and lymphocytes led to a spectacular lowering in BLV prevalence within a little year (Ruggiero et al., 2019; Bartlett et al., 2020).

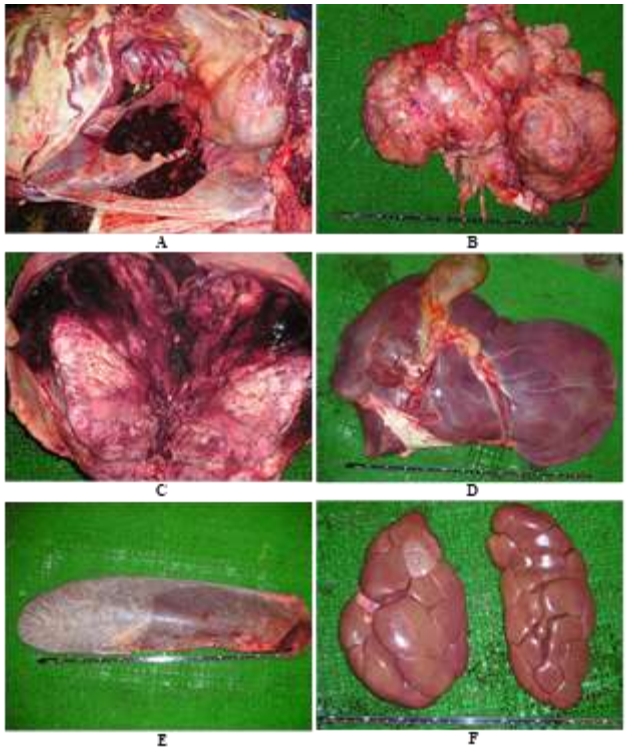

Fig. 7: Postmortem examination in a Holstein cow with enzootic bovine leukosis. Four large pelvic masses were found (**A, B**) with hemorrhagic cut section (**C**). The liver (**D**), spleen (**E**) and kidneys (**F**) are enlarged.

Sporadic Bovine Leukosis

Calf or Juvenile form

This form affects animals from three to six months, but juvenile lymphoma can be detected in calves as young as one month or in cows as old as three years of age. Calves always presented with a complain of weight loss, slight or moderate depression, weakness (principally in elder calves and in spite of perfect appetite), or lymphadenopathy (fundamentally in younger animals) (Fig. 8). The appearance of symptoms can be abrupt (within a few days). Clinical examination may show generalized enlargement of the superficial lymph nodes which tend to be smooth and firm but are not painful or hot. Visible mucosae are always pale due to anemia. In addition, tachycardia, hyperpnea, tachypnea, harsh respiratory sounds and cough may be apparent on thoracic auscultation. Other symptoms less frequently noted include ruminal tympany, fever, ataxia, diarrhea and hepatomegaly (Tawfeeq et al., 2012; Smith, 2015; Constable et al., 2017).

Hematologic abnormalities include a hypochromic, microcytic anemia, low PCV, low hemoglobin and leukocytosis caused by lymphocytosis. Hypoglobulinemia and elevated AST may also be encountered in diseased calves. Tumor involvement of

different organs including heart, spleen, liver, kidney, uterus, pancreas and thymus may occur. Sub-periosteal tumor infiltration may lead to compression of the spinal cord leading to paresis. The disease is speedy progressive becoming fatal within two to eight weeks of its onset (Smith, 2015; Constable et al., 2017). Postmortem examination usually detects the enlarged deep-seated lymph nodes (Fig. 9).

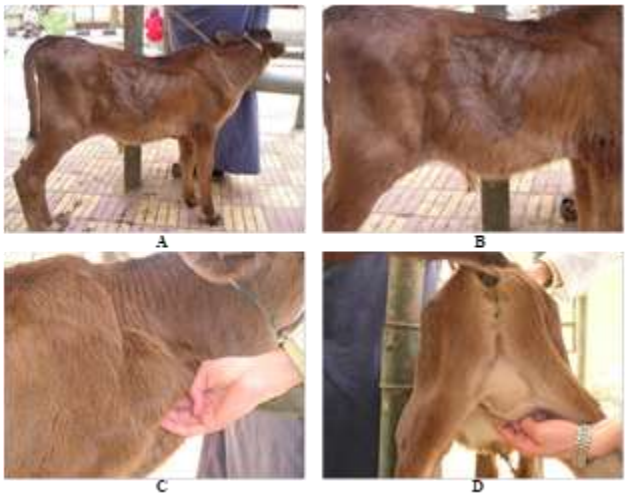

Fig. 8: Enlargement of the superficial lymph nodes in a male calf with juvenile-type bovine leukosis (**A**). Prefemoral (**B**), prescapular (**C**) and superficial inguinal (**D**) lymph nodes are enlarged.

Fig. 9: Necropsy findings in a calf with juvenile-type bovine leukosis. The lymph nodes prescapular (**A**), prefemoral (**B**), inguinal (**C**) and mesenteric (**D**) are enlarged.

Thymic or Adolescent Form

This form is extremely rare. It is usually observed in animals aged from six to twenty-four months but it may also occur in neonatal calves and adult cattle up to four years. Clinical manifestations are apparent due to the presence of neoplastic lesions in the thorax or neck. Cattle with thymic lymphosarcoma typically present with pre-sternal enlargement or firm swelling in the brisket together with doughy edema. Rumen tympany, loss in body condition and dysphagia are common presenting findings (Fig. 10). A tumorous lesion around the esophagus can cause bloat secondary to the animal's inability to eructate ruminal gas. Although generalized form of lymphadenopathy is not common in this type, enlarged prescapular and cervical lymph nodes are usually seen. Jugular veins are always distended but not pulsating. Muffling of the heart sounds as well as decreased resonance on percussion of the thorax may be fined. Dyspnea, tachycardia, coughing, or respiratory distress may also be observed (Tharwat and Abd El-Rahim, 2012).

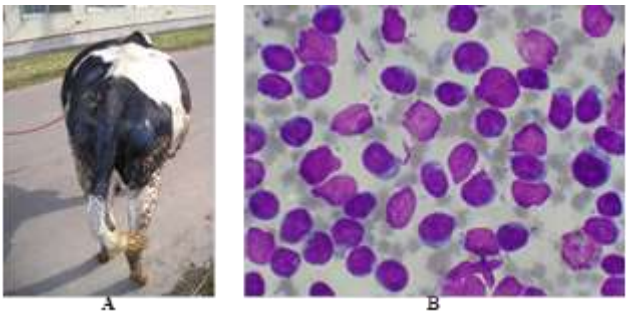

Fig. 10: Severe tympany in a 19-month-old heifer with thymic lymphoma. The heifer was presented with a history of anorexia, weight loss, cervical swelling and severe ruminal tympany (**A**). Clinical examination showed distended jugular veins, right-sided heart failure, difficult breathing, ataxia and muffled heart sound. There was enlargement of the pre-scapular lymph node. Values of rectal temperature, pulse rate and heart rate were 40.5, 105 and 35, respectively. Other signs included pale mucous membranes. Image **B** shows fine needle aspiration biopsy of the cervical mass where numerous lymphocytes (typical and atypical) are apparent (Giemsa stain).

Hematologic changes are generally not clear. Lymphocytosis is only observed occasionally and anemia is not a constant feature. The path of the disease starting from the time of detection ranges from two to nine weeks, but poor condition may have been present for several months before presentation. The disease is deadly, often as a complication of severe rumen bloat (Smith, 2015; Constable et al., 2017). Postmortem examination usually confirms the enlargement of the thoracic and cervical parts of the thymus gland (Fig. 11).

Skin or Cutaneous form

This form is not as age linked as the other types of sporadic lymphoma and may affect cows between one and three years. Presenting complain may detect a starting period of one to three months where skin swellings are noted around the vulva, anus, shoulders, escutcheon or flank. These lesions may diminish and thereafter recur. The lesions tend to be elevated and can be ulcerated. They are mostly about two to three cm in diameter with a characteristic necrotic core that may be painful on palpation (Fig. 12). Other clinical manifestations depend on invading of the tumor to other organs and may include cardiac involvement, with jugular pulsation and pre-sternal edema expanding along the ventral abdomen. Respiratory and pulse rated may be increased as a result of observed anemia. The prescapular, mandibular, prefemoral and mammary nodes are always enlarged. At postmortem examination, different organs may be affected, including spinal cord, brain, heart, skin, kidney, liver, abomasum and lungs. The enormous lymphoid infiltration of the integumentary system looks like the clinical symptoms of mycosis fungoides of human beings (Smith, 2015; Constable et al., 2017).

Fig. 11: Postmortem examination in a heifer with thymic lymphoma. Image **A** shows that both the cervical (white arrow) and thoracic (black arrow) parts of the thymus are enlarged. The liver is enlarged and the hepatic veins are severely distended (**B**) and abomasum is ulcerated (**C**).

Fig. 12: Early cutaneous form lesions of bovine leukosis in a Japanese black cow. Skin swellings are observed as raised lesions on the right (**A**) and left (**B**) sides of the body. Bilateral exophthalmia was also observed in this cow (**A**).

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

MT: conceived, designed the review article, collected the materials, wrote the manuscript draft, and prepared the Figures. **MM** and **AA**: revised the manuscript draft. Both authors re-read, revised and approved the manuscript.

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