Cytopathology of Extragenital Transmissible Venereal Tumour in a Dog in Lusaka, Zambia

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ABSTRACT

Transmissible Venereal Tumour (TVT), a round cell tumour probably originating from genetic alteration of canine histiocytes, is a horizontally and sexually transmitted disease that affects dogs and other canids. There is no published report on extragenital TVT among dogs in Zambia. The present case reports the cytopathology and haemato-biochemistry of an extragenital TVT, without primary genital involvement in a 4-year-old male dog in Lusaka, Zambia. There were a total of 18 cutaneous immobile nodules ranging in size from 25 to 57 mm on the dorsal and ventral aspects of the body, and no ulceration or bleeding was observed. Haemato-biochemistry revealed anaemia, thrombocytopaenia, hyperproteinaemia, increased blood creatinine and urea nitrogen. Based on cytology, TVT of the lymphoid subtype was diagnosed, ruling out suspicion of cutaneous lymphoma or histiocytoma. Definitive diagnosis was easily based on physical examination and cytological findings of typical TVT cells in exfoliated cells through FNAC, which is a rapid, reliable, efficient, cost-favourable and more conclusive hastening intervention than waiting for histopathology. Our findings emphasise the need to consider TVT on the differential diagnosis list for masses in extragenital sites in dogs from geographic regions where TVT is prevalent.

Keywords: Extragenital TVT, Dog, Haemato-biochemistry, Cytopathology, Diagnosis, Zambia

INTRODUCTION

Transmissible Venereal Tumour (TVT) is a horizontally and sexually transmitted among round cell tumours believed to be originating from specific genetic

alteration of canine histiocytes affecting dogs and other canids (1, 2), also known as canine transmissible venereal sarcoma, venereal granuloma, transmissible lymphosarcoma, or sticker tumour of mesenchymal origin (3). TVT can easily transmit from animal to animal during copulation by viable tumor cells by licking, biting and direct contact with the tumor and it frequently affects dogs of either sex (4). TVT mostly affects genitalia, including the vagina in females and the penis and prepuce in males and occasionally extragenital sites such as skin, mouth, eye, nasal, mammary gland and other sites (5, 6).

TVT is distinguished morphologically into three cyto-morphological subtypes, namely;

i.) Lymphocytic type, having granular cytoplasm with a centrally placed nucleus and a number of intracytoplasmic vacuoles.
ii.) Plasmacytic type, having eccentric nuclei with many cytoplasmic vacuoles, and
iii.) Mixed type, characterised by both lymphocytic and plasmacytic types (7).

The histological features of TVT resemble histiocytoma and cutaneous lymphoma, whereas, on impression, the cytology of TVT, cytoplasm reveals a number of punctuate vacuoles in most cells that differ from histiocytic tumour and other round cell tumours (8). TVT displays histological resemblance to canine cutaneous histiocytomas and other round cell tumours, thereby presenting difficulties for pathologists in their differentiation. Genital organs involving TVT have been reported among dogs in Zambia (9). However, there is no published report on extragenital TVT in Zambia. According to the literature, TVT occurs predominantly in stray dogs with tropical and sub-tropical climates involving genital organs of both sexes (10). Therefore, the present case reports the cytopathology and haemato-biochemistry of an extragenital TVT without primary genital involvement in a dog in Lusaka, Zambia.

CLINICAL EXAMINATION AND METHODOLOGY:

A four-year-old Boerboel cross, an intact male dog, was presented to the Small Animal Clinic of the School of Veterinary Medicine, University of Zambia, Lusaka, Zambia on 10 July 2023. The dog was reported to have a history of dullness, reduced appetite, and weight loss for the last two months with multiple small nodules-like growth over the dorsal and ventral aspects of the body. On physical examination, physiological parameters were within normal range. There were a total of 18 cutaneous immobile nodules ranging in size from 25 to 57 mm on the dorsal and ventral aspects of the body, but no ulceration or bleeding observed (Figure 1). The largest nodular growth was seen medial to the right thigh on the first day of examination (Figure 2). Based on clinical examination, the dog was initially suspected to be a case of histiocytoma or cutaneous lymphoma while awaiting cytology. There were no lesions on the genital organ when the penis and prepuce were examined on the next visit within 4 days of the first visit. Blood was collected both in EDTA and without EDTA tube for hemato-biochemical analysis using ProCyte Dx Haematology Analyser and Catalyst Chemistry Analyser. The owner had

reported that the dog was maintained in enclosed premises, except it went out for once a month to a friend's house for breeding purposes. No information was available about the bitch that was bred with this dog. Fine needle aspiration (FNA) puncture was performed on nodule using, a 22G needle coupled to a 5 mL syringe, smears were prepared, dried, fixed in methanol, stained with Giemsa stain and examined using light microscopy. A small nodule mass was collected and fixed in 10 per cent neutral buffer formalin for histopathological examination as per standard protocol.

Figure 1 Dog Showing Multiple Cutaneous Nodules of Varying Sizes on the Back

Figure 2: Dog Showing Large Nodular Growth Medial to Right Thigh with no Lesion on the Penis or Prepuce

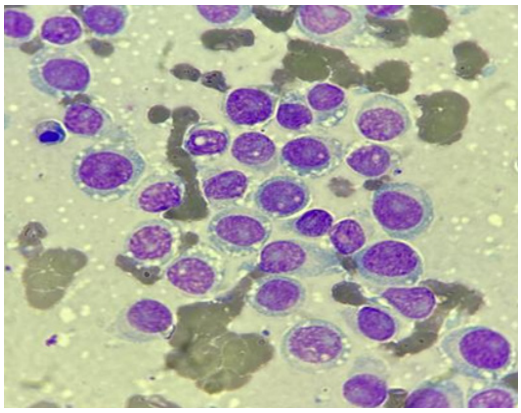

Figure 3: FNAC of cutaneous nodule showing many round TVT cells, large deeply blue stained round nuclei, large nucleoli with many vacuoles within the cytoplasm, and high N: C ratio Giemsa stain; ×100

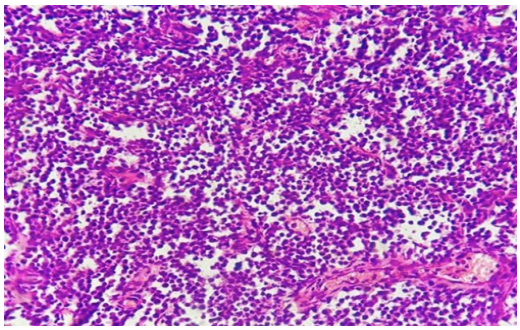

Figure 4: Cutaneous TVT nodular mass section on H/P showing uniform sized, round to oval cells resembling lymphocytes along with the presence of fine cutaneous fibro-vascular strands H and E stain x 40

RESULTS AND DISCUSSION

FNAC impression smear cytology showed round or ovoid tumour cells containing single large round nuclei deeply stained of variable size, coarse nuclear chromatin, large nucleoli and abundant vacuolated cytoplasm with mitotic figures and high nuclear-cytoplasmic ratio (Figure.3). Based on cytology TVT of lymphoid subtype was diagnosed ruling out cutaneous

lymphoma or histiocytoma. In clinical practice, the diagnosis of extragenital TVT can be challenging. It is accurately diagnosed, differentiating it from other round cell tumours, using techniques such as cytology, histopathology or even immunohistochemistry (1, 2). In this report, the fine needle aspiration cytology and H/P technique were used. Through cytological examination, TVTs can be sub-classified as lymphocytic, plasmacytic or mixed type, and the present case being lymphocytic type; an interesting finding is the presence of extragenital lesions without primary genital involvement.

Table 1 : Haemato biochemical observation in the Dog with Extragenital TVT

Parameters	Observed Value	Range Value
Haematological		
Total RBC (x10^6 / ul)	5.30	5.5-8.5
PCV %	35.4	37-55
Haemoglobin g/dl	11.5	13.8-20.3
MCV (fl)	65.0	61-75
MCH (pg)	21.7	19-24
MCHC g/dl	33.0	30-35
Reticulocytes %	1.1	-
Total WBC (x10^3 / ul)	8.1	6-17
Neutrophils %	73	60-77
Lymphocytes %	20	15-30
Eosinophils %	2	2-10
Monocytes %	5	3-5
Basophils %	0	rare
Total Platelets (10^3/ul)	156	200-500
Biochemical		
Glucose	4.85	4.11-7.95 mmol/l
Blood Creatinine	242	44-159 um-mol/l
Blood Urea	22.7	2.5-9.6 mmol/l
BUN: Creatinine	23	-
Total Protein	110	52-82 g/l
Albumin	25	23-40 g/l
Globulin	75	25-45 g/l
A: B Ratio	0.3	-
AST	169	0-50 u/l
ALP	97	23-212 u/l
GGT	12	0-11 u/l
LDH	171	40-400 u/l
Creatine Kinase	34	10-200 u/l

Haematological examination (Table 1) revealed slight anaemia and thrombocytopenia. In the blood count, the alterations are usually influenced by many factors, such as negligence in health care by the owners and the location of the tumours and other underlying conditions. In the present case, the dog had a loss of appetite and weight, and other underlying conditions might have contributed to anaemia. Thrombocytopaenia and anaemia observed in the current case have also been reported in TVT (11, 12).

Serum biochemistry revealed increased creatinine and blood urea nitrogen (BUN) (Table 1), which was in concordance with the findings of Behera *et al.,* (13) and Komnenou *et al.* (14). High BUN level is also

associated with glomerular filtration rate (GFR) or enhanced protein catabolism due to tumour cell necrosis and metabolic side effects, respectively, leading to renal incapacity. We also observed hyperproteinaemia and hyperglobulinaemia as observed by Girmabirhan *et al.,* (12) and Behera *et al.,* (13), although Albanese *et al.,* reported normal levels of total protein, BUN and serum creatinine (15).

Histopathological examination of the cutaneous nodular mass revealed a large number of uniformly sized, round to oval cells along with the presence of fine cutaneous fibro-vascular strands resembling lymphocytes (Figure 4). These cells also displayed hyperchromatic nuclei, prominent nucleoli and punctate cytoplasm. The neoplastic cells had cytoplasmic projections, enhancing attachment with each other. Hence, TVT is also called a sticker cell tumour (14,16). Similar histological features were reported by Sritrakoon et al (4) and Gupta and Sood (5). It was difficult to differentiate histiocytoma or lymphoma from TVT on H and E stained sections.

Based on the observations of an unusual extragenital TVT in the above dog and its investigation, it is concluded that the diagnosis of TVT by fine-needle aspiration cytology is rapid, reliable, efficient, cost favourable and more conclusive than histology. There is less or no distortion of cells in the cytological smear, and cytology is a non-invasive technique, hence causing less pain to the animals (17). The diagnosis can be made within two hours after receiving the impression smears. In addition, histology alone may make it difficult for TVT cells to differentiate from other round cell tumours of extragenital locations (12).

The histopathological facility of animal tissues is only available at the University of Zambia, School of Veterinary Medicine in Lusaka, and it takes a minimum of not less than one week to give the results, hence delaying the treatment intervention. While cytology can be done in any simple laboratory, TVT can easily be diagnosed wherever a blood smear examination facility with expertise in cytology is available fairly quickly. Further, TVT displays histological resemblance to canine cutaneous histiocytomas and other round cell tumours, thereby presenting difficulties for pathologists in their differentiation (18). The diagnosis of TVT by cytology has been preferred over histopathology (19). This is due to the fact that the histopathology of TVT is difficult to differentiate from other round cell tumours such as lymphoma, histiocytoma, and mast cell tumour (19, 20). So, the definitive diagnosis could be based on physical examination and cytological findings of typical TVT in exfoliated cells obtained by impression smear, swabs, and fine needle aspiration, avoiding expensive time-consuming histopathology unless absolutely necessary in Zambia and other poor developing countries.

After TVT was diagnosed cytologically and histo-pathologically, vincristine injection 0.025 mg/kg body was given I/V. Unfortunately, the dog died after two days post-injection, and the carcass was not available for necropsy. Vincristine toxicity cannot, therefore, be ruled out as a possible cause

of death, or underlying causes might have aggravated the survival of the dog. High creatinine and urea blood level, anaemia and thrombocytopenia may have contributed towards the mortality of dogs as vincristine, although a drug of choice has side effects including death too, needing regular blood monitoring and supportive treatment (21).

This is the first observation of extragenital TVT in a dog in Lusaka, Zambia. This dog most likely contracted the TVT through physical or sexual contact with a female dog that it was with, in the past, for about a month for breeding. Our findings highlight the need to consider TVT for differential diagnosis of the extragenital mass or growth in dogs from geographic regions where TVT is prevalent.

CONCLUSION

The present case reports the cytopathology and haemato-biochemistry of an interesting extragenital Transmissible Venereal Tumour without primary genital involvement in a 4-year-old male dog in Lusaka, Zambia. Our findings highlight the need to consider TVT for differential diagnosis of the extragenital mass or growth in dogs from geographic regions where TVT is prevalent. It is concluded that the diagnosis of TVT by fine-needle aspiration cytology is a rapid, reliable, efficient, cost-favourable and conclusive hastening intervention.

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Conflict of Interest

The authors declare no conflict of interest.

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