A Review on Canine Transmissible Venereal Tumor: from Morphologic to Biochemical and Molecular Diagnosis

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Abstract: Canine transmissible venereal tumor (CTVT) is a neoplasm transmitted by the physical transfer of viable tumor cells by direct contact with injured skin and/or mucous tissue. This review paper is aimed to provide a compiled and enough information about cytogenic origin, immunology and morphological, biochemical and molecular diagnosis of CTVT. These cells can transpose across histocompatibility barriers into unrelated hosts. The progression of this tumour is unique in that, it follows a predictable growth pattern includes progressive growth phase, stable phase and regression phase and this is followed by transplantation immunity in immunocompetent adults, while metastasis occurs in puppies and immunosuppressed dogs. Dogs that have recovered from CTVT have serum transferable immunity to re-infection. The etiology appears to be cell transplant from affected to unaffected dogs. There is a remarkable aberration in the numbers and morphology of the chromosomes of the constituent cells of CTVT. Gross findings of small nodule like lesions which are hyperaemic the most consistent clinical finding. Smears made from the tumor reveal round cells with vacuoles and mitotic figures. Histologically, CTVT cells exhibit radially arranged around blood and lymphatic vessels and have a high nucleus to cytoplasm ratio with around nucleus. Most CTVT have been immunocaracterized using several tumor markers making it easy and, antibodies against CD3, surface immunoglobulins G and M, κ-light chains and λ-light chains are useful for differentiating CTVT from lymphomas and plasma cell tumor. The long interspersed element (LINE) insertion near c-myc has been found a diagnostic marker to confirm that a tumor is a CTVT. So, more study should be done on hematological and biochemical nature of CTVT, role of the tumour stroma in the progression of CTVT and also analysis of the temporal expression of extracellular matrix.

Key words: Canine Transmissible Venereal Tumor · Dog. Morphology · Biochemical · Molecular

INTRODUCTION

Cancer arises when a single cell lineage acquires somatic mutations that promote it toward a program of continued proliferation. Natural selection favors the most prolific sub clones, often steering the cancer toward a more aggressive phenotype. By its nature, cancer is counters elective and often lethal to its host and thus cancer is usually an ultimately short-lived and self-destructive entity [1].

The canine transmissible venereal tumor (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 [2].

Also known as Sticker’s sarcoma, this tumor was first reported in 1820 by Hüzzard and was then later reported by Delabere-Blaine (1928). However, it was best characterized by Sticker between the years of 1905 – 1906, leading to its designation as Sticker Tumor for many years. Sticker described this neoplasia in detail and found that it was a transmissible neoplasia predominantly localized to the genital region [3, 4].

This tumour is unique in oncology because it was the first tumour to be transmitted experimentally, this being achieved by the Russian veterinarian Nowinsky in 1876. This stimulated a lively interest among scientists and became a new starting point for the study of oncology [5]. Due to the unique nature of transmission by sexual
contact, naturally occurring CTVT generally develops in the external genitalia [6]. Less commonly, the tumor may also be transmitted to the nasal or oral cavities, skin and conjunctive and the rectum by sniffing or licking [7, 8]. With genital CTVT, probably as a consequence of social behaviors [6]. More rarely, they may be found in other areas, including the lips, oral mucosa and peritoneum, or in organs such as the tonsils, eye, liver, spleen, kidney, lung and musculature [9].

Dogs of any breed, age or sex are susceptible [5]. Although dogs over one year of age are at high risk in endemic areas, most common in dogs 2 to 5 years old [5, 1].

The tumour is never found in virginal females and females found to be more susceptible than males. Naturally occurring disease may be more common in females because one infected male often mates with numerous females; single CTVT affected male dog spread the disease to 11 of 12 females [5, 10, 11].

Metastasis of CTVT is uncommon, only occurring in puppies and immunocompromised dogs. Most of the reported metastasis cases actually are mechanical extensions of growth or transplantation [12].

Young dogs, stray dogs and sexually active dogs are most frequently affected by this neoplasm [5, 13]. It has a worldwide distribution and the incidence in highest in tropical and subtropical regions. This tumor affects dogs (Canis familiaris) and can also infect other canids, such as foxes, coyotes and wolves [13-15].

Limited reviews on diagnosis of CTVT have been published. The aim of this paper is to review the current knowledge of the cytogenetic feature and origin, immunology of CTVT, morphological characteristic of CTVT and confer its biochemical and molecular diagnosis and to discuss how these tumour characteristics are involved in the unique behavior of this tumour.

Etiopathogenesis of CTVT: 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 [5, 15, 16].

CTVT transmission may be enhanced both by the extended period of canine sexual intercourse, which involves the mates being ‘tied’ due to the expansion of the penis within the female genital tract and by the injuries to the genital mucosa that are frequently incurred as mates attempt to separate [17].

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 [4,16].

Study done on examining the transplantation of CTVT cells into mice, revealed that this tumor could only be transferred between healthy animals that shared the same MHC or into immunocompromised recipients, as the CTVT cells induce an immune response in healthy recipients [4]. These studies were guided by the transplantation theory and etiology of CTVT. The transplantation theory is based on the observation that experimental tumor transplantation can only occur using living tumor cells [9]. Other studies have established that CTVT cells can be derived following mutations induced by viruses, chemicals or radiation of lymphohistiocytic cells and that these clones of tumor cells can then be disseminated by allogegeneic transplantation [4].

Studies using immunohistochemical techniques demonstrated that this neoplasm was positive for lysozyme and alpha-1-trypsin and suggested that CTVT is of mesenchymal and histiocytic origin [9,18, 19].

The clonal transmission of this tumor is confirmed when studies revealed that the pattern of microsatellite polymorphisms in CTVT from different regions of the world showed evidence of monophyletic origin. Mitochondrial and MHC differences suggest that many modern CTVT clones belong to two groups distributed around the world [4, 10].

Experimentally transferred CTVT tumors have three distinct phases of growth, described as progressive, stable and regressive [20, 21]. Tumors generally become palpable 10 to 20days following experimental transfer. The initial progressive phase, which generally lasts for a few weeks, is characterized by a rapid increase in tumor volume with a doubling time of between 4 and 7 days and an estimated loss of 50% of cells [20]. During the subsequent stable phase, there is markedly slower tumor growth with a doubling time of approximately 20 days and an estimated cell loss of 80 to 90% [22]. Following the stable phase, which can last from weeks to months to indefinitely, up to 80% of CTVT tumors enter a regressive phase during which the tumor shrinks and eventually disappears [20, 21].

The regressive phase generally lasts between 2 and 12 weeks, during which time tumors as large as 100 cm can disappears completely. Alternatively, rather than entering the regressive phase, between 1 and 20% of transplanted tumors enter a second phase of rapid growth which progresses to metastasis [1, 20]. Spontaneous regression of the tumor can occur, probably due to a response from the immune system [21].
The life history of naturally occurring CTVT is less well understood. Although an initial progressive phase and subsequent stable phase may be observed, spontaneous regression has not been well documented in naturally occurring CTVT [6, 22].

**Immunology of CTVT:** Immune response against the tumor plays a major role in determining the course of the disease, with disease manifestation representing the outcome of tumor immune evasion strategies balanced against host immune responses [6, 17].

In immunocompromised animals experimentally infected with viable CTVT cells, disease progression and metastasis was observed; however, those dogs who quickly recovered acquired immunity against subsequent implantations [22]. Conversely, dogs that have recovered from CTVT have serum transferable immunity to re-infection and puppies born to mothers that have been exposed to CTVT are less susceptible to the disease [17].

Cyto genetic studies on spontaneous and experimentally transplanted CTVT have been done by several researchers in different geographical areas of the world and these studies have demonstrated that tumours from different geographical regions (France, Nigeria, Uganda and USA) have a similar karyotype in terms of chromosome number (59 chromosomes), the frequency of metacentric chromosomes and the incidence of marker chromosomes and these are clearly distinguishable from that of the normal canine cell [6, 23-25]. All dog chromosomes except X and Y are acrocentric, having an acrocentromere very near to the end of the chromosome, while many of the CTVT chromosomes are metacentric or submetacentric, having a centromere nearer to the middle [26].

The demonstration that the karyotypes of CTVTs from different geographical regions are similar suggests that CTVT is transferred from one animal to another by transplantation of viable cells [4, 10].

According to Rebbeck et al. [10] the genetic ancestor of CTVT was probably arose from a dog or wolf rather than from a distant member of the canid family. Furthermore, Murgia et al. [4] used microsatellite polymorphisms to compare CTVT with normal tissues of 85 breeds of dogs and eight species of wolves and found that CTVT showed strong identity with wolves. MHC variants found in the tumor cells also showed a significant phylogenetic relationship with wolves.

The research using microsatellites to determine the timing of the origin of CTVT indicated that CTVT probably arose from a single wolf approximately 7,800 to 78,000 years ago. More recently, a single clone became dominant and then divided into two groups with a worldwide distribution. This study this evidence indicates that CTVT is the oldest transplantable somatic cell clone known [10, 17].

**Cytogenetic Features and Origin of CTVT:** The normal diploid number of chromosomes in the somatic cell of the dog (Canis familiaris) is 78 and 76 of these, the autosomal chromosomes are acrocentric, while two, the sex chromosomes are metacentric [4, 6]. There is a remarkable aberration in the numbers and morphology of the chromosomes of the constituent cells of CTVT [5].

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The frequent spontaneous regression of both natural and experimentally transplanted CTVT associated with the infiltration of lymphocytes and plasma cells and with necrosis and apoptosis [15]. The transition from progressive to regressive phases of CTVT growth is accompanied by a marked increase in immune cell infiltration [6, 15, 22, 27].

The major histocompatibility complex (MHC) class I and II molecules are either not expressed or are present on only a small subset of neoplastic cells during the progressive phase [4, 17, 27]. Interestingly, a significantly greater proportion of CTVT cells express MHC class I and II in the regression phase [17, 15]. In addition, Hsiao et al. [27] showed CTVT cells can be induced to express MHC by exposure to supernatant of cultured regressive phase CTVT cells and tumor infiltrating lymphocytes, but not by progressive phase CTVT cells and tumor infiltrating lymphocytes.

Peripheral blood lymphocytes (PBL) of dogs in which CTVT had regressed, have been shown to be cytotoxic to the tumor cells in contrast to PBL of normal dogs and animals during progressive tumor growth [6]. Liao et al. [21] showed that the proportion of B lymphocytes in the peripheral blood decreased dramatically with CTVT growth. The destruction of B lymphocytes was caused by substances released by the tumor cells, such as cytotoxic proteins and other circulating substances. These cytotoxic substances cause B lymphocyte apoptosis during the neoplastic progression phase.

Tumors are frequently infiltrated by T-lymphocytes and natural killer (NK) cells. Increased numbers of tumor infiltrating lymphocytes (TILs), particularly T-lymphocytes, are associated with tumor regression in CTVT [6]. The T-cell cytotoxicity is believed to be associated with apoptosis and apoptosis is increased in regressing CTVTs [15].
Fig. 1: Model for canine transmissible venereal tumor immune evasion. CTVT has distinct phases of growth, progressive and regressive. During the progressive phase, the tumor cells do not express MHC class I or class II and the tumor secretes transforming growth factor-β (TGF-β), a cytokine that inhibits tumor-infiltrating lymphocyte (including natural killer cell) cytotoxicity. Tumor cells may also inhibit some types of antigen-presenting cells. Tumor-infiltrating lymphocytes are present in low numbers. During the regressive phase, tumor-infiltrating lymphocytes increase in number and their secretion of IFN-γ and interleukin-6 (IL6) counteracts the repressive effects of tumor-derived TGF-β and induces MHC class I and class II expression in tumor cells. MHC expression reveals CTVT as an allograft and it is rejected by both antibody-dependent and -independent cytotoxic processes. Source: Murchison [17].

Hsiao et al. [28], found that CTVT cells produce transforming growth factor β1 (TGF-β1) and showed that this cytokine inhibited NK cell activity, as well as tumor infiltrating lymphocyte cytotoxicity. The suppressive effects of TGF-β1 on NK cell killing activity could be counteracted by the pro-inflammatory cytokine interleukin-6 (IL6), which is secreted by tumor infiltrating lymphocytes [17, 28].

The host derived IFN-γ acted synergistically with IL6 to induce MHC expression in CTVT cells. In addition, IL6 induced MHC expression in CTVT in vitro [29] and could induce MHC expression in combination with IL15 in vivo [30].

There is convincing evidence that humoral immunity plays a role in CTVT progression. Antibodies recognizing CTVT antigens can be detected in the sera of CTVT affected animals [6]. Although serum antibody levels did not correlate strongly with tumor volume, they were undetected in the serum of puppies with metastatic CTVT and few cells in CTVT metastases could be labeled with anti-CTVT antibodies [15, 17].

CTVT is antigenic in dogs and provokes both cell-mediated and humoral immune responses. The tumor is able to escape immune rejection by down regulating MHC, suppressing NK cells, killing B cells and preventing the maturation of dendritic cells [17, 28, 31]. However, CTVT may often eventually succumb to host defense system and its final regression is accompanied by subsequent immunity. Although the triumph of the immune system over CTVT may reveal an inherent weakness in the tumor’s defense strategy, it has also been suggested that natural regression may be an adaptation to maintain the viability of the host population [4].

Clinico-pathological Characteristics: In the male dog, the tumour is usually located on the caudal part of the penis, from the crura to bulbis glandis or the area of the glans penis and occasionally on the prepuce [5, 32]. In the bitch, the neoplasmis usually found in the posterior part of the vagina, often at the junction of the vestibule and the vagina. It sometimes surrounds the urethral orifices and, if it is just within the vagina, it may protrude from the vulva [15, 32].
The vast majority of cases are sexually transferred, CTVT on the external genitalia of both sexes appear initially as small hyperaemic papules that later progress to nodular, papillary multilobulated, cauliflower-like or pedunculated proliferations. The tumor size can vary from 3 to 12 cm in diameter. The mass is firm but friable and the superficial part is commonly ulcerated and inflamed [5, 9, 32, 33].

During rapid tumour growth, the colour is bright red owing to extensive vascularization. The tumour often oozes a serosanguinous, simple haemorrhagic fluid or preputial discharge and eventually becomes ulcerated, with a necrotic appearance [5]. The peculiar odour of the neoplastic lesions discharge, which after secondary bacterial infection became particularly unpleasant and the excessive licking of the genitalia [34]. The continuous discharge from the external genitalia, soiling the floor, carpet and even clothes, is a great nuisance for the owner. The bloody discharges may be confused with oestrus, urethritis or cystitis and in the male, with prostatitis. In older dogs, the differential diagnosis Must also include urinary bladder and urethral neoplasms [13, 35]. When cases become complicated it may cause Phimosis or paraphimosis in the male and few cases are record where this neoplasm has caused actual Mechanical obstruction to the flow of urine, or has produced dystocia in whelping females [5, 34].

CTVT may also develop in extragenital sites such as skin, subcutaneous tissues and around and in the oral and nasal cavities. Extragenital tumors are well circumscribed and can measure 2-5 cm [5, 15]. Metastases are rare in CTVT, yet they can occur, especially in puppies and immunocompromised dogs. These metastases are often considered mechanical extensions of the primary tumor; however, metastases have been reported in inguinal lymph nodes [5, 9, 15]. Brain, liver and eye [12].

Many of the reported cases of metastasis are actually mechanical extension of the growth or either auto- or hetero-transplantation to the skin, cervix, uterus and fallopian tubes from the tumor on the external genitalia [36].

**Histopathology Characteristics:** On histopathologic examination, CTVT cells exhibit a round to polyhedral shape, arranged or grouped in strings, interspersed with delicate conjunctival stroma when stained with hematoxylin and eosin. The tumor cells are usually arranged radially around blood and lymphatic vessels and...
have a high nucleus to cytoplasm ratio with a round nucleus and chromatin ranging from delicate to coarse and prominent nucleoli and cytoplasmic vacuoles [9, 15, 38]. These cells contain a large amount of cytoplasm that is slightly acidophilic with poorly-defined limits [6, 17]. There is also frequently an infiltration of lymphocytes, plasma cells and macrophages which suggest a role of immune mediated control [39].

**CTVT Undergoes a Predictable Cycle:** the initial growth phase of four to six months (P phase), a stable phase and a regression phase (R phase), although not all CTVTs will regress [21]. The progression phase presents as round cells 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 [6, 21]. The final regression phase involves collapse of the neoplastic tissue and the frequent presence of apoptotic bodies [6, 15]. CTVTs should be differentiated from mastocytomas, histiocytomas or malignant lymphomas [5].

Further, CTVT displays histological resemblance to canine cutaneous histiocytomas and other round cell tumours, thereby presenting great difficulties for pathologists in their differentiation [40]. So, definitive diagnosis could be based on physical examination and cytological findings typical of TVT in exfoliated cells obtained by swabs, fine needle aspiration or imprints of the tumours [41]. This tumour could easily be distinguished from other round cell tumours by a simple algorithm.

**Cytopathological Characteristics:** Cytology must be the method of choice for diagnosis of suspected CTVT, since the technique is simple, cheap, minimally invasive and painless and, furthermore, produces much less distortion of cell morphology than biopsy samples fixed in formalin [8, 42]. When subjected to Romanovisky staining, both genital and extra-genital neoplasias present characteristic round cells with distinct cytoplasmic borders. The nuclei are oval or round and centrally-located, with delicate chromatin and large nucleoli; the cytoplasm is slightly acidophilic and contains finely granular, delicate vacuoles and cells do not display anisokaryosis, anisocytosis, hyperchromasia or nuclear macrokaryosis [43].
Fig. 4: An algorithm used in evaluating and differentiating TVTs from other round cell tumours (a) Round cells with eccentric nuclei + Cytoplasmic granulation; b. Round cells with centrally placed nuclei + Cytoplasmic vacuolation; (c) Round cells without cytoplasmic granulation or vacuolation + Bean shaped nucleus; (d) Round cells without cytoplasmic granulation or vacuolation + Multinucleated giant cells. Source: Thangathurai et al. [41].

Cytological samples of CTVT are generally multicellular and contain round or oval cells that vary between 14 and 30 µ in diameter, with well-delimited cytoplasmic borders. The nucleus, round or oval, is frequently eccentric, of variable size, with rough and granular chromatin and with one or two prominent nucleoli and the nucleus to cytoplasm ratio is relatively high [8].

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 and round nuclei with coarse chromatin and the presence of one or two evident nucleoli [44]. 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 [45].

Many times the cellular aspect can vary between the primary tumor and the metastasis or can be atypical in cases of old and are indicative of proliferation of tumor cells [15, 45]. Apoptotic bodies of are also observed by cytological exam and are present in higher quantities in CTVT in the regression phase [38]. Inflammatory cells such as lymphocytes, plasma cells, macrophages and neutrophils are observed regardless of the stage of neoplastic development [15].

**Diagnosis of CTVT:** Diagnosis is based on the environmental history, clinical and cytological findings. Biopsy for histological examination is the most reliable method for diagnosis. Many of the diagnostic difficulties previously faced by pathologists have, however, been eased by the introduction of novel techniques such as immunohistochemistry and/or immunocytochemistry and molecular biology [5, 46].

**Hemato-biochemical Diagnosis of CTVT:** Studies using Hemato-biochemical observations in the mongrel dog demonstrated that this neoplasm shows markedly elevated with neutrophilia, lymphopenia and thrombocytopenia. Serum chemistry was indicative of hypoproteinemia, hypoalbuminemia, hypoglobulinemia with higher levels of blood urea nitrogen and creatinine. Increased activities of alanine aminotransferase and alkaline phosphatase were also observed probably due to metastasis to these organs affecting the organ function [36].
Fig. 5: Cytological samples of transmissible venereal tumor of different cytomorphological types: (A) lymphocytic pattern (Predominance of round cells, scarce cytoplasm and high nucleus:cytoplasm ratio); (B) plasmacytic pattern (Predominance of ovoid cells, ample cytoplasm and eccentric nucleus); (C) mixed pattern (Presence of both morphological types without predominance of either. Giemsa, bar = 20µm, Source: Amaral et al. [8].

Free radicals are highly reactive molecules produced during normal metabolism in the body, or after exposure to environmental factors, which are kept in equilibrium by the body through endogenous antioxidant defense mechanisms (Comprising of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, glutathione reductase and glutathione (GSH) etc. If this balance is disturbed, it may result in oxidative stress, leading to enhanced lipid peroxidation, DNA strand breaks and protein damage [47]. The role of oxidative stress in carcinogenesis has been delineated in dogs with lymphoma [48]. CTVT in bitches [49]. And canine mammary tumors [50].

Hemato-biochemical study by Behera et al. [36] revealed a marked increase in the level of lipid peroxidation (LPO, 3.84 µmol MDA/mg Hb; control: 0.94), a decreased level of reduced glutathione (GSH, 0.76 µmol/mg Hb; control: 1.14), reduced activities of superoxide dismutase (SOD, 0.73 Units/mg Hb; control: 1.29) and catalase (CAT, 74.37 Units/mg Hb; control: 132.25). These alterations in oxidant-antioxidant status might be due to direct injury by tumor cells or inflammation and/or necrosis which needs further validation in similar types of cases.

Immunohistochemical Diagnosis of CTVT:
Most canine round cell tumours have been immunocharacterized using several tumour markers making it easy for accurate diagnosis and classification [37]. Although CTVT has been subjected to immunohistochemical studies with several tumour markers, its origin and the immunophenotype still remain uncertain. However, immunohistochemical studies with a panel of several antibodies have managed to rule out some of the early suggestions [6].

CTVT cells are negative for keratins, α-smooth muscle actin, desmin, CD3, immunoglobulins G and M, λ-light chains and κ-light chains and this means that an epithelial, smooth muscle and T and B lymphocyte origin can be ruled out [4, 51].

Antibodies against CD3, surface immunoglobulins G and M, λ-light chains and κ-light chains are useful for differentiating CTVT from lymphomas and plasma cell tumours [44]. Lysozyme and alpha-antitrypsin (AAT) have been considered good markers for benign and malignant histiocytes and these antigens are not expressed by other mesenchymal cells [5, 15].
The phenotypic origin of CTVT is not yet clear, but the expression of ACM1, lysozyme and AAT suggest a histiocytic origin because these antigens are not expressed by other mesenchymal round cells, except those of histiocytic origin [6, 37].

**Molecular Diagnosis of CTVT:** Molecular biology can also be useful in the diagnosis of canine TVT. The \textit{c-myc} oncogene is rearranged in this tumour by insertion of a transposable sequence, known as the long interspersed element (LINE), 5' to the first exon [21]. This genomic rearrangement has been identified in a large set of globally distributed CTVT tumors, but not in any other canine tissue and is now considered diagnostic evidence for CTVT [4, 9, 10, 15]. It is possible that this rearrangement was present in the germ line of the CTVT founder, that it occurred somatically during the development of the founding CTVT tumor or that it occurred somatically in a CTVT clone that has subsequently achieved global distribution [17].

Several treatments including surgery, radiotherapy and chemotherapy have been used to treat CTVTs. Surgery has been used extensively to treat small, localised TVTs, although the recurrence rate can be as high as 50–68% in cases of large invasive tumours. Chemotherapy using antimitotic agents such as vincristine, cyclophosphamide, methotrexate, vinblastine and doxorubicin is the most effective TVT treatment [1,5].

**CONCLUSION**

CTVT is the most prevalent neoplasia of the external genitalia of the dog in tropical and sub-tropical areas. The most frequent owner’s complaint is the hemorrhagic discharge. Diagnosis is based on typical physical, cytological, histochemical and molecular findings.

Therefore, based on these facts, the following recommendations are forwarded; more study should be done on hematological and biochemical nature of CTVT, role of the tumour stroma (Fibroblasts, vessels and extracellular matrix components) in the progression of CTVT and also analysis of the temporal expression of extracellular matrix components like tenascin, hyaluronan and versican that play an important role in tumour progression in CTVT. And dog licensing laws, controlling the pool of potentially infected, owner less dogs roaming wild, will control the incidence of the disease.

**REFERENCES**


