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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISATION OF T-CELL LYMPHOMA IN A CAMEL

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ABSTRACT

A ten-year-old single-humped male camel was found dead after prolonged illness. On necropsy, marked body cavity effusion was found with multiple, off white, umbilicated, well demarcated, nodules of variable size on the liver and spleen. Multiple lymph nodes within the abdominal and thoracic cavities were enlarged. Histologically, a pleomorphic small to medium sized noncohesive population of neoplastic lymphocytes infiltrated various organs and effaced normal architecture. Neoplastic cells were strongly positive for CD3 but negative for CD79, MUM1, CD68 and lysosomes. Based on the histopathology and immunohistochemistry, it was diagnosed as T-cell lymphoma.

Key words: Camel, lymphoma, immunohistochemistry, T-cell, CD3

Lymphoma is a malignant neoplasm of either B or T lymphocytes, arising from lymphoid tissue. In humans, lymphoma can be classified into Hodgkin lymphoma (HL) and Non-Hodgkin lymphoma (NHL). NHL is the sixth most common cancer in males and the seventh most common cancer in females in the US.

The characteristic neoplastic cell of Hodgkin lymphoma, the Reed-Sternberg cell, is rarely recognised in animals (Parodi, 2001; Jacobs *et al,* 2002). NHL in animals develops from mature or immature B- or T-cells. Among animals, lymphoma is the most common neoplasm of cats, frequently reported in dogs and cattle, and less frequently reported in horses, swine, camel, sheep and goats (Jacobs *et al,* 2002).

According to the World Health Organisation (WHO), B- or T-cell lymphomas have been classified into many subtypes. Simple histopathological examination is inadequate for confirmatory diagnosis of subtypes of B- of T-cell lymphoma; therefore, immunohistochemistry has become useful for differentiating T and B lymphocytes. Several markers used for such purpose are CD3 (T-cell marker), CD79 (B cell marker), MUM1 (plasma cell marker) and CD68 (histiocytic marker) (Zhang (Mary) and Aguilera, 2011).

Few published reports exist for lymphoma in single-humped camels (*Camelus dromedarius*) (Simmons *et al,* 2005) in particular, and Camelidae family (Pusterla *et al,* 2006; Twomey *et al,* 2008) in general, with immunophenotyping of the tumour. There is no age predisposition for lymphoma in camelidae. It has been reported in young (≤2 years) (Potter and Young, 1994; Cebra *et al,* 1995; Irwin, 2001; Hemsley *et al,* 2002; Sartin *et al,* 2004; Pusterla *et al,* 2006) and old camelids (Underwood and Bell, 1993; Cebra *et al,* 1995; Sartin *et al,* 2004). This report describes the gross, histopathological and immunohistochemical findings of a case of T-cell lymphoma in a single-humped male camel.

Materials and Methods

The ten-year-old intact male camel had pica and decreased food intake for two months. Anorexia and weight loss developed in last week prior to death. Symptomatic treatment was attempted but there was no improvement. After natural death, the camel was presented to the Department of Veterinary Pathology, College of Veterinary Science, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India, for necropsy.

Representative samples of the abdominal masses and other affected organs were collected during necropsy, fixed in 10% buffered formalin, and processed routinely. Histologic sections of the mass were stained with haematoxylin and eosin

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(H&E). Formalin-fixed paraffin-embedded spleen and lymph node around the abdominal aorta were submitted to the Joint Pathology Centre (JPC), USA for immunophenotyping. Immunohistochemistry was performed for CD3, CD79a, MUM1, CD68 and lysozyme. The primary and secondary antibodies details are summarised in Table 1.

Result and Discussion

External examination revealed animal with poor body condition, dehydration, and markedly worn teeth. On necropsy, the abdominal cavity contained approximately 15 L of straw coloured fluid admixed with fibrin clots. All lobes of the liver contained asymmetrical, randomly distributed, multifocal, off-white, raised, umbilicated, firm, well-demarcated nodules of variable size ranging from 2-10 cm in diameter (Fig 1). A focal, off-white, firm, lobulated mass of about 15 cm in diameter was also found near the duodenum. An approximately 4 cm diameter mass (similar morphology to that found in liver) was also found in spleen. Both kidneys showed focal extensive subcapsular haemorrhage. Multiple small nodules were also found on the serosal surface of the urinary bladder, which also had a thickened wall. There was a marked bilateral hydrocele with mild atrophy of testis. Multifocal ulcers were evident in abomasum. A focal 20 cm diameter mass was found around the abdominal aorta. The mediastinal lymph nodes were severely enlarged with multifocal haemorrhages in the cortex. The pericardial sac was filled with approximately 500 mL of straw coloured fluid admixed with fibrin clots.

On the basis of H&E staining and immunohistochemistry, the neoplasm was diagnosed as a T-cell lymphoma. Histologically, in all affected organs, the neoplasm was composed of pleomorphic round to polygonal noncohesive population of neoplastic lymphocytes. These neoplastic cells effaced normal architecture of the organs in which they infiltrated (Fig 2). Neoplastic cells were small to medium and had scant to variable amounts of cytoplasm with distinct cell borders. The nucleus was round, oval or indented and occasionally band, convoluted or bilobed. The nucleus contained clumped or coarsely stippled chromatin with inconspicuous nucleoli. Nuclei showed marked pleomorphism, with marked anisocytosis and anisokaryosis (Fig 3). Occasionally multinucleated cells were also present. Neoplastic cells were separated by delicate collagenous fibrovascular stroma. An average of two mitotic stages was found per field. There was scattered single cell necrosis of neoplastic cells. Hepatocytes adjacent to the border of the neoplasm showed macrovesicular fatty changes. Multifocally, neoplastic cells were also present within vessels and sinusoids of the liver. Multiple lymph nodes were completely effaced by the neoplastic cells and admixed with variable degrees of haemorrhage.

Immunohistochemically, neoplastic cells of spleen and lymph node (peri- abdominal aortic mass) were strongly positive for CD3 (Fig 4), but negative for CD79, MUM1, CD68 and lysozyme. Strong positive CD3 immunostaining confirms the diagnosis of T-cell lymphoma.

In the present study, haematology and clinical biochemistry data were not available. Reported incidence rate of lymphoma in cats, dogs, and cattle are 200, 13 to 24, and 18 per 100,000, respectively (Volli, 2007). For camel, such information is not available. Limited reports of lymphoma in the camelidae family are available and no gender predisposition has been reported (Hemsley *et al,* 2002; Pusterla *et al,* 2006; Twomey *et al,* 2008). Lymphoma in adult cats, cattle, dogs and sheep is associated with the presence of a retrovirus (Volli, 2007). In camelidae, only one study was conducted to identify the possible role of a retrovirus in the pathogenesis of lymphoma. Cebra *et al* (1995) examined tissue from four llamas and one alpaca ultra structurally, but no retroviral particles were seen in tissues.

Literature regarding immunophenotyping of lymphoma in camelidae is very meagre. For differential diagnosis of various subtypes of lymphoma, immunohistochemistry is an essential

Tabel 1. Primary and secondary antibodies used in this study.

Antibody	Clone	Species	Company	Catalog #	Dilution
CD3	2GV6	Rabbit Monoclonal	Ventana Medical Systems	790-4341	Ready to Use
CD79a	SP18	Rabbit Monoclonal	Ventana Medical Systems	790-4432	Ready to Use
Lysozyme	N/A	Rabbit Polyclonal	Ventana Medical Systems	760-2656	Ready to Use
CD68	KP-1	Mouse Monoclonal	Ventana Medical Systems	790-2931	Ready to Use
MUM-1	MRQ-43	Rabbit Monoclonal	Ventana Medical Systems	760-4529	Ready to Use
UltraView Universal DAB Detection Kit	–	–	Ventana Medical Systems	760-500	Ready to Use (Biotin free)

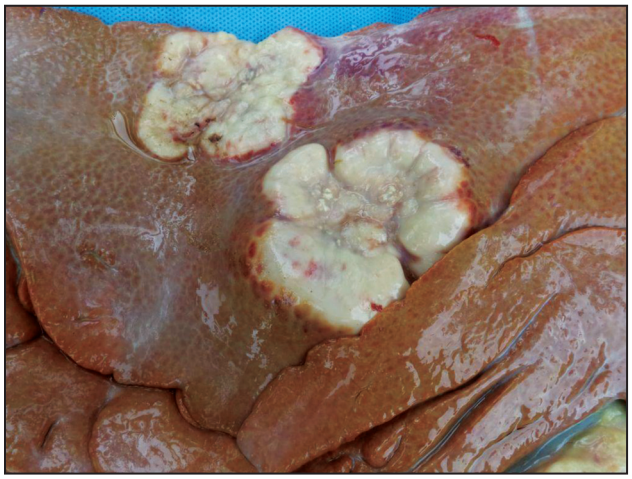

Fig 1. Well demarcated, off white, raised, umbilicated, lobulated nodules in liver.

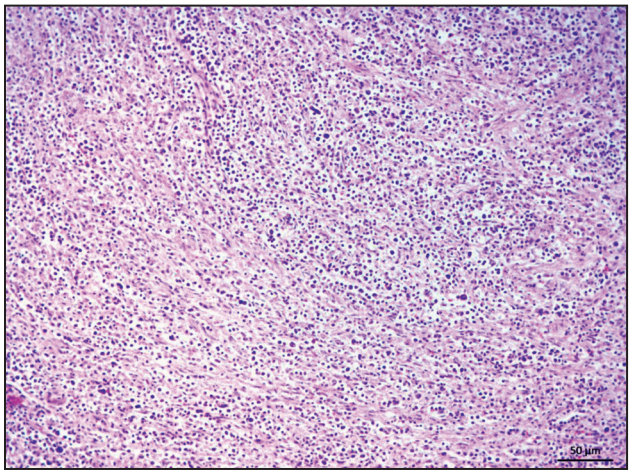

Fig 2. Normal architecture of liver is effaced by pleomorphic round to medium to large noncohesive population of neoplastic lymphocytes and separated by collagenous fibrovascular stroma, Liver (HE X 100).

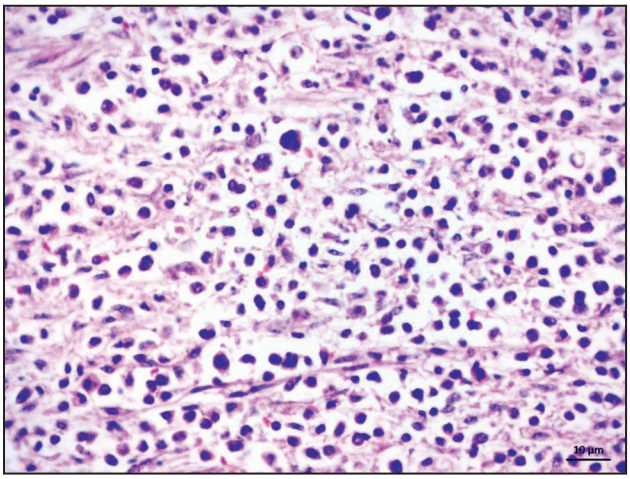

Fig 3. Higher magnification of Fig 2. Medium to large noncohesive neoplastic lymphocytes show marked variation in shape of nucleus, anisokaryosis and anisocytosis. Neoplastic cells are separated by a delicate fibrovascular stroma, Liver (HE X 200).

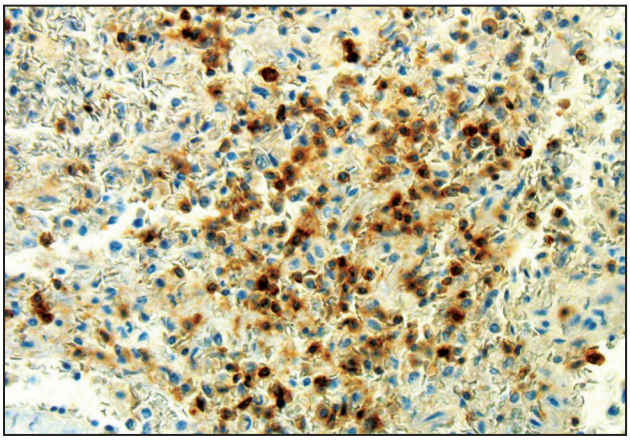

Fig 4. Neoplastic cells show strong positive cytoplasmic immunoreactivity to CD3, Spleen Immunohistochemistry (HE X 200).

tool. CD3 is the most commonly used pan–T-cell antigen and is normally expressed at the second stage of thymic differentiation and beyond. CD79 is an excellent marker for B-cell lineage (Higgins *et al,* 2008; Valli *et al,* 2011). Immunophenotyping of six cases of lymphoma in camelidae was performed by Twomey *et al* (2008), and it was found that four cases were of T-cell lineage (positive for CD3), one case was B-cell lineage (positive for CD79a), and one case showed positive reaction for both CD3 and CD79 which was diagnosed as mixed tumour. Such dual positive reaction was also reported by Hemsley *et al* (2002) in one out of two cases and another case showed positive reaction to only CD79 (B-cell lymphoma). Sartin *et al* (2004) also reported B-cell lymphoma in three alpacas. Pusterla *et al* (2006) reported one case of T-cell lymphoma in an alpaca. In humans and animals T-cell lymphoma is less common and has a poorer survival profile and response to therapy than B-cell lymphoma (Volli, 2007; Gavazza *et al,* 2013). More studies are required to report the most prevalent subtype of lymphoma in camelidae.

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