Cytological and Histopathological Characteristics of Canine Transmissible Venereal Tumour in Male and Female Dogs Before and After Vincristine Treatment

J. T. Katjimune[1] and C. Mathew[1*]

[1]College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3021, Chuo Kikuu, Morogoro, Tanzania,

[*]Corresponding author: colethamat@sua.ac.tz

SUMMARY

Canine transmissible venereal tumour (CTVT) is a neoplasia naturally transmitted in susceptible dogs through coitus. CTVT has a worldwide distribution, with a high prevalence in tropical and sub-tropical urban environments. The study aimed at evaluating CTVT lesions in local breeds of dogs and to assess morphological changes based on sex before and after administration of vincristine sulphate. Clinical and gross morphology, fine needle aspirates cytology (FNAC) and routine histopathology methods were used. Two FNAC and histopathological microscopic slide sections from each of the seven sampled dogs were stained with Giemsa stain and Hematoxylin and Eosin. All dogs were treated with vincristine once weekly over a six weeks period after which clinical morphological and histopathological changes were assessed. Grossly, before treatment the tumour masses appeared irregular, cauliflower like with tendency to bleed, sizes ranged from ≥5cm to ≤2cm with or without metastasis to regional lymph nodes. Cytologically, the tumours had homogenous, sheet-like cellular mass. Cytoplasm with punctate vacuoles, anisokaryosis with anisonucleoliosis and coarse to reticulate nuclear chromatin were seen. Lymphocytoid cell pattern was dominant cell type. Histopathology showed sheets of round cells with nuclear and cytoplasmic variations. Histopathology of the treated dog revealed hyper-cellularity, absence of nucleoli, prominent mitotic figures, reduced cell size and presence of inflammatory cells. There was no difference on the cellular changes after vincristine treatment between female and male dogs. Cytology and histopathology showed that vincristine sulphate suppresses the development of tumour through alteration of cellular morphology with no difference between male and female dogs.

Key words: fine needle aspirates cytology, histopathology, canine transmissible venereal tumour

INTRODUCTION

The canine transmissible venereal tumour (CTVT) is a contagious and sexually transmitted neoplasia of unknown origin. This tumor affects dogs (*Canis familiaris*) and can also infect other canids, such as foxes, coyotes and wolves. Under natural conditions, CTVT only affects dogs, and experimentally, other species. It was first described by Hujard in 1820 in Europe (Ganguly *et al.*, 2013; Madewell, 2001). CTVT was first described as a transmissible and transplantable tumour in 1994 (Booth, 1994) and has been transplantable since the first description. This neoplasia is worldwide distributed (Strakova & Murchison, 2014), but the incidence and prevalence are highest in tropical and subtropical climates (Ferreira *et al.*, 2000). This includes mainly countries with large populations of mongrel street dogs. CTVT is a neoplasia naturally transmitted in susceptible dogs by transplantation of viable tumor cells especially if there are abrasions or loss of integrity on the surface. Due to the

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unique nature of transmission by sexual contact, naturally occurring CTVT generally develops in the external genitalia.

Less commonly, the tumor may also be transmitted to the nasal or oral cavities, skin and conjunctiva and the rectum by sniffing or licking. Dogs of any breed, age or sex are susceptible; however, females are infected more often than males because one infected male met with several females especially in stray dogs (Ganguly *et al.*, 2013). Although dogs over one year of age are at high risk in endemic areas, the condition is most common in dogs of 2 to 8 years old (Pandey *et al*., 1977. CTVT may metastasize in puppies and immuno-compromised dogs to regional lymph nodes and can also be seen in the skin brain, eyes, liver, spleen, testicles, rectum and muscles. Although reports on responses to vincristine treatment by gender suggest equal responses among male and female dogs, information on how the tumor cells responds and changes morphologically following vincristine treatment is limited. The present study was done to evaluate morphological changes of CTVT based on sex before and after administration of vincristine sulphate by consideration of using biopsy or cytological examination of fine needle aspirates or impression smears as previously described (Birhan & Chanie, 2015). The two tests complement each other to increase diagnostic accuracy and distinguish reactive dysplastic changes from neoplastic changes.

MATERIALS AND METHODS

Study location

The study was permitted and conducted at Sokoine University of Agriculture (SUA), College of Veterinary Medicine and Biological Sciences' Animal Teaching Clinic and two dog dipping sites (SUA and Kihonda) within Morogoro municipal area, where samples were collected and then processed at Sokoine University of Agriculture, College of Veterinary Medicine and Biological Sciences, Department of Veterinary Anatomy and Pathology for various laboratory procedures. Samples were collected after obtaining consent from owners of the respective patients.

Study design, sample size and sample collection

A cross sectional study design was used and sample size was calculated using formula described by Thrusfield (2005) and the assumption that: prevalence (p) of CTVT within Morogoro Municipal area is 7% (Sokoine University of Agriculture Animal Hospital Records., 2018), with 95% confidence level. Calculation of sample size is given using the formula below:

$n = \frac{1.96^2 P_{exp}(1- P_{exp})}{d^2}$, where n = required sample size; P_{exp} = expected prevalence; d = desired precision. With the estimated dog population of 10,800 and a prevalence of 7%, one hundred dogs (n=100) presenting CTVT is the desired sample size. Ten (10) dogs were sampled and used for piloting the study. Convenient non-probability sampling was used to obtain 10 dogs for the later and owner's consent was obtained before their animals could be enrolled for study. Participating animals were selected regardless of age, breed and sex bu these parameters were recorded. Key enrolment criteria was the presence of grossly notable masses resembling CTVT (Supplementary Figure 1) or swelling on or around the vulva or penis which were later confirmed through individual clinical evaluation.

Biodata collection, gross and clinical examination of dogs

All clinical information and data from selected dogs were recorded in the data collection sheet. Clear photos of the lesions were taken and gross description of the appearance of the lesions was done. Tumor were classified and staged grossly according to criteria as described in (Hataka, 2004).

A TNM (where T describes the size of the tumor and any spread of cancer into nearby tissue; N describes spread of cancer to nearby lymph nodes; and M describes metastasis (spread of cancer to other parts of the body) was done. Staging T1=≤ 2cm (tumour less than or equal to two centimeters), T2=≥2cm≤5cm (tumour larger than two centimeters but less than five centimeters), T3=≥5cm (tumour larger than five centimeters), N0=No metastasis to regional lymph nodes, N1=Metastasis to regional lymph nodes, M0=No metastases to other organs, M1=Metastases to other organs except regional lymph nodes; was used in this study.

Cytological and histological examination before and after vincristine treatment

Fine needle aspirates (FNA), were collected with a 21-gauge needle and prepared on two microscopic slides, fixed with 90 % methanol and stained, one by Giemsa staining and the other by H&E staining. Both prepared slides were then examined under microscope at 40x to observe the cells individually. Cells were counted from ten microscopic fields and cytological observations were recorded in a data recording sheet. For histopathological examination, one tumour pieces of about 0.5 cm^3 was collected directly in tissue cassette and then placed into a container with 10% neutral buffered formalin. The collected tissue was then divided into two approximate halves. One half of the collected tissue was then processed for routine histopathological examination and stained with Hematoxylin and Eosin staining and another half stained with Giemsa. Both slides were examined under the microscope at 40x to observe individual cells and detailed histopathological features and results recorded in a data recording sheet. CTVT cells were classified as plasmacytic (P): if at least 60% of TVT cells were ovoid, and contain large amount of cytoplasm and eccentric nucleus. Cells were considered lymphocytic (L): if at least 60% of TVT cells were round, have round nuclei, 1-2 nucleoli, scarce cytoplasm and high nucleus to cytoplasm ratio.

Cells were classified as mixed posses characteristics of both plasmacytic and lymphocytic cell types (Amaral *et al.*, 2007). Each of the two main cell type was counted and the lymphocytic plasmocytic (L:P) ratio calculated by obtaining the ration of the two types. After pre-treatment evaluation, all participating dogs were treated once weekly over a period of four (4) to six (6) weeks, with vincristine sulphate at a dose of $1mg/m^2$, where body weight in kg is correlated to tumour surface area in m^2 (e.g. 2kg ≈ 0.16 m^2; 10kg ≈ 0.47 m^2) (www.vetoncologyconsults.com). After six weeks of treatment sampling of tumour was done and prepared as described above for histopathological evaluation.

RESULTS

Out of 10 dogs, seven dogs were confirmed to have CTVT and selected independently of age and breed (Table 1). Presence of CTVT was judged based on the presence of extra-genital red irregularcauliflower-like mass (Supplementary Figure 1). These features were present in all the seven (7) dogs selected for the study. In all dogs, tumours were located on the extra genitalia, whereas metastasis to regional lymph nodes was noted in a four-year old male and a four-year old female. The size of extragenital tumors was variable in all dogs. One female dog had a tumor size of ≤2cm, three dogs (2 female and 1 male) had tumor size between ≥2cm ≤5cm and three other dogs (1 female and 2 males) had tumors ≥5cm in diameter. The staging was from T3N1M0 being worse with a tumour larger than 5cm and

metastasis to regional lymph nodes in two dogs, to T1N0M0 being less severe with a tumour less than 2 cm and no metastasis to regional lymph nodes in one dog. Cytological evaluation with Giemsa staining revealed hyper-cellularity of both lymphocytoid cell pattern (characterized by round morphology, rare/scanty cytoplasm and central nucleus) and plasmacytoid cell

pattern (characterized by oval morphology, abundant cytoplasm and eccentric nucleus), presence of large numbers of round to ovoid cells with punctate vacuoles in the cytoplasm; (Table 2). The nuclear to cytoplasm ratio is found to be higher and few mitotic figures were observed in the nucleus of the cells.

Other cytological changes include clumping of chromatin material which leads to granular appearance of the karyoplasm and prominent nucleoli. Cellular morphology was the same in both male and female dogs. In terms of cell type, all male dogs had lymphocytoid cells predominate the cases while in female dogs two of the four dogs had a mix of lymphocytoid and plasmacytiod and the remaining two female dogs, one had dominance of lymphocytoid and another one plasmacytoid (Table 2). The ratio of lymphocytoid to plasmacytoid cells (L:P ratio) was consistently higher in male dogs but variable in female dogs (Table 2). Both Giemsa-stained and H & E-stained histopathological sections revealed massive proliferation and hyper cellularity of pleomorphic cells (round, oval to no distinct shape) separated by pocket-like fibrovascular stroma with large prominent nucleus and large ovoid nucleoli, presence of inflammatory cells, furthermore the cells were observed to have mitotic figures in the nucleus; (Table 3). Male dogs had lymphocytoid as predominant cell type while females had a mixture of both with two female dogs having both Lymphocytoid and plasmacytoid in the in the same section (Table 3).

Table 1: Biodata, clinical and gross data from sampled dogs

Case number	Sex	Age (years)	Ownership Status	Status	Tumour Location	TNM
TK/001	M	4	Ow	En	EG	T3N1M0
TK/002	F	5	Ow	En	EG	T2N0M0
TK/003	F	1	Ow	En	EG	T2N0M0
TK/004	M	2	Ow	En	EG	T3N0M0
TK/005	F	3	Ow	En	EG	T1N0M0
TK/006	F	4	Ow	En	EG	T3N1M0
TK/007	M	9	Ow	En	EG	T2N0M0

M= Male**, F** = Female, **Ow** = Owned, **En** = Entire, **EG** = Extra Genitalia, **T1** = ≤2cm, **T2** = ≥2cm≤ 5cm, **T3** = ≥5cm, **N0** = No metastasis to regional lymph nodes, **N1** = Metastasis to regional lymph nodes, **M0** = No metastases to other organs, **M1** = Metastases to other organs. T: Size, N: Spread to nearby lymphnode, M: Metastatsis

Table 2: Cytological data from sampled dogs

Case Number	Sex	Age	Cell Type	FNA stained with Giemsa: N(L:P)
TK/001	M	4	L	110 (70:40)
TK/002	F	5	Mx	93 (41:52)
TK/003	F	1	Mx	135 (73:62)
TK/004	M	2	L	214 (143:71)
TK/005	F	3	P	147 (42:105)
TK/006	F	4	L	212 (151:61)
TK/007	M	9	L	127 (85:42)

M = Male**, F** = Female, **Age in years**, **L** = Lymphocytoid (≥60% round cells predominance, scarce cytoplasm and central nucleus), **P** = Plasmacytoid ((≥60% ovoid cells predominance, abundant cytoplasm and eccentric nucleus), **Mx**= Mixed cellularity (neither lymphoid or plasmacytoid surpassed 59% of the total). FTNA: Fine needle aspiration. N=total number of cells

Table 3: Histopathological data from sampled dogs

Case Number	Sex	Age	Cell Type	Giemsa stained Histological section N(L:P)	Haematoxylin and Eosin stained histological section N(L:P)
TK/001	M	4	L	171 (121:50)	142 (112:30)
TK/002	F	5	M	139 (64:75)	128 (64:64)
TK/003	F	1	M	68 (45:23)	88 (44:44)
TK/004	M	2	L	172 (91:81)	134 (93:41)
TK/005	F	3	P	94 (31:63)	77 (22:55)
TK/006	F	4	L	137 (95:42)	163 (102:61)
TK/007	M	9	L	223 (142:81)	154 (92:62)

M= Male**, F** = Female, **Age in years**, **L** = Lymphocytoid (≥60% round cells predominance, scarce cytoplasm and central nucleus), **P** = Plasmacytoid ((≥60% ovoid cells predominance, abundant cytoplasm and eccentric nucleus), **M** = Mixed cellularity (neither lymphoid or plasmacytoid surpassed 59% of the total). N=total number of cells.

DISCUSSION

Results obtained from this study revealed extra genital red irregular cauliflower-like mass with a tendency to bleed in all of the seven sampled dogs and this is in conformity with information available in the literature (Brown *et al.*, 1980; Das & Das, 2000). The age range of affected dogs was from 1 to 9 years which was expected as the dogs at this age are sexually active; given TVT mode of transmission through coitus (Ganguly *et al*., 2013) The predominant tumor localization mostly at extra genital sites was similar to previous reports for naturally occurring TVT in male and female dogs (Rogers, 1997).

CTVT metastasis to regional lymph nodes has been described (Ferreira *et al.,* 2000; Park *et al.*, 2006) and metastases were found in two of the 7 cases in our study (28.6%) and variable tumor shape and sizes recorded was not different to previous observations (Cohen, 1985; Das & Das, 2000). Moreover, cytological and histopathological pattern of the CTVT observed before vincristine sulphate treatment is in agreement with earlier findings with massive proliferation and hyper-cellularity of pleomorphic cells (Birhan & Chanie, 2015, Ganguly *et al.,* 2013). Likewise, the decrease in tumor cell size and presence of intercellular edema after vincristine treatment found in the present study has been observed before (Tella *et al.,* 2004). Proteinaceous material was found in tumours in histopathological section of the dog treated with vincristine sulphate, in our study which is an indication of better prognosis after chemotherapy. Decreased mitotic figures after vincristine treatment indicate the direct effect of vincristine on cell division as reported by other authors (Zhang *et al.,* 1992). Presence of inflammatory cells after vincristine treatment indicates the role of immunity complementing the effect vincristine which as previously reported (Tella *et al.,* 2004).

However, before vincristine treatment in male dogs the predominant cell type was lymphocytoid while in female it was both lymphocytoid and plasmacytoid (Table 2 and 3). However, this difference in cell type was not obvious after vincristine treatment. Regardless of the sex, de Olivera Lima *et al* (2013) and Amaral *et al* (2007) reported plasmacytoid to be the dominant cell type in CTVT. Contrary to these two reports, the present study female dogs were found to have a mixed cell types and in male dogs lymphocytosis predominated. However, after treatment in both male and female dogs the cells were mainly lymphocytoid type. Lack of differences after vincristine treatment could be related to tumor regression due to differences in sensitivity to vincristine between the two cell types as reported elsewhere that plasmocytoid are less sensitive to chemotherapy compared to

lymphocytoid and that plasmacytoid are found in tumour which are more aggressive (Gasper *et al.,* 2010).

Nevertheless, Canine transmission venereal tumour remains prevalent among mongrel dogs. Although some differences were observed to exist in terms of cellular composition before vincristine treatment, this difference is not maintained after vincristine treatment. It is not clear whether the cytological differences have any influence on the tumor regression following vincristine treatment. The cause of the observed pre-vincristine treatment is not known but most likely could be caused by differences in immune status of the animal.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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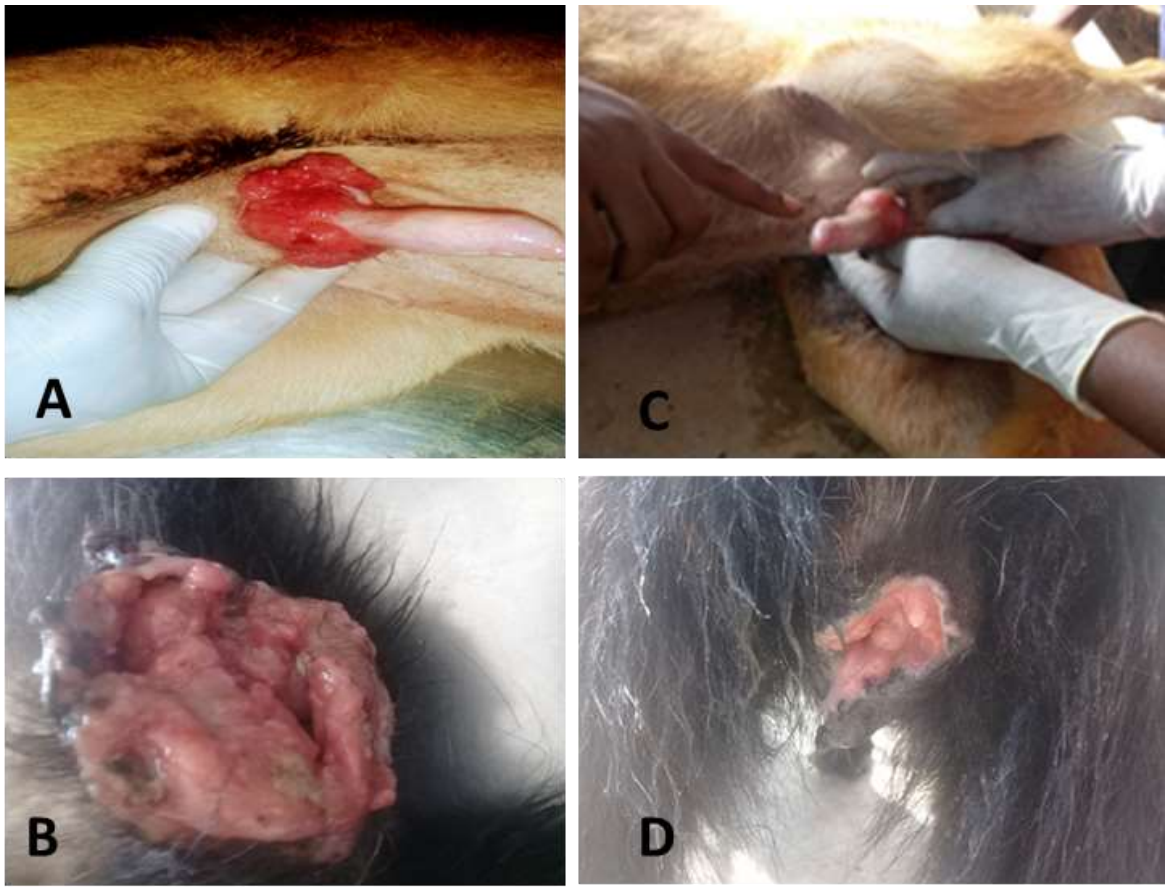

Supplementary Figure 1: Gross characteristics of CTVT before (**A and B**) and after **C and D**) vincristine treatment **A:** Male dog with characteristic reddish irregular cauliflower-like lesions at the base of the penis. **B:** A female dog with a red cauliflower-like mass with greenish fibrinous deposits on the vaginal mucosa, extruding through the vulva before. **C and D:** Male and female dogs after vincristine treatment note the regression of the tumor mass.

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