Review Article

An appraisal of Canine Transmissible Venereal Tumour with emphasis on molecular biology and pathology

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Abstract

Canine transmissible venereal tumour (CTVT) is a contagious tumour of dogs, transmitted via coitus or coital behaviour which in some cases spreads by metastasis but primarily, CTVT appears as reddish soft nodules or papilla, protruding from the surface of the penis, prepuce, vagina and vulva but sometimes appears in locations outside the genitals. There was early evidence of CTVT more than 10,000 years ago and this disease has been reported in at least 90 countries across the continents of the world, especially in third world countries, where there are high numbers of stray dogs. CTVT natural infection occurs only in dogs but this disease can be experimentally inoculated into other species of the family, *Canidae*. Macroscopic lesions are mainly cauliflower-like, papillary or multilobulated which most times immunologically regress but occasionally may progress to malignancy. Due to the uniqueness of this tumour in its transmission, broad geographical, sex and breed distribution and because CTVT is the world's oldest known neoplasm, this disease has attracted great global research interest. Diagnostic techniques including clinical examination, histopathology, cytology, immunohistochemistry, cytogenetic, computer tomographic imaging and molecular diagnostic methods such as Polymerase chain reaction (PCR) have been invaluable in the diagnosis of CTVT. Therefore, this review casts a searchlight on the aetiology, structure, epidemiology, disease status, transmission, pathogenesis, molecular biology, macroscopic and microscopic pathology, immunology, diagnosis, prevention, treatment and control of this unique tumour.

Keywords: Canine, Diagnosis, Immunopathology, Molecular biology, Transmissible, Tumour

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Introduction

Canine transmissible venereal tumour (CTVT) is a contagious venereal tumour with round cells of mesenchymal origin (Ganguly *et al.,* 2016; Ostrander *et al.,* 2016; Hiblu *et al.,* 2019). It is commonly observed in dogs that are in close contact with one another or in stray and wild dogs that exhibit untrammelled sexual activity (Chiti *et al.,* 1996; Ganguly *et al.,* 2016). It a solid tumour found in dog populations across the globe and is the oldest and most prolific cancer lineage known in nature (Murchison *et al.,* 2014; Strakova and Murchison, 2014; Strakova and Murchison, 2015; Metzger and Goff, 2016). Canine transmissible venereal tumour (CTVT), also called transmissible venereal sarcoma, Sticker's sarcoma, venereal granuloma, transplantable lymphosarcoma and infective sarcoma, was first described by Hujard in 1820 in Europe (Boscos and Ververidis, 2004). The year 1876 was the first-time history of oncology when the Russian veterinarian, Novinsky, demonstrated the transplantation of the tumour from one dog to another by infecting them with tumour cells (Ganguly *et al.,* 2016).

CTVT appear in papillary or nodular form, showing a pinkish to reddish outlook, sticking out from the surface of the penis, vagina or vulva. The masses are soft and have a great tendency to bleed (Ajayi *et al.,* 2018). CTVT is one of the the world's primeval known cancers whose metastatic spread through its global host population provides unique insights into evolutionary processes operating in cancer (Strakova *et al.,* 2016).

Aetiology

CTVT represents a unique but unusual, naturally transmissible, contagious tumour, where the mutated tumour cell itself is the causative agent and perpetuates as a parasitic allograft in the host (Ganguly *et al.,* 2016). Therefore, the causative agent causing canine transmissible venereal tumour (CTVT) is not a bacterium, virus or fungal agent but is thought to be the tumour cell itself (Murgia *et al.,* 2006; Murchison *et al.,* 2014).

Tumour cellular structure: The tumour's nuclear chromosomal outlook is aneuploid but has characteristic marker chromosomes (Murray *et al.,* 1969; Oshimura *et al.,* 1973; Murgia, 2006; Murgia *et al.,* 2006). The majority of the CTVT chromosomes are metacetric or submetacentric, instead of the acrocentric nature found in other dogs and CTVT chromosomes have a centromere nearer to the middle (Hasler and Weber, 2000). It also has a long interspersed nuclear element (LINE-1) insertion near c-*myc* (Katzir *et al.,* 1985; Murgia *et al.,* 2006), and this feature can be used as a diagnostic marker to confirm CTVT (Liao *et al.,* 2003; Park *et al.,* 2006). Zayas *et al*. 2019, reported that LINE-c-myc insertion in the isolated CTVT cell line at 550 bp was not detected. However, a 340-bp band was amplified. The Dog Leucocyte Antigen (DLA) genotyping indicated that the class II genes found in CTVT are either homozygous or hemizygous, except for the *DRB1* gene, which possesses two alleles that differ by one non - synonymous substitution distant from the peptide binding groove (Murgia *et al.,* 2006). It is important to highlight that, in spite of 13 decades of research on CTVT, several characteristics of this transmissible cancer remain poorly understood (De La Sota *et al.,* 2000; Antonov, 2015) and no vaccine is available yet. The literature regarding the *in vitro* culture of CTVT cells is limited (Zayas *et al.,* 2019).

Epidemiology: CTVT is most common in dogs aged 2 to 5 years and there is no breed or sex predisposition constituting a compulsory financial burden on dog owners (Das and Das, 2000; Park *et al.,* 2006; Zayas *et al.,* 2019). CTVT first arose several thousand years ago and has been reported in feral dog populations in all the continents of the world except Antarctica (Das and Das, 2000; Boscos and Ververidis, 2004; Strakova and Murchison, 2014, Metzger and Goff, 2016). It is common in tropical and subtropical regions of the world, where there is a high population of stray and malnourished dogs (Kabuusu *et al.,* 2010; Milo and Snead, 2014; Ganguly *et al.,* 2016), particularly in the southern parts of the United States, Central and South America, South-East Europe, Ireland, China, the Far East, Middle-East and parts of Africa (Ganguly *et al.,* 2016), but is rarely reported in North and Central Europe and North America, simply because of the effective control of stray dog populations, thorough pre-breeding examinations and the effective treatment of clinical cases (Ganguly *et al.,* 2016). However, there is valid proof that CTVT was present prior to 1910 in the United Kingdom (White, 1902), Germany (Sticker, 1902), the United States (Beebe and Ewing, 1906) and France (Borrel, 1907).

CTVT however, remains enzootic in the rest of the world, with reports in the following countries: Grenada, Puerto Rico, Jamaica, Brazil., Papua New Guinea and south-west France (Thorburn *et al.,* 1968; Chikweto *et al.,* 2013; Ganguly *et al.,* 2016; Castro *et al.,* 2017). In the Bahamas, Pakistan, Japan and India, it is the most common tumour of dogs (Tateyama *et al.,* 1986; Singh *et al.,* 1991; Ganguly *et al.,* 2016; Awan *et al.,* 2017). It has also been reported in Africa, in Uganda (Wright *et al.,* 1970), Tanzania (Batamuzi *et al.,* 1992), Zambia (Chiti *et al.,* 1996; Nalubamba, 2015), Nigeria (Tella *et al.,* 2004; Ajayi *et al.,* 2009; Ajayi *et al.,* 2018) and Kenya (Abuom and Mande, 2006).

Disease status: Strakova and Murchinson (2014), reported evidence of CTVT on all six continents where humans live and it has been in existence from 1810 till today. This study revealed that CTVT is endemic in at least 90 countries worldwide, therefore CTVT has worldwide distribution, with a higher incidence in tropical areas and has been mostly reported in dogs (*Canis familiaris*) and foxes (Carreria *et al.,* 2014). Prevalence shows that the presence of free-roaming dogs in a poorly controlled area as seen in most tropical and subtropical countries is associated with increased CTVT prevalence, while dog spaying and neutering is associated with reduced CTVT prevalence as practised in Temperate European countries (Das and Das, 2000; Strakova and Murchinson, 2014). A prevalence of CTVT is estimated to occur at between 1 and 10% in dogs in at least 13 countries in South and Central America, at least 11 countries in Africa and 8 countries

in Asia. With recent reports in African countries like Zambia (Nalubamba, 2015), Nigeria (Ajayi *et al.,* 2018), Libya (Hiblu *et al.,* 2019) and in the following Asian countries; India (Shiju Simon *et al.,* 2016), Thailand (Setthawongsin *et al.,* 2016) and Pakistan (Awan *et al.,* 2017). In the United States and Australia, CTVT has been reported to be endemic only in remote indigenous communities. Comparison of current and historical reports of CTVT indicate that its prevalence has declined in Northern Europe, possibly due to changes in dog control laws over the last two centuries. Evaluation of risk factors influencing CTVT prevalence show that the presence of free-roaming dogs is associated with increased CTVT prevalence, while dog spaying and neutering are associated with reduced CTVT prevalence (Strakova and Murchinson, 2014).

The highest estimated CTVT prevalence that has been recorded was in Belize, where the average CTVT prevalence was estimated to be between 10 and 20%. Countries like Canada, the Czech Republic, Finland, the Netherlands, New Zealand, Sweden, Switzerland and the United Kingdom are consistently reported to be free of endemic CTVT; in above listed countries, the only CTVT cases were specifically reported to be imported from abroad (Strakova and Murchinson, 2014). CTVT was reported as absent from many regions of the United States and Australia but was present in remote indigenous communities, including Indian reservations in Arizona and North Dakota, as well as in Australian Aboriginal communities in the Northern Territory and Western Australia. There has also been geographical variation in estimated CTVT prevalence in Europe; the disease has been reported to be absent except for occasional imported cases in many countries of Northern and Western Europe but has been estimated to be present at less than ten percent prevalence in countries in Southern and Eastern Europe (Strakova and Murchinson, 2014).

Transmission and associated risk factor: Transmissible tumours are a class of tumour that can be transmitted between individuals through living cells and CTVT is one of them (Yin *et al.,* 2015; Fassati, 2018). It is a transplantable and contagious neoplasm that is naturally transmitted between dogs by the allogeneic transfer of living cancer cells during coitus (Strakova and Murchinson, 2014; Castro *et al.,* 2017). During coitus there is a physical transfer of viable tumor cells by direct contact with injured skin and/or mucous tissue (Stockman *et al.,* 2011). It is common among sexually active dogs, where sexual behaviour is not under control. In this way it is transmitted from dog to dog by living cells rather than by the transformation of cells in the affected dog (Nak *et al.,* 2005; Agnew and MacLachlan, 2017). CTVT may also spread through licking, biting and sniffing tumour-affected areas (Das and Das, 2000; Murgia *et al.,* 2006). CTVT can also be transmitted from mother to the offspring during social interactions such as grooming and other maternal behaviour (Das and Das, 2000; Awan *et al.,* 2017). CTVT cells can be transmitted only across abraded mucosa with broken epithelium (Das and Das, 2000; vonHoldt and Ostrander, 2006).

Transmissible Venereal Tumour mainly affects the external genitalia of sexually active animals and its transmission is more frequent during intercourse or sexual behaviour (Rocha *et al.,* 2014; Setthawongsin *et al.,* 2016). However, Rocha *et al*. (2014) reported an unconventional mode of transmission animals where verrucous and ulcerated lesions on the vulva of its mother during pregnancy and childbirth infected the immature animal, even though, sexually immature animals without sexual contact to the street dogs, rarely have CTVT. This was identified as a transmissible disease and was first experimentally transplanted from dog to dog in 1876 (Metzger *et al.,* 2016). However, experimentally transplanted CTVT usually regresses after a few months but naturally acquired disease does not always regress (Cohen *et al.,* 1985; Metzger and Goff, 2016). CTVT rarely metastasizes, only occurring in puppies and immune-compromised dogs however, less than 5-17% of metastatic cases have been documented (Gurel *et al.,* 2002; Mukaratirwa and Gruys, 2003; Ajadi *et al.,* 2010; Ajayi *et al.,* 2009) but when metastases occurrs, it is usually observed in extra-genital regions (Mascarenhas *et al.,* 2014) such as the eye, brain, nasal cavity, lips, oral cavity; mammary gland, skin, tonsils, liver, spleen, kidney, lung, bone and musculature etc (Ferreira *et al.,* 2000; Abuom and Mande, 2006; Park *et al.,* 2006; Komnenou *et al.,* 2015; Gupta and Sood, 2012; Ajayi *et al.,* 2018). CTVT can be transplanted into other dogs by subcutaneous or intra-organ injection using viable cells, and this (CTVT) has been widely used as an experimental model in pioneer studies of many cancer therapies (Yang *et al.,* 1973; Chou *et al.,* 2009; Chuang *et al.,* 2009; Schwartz *et al.,* 2009; Ahrar *et al.,* 2010). Although naturally occurring tumours have been described only in dogs, the tumour can be experimentally transmitted by inoculation in other species of the *Canidae* family such as: foxes, wolves, jackals and coyotes (Murgia, 2006). Management practices that allow unrestrained contact between dogs are among the important risk factors; compromised biosecurity and increased stray dog population are other risk factors which favour the rapid spread of CTVT (Batamuzi *et al.,* 1992; Awan *et al.,* 2017).

Pathology

Clinical signs: In male dogs the tumours are located in the caudal region of the penis or prepuce and in females it is present on the vagina or labia, mostly around the vestibulo-vaginal junction (Das and Das, 2000; Kabuusu *et al.,* 2010; Sreekumar *et al.,* 2015). The tumours can be multifocal cutaneous nodules and plaques, haemorrhagic gingival masses and, apart from these, there can also be the presence of these clinical signs namely: anorexia peripheral lymphadenopathy, serosanguinous clotted bloody discharge, lethargy and haemorrhagic vaginal discharge without obvious genital masses (Laporte *et al.,* 2016; Awan *et al.,* 2017). Clinical signs in Extra-genital areas have been reported such as Extra-genital primary ophthalmic TVT (Komnenou *et al.,* 2015), there could be haemorrhagic epiphora with moderate brown-black crusts around the eyes (Milo and Snead, 2014). In male dogs, phimosis or paraphimosis may occur as a complication (Das and Das, 2000).

Pathogenesis: A TVT is unique in its pathogenesis compared with other neoplasms since it does not arise spontaneously but is transmitted from one animal to another (Das and Das, 2000). The transmissible nature is suggestive of an infectious aetiology; however, no infectious particles have ever been detected within the tumour cells (Das and Das, 2000; Ibrahim and Porter, 2012). These tumour cells are not the patient's own cells transformed into cancer cells. The TVT grafts itself from one dog's body onto another dog's body (Rodrigues *et al.,* 2001).

Gross Pathology: The tumour is cauliflower-like, pedunculated, nodular, papillary or multilobulated. It ranges from a small nodule (5 µm) to a large mass (up to 15 cm) and is firm, although sometimes appears friable. The surface is often ulcerated and inflamed and may be haemorrhagic and infected (Brown *et al.,* 1980; Hiblu *et al.,* 2019). The tumour may be solitary or multiple and is almost always located on the external genitalia (Figure 1i&ii), although it may occur in adjacent skin and oral, nasal and conjunctival mucosae leading to ulcerative keratitis (Ferreira *et al.,* 2000; Papazoglou *et al.,* 2001; Kabuusu *et al.,* 2010; Komnenuo *et al.,* 2015). Incidence varies from relatively high in some parts of the body to rare in others. The tumour may arise deep in the prepuce or vagina and be difficult to see. This may lead to misdiagnosis if bleeding is compounded with oestrus, urethritis, cystitis or prostatitis (Ganguly *et al.,* 2016). In cases with extra-genital localization of the TVT, clinical diagnosis is usually more difficult because TVTs cause a variety of signs depending on the anatomical localization of the tumour, e.g. sneezing, epistaxis, epiphora, halitosis, dental fistula, exophthalmos, skin bumps, facial or oral deformation along with regional lymphadenopathy (Papazoglou, 2001; Ganguly *et al.,* 2016).

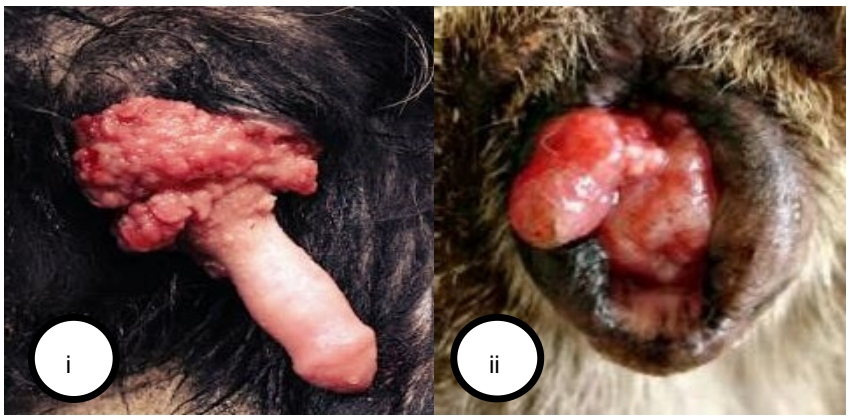

Figure 1 i&ii- multifocal haemorrhagic, cauliflower-like and nodular growths on the base of the penis and the vagina of dogs diagnosed with CTVT (Carreira *et al.,* 2014).

Histopathological and Histochemical findings

Histopathological findings show early massive lymphocytic infiltration resulting in tumour necrosis (Pia *et al.,* 2011), there are inflammatory cell infiltrates within the subepithelial stroma and these cells are mainly lymphocytes, plasma cells, macrophages and a few neutrophils (Carreira *et al.,* 2014). These CTVT cells contain large slightly acidophilic cytoplasm (Mukaratirwa and Gruys, 2003). The tumour can be classified into progression and initial and final regression phases according to developmental stages. The progression phase presents as round cells arranged diffusely, interspersed by delicate conjunctival stroma and the frequent presence of mitotic structures, the presence of greater mast cell counts and micro-vessel counts at the invasive edges of the tumours, in the Progressive phase (P-Phase), tumours consist of round cells with microvilli and few infiltrating lymphocytes, whereas those in the stable/ stationary phase consist of cells undergoing transition from round cells to spindle-shaped fibroblasts, there are fewer cancer cells in mitosis, and more apoptotic cells and infiltrating lymphocytes (Yang, 1988; Mukaratirwa and Gruys, 2003; Mukaratirwa *et al.,* 2006; Frampton *et al.,* 2018). In the initial phase of regression, tumour-infiltrating lymphocytes (TILs) appear and are widely distributed or associated with the conjunctival stroma (Liao *et al.,* 2003; Mukaratirwa and Gruys, 2003; Frampton *et al.,* 2018). Regressing tumours in the Regression Phase (R-Phase) contain higher numbers of lymphocytes that is the TILs, most of which are T-cells, they also have spindle-shaped cells with intracellular collagen bundles or collagen deposition within the vacuoles and the tumour stroma collapses (Yang, 1988; Mukaratirwa *et al.,* 2004; Frampton *et al.,* 2018). Metastasis is rare, but when it occurs, it is usually to the regional lymph nodes including external iliac, mesenteric, submandibular, cervical and inguinal lymph nodes but may also be seen in the kidney, spleen, liver, eye, tonsils, brain, pituitary, skin and subcutis, maxillary bone, ovaries, nasal cavity, eye orbit, spleen, liver, skin, ribs and peritoneum (Ferreira *et al.,* 2000; Abuom and Mande, 2006; Chikweto *et al.,* 2013; Ganguly *et al.,* 2016). The following can be seen microscopically as shown in Figure 2, neoplastic cells arranged in solid sheets, clusters or cords entwined by connective-tissue dermoplastic stroma (Gupta and Sood, 2012; Hiblu *et al.,* 2019). The presence of round cells, with abundant cytoplasm that was either clear or finely granular and had a large nuclear-cytoplasmic ratio. Hyperchromatic pleomorphic nuclei show chromatin clumping and contain one or more prominent nucleoli (Gupta and Sood, 2012; Milo and Snead, 2014; Hiblu *et al.,* 2019).

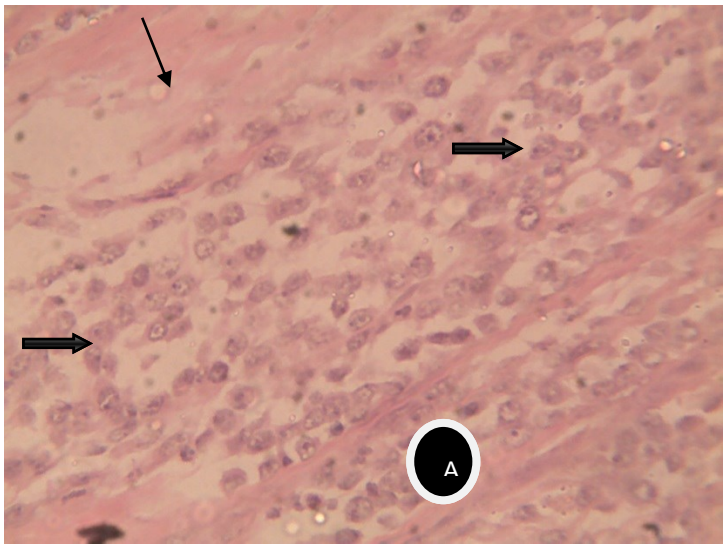

Figure 2 Photomicrograph of transmissible venereal tumour showing neoplastic cells with a few giant-like cells (A), collagen deposition (Small arrow) few mitotic figures (Thick arrow) and infiltrating lymphocytes. H&E Stain X 100.

Haematological and Serum Biochemical findings

Clinical and laboratory findings suggest that the general health of affected animals is not impaired unless the lesions turn necrotic and become infected. There is an obstruction in the urethral orifice or metastasis occurs. Haematocrit values are slightly lower than normal in less than 10% of the affected dogs but no severe anaemia is found. In about 30% of the cases, a mild-to-moderate leukocytosis may be apparent, probably caused by the inflammation of the tumour surface and the differential white blood count picture in affected dogs shows elevation with neutrophilia, lymphopenia and thrombocytopenia (Behera *et al.,* 2012; Ganguly *et al.,* 2016). The serum chemical profile in affected dogs shows hypoproteinemia, hypoalbuminemia, hypoglobulinemia with higher levels of blood urea nitrogen and creatinine. Elevated levels of alanine aminotransferase and alkaline phosphatase are also observed probably due to metastasis to liver (Behera *et al.,* 2012; Birhan and Chanie, 2015).

Cytology findings

Cytologically, TVT cells have a very distinct appearance. They are round to oval and often contain mitotic figures, with chromatin clumping and a few prominent nucleoli. The most distinguishing cytological finding is the presence of multiple clear cytoplasmic vacuoles (Figure 3i), this vacuolation increases during early stages of regression as TVT cells undergo degeneration. During degeneration, amounts of endoplasmic reticulum and ribosomes also increase, the same as the swelling of mitochondria. Degenerating cells often contain numerous membrane-bound granules and clusters (Ganguly *et al.,* 2016). There is also the presence of some inflammatory cells mostly neutrophils and some macrophages. Features of malignancy in the round cell population include mild to moderate anisocytosis, anisokaryosis, numerous mitotic figures (Figure 3ii), and abundant faint basophilic cytoplasm (Milo and Snead, 2014). It is important to point out that CTVT has three cytomorphological types namely, plasmacytic, lymphocytic and mixed type (Setthawongsin *et al.,* 2018).

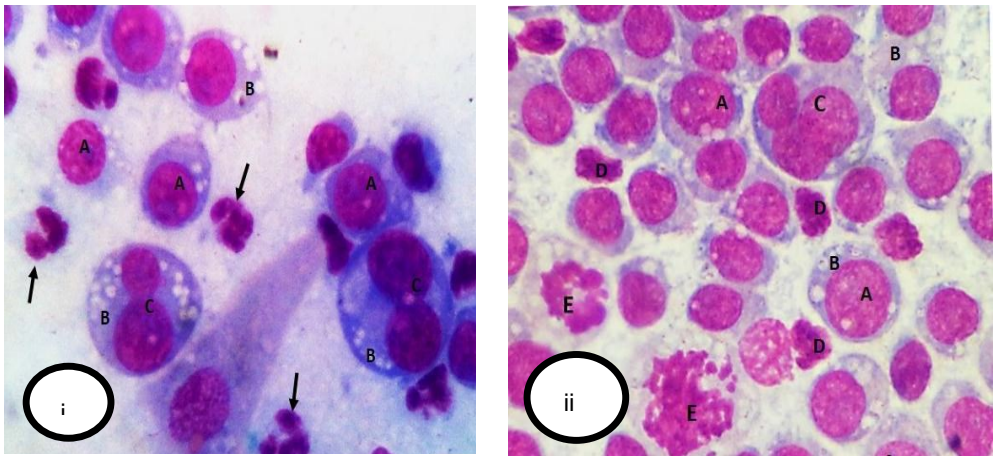

Figure 3 i- Fine needle aspirate of transmissible venereal tumour showing intra-nuclear (A) and intra-cytoplasmic (B) vacuolations, two neoplastic cells undergoing nuclear budding (C) and few neutrophils (arrows). Giemsa Stain ; ii- Fine needle aspirate of transmissible venereal tumour showing intra-nuclear (A), intra-cytoplasmic (B) vacuolations and a three nuclear-budded giant cell (C) and few neutrophils (D) with two mitotic figures (E). Giemsa Stain (Ajayi *et al.,* 2018).

Immunology and Immunopathology

CTVT is transmitted as an allograft by cell transplantation and the tumour grows in an autonomous manner from the cell growth pattern of the original host. TVT behaves more like a parasite and can evade host immune-detection (Mukaratirwa and Gruys, 2003; Belov, 2012). This kind of tumour develops only dogs, probably because during coitus there is extensive abrasions and bleeding of the penile mucosa and vagina, making transplantation of the tumour uncomplicated and straightforward (Mukaratirwa and Gruys, 2003). This transmission is possible through live tumour cell inoculation and TVT spontaneously auto-regresses, this behaviour is closely related to host immune response, and *CCL5 has been suggested* as a possible driver of CTVT regression (Pai *et al*., 2011; Frampton *et al*., 2018). Spontaneous and experimentally transplanted CTVT shows an initial stage of rapid tumour growth followed by a regressive stage which is an immune response in affected animals. Several reasons supported by both *in vitro* and *in vivo* studies have been proposed as to how CTVT initially evades the immune system and how the immune system later succumbs (Mukaratirwa and Gruys, 2003). To avoid this immune response of CTVT regression, the tumour uses a variety of immune escape strategies that have similarities, such as, Major Histocompatibility Complex (MHC) loss and the expression of immunosuppressive cytokines. CTVT appears to have a complex interaction with the immune system of the host, which has evolved over the long life of the tumour. According to current models of the interaction of CTVT with host immune cells, tumour cells lack MHC molecules and release transforming growth factor-p (TGF- 9), which suppresses T cells and Natural Killer (NK) cells during the growth phase (Hsiao *et al*., 2004; Liu *et al*., 2008; Tez and Kanca, 2018). Although, Tumour infiltrating lymphocytes (TlLs) produce high levels of lnterferon (lFN)-γ which promotes MHC expression, lFN-y activity is inhibited by TGF-P. However, during regression TlLs produce high concentrations of lnterleukin (lL)-6, antagonizing TGF-P. The levels of (lFN)-γ and MHC expression increase, leading to cytotoxicity by T cells and NK cells (Tez and Kanca, 2018). The immunologic interactions between host and CTVT involving TGF-, IL-6, IFN-y, MHC expression and DC activities makes CTVT, a reasonable model for the study of the immunologic interaction between host and cancers (Hsiao *et al*., 2004; Liu *et al*., 2008). Although CTVT samples have been subjected to immune-histochemical analysis for several tumour markers, including keratin, vimentin, desmin, CD3, α-smooth muscle actin, immunoglobulins G and IgM, λ-light chains, κ-light chains, lysozyme, ACM1, and A-1-antitrypsin, its origin and immune-phenotype remain vague (Mukaratirwa and Gruys, 2003; Zayas *et al*., 2019). However, immune-histochemical staining of CTVT reveals that the neoplastic cells were positive for lysozyme, ACM1 and A-1-antitypsin and shows strong cytoplasmic reactivity to vimentin but were negative for cytokeratin, S-100, desmin, CD3, p63 and CD79a (Mozos *et al*. 1996; Marchal *et al*., 1997; Mukaratirwa and Gruys 2003; Park *et al*., 2006; Gupta and Sood, 2012). PCNA, Ki-67 and c-myc, the oncogenes Rb and cyclin D1 can be immune-histochemical markers of CTVT (Gupta and Sood, 2012).

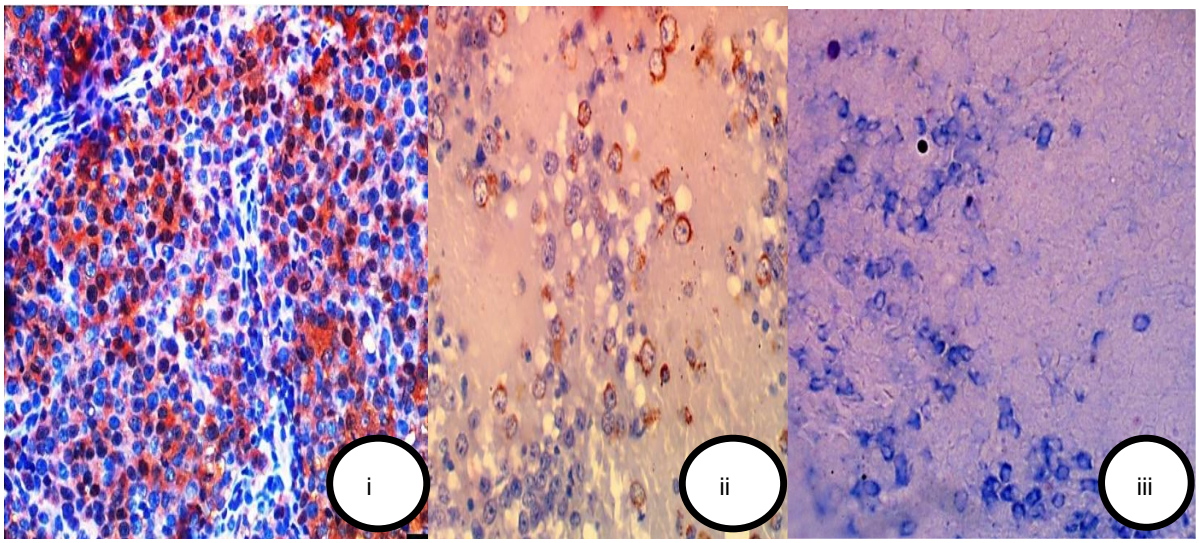

Figure 4 i- Photomicrograph of transmissible venereal tumour, vagina, dog. Numerous neoplastic cells showing moderate cytoplasmic immunoreactivity to S-100 protein in most of the neoplastic cells. Biotin Streptavidin peroxidase, DAB X 100; Figure 4ii- Photomicrograph of transmissible venereal tumour, vagina, showing a few cells with mild cytoplasmic expression of vimetin protein. Biotin Streptavidin peroxidase, DAB X 100; Figure 4iii-Photomicrograph of transmissible venereal tumour, penis, dog. Numerous neoplastic cells showing non-immunoreactivity to anti-desmin protein. Biotin Streptavidin peroxidase, DAB X100 (Ajayi *et al*., 2018).

Molecular Biology of CTVT

Major Histocompatibilty Complex (MHC) class I and II are expressed by the CTVT cells in the regression phase (Yang *et al*., 1998, Perez *et al*., 1998; Hsaio *et al*., 2002). Liao *et al*. (2003) reported that the proportion of B lymphocytes decreased dramatically with CTVT growth. This destruction of B lymphocytes was caused by substances released by the tumour cells, such as cytotoxic proteins and other circulating substances. These cytotoxic substances cause B lymphocyte

apoptosis during the neoplastic progression phase (Liao *et al.,* 2003). Hsiao *et al*., (2004) showed that CTVT cells elicit the cytokine transforming growth factor β1 (TGF- β1), which inhibits the activity of natural killer cells (NK) and the infiltration of cytotoxic lymphocytes. This suppressive action of TGF β1 on NK cell activity can be balanced by the effect of a pro-inflammatory cytokine called interleukin 6 (IL-6), which is secreted by tumour lymphocytes (Hsaio *et al*., 2008). The expression of MHC by CTVT cells in vitro can be induced by synergy between interferon-γ and IL-6 (Hsaio *et al.,* 2008). IL-6 can induce the expression of MHC by CTVT cells in vitro and in vivo. In the latter case, MHC is induced by the presence of IL-15 (Chou *et al*., 2009).

TP53 is one of the most important tumour suppressor genes involved in the development of neoplasia (Veldhoen and Milner, 1998; Tomita *et al*., 2006; Stockmann *et al*., 2011a). The important role TP53 plays, its functional polymorphisms can profoundly affect the development of tumours (Oren, 1999; Toledo and Whal, 2006). TP53 encodes a 393 amino acid nuclear protein, p53, able to bind to specific DNA sequences and act as a transcription factor (Liu *et al*., 2008; Stockmann *et al.,* 2011a). More than 10 mutations in the TP53 gene have been described in canine neoplasias (Oren, 1999; Setoguchi *et al*., 2001). Recently, TP53 mutations have also been reported in CTVT (Choi and Kim, 2002; Sánchez-Servín *et al*., 2009, Stockmann *et al*., 2011a).

The Bcl-2 family is a group of proteins that induce or inhibit cell death by apoptosis (Borner, 2003). Some members of the Bcl-2 family, including Bcl-2 and BclxL, are anti-apoptotic regulators preventing the release of cytochrome C from the mitochondria. (Kirkin *et al*., 2004). Cells of TVTC show overexpression of Bcl-2 protein independent of the stage of tumour development (Stockmann *et al*., 2011b). Bcl-2 and its family members participate in carcinogenesis, but their contribution remains puzzling because their expression can be associated with resistance to drugs and radiotherapy (Stockmann *et al*., 2011a).

Diagnosis

Clinical history, signalment and cytological features are often obvious for establishing a diagnosis though biopsy and histological examination may be needed in atypical cases (Ganguly *et al*., 2016). In areas where CTVT is highly prevalent it is important to emphasize the need to consider TVT as one of the differential diagnoses for masses in extra-genital locations in dogs from regions where TVT is prevalent (Chikweto *et al*., 2013). Diagnosis is established based on History, signalment, clinical findings, cytology and histology, immunohistochemistry, cytogenetic and molecular techniques may also be applied (Park *et al*., 2006; Ganguly *et al*., 2016). In female animals with confirmed extra-genital TVT, a digital vaginal examination and possibly vaginoscopy could aid in making a diagnosis (Das and Das, 2000). TVT cells can be examined after making a fine-needle aspiration or impression smear cytology of the tumour mass (Erünal-Maral *et al.,* 2000; Milo and Snead, 2014; Rocha *et al.,* 2014; Gangul *et al.,* 2016). Cytology gives better evidence of CTVT together with histology. The histological appearance of transmissible venereal tumour may be difficult to distinguish from other round cell tumours such as histiocytoma, lymphosarcoma or mast cell tumours; especially so when the tumours are seen in extra-genital locations. Upon reticulin staining, TVT cells show the invasion of the inter-alveolar spaces by reticulum fibres, characteristic of alveolar soft-part sarcoma (Das *et al*., 1990).

For the diagnosis of TVT by immunohistochemistry a panel of antibodies is required but this technique is of great use in the diagnosis of CTVT. It involves anti-vimentin, anti-lysozyme, anti-alpha 1 antitrypsin, anti-CD3 and anti-CD79α antibodies have been used for characterization of primary cell culture in the transmissible venereal tumor (TVT) (Duzanski *et al*., 2017; Florez *et al*., 2016; Mascarenhas *et al*., 2017). TVT shows the immuno-histochemical staining characteristics of histiocytic cells and the differentiation between these two tumour types should, therefore, be based on clinical and histopathologic criteria (Marchal *et al*., 1997; Mascarenhas *et al*., 2017). Polymerase chain reaction (PCR) can also be used for the molecular diagnosis of this disease (Setthawongsin *et al*., 2016, Castro *et al*., 2017). Cytogenetics is of decisive advantage in the definitive diagnosis of the CTVT because of the highly significant karyotypic differences that exist between normal and cancerous cells (Cangul, 2001; Ganguly *et al*., 2016). Cytomorphometry, a method of computerized image analysis, has been used recently in Veterinary Medical practice. It involves comparison between nuclear and cellular morphometric parameters such as radius, diameter, perimeter and cell or nuclear area of different types of CTVT (Setthawongsin *et al*., 2018). Other imaging diagnostic techniques include computer tomographic imaging and Transmission Electron Microscopy, which have also been used in the diagnosis of CTVT (Ferriera *et al*., 2000; Mascanrenhas *et al*., 2017; Ojeda *et al*., 2018). Single-cell gel electrophoresis, a visual technique to analyze and measure DNA breaks in mammalian cells that is also known as the "comet test", demonstrate that CTVT cases with a plasmacytoid morphology exhibit fewer DNA breaks, which is probably an evasive mechanism for the elimination of the CTVT cells (Amaral *et al*., 2011; Duzanski *et al*., 2017)

Disease prevention, control & treatment

Treatment can be attempted by excisional surgery, chemotherapy, radiotherapy, immunotherapy or a combination thereof (Ganguly *et al*., 2016). A high rate of spontaneous regression warrants proper caution in the evaluation of the success achieved with different therapeutic approaches (Das and Das, 2000). Vincristine chemotherapy has been proved to be very effective and, after treatment, the tumour regresses (Milo and Snead, 2014; Komnenuo *et al*., 2015; Fassati, 2018; Tez and Kanca, 2018). Most cases are curable with three intravenous injections of vincristine sulphate at weekly intervals (Ganguly *et al*., 2016) Vincristine sulphate, a mono-chemotherapy for TVT has direct effects on tumour cell division and bacterial

multiplication (Tella *et al*., 2004; Setthawongsin *et al*., 2019).

Although there are reports that are some degree of resistance to treatment with vincristine sulphate, resistant CTVT has emerged as a intriguing problem for clinical management, even leading to eventually peacefully putting suffering animals to sleep (Arcila Villa *et al*., 2018). Therefore, in addition to Vincristine chemotherapy, Interleukin 2 has been used with great success (Den Otter *et al*., 2015). Nak *et al*. 2005, reported some success with combined treatment using vincristine and doxorubicin. There are also reports of the combined therapy of L-asparaginase (LAP) and Vincristine with reported appreciable success and also decreasing treatment time. (Setthawongsin *et al*., 2019). LAP is an enzyme which inhibits the protein synthesis and induces tumour necrosis. The major advantage of using LAP is that no dogs treated with LAP show any adverse side effects. Moreover, LAP is well-tolerated as an effective drug with a unique mechanism of action (Saba *et al*., 2007 and 2009). Immunotherapy is considered as a useful adjunct therapy for cancers and has been used with appreciable success in the treatment of CTVT (Casal and Haskins, 2006; Pia *et al*., 2011). The role of stray and wild dogs makes the CTVT difficult to control and necessitates sustained animal birth control in stray dogs along with prompt therapy of the affected dogs and in addition to this, dog spaying and neutering have been linked to reduced CTVT prevalence (Strakova and Murchinson, 2014; Ganguly *et al*., 2016).

In conclusion, canine transmissible venereal tumour has been extensively studied in the last decade because of its inherent pathogenic and metastatic capabilities and because it is an excellent model to investigate and study unique insights into evolutionary trends operating in cancer. It is also of great importance in understanding the mechanisms of CTVT infection and experimental infection in canids, most importantly in the development of other chemotherapeutic means of treatment of this unique tumour apart from the use of Vincristine. This review thus, focuses on the aetiology, cellular structure, epidemiology, pathology, immunology, diagnosis, prevention, treatment and control of this contagious tumour in domestic and wild canids which were studied to better understand this distinct neoplasm.

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