CHAPTER I

INTRODUCTION

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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*., 2002a 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 immunosupresssed 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 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 appear in a diagnosis. From all circumferential and pathological evidence the wound was diagnosed as progressive form of Canine transmissible venereal tumor (CTVT).

The objectives of the present study were as follows:

1. To identify the cause of tumor like lesion and in the venereal region in the present case.
2. To know the clinicopathological and histopathological features of the tumor lesions.

CHAPTER II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

The literature relevant to the present study has being reviewed and presented in this chapter under different headings and subheadings.

2.1. Canine Transmissible Venereal Tumor (CTVT).

The canine transmissible venereal tumor (CTVT) which is also known as Sticker´s sarcoma is a naturally occurring neoplasm of mainly sexually active mature dogs (Rogers, 1997). It is naturally transmitted to dogs by transplantation of viable tumor cells (Chu *et al*., 2001). The CTVT tumor cells are classified as round cell neoplasms, mastocytomas, histiocytomas, plasmacytomas and lymphomas (Vermooten, 1987).

2.2. Etiopathology.

CTVT is usually transmitted to genital organs during sexual intercourse but can affect the skin via the direct implantation of tumor cells during contact between skin and tumor masses (Cohen 1985, Das and Das, 2000, Murgia *et al*., 2006 and Liu *et al*., 2008). A consensus view is that, CTVT arise from allogenic cellular transplants (Richardson *et al*., 1981) and that the abnormal cells of the neoplasm are the vectors of transmission. The exfoliation and transplantation of neoplastic cells during physical contact provide the main mode of transmission onto genital mucosa, and also onto nasal or oral mucosa, during mating or licking of affected genitalia, respectively (Cohen, 1985 and Johnston, 1991). The implantation of the tumor is facilitated by the presence of any mucosal lesion or by the loss of mucosal integrity (Vermooten, 1987).

Previously it has been demonstrated that the tumor could be transplanted from one susceptible host to another by inoculating it with tumoral cells (Richardson, 1981). Cytoplasmatic inclusions found in these tumor cells caused in this neoplasia was considered to be attributed by viral agent in some reports (Cockrill *et al*., 1975), although the tumor could not consistently be transmitted by cell free extracts (Calvet, 1983). Transplantation occurs when intact host tumor cells lose the expression of major histocompatibility complex (MHC) class I and II molecules, enabling transposition of the

tissue to a healthy animal by contact between skin and damaged mucosa (MacEwen, 2001 and Murgia *et al*., 2006).

Chromosomal predilection might have attributed to the tumor containing an abnormal number of chromosomes ranging from 57 to 64 and averaging 59, in contrast to the normal 78 of the dogs. Surface antigen characteristics suggest that all CTVTs arose from a single original canine tumor (Rogers, 1997). The capacity of immunologic response of the host has a main role in the expansion of such tumors (Cohen, 1985) with an increase in severity seen in immunologically compromised animals.

CTVTs are immunogenic tumors, and it has been demonstrated that the immune system of the host plays a main role in inhibiting tumor growth and metastasis (Cohen, 1985). Immunological studies have demonstrated that the tumor is transplanted through barriers of major histocompatibility complexes (MHC) (Yang and Chandler, 1987). Presence of Immunocomplexes has been described in serum samples of dogs with TVT (Palker *et al*., 1985). Tumor cells in the rapid growth phase do not express type I and II MHC antigens, while 30% to 40% of cells in the initial regression phase.

2.3. Distribution and Epidemiology.

Although CTVT has a cosmopolitan distribution, it is most frequently encountered in tropical and subtropical zones (Rogers, 1997). It is the most prevalent neoplasia of the external genitalia of the dog in tropical and sub-tropical areas and usually transmitted during coitus (Calvet, 1983). It is estimated to be more prevalent in temperate climates (Ndirty *et al*., 1977 and Rogers, 1997). A large number of reports have been produced in India (Pandey *et al.* 1977, Chaudhary and Rao, 1982, Nayak *et al*., 1987, Padile *et al*., 1988, Chauhan *et al*., 1991, Das *et al*., 1991, Tiwari *et al*., 1991, Dinesh *et al*., 1993, Gandotra *et al*., 1993, Hoque *et al*., 1995, Maiti *et al*., 1995 and Jain *et al*., 2002a, 2002b). In India TVT is known to be the most frequently reported tumor in dogs ranging from 23-43 % of the total number of tumors in canine population (Chaudhary and Rao, 1982 and Gandotra *et al*., 1993).

CTVT occurs at same frequencies in both male and female dogs (Moulton, 1978 and Smith and Washbourn, 1998). Young dogs (*Canis familiaris*), stray dogs and sexually active dogs are most frequently affected by this neoplasm (Dominguez *et al.*, 1996, MacEwen, 2001 and Varaschin *et al*., 2001). Uncontrolled sexual behavior and a large

stray dog population appear to be one reason for such a high incidence of CTVT. An age related incidence has been shown for CTVT (Higgins, 1966 and Pandey *et al*., 1989) where the tumor was common at 2-5 years of age. Wild carnivores such as foxes, coyotes and wolves (Das and Das, 2000 and Chu *et al*., 2001) can also infected by this neoplasm.

2.4. Pathological lesions.

Grossly, 1 mm to 3 mm diameter **s**mall pink to red nodules can be observed in the genital area primarily superficial dermoepidermal or pedunculated. Multiple nodules fuse together forming larger, red, hemorrhagic, cauliflower-like, friable masses which bleed easily, ulcerate and become contaminated (Aprea *et al.*, 1994). In males, lesions usually localize cranially on the glans penis, on preputial mucosa or on the bulbus glandis. Tumoral masses often protrude from the prepuce (Higgins, 1966) resulting phimosis as a complication (McEnvoy, 1987). In bitches the tumors are of similar gross appearance as in male dogs and can be localized in the vestibule and/or caudal vagina, protruding from the vulva and frequently causing a deformation of the perineal region. In rare cases they interfere with micturition. A considerable hemorrhagic vulvar discharge may occur which can cause anemia in persistent cases. This discharge of the bitch can attract males and can be mistaken for estrus by the owners. Infrequently, TVTs can localize in the uterus (Aprea *et al*., 1994).

Extra genital lesions have been reported to occur both in isolation and in association with the genital lesions (Richardson *et al*., 1981). Extra genital lesions causing neoplastic foci might be found in one in every 500 cases (Boscos and Ververidis, 2004). Although, spontaneous remission has been described in experimental transplantation it has not been confirmed in natural cases (Richardson *et al*., 1981 and Vermooten, 1987).

Histologically, CTVTs are made up of a homogenous tissue with a compact mass of cells that are mesenchymal in origin and the borders of which cannot easily be differentiated (Richardson, 1981, Johnson, 1994 and Rogers, 1997). Bari *et al.* (1983) demonstrated the characteristics of CTVT tumor mass using histochemistry and suggested it as mesenchymal in origin. The tumor is seen abundant with round cells, arranged or grouped in strings and interspersed with delicate conjunctival stroma when stained with hematoxylin and eosin. The cells are usually arranged radially around blood and lymphatic vessels and have a high nucleus: cytoplasm ratio. The cells were with large

round nucleus and chromatin ranging from delicate to coarse and prominent nucleoli (Das and Das, 2000, Santos *et al*., 2005 and Park *et al*., 2006). These cells contain a large amount of cytoplasm that is slightly acidophilic with poorly-defined limits (Mukaratirwa and Grays, 2004).

According to developmental stages the tumor shows different histological features and can be classified into progression phage and initial to final regression phases. In the progression phase presents as round cells which are arranged diffusely, interspersed by delicate conjunctival stroma and the frequent presence of mitotic structures. In the initial phase of regression, tumor-infiltrating lymphocytes (TILs) appear and are widely distributed or associated with the conjunctival stroma (Liao *et al*., 2003 and Mukaratirwa and Grays, 2004). The final regression phase involves collapse of the neoplastic tissue and the frequent presence of apoptotic bodies (Mukaratirwa and Grays, 2004). Regressing tumors have a high number of T lymphocytes (Yang *et al.,* 1976 and Hill *et al*., 1984) and it is thought that substances secreted by the lymphocyte infiltrate are responsible for the tumor's regression by inducing cellular differentiation (Yang, 1988). Differences in cell types have also been found between stages of tumor progression (Yang and Chandler, 1987). The round cells of progressive growth are characterized by having microvilli, on the other hand regressing tumors presents transitional rather fusiform cells (Yang, 1988).

Metastasis has been reported in many cases although CTVT is usually a benign reticuloendothelial tumor (Rogers, 1997). Metastasis in TVT is rarely occurs in less than 5-17% of cases, but it is reported to be high in puppies and immunocompromised dogs (Richardson, 1981; Rogers, 1997 and Dominguez *et al*., 1996). The tumor metastasizes mostly to regional lymph nodes and less commonly to abdominal viscera, eyes, brain, tonsils, liver, spleen, oral mucosa, hypophysis, peritoneum and bone marrow (Moulton, 1978, Brown *et al*., 1981, MacEwen, 1989 and Tinucci-Costa *et al.*, 1997).

2.5. Cytopathological characteristics.

Cytological examination is a quick, efficient, inexpensive and relatively simple tool for the diagnosis of CTVT (Greatti *et al*., 2004). When subjected to Romanovisky staining, both genital and extra-genital neoplasia presents characteristic round cells with distinct cytoplasmic borders. In cytological examination the typical CTVT cells appear round to slightly polyhedral in shape, with rather eosinophilic vacuolated thin cytoplasm and a

round hyperchromatic nucleus with a nucleolus and a moderate number of mitotic figures (Tasqueti *et al.*, 1999). The nucleus-cytoplasm ratio is large. There is frequently an infiltration of lymphocytes, plasma cells and macrophages (Tinucci-Costa, 1999). The cytoplasm is slightly acidophilic and contains finely granular, delicate vacuoles, and cells do not display anisokaryosis, anisocytosis, hyperchromasia or nuclear macrokaryosis (Erunal *et al*., 2000 and Denicola, 2007).

Mitoses are frequent, may be typical or atypical, and are indicative of proliferation of tumor cells (Amaral *et al*., 2004). Apoptotic bodies are also observed by cytological exam and are present in higher quantities in CTVT in the regression phase (Santos *et al*., 2005). Inflammatory cells such as lymphocytes, plasma cells, macrophages and neutrophils are observed regardless of the stage of neoplastic development (Wellman, 1990, Boscos *et al*., 1999, Erunal *et al*., 2000, Das and Das, 2000 and Santos *et al*., 2005).

Upon cytopathological exam, it is possible to classify the CTVT tumor based on the predominant cell type as lymphoid, plasmacytoid or mixed. The lymphoid type of tumor predominantly includes cells with a rounded morphology, scant and finely granular cytoplasm, the presence of vacuoles, round nuclei and coarse chromatin material with presence of one or two evident nucleoli. In plasmacytoid tumors, most cells have an ovoid morphology, a smaller relative nucleus: cytoplasm ratio and eccentrically-located nuclei whereas the mixed type of tumor exhibits mixed cellularity (Amaral *et al*., 2004 and Greatti *et al*., 2004).

CHAPTER III

MATERIALS AND METHODS

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3.1. Case History.

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.

3.2. 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® 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 guessed out from the prepuce on palpation of the penis. Rectal temperature was 38.5^0 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.

3.3. 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 shinny 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 tarter 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). Form the above procedure the dog studied in this case was found adult and having good health.

3.4. Fine needle aspiration 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.

3.5. 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).

3.6. 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.

3.6. Histopathological study.

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 µm

thickness and stained with hematoxylin and eosin as per standard method (Luna, 1968) for microscopic examination.

3.7.1 Equipment and appliances for histopathology.

- Sample from animals(tumor mass, regional lymphnodes, liver and spleen).
- 10% neutral buffered formalin.
- Chloroform.
- Paraffin.
- Alcohol.
- Tape Water.
- Xylene.
- Hematoxylin and Eosin Stain.
- Distilled water.
- Clean Slides.
- Cover slips.
- Mounting media (DPX).
- Microscope.

3.7. 2. Collection of samples and processing.

During tissue collection the following point 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 by following protocol.

Fixation: 10% neutral buffered formalin was added in the plastic container. (10 folds of the tissue size and weight) and fixed for 3-5 days.

Washing: The tissues were trimmed into a thin section and washed over night in running tape water to remove formalin.

Dehydration: The tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per following schedule. The tissues were dehydrated in 50%, 70%, 80%, 95%, 100%, 100% ethanol one hour in each.

Cleaning: The tissues were cleaned in chloroform for 3 hours to remove ethanol (two changes; one and half hr in each).

Impregnation: Impregnation was done in melted paraffin (56- 60°c) for 3 hours.

Sectioning: Then the tissues were sectioned with a microtome at 5-µm thickness. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The sections were allowed to spread on warm water bath at 40-42°C. Then the sections were taken on grease free clear slides.

Drying: The slides containing section were air dried and kept in cool place until staining**.**

3.7.3. Routine hematoxylin and eosin staining procedure.

The sectioned tissues were stained as described below:

1. The sectioned tissues were deparaffinized in three changes of xylene (three minutes in each)
2. Then the sectioned tissues were rehydrated through descending grades of alcohol (three changes in absolute alcohol, three minutes in each; 95% alcohol for two minutes; 80% alcohol for two minutes; 70% alcohol for two minutes) followed by distilled water for five minutes.
3. The tissues were stained with Harris hematoxylin for fifteen minutes.
4. Washed in running tap water for 10-15 minutes.
5. Then the tissues were differentiated in acid alcohol by 2 to 4 dips (1 part HCL and 99 parts 70% alcohol).
6. Washed in tap water for five minutes followed by 2-4 dips in ammonia water until sections were bright blue.
7. Stained with eosin for one minute.
8. Differentiated and dehydrated in alcohol (95% alcohol: three changes, 2-4 dips each; absolute alcohol: three changes 2-3 minutes for each).
9. Cleaned in xylene: three changes (five minutes each).
10. Tissues were mounted with cover slip by using DPX.
11. The slides were dried at room temperature and examined under a low (10X) and high (40X, 100X) power objectives.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1. Cytology of the 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 (Fig. 4.1.a).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 (Fig. 4.1.b).

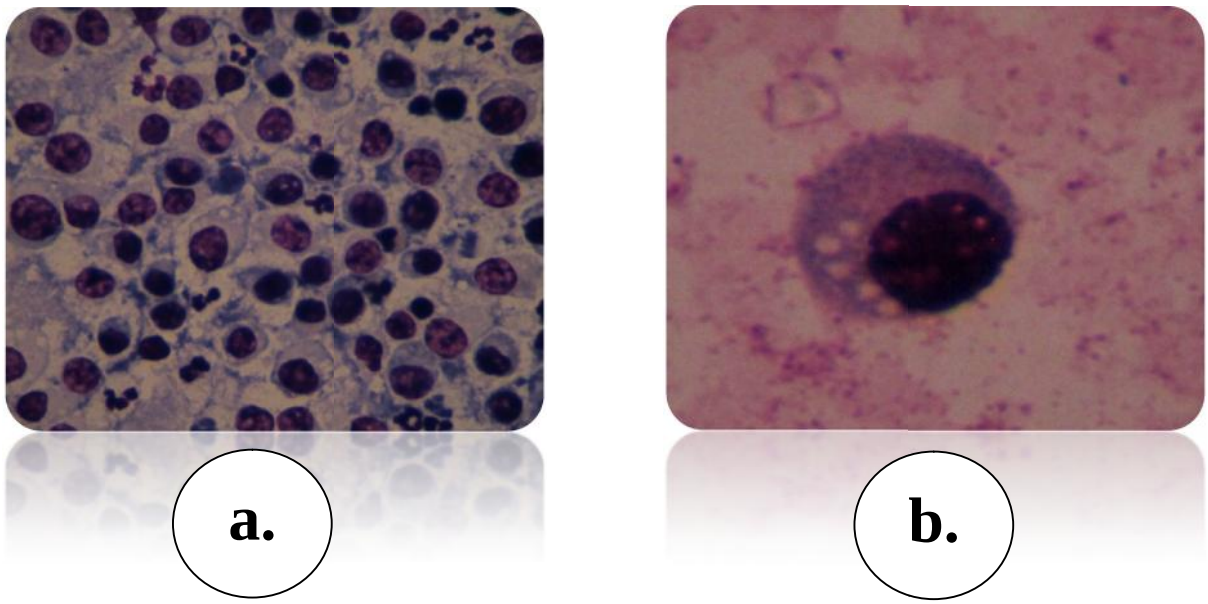

Fig. 4.1: Cytological features of round cells in CTVT mass (FNAB).

- **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).

4.2. Gross Pathology of the suspected tissue.

At necropsy, multiple (12) rounds to oval, firm nodular mass, ranging from 5 cm to 9 cm diameter (Fig. 4.2.d) 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 (Fig. 4.2.b) with considerable blood tinged discharge in penile region. The average diameter of the growth was 5-9cm and appear protruded from the preputial mucosa (Fig. 4.2.a). Multilobular subcutaneous lesions were found. There was no gross changes observed anywhere other than penile region in the infected dog.

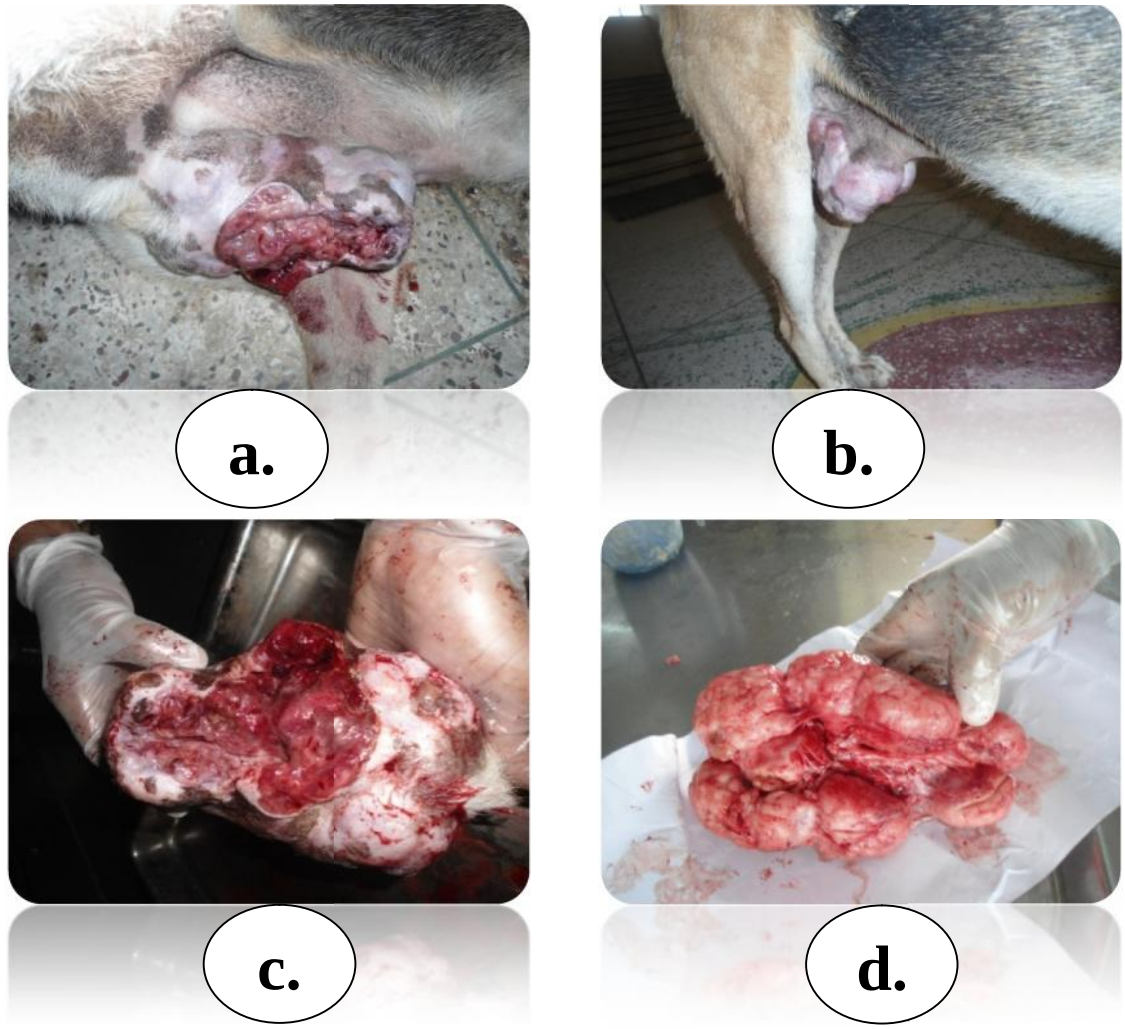

Fig: 4.2: Gross lesions observed in CTVT of infected dog.

- **a.** Large cauliflower-like growth, along with bloody discharge from penile region.
- **b.** Tumor growth induced deformation in the penile region.
- **c.** Multilobular subcutaneous mass found at necropsy.
- **d.** Multiple round to oval, encapsulated nodular mass observed at necropsy.

4.3. Histopathological Evaluation.

Hematoxylin and Eosin stained histopathological slides revealed confluent sheets of tumor cells arranged in grapes like or “grouped in strings” appearance (Fig.4.3.a) in loose connective tissue stroma as an evidence of progressive tumor (Fig.4.3.b). 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.

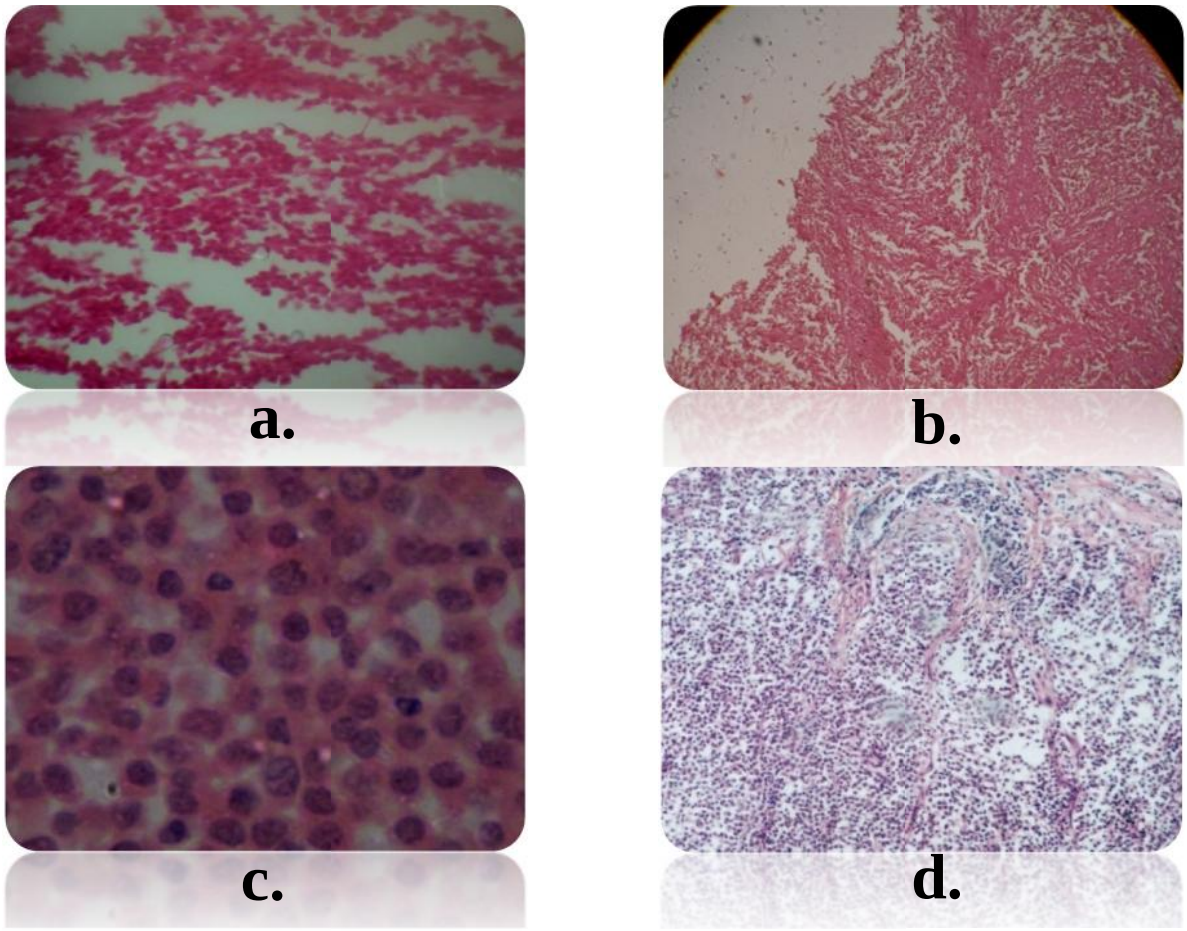

Fig. 4.1: Histopathological features of CTVT mass.

- **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).
- **c.** Large nucleus-cytoplasm ratio and increased mitotic figures observed in higher magnification (H&E, 40X).
- **d.** Scanty amount of connective tissue stroma indicate progressive stage of CTVT.

CHAPTER V

DISCISSION

CHAPTER V

DISCUSSION

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 phage 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 phage 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 phage 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, TVT are usually progressive and are treated accordingly. In the present case, as the dog was a stray and municipal authority has decided to perform euthanasia instead of any treatment approaches no such attempt was taken. But surgery has been extensively used for the treatment of TVT 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).

CHAPTER VI

CONCLUSION

CHAPTER VI

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 phage and histopathological examinations are essential for determining the actual state. The histopathological features of the tumor lesions in present the case confirmed a progressive phage 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 immunohistochemistry study. The establishment of treatment facility for such cases is also an emerging demand particularly for the pet and community dogs.

CHAPTER VII

REFERENCES

CHAPTER VII

REFERENCES

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APPENDIX

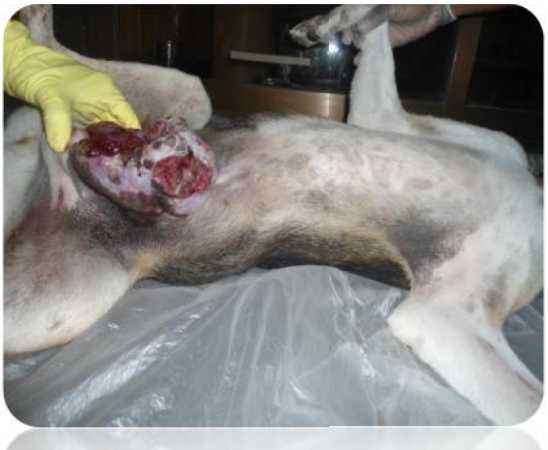


Fig: Necropsy of the infected dog.

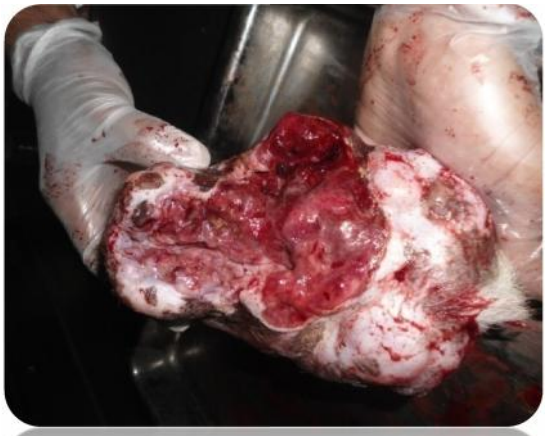


Fig: Collection of tumor mass.

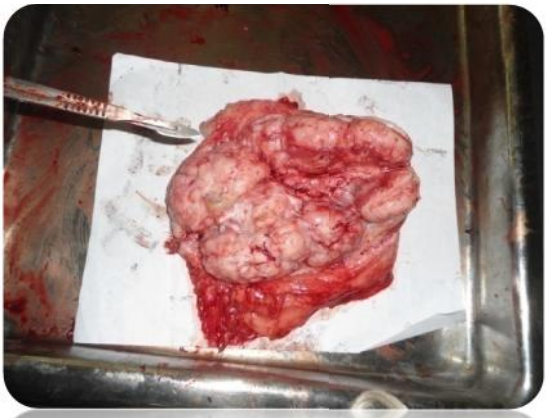


Fig: Cauliflower like subcutaneous nodule.

Fig: Blocks are made from collected sample.

Fig: Sectioning of blocks.

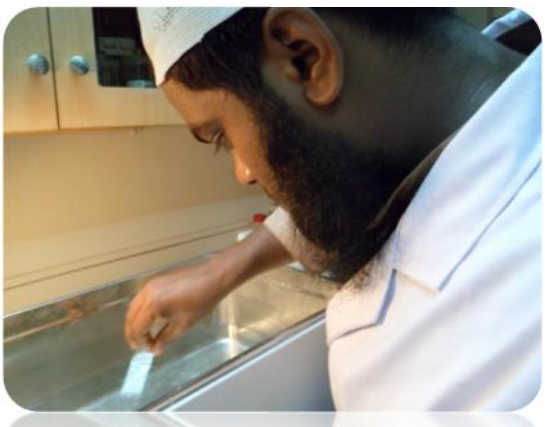
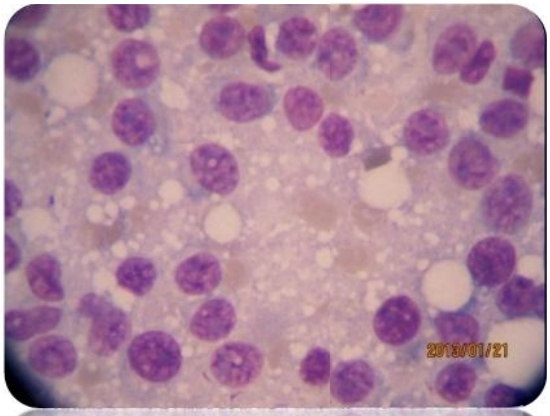


Fig: Preparation of histopathological slide.

Fig: Staining of histopathological slide.

Fig: Microscopic examination.



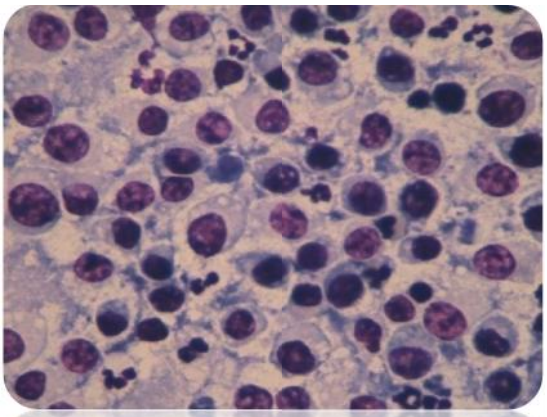
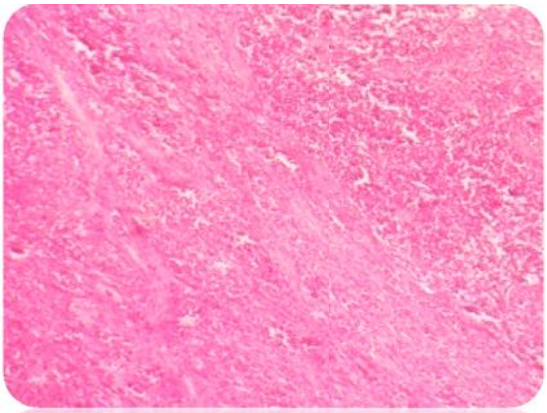


Fig: Cytology shows numerous round cells with coarse eccentric nuclei.



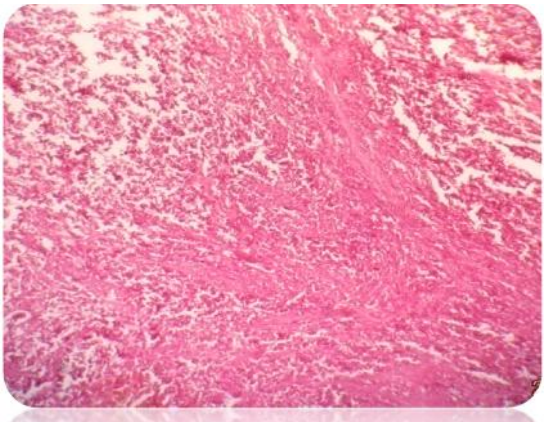


Fig: Histopathology shows Confluent sheets of round cells arranged in grapes like or strings like appearance in loose stroma.