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Immunohistochemical diagnosis of cutaneous T-cell lymphoblastic lymphoma in cattle

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Abstract
A four year old Jersey Cross cow was presented with the raised, spherical alopecic, non-ulcerated multiple varying sized masses on left inguinal region. Fine needle aspiration biopsy (FNAB) was done for cytological analysis. Cell block preparation made from the aspiration fluid and was used for Immunodiagnosis. Cutaneous form of T cell granular lymphoblastic lymphoma was diagnosed

Keywords: Immunohistochemical diagnosis, cutaneoust cell lymphoblastic lymphoma, cattle

Introduction
Cutaneous lymphoma or lymphosarcoma in cattle is comprising of two different forms: enzootic bovine leukosis (EBL) and sporadic bovine leukosis (SBL). Enzootic bovine leukosis, the "adult form," is directly related to infection with the *Bovine leukemia virus* (BLV). Sporadic lymphoma, is a disease of younger animals (<3 y) and occurs independently of BLV infection. Three forms of SBL have been described: calf, thymic, and cutaneous [1]. Based on the degree of epidermal involvement, the cutaneous form has 2 subsets, nonepitheliotropic and epitheliotropic [2]. Epitheliotropic tumours are usually primary to the skin and confined to it and the superficial lymph nodes. Non epitheliotropic tumours are mostly multicentric and eventually spreading to skin. Enzootic bovine leukosis is a neoplasm of B-lymphocytes, while SBL may originate from the malignant transformation of either B- or T-lymphocytes [3].

Materials and Methods
A four year old Jersey Cross cow was presented to the Veterinary Dispensary, Kalambur, Thiruvannamali District, Tamil Nadu, India with the raised multiple varying sized masses on left inguinal region. One large nodule (Fig.1) was spherical, alopecic, ulcerated measuring about 15cm in diameter. Other masses were like raised plaques. The large mass was slightly hard in consistency, generally, and soft in the middle portion. On physical examination, the cow was active and alert but poor in bodily condition. Rectal temperature, pulse rate and respiratory rates were within the normal range. This mass was growing slowly for the past six months and evinced no pain on palpation. Lymphadenopathy of the left inguinal lymph node was observed.
The animal was treated with broad spectrum antibiotics and non-steroidal anti-inflammatory drugs for 3 days, but no improvement was seen.
Blood was submitted for a complete blood count (CBC), biochemical analysis. Routine FNAB was performed using a 23 gauge needle attached to a 20 mL syringe. Two number of air-dried smears were collected and stained by May-Grünwald-Giemsa stain (MGG) for cytological examination under light microscopy. Cell blocks were prepared from the centrifuged aspirated material after fixation in 10% buffered formalin. Antigen retrieval was carried out with citrate buffer 10 mM pH 6.0 in a pressure cooker, for 3 min, at 120 °C. Endogenous peroxidase was blocked with 6% hydrogen peroxide for a minimum of 30 min. Primary antibodies used were CD3 (Rabbit polyclonal, PathnSitu, USA) and CD20 (Mouse monoclonal, PathnSitu, USA). Cell block specimens for immunocytochemistry were stained by a streptavidin-horseradish peroxidase method according to the manufacturers instruction (PathnSitu, USA). The samples were, then, counterstained with Harris Hematoxylin for 30 s at room temperature, followed by dehydration and slide mounting with DPX mountant.

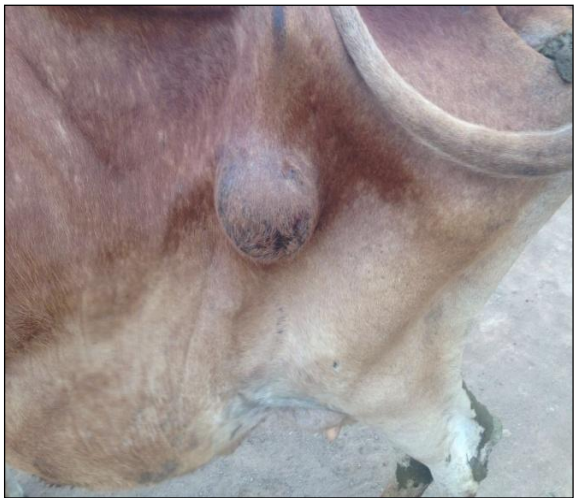

Fig 1: Cutaneous lesions, one large mass and few small plaques

Results

The values of CBC and biochemistry were within the normal range. Cytological analysis of the fine needle aspirates showed high cellularity with high cell fragility. Smear revealed a monomorphic population of large lymphoblasts with high nuclear to cytoplasmic ratios, moderate basophilic cytoplasm, single to multiple distinct nucleoli, and a finely stippled chromatin pattern (Fig.2). The nuclei of the large lymphocytes was varied from round to oval to kidney shaped or had a deep indentation. Most of the lymphoblasts contained numerous eosinophilic granules in the cytoplasm and other few cells were bi/tri nucleated. Smears showed high numbers of dark blue lymphogandular bodies in the background. More numbers of mitotic figures and tingible body macrophages (TBM) were also observed (Fig. 3). Immunocytochemically, the neoplastic cells were positive for CD3 (Fig.4) and negative for CD20. A diagnosis of cutaneous T cell granular lymphoblastic lymphoma was made.

Within 45 days after the confirmation of lymphoma, the animal became very dull, weak, decreased in weight and emaciated. The owner disposed the animal without our notice. Hence, it was impossible to perform postmortem examination and to collect samples. We also could not diagnose the seropositivity of BLV, because of the unavailability of laboratory facilities.

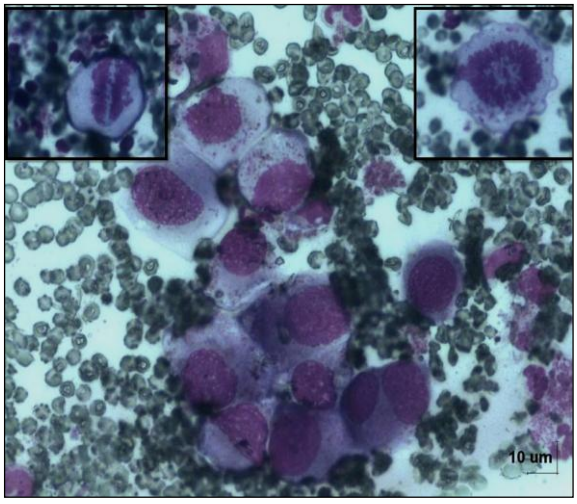

Fig 2: Skin mass-FNAC: Lymphoblasts with cytoplasmic granules. Insets: Mitotic figures. MGG bar=10µm

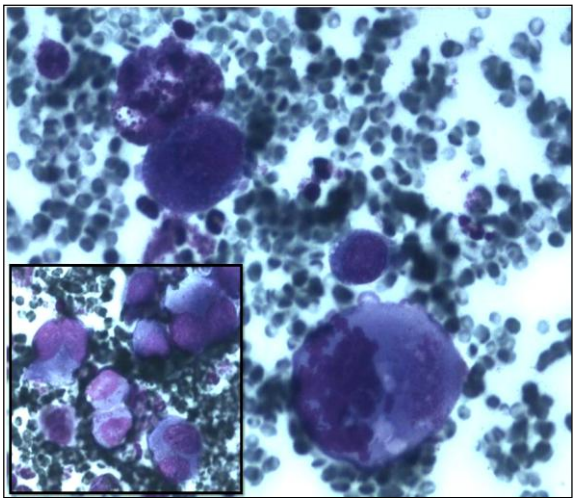

Fig 3: Skin mass-FNAC: Mitototic figure (arrow) and TBM (arrow head). Inset: Binucleate cells. MGG bar=10µm

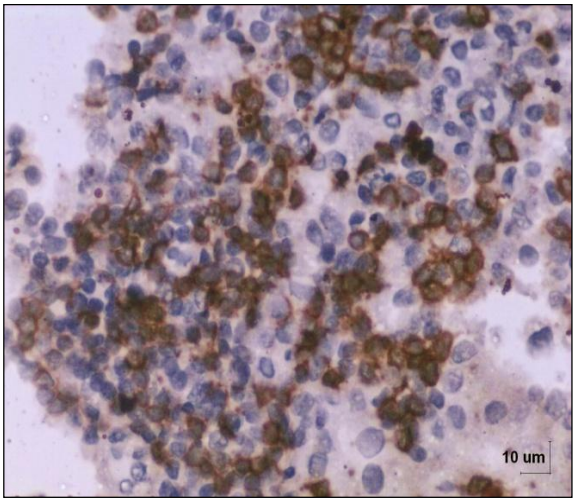

Fig 4: Skin mass-Cell block-Lymphoid cells positive for CD3 antigen. DAB, bar=10µm

Discussion

The pathogenesis of cutaneous lymphomas most likely involves multiple steps, has a multifactorial nature and starts as a hyper-reactive, inflammatory process due to various stimuli that may be genetic (numerical and structural chromosomal abnormalities), environmental, infectious (herpes- and retroviruses, bacteria, chlamydia) or immunological (vaccination or allergen hypersensitivity, drugs or arthropod reactions) [4]. Though, the cutaneous form of SBL primarily affects young adults of 2-3 years old [5], it was also recorded in a 9 months old bullock [6]. Cutaneous lesions were described to be discrete, alopecic, intradermal plaques or nodules of varying sizes that often become ulcerated and bleed, particularly in the advanced stages of disease [7, 8]. Reportedly, the lesions can regress over a period of time [1], but it is thought that recurrences occur with subsequent systemic involvement. Of peripheral lymph nodes and internal viscera, including the heart, kidneys, liver, and spleen, has been reported to occur in 50% of cases [9]. It has been postulated that nonepitheliotropic forms are of B-cell lineage, whereas the epitheliotropic forms, which are less common, are of T-cell origin [10] as recorded in this study. The gross appearance of the lesions is similar in both forms, though microscopically there are differences. Epitheliotropic lymphosarcomas demonstrate an affinity for Langerhans' cells and follicular epithelium of hairs, with progressive

accumulation of neoplastic lymphocytes developing within these sites. There tends to be more severe destruction of adnexal structures [8-10]. Disease progression in epitheliotropic and nonepitheliotropic lymphosarcoma also differs, in that, epitheliotropic forms appear to be confined primarily to the skin and superficial lymph nodes, whereas nonepitheliotropic forms develop visceral involvement late in the course of the disease [2].

Cutaneous lymphoma is a less common form of lymphoma and not associated with enzootic bovine leucosis (EBL) [6]. The skin or cutaneous form of BLS is a rare condition seen in cattle from one to three years of age, but is not as age specific as other forms of BLS. The skin form is characterized by the development of tumors beneath the skin surface. Often there is an initial period of one to three months during which cutaneous swellings are found around the anus, vulva, escutcheon, flank and shoulders. Lesions can be raised and ulcerated with necrotic centers. These areas may be painful on palpation. The tumors themselves may resolve or metastasize, producing other lesions such as cardiac insufficiency, ventral eden, elevated pulse and respiration and enlarged Iymph nodes. Anemia and atypical lymphocytes are common clinical pathologic findings with the skin form. This disease is fatal if metastasis occurs. On necropsy a variety of organs may be involved [11].

The nuclear polymorphism especially prominent in immature or undifferentiated reticulum and lymphoid tumor cells may have caused the formation of the nuclear inclusion-like masses of cytoplasm.

Based on cytological features and immunocytochemical studies this case was diagnosed as cutaneous form of T cell granular lymphoblastic lymphoma.

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