

## Progressive type canine transmissible venereal tumor (CTVT) in a male stray dog

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### ABSTRACT

An adult male stray dog of non descriptive breed with a cauliflower like growth in the penile region was admitted in S. A. Quaderi Teaching Veterinary Hospital (SAQTVH), Chittagong Veterinary and Animal Sciences University (CVASU). A detailed clinicopathological and histopathological examination of the tumor mass were performed to diagnose the case. The results of fine needle aspiration biopsy (FNAB) and histopathology confirmed the case as canine transmissible venereal tumor (CTVT). Under light microscopes, the tumor mass showed sheets of numerous neoplastic round cells with characteristic features and hyperchromatic nuclei. No evidence of metastasis was observed as all other visceral organs and lymphoid tissues of the vicinity were found free from neoplastic changes. Besides, depending on the histological features of the tumor mass the case was designated as progressive phase of CTVT.

### INTRODUCTION

The canine transmissible venereal tumor (CTVT) is a naturally occurring neoplasm of mainly young sexually mature dogs (Rogers, 1997) and is usually transmitted during coitus (Calvet, 1983). It has a worldwide distribution but seen mainly in tropical and subtropical countries (Roger, 1997). CTVT is a benign reticuloendothelial neoplasm producing cauliflower like, firm to friable nodular mass on the external genitalia of either male or female dogs and it occur at the same frequencies in both sexes (Rogers, 1997). It is known to be the most frequent tumor in ownerless stray dog population that usually run and copulate freely (Chaudhary and Rao, 1982, Gandotra et al., 1993 and MacEwen, 2001). It has been reported in many regions and several countries all over the world including Bangladesh and India (Chauhan et al., 1991, Jain et al., 2002 and Islam, 2010). Young dogs, stray dogs and sexually active dogs

are most frequently affected by this neoplasm but this tumor affects dogs (*Canis familiaris*) and can also infect other canids, such as foxes, coyotes and wolves (Daniela Stockmann et al., 2011).

CTVT is also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or Sticker tumor which mainly affects the external genitalia and occasionally the internal genitalia, as CTVT is usually transmitted during coitus (Tella et al., 2004). In the male dog, the tumor occurs frequently on the glans penis and prepuce but may also involve the scrotum and perineum. In the female, the tumor affects the vagina and may protrude from the lips of the vulva (Amber and Henderson, 1982). The tumor may be single or multiple, nodular or pedunculated, ranging from a small nodule less than a centimeter to over ten centimeter. During the initial growth they appear small raised and

hyperemic but later become cauliflower-like and very friable as they enlarge (Deborah, 1995).

The immune system of the host plays a role in the growth pattern of the tumor with the tumor undergoing spontaneous regression in healthy dogs (Cohen, 1985). It is transplanted during coitus with intact viable cells across major histocompatibility complex (MHC) barriers within the same species (Mukaratirwa and Gruys, 2003). TVT cells contain an abnormal number of chromosomes ranging from 57 to 64 and averaging 59, in contrast to the normal 78 of the species. This kind of tumor developed only in the dog, probably because during coitus there is extensive abrasive abrasions and bleeding of the penile mucosa and vagina, making transplantations of the tumor easy (Cohen, 1973).

CTVT can be diagnosed histopathologically by appreciating round, ovular polyhedral cells with indistinct poor cytoplasmic boundaries. Characteristic multiple cytoplasmic vacuoles and large foamy nuclei are the common cytological features of such tumor cells (Tasqueti et al., 1999). Metastasis in CTVT occurs rarely but found as sequelae of primary lesions particularly in malnourished and immunosuppressed scavenging population (Yang, 1988). CTVT tumor cells can be classified as round cell neoplasms, mastocytomas, histiocytomas, plasmacytomas and lymphomas.

CTVT affected animals are usually brought by their owners to the hospital because of a mass on the external genitalia or blood stained discharge from the prepuce or vulva. In this case study, an adult intact male stray dog of indigenous non descriptive breed was brought to S. A. Quaderi Teaching Veterinary Hospital (SAQTVH) with history of tumorous growth in the penile region with blood stained discharge. The penile structure of the dog became obscure due to the abnormal swelling and the infected part produced extensive bad odor resulting public nuisance. Therefore, the local municipal authority decided to get rid of the dog by performing euthanasia with the aid of registered veterinarian. Before euthanasia gross examination of the wound was performed by experienced veterinary surgeon followed by

cytological and histopathological study to diagnose the disease.

## **MATERIALS AND METHODS**

An adult intact male, indigenous (non descriptive breed) stray dog of 30 kg body weight was brought to SAQTVH having prolonged history of cauliflower like growth and blood stained discharges from the penile region. In distant physical examination, the penile sheath of the dog was found obscure and extensively swelled. Besides, the blood stained flesh was visible with bad odor from a distance. The local municipal authority and the expert veterinarians of SAQTVH have jointly decided to perform euthanasia of the dog to cease the public nuisance by the dog and to prevent further transmission of the disease condition among other pet and community dogs around the vicinity.

### **Physical examination of the dog**

Before performing euthanasia the dog was sedated by using intravenous injection of Diazepam @ 0.5 mg/ kg body weight (Sedel<sup>®</sup> 5ml). In physical examination the rapidly growing mass throughout the penile was palpated. The dog was restrained in standing position and complete physical examination was performed by careful palpation of the penile region which revealed firm swollen region around penis and scrotum on palpation. A considerable bloody discharge gushed out from the prepuce on palpation of the penis. Rectal temperature was 38.5<sup>0</sup> C, respiratory rate was 20 breaths/min, heart rate was 102 beats/min and pulse rate was 102 beats/min. All these parameters were within the normal range.

### **Determination of approximate age and body condition**

Demographic information (sex, approximate age and body condition) was carefully recorded. The approximate age of the stray dogs was estimated by examining the teeth following the method described in The Merck's Veterinary manual (2011). Dogs having all white and shiny permanent teeth without worn off cusps on the incisors were considered as young (below one year old), while dogs having teeth showing yellowish

discoloration and tartar formation with worn cusps on the incisors were considered over one year old (adult), whereas the particular stray dog was adult. Dog's body conditions were documented according to the guideline of Laflamme (1997). From the above procedure the dog studied in this case was found adult and having good health. Biopsy and cytology of the tumor mass

The observed tumor mass were examined for cytology using fine needle aspiration biopsy following the procedures described in Cowell et al. (2008). With this technique, cell suspension was obtained from the tumor mass by using a small gauge (23G) needle coupled to a 10 ml sterile, dry, plastic syringe. The tumor mass was stabilized in one hand while the needle, with syringe attached was introduced into the center of the mass. Strong negative pressure was applied by withdrawing the plunger to collect tissues. After several areas were sampled, the negative pressure was released and the obtained tissues in the barrel of syringe and hub of the needle was expelled onto the middle of a microscopic slide and slide on slide or "Squash" smears were prepared. Then the prepared smear was stained with Giemsa stain for 20 minutes, washed, air dried and examined under light microscope.

### Performing euthanasia of the dog

After complete sedation of animal the 30ml of saturated  $MgSO_4$  was injected intravenously in radial vein for performing euthanasia. The whole euthanasia procedure was performed following the method described in The Merck's Veterinary manual (2011).

### Postmortem examination

The dog was necropsied at the pathology laboratory of Department of Pathology, and Parasitology, CVASU at the earliest possible time of euthanasia. Necropsy was conducted as per standard method described in Coles (1986). At necropsy, gross tissue changes were observed carefully and recorded. Gross pathological changes in the suspected tissue with gross lesions and also the vital organs (lung, liver, spleen, kidney, lymph nodes, brain etc.) were carefully observed and smaller tissue sections were fixed in

plastic jar containing 10% neutral buffered formalin for histopathological study.

### Histopathological study

During tissue collection the following points were taken into consideration; the tissues were collected in conditions as fresh as possible. Normal and diseased tissues were collected side by side. The thickness of the tissues were as less as possible (5mm approximately). Formalin fixed tissues were processed as per standard protocol

For histopathological study formalin fixed tissue samples were washed and dehydrated in graded ethanol and embedded in paraffin wax. Fixed tissues were sectioned at 5  $\mu$ m thickness and stained with hematoxylin and eosin as per standard method (Luna, 1968) for microscopic examination.

## RESULTS AND DISCUSSION

### Cytology of tumor mass

Giemsa stained smear from fine needle aspiration biopsy of the affected region yielded numerous round cells with moderate amount of pale finely granular cytoplasm with poorly defined outline (Figure 1A). The nuclei of the cells seem slightly eccentric and coarse with numerous mitotic figures. Each of the nuclei had distinct chromatin clumps and one or two prominent nucleoli. Sharply defined multiple vacuoles within the round cells confirm them as CTVT round cells by making clear distinction with other types of neoplasms (Figure 1B).

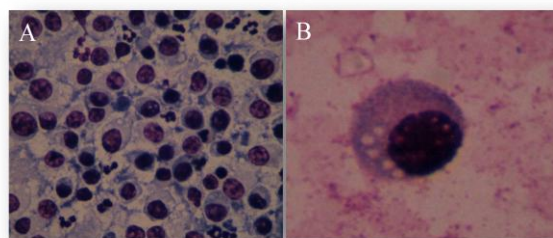


Figure 1  
Cytological features of round cells in CTVT mass (FNAB). (A) Numerous round cells with coarse eccentric nuclei and pale finely granular cytoplasm with poorly defined outline in Giemsa stained

smear (40X). (B) Multiple sharply defined vacuoles seen within the round cells cytoplasm (100X).

### Gross pathology of suspected tissue

At necropsy, multiple (12) rounds to oval, firm nodular mass, ranging from 5 cm to 9 cm diameter (Figure 2B) were noted throughout the subcutis, mainly at the preputial mucosa and cranial to the glans penis. The masses were found encapsulated and attached to the subcutaneous tissue. The infected dog exhibited a friable cauliflower-like growth (Figure 2A) with considerable blood tinged discharge in penile region. The average diameter of the growth was 5-9cm and appear protruded from the preputial mucosa (Figure 2A). Multilobular subcutaneous lesions were found. There was no gross changes observed anywhere other than penile region in the infected dog.

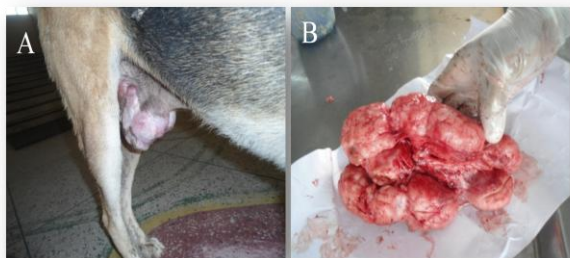


Figure 2

Gross lesions observed in CTVT of infected dog. (A) Large cauliflower-like growth, along with bloody discharge from penile region. (B) Multiple round to oval, encapsulated nodular mass observed at necropsy.

### Histopathological evaluation

Hematoxylin and Eosin (H&E) stained histopathological slides revealed confluent sheets of tumor cells arranged in grapes like or “grouped in strings” appearance (Figure 3A) in loose connective tissue stroma as an evidence of progressive tumor (Figure 3B). Large nucleus-cytoplasm ratio and abnormal mitotic figures were evident in H&E stained round cells. No extra-genital lesions were found with this infection and the histological features of regional lymphnodes,

spleen or viscera of the infected dogs did not show any evidence of metastasis.

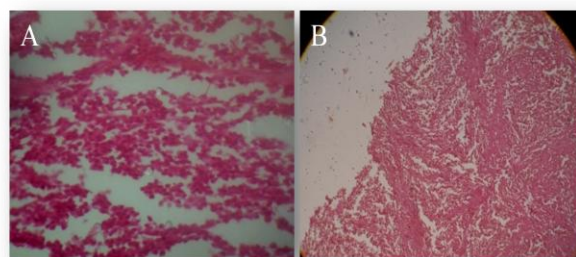


Figure 3

Histopathological features of CTVT mass. (A) Confluent sheets of round cells arranged in grapes like or strings like appearance in loose stroma; observed in the progressive stage of CTVT (H&E, 10X). (B) Rounded cells loosely attached with stroma (H&E, 4X).

In the present study the infected dogs was observed with multilobular tumorous lesions in penile region. The cauliflower like protruding tumor mass in penile region is one of the commonest gross appearances of the CTVT and these lesions have been described by several previous researchers (Amber and Henderson, 1982; Cohen, 1985; Vermooten 1987; Deborah, 1995 and Das and Das, 2000). The size of the friable tumor mass was within the range described by Park et al. (2006) and Brown et al. (1981) who reported that the tumor size can vary from 3 to 15 cm in diameter. CTVT may be solitary or multiple and are almost always located on the genitalia and the surface may often ulcerate and inflamed which bleeds easily. The tumor may also arise deep within the prepuce or vagina and be difficult to see during cursory examination. This may lead to misdiagnosis if genital bleeding is incorrectly assumed to be hematuria (Kisani and Adamu 2009) Therefore, cytological examination provides more authentic and confirmatory approaches for the diagnosis of this type of tumor.

In this study aspiration biopsy of the tumor mass exhibited numerous round cells with characteristic cytological features. Several authors demonstrated the microscopic features of these cells having round to oval shape, slightly granular basophilic cytoplasm, slight eccentric nucleus with thick



granular chromatin and 1 or 2 prominent nucleoli (Tasqueti et al., 1999; Tinucci-Costa, 1999; Erunal et al., 2000; Greatti et al., 2004 and Denicola, 2007). The presence of multiple cytoplasmic vacuoles with sharp outline indicates that those were lymphoid or mixed type of tumors. Because of their homogenous populations of large, round cells with distinctive centrally located nucleoli, CTVT are usually easily diagnosed by cytological examination of fine-needle aspirates or impression smears or by histopathologic evaluation of biopsies. (Kisani and Adamu 2009)

From the excised tumor mass histopathological examination revealed sheets of large round cells resembling lymphoblast. However, the nuclei of the cells are larger than those of lymphoid cells. The round or slightly indented nuclei stain more hyperchromatically than those of lymphoblasts and Individual neoplastic cells and their nuclei showed pronounced variation in size. Besides, numerous mitotic figures are seen in the neoplastic cells. These four histopathological findings are typical of a progressive type transmissible venereal tumor and appear in agreement with the findings of several other pathologists (Das and Das, 2000, Santos et al., 2005 and Park et al., 2006). In the progression phase of CTVT the round cells remain diffusely arranged in scanty delicate stroma (Liao et al., 2003 and Mukaratirwa and Grays, 2004). The cells associated with CTVT may be difficult to distinguish from other round cell tumors, particularly lymphosarcomas, when they occur in extragenital locations. (Thangathurai et al., 2008) According to developmental stages the tumor shows different histological features and can be classified into progression phase and initial to final regression phases. The progression phase represents numerous round cells which are arranged diffusely, interspersed by delicate connective tissue stroma and frequent mitotic structures. On the other hand, in the phase of regression tumor-infiltrating lymphocytes (TILs) appear and are widely distributed or associated with the conjunctival/Connective tissue stroma (Liao et al., 2003 and Mukaratirwa and Grays, 2004). Regression is associated with increased numbers of tumor- infiltrating lymphocytes and is characterized by increased apoptotic tumor cells and fibrosis. As suggested by the histopathological features in the present case, there is minimal

involvement of tumor infiltrating lymphocytes and high numbers of mitotic figures which designate it as progressive phase of CTVT.

Several authors reported that metastasis in CTVT is comparatively rare and occurs in less than 5-17% of cases (Richardson, 1981; Dominguez et al., 1996 and Rogers, 1997). Yang (1988) reported metastasis occurs more frequently in puppies and immunocompromised dogs. When metastasis occurs, it is usually to the regional lymph nodes, but kidney, spleen, eye, brain, pituitary, skin and subcutis, mesenteric lymph nodes, and peritoneum may also be sites. In this present study metastasis was not found in any tumor surrounded organs, tissues or lymphnodes did not show any neoplastic change.

The dog probably had acquired the infection since as it was a stray dog and the dog freely roamed around the urban area. This allows for easy contact and transmission of the disease since the dog interacts with other stray dogs some of which might already have been infected. Usually the tumor is transplanted from site to site and dog to dog by direct contact with the mass (Daniela Stockmann et al., 2011). This is even more important given the contagious nature of the disease. As CTVT spreads via coitus it is well established that adult sexually active animals of 2-5 years age become commonly infected (Higgins, 1966 and Pandey et al., 1977).

Although spontaneous regression can occur, CTVT are usually progressive and are treated accordingly. But surgery has been extensively used for the treatment of CTVT even though recurrence rate is said to be high (Amber and Henderson, 1982 and Rogers, 1997). Complete surgical excision, radiation therapy, and chemotherapy are effective treatments; however, chemotherapy is considered the treatment of choice. The prognosis for total remission with chemotherapy or radiation therapy is good, unless there is metastatic involvement of organs other than skin. Complete surgical excision often cannot be achieved because of the anatomic location of many of these tumors. Recurrence is likely in such cases unless adjunct radiation or chemotherapy is used (Merck Veterinary Manual, 2011).

## CONCLUSION

CTVT is the most prevalent neoplastic condition of the external genitalia of dogs particularly in stray dogs in tropical and sub-tropical regions. In common practice, diagnosis is based on typical gross pathological lesions and cytological features. Such tumors may become regressive after a rapidly growth phase and histopathological examinations are essential for determining the actual state. The histopathological features of the tumor lesions in present the case confirmed a progressive phase CTVT in penile region without any evidence of metastasis. However, detailed analysis of the origin of these round (tumor) cells types was not determined. Besides, the dog was euthanized without exploring the options for the treatment. Further investigation is suggested to find out the specific cell type involvement using immune histochemistry study. The establishment of treatment facility for such cases is also an emerging demand particularly for the pet and community dogs.

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