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Canine transmissible venereal tumor

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Abstract
A naturally occurring tumor known as a transmissible venereal tumor (TVT) in dogs is spread from one animal to another during mating by viable tumor cells. This cancer, which affects dogs, is also referred to as Sticker's sarcoma or Canine Transmissible Venereal Tumor (CTVT). The internal genital organs may also rarely be affected; it mainly affects the external genitalia. Hemorrhagic discharge is frequently associated with TVTs located on mucosal or membrane-based surfaces such as the genital, oral and nasal cavities. Cytological examination revealed presence of pleomorphic cells with round and eccentric nucleus, moderate anisocytosis, high N:C ratio and vacuolated cytoplasm with distinct cell borders. In histopathological examination, neoplastic cells arranged in solid sheets or in cords interlaced by connective tissue stroma, cells are round in shape with prominent nucleoli, clear or finely granular cytoplasm and coarse chromatin. After diagnosis follow-up appointments for treatment will ultimately aid in the tumor's eventual elimination from the affected area. Treatment usually works well for it and dogs that have spontaneous regression develop immunity against more tumor threats in the future. In tropical and sub-tropical metropolitan regions, dogs' external genitalia are most frequently affected by this kind of tumor. Because of its high contagiousness among dogs and its ability to spread to other wild canids like foxes, jackals and coyotes, its geographical frequency among stray dog populations in these locations is noteworthy. Strict control measures for stray dog populations should be implemented by government policies to successfully reduce the spread of disease. Its high occurrence can be attributed to unrestricted sexual behavior and a sizable stray dog population. The majority of cases of this tumor are seen in sexually active canines, both male and female, usually between the ages of 2 and 8 years.

Keywords: Cancer, canine. transmissible, tumor

Introduction
Cancer develops when a single lineage of cells accumulates mutations that drive continuous cell division. Natural selection tends to favor the most prolific subpopulations, often leading to a more aggressive form of cancer. The canine transmissible venereal tumor (CTVT), also known as Sticker's sarcoma, is a unique form of neoplasia that can be naturally transmitted among susceptible dogs through the transfer of viable tumor cells, especially when there are abrasions or breaches in the skin surface (Chu *et al*., 2001) [9]. Its first documented occurrence dates back to 1820 when Hüzzard reported it, and subsequent researchers, including Delabere-Blaine in 1928, provided further insights. However, Sticker's comprehensive characterization of this neoplasia between 1905 and 1906 led to its long-standing association with his name. Sticker's research revealed that this transmissible neoplasia predominantly affects the genital region (Murgia *et al*., 2006) [24].

CTVT stands out in oncology as it was the first tumor to be experimentally transmitted, an achievement credited to the Russian veterinarian Nowinsky in 1876. This groundbreaking event sparked significant scientific interest and marked a turning point in oncology research (Das and Kumar 2000) [10]. Due to its unique transmission through sexual contact, naturally occurring CTVT typically develops in the external genitalia (Mukaratirwa and Gruys 2004) [23]. Less frequently, it may spread to the nasal or oral cavities, skin, conjunctiva, and the rectum through sniffing or licking (Amaral *et al*., 2007) [5]. With genital CTVT, social behaviors likely contribute to its prevalence. In rarer cases, it can manifest in other areas, including the lips, oral mucosa, peritoneum, or various organs like the tonsils, eye, liver, spleen, kidney, lung, and musculature (Park *et al*., 2006) [26]. Dogs of all breeds, ages, and sexes can be affected, with a higher risk for dogs over one year of age in endemic regions, particularly those aged 2 to 5 years (Das and Kumar 2000) [10]. Interestingly, this tumor is not found in virginal females, and it affects females more often than males. This disparity may be due to one infected male spreading the disease to multiple females during mating (Rebbeck *et al*., 2009) [27].

Metastasis of CTVT is infrequent and primarily occurs in puppies and immuno-compromised dogs. Most reported cases of metastasis are, in fact, mechanical extensions of the tumor or transplantation (Singh *et al*., 1996) [31]. Young dogs, stray dogs and sexually active dogs are the most commonly affected by this neoplasm (Nak *et al*., 2005) [25]. CTVT has a global distribution, with the highest incidence in tropical and subtropical regions. Notably, this tumor can affect various canids, including foxes, coyotes and wolves, in addition to dogs (Canisfamiliaris) (Birhan and Chanie 2015) [7].

Distribution

During the 20th century, TVT has been documented on all continents, including Asia, America, Africa, and Australia. However, this does not imply its absence in these regions prior to the 20th century. The disease is globally prevalent, with a particular concentration in sub-tropical regions. Reported cases span across North, Central, and South America, Europe, the Middle and Far East, Asia and certain parts of Africa (Kabuusu *et al*., 2010) [18]. Naturally occurring cases of TVT have been observed in 33 different countries worldwide. It is considered endemic in many tropical and subtropical regions where there are populations of free-roaming or stray dogs, facilitating local propagation due to the uncontrolled breeding of such dogs and the limited availability of effective treatments. Typically, sexually mature dogs, those older than two years, are the most commonly affected (Das and Das 2000) [10].

The occurrence of this tumor is evenly distributed between males and females (Smith, 1998) [32] and is most prevalent in dogs aged 2 to 5 years (Higgins, 1966) [15]. TVT appears to be favored by temperate climates (Withrow and McEwen, 1996; Rogers, 1997) [35, 28]. The tumor is often associated with dogs in close contact with one another, including stray and wild dogs engaged in unrestrained sexual activity.

In India, TVT is the most common tumor in dogs due to uncontrolled breeding practices. Its prevalence has been reported to range from 23.5% to 28.6% of all tumors in canine patients in Punjab. A similar prevalence pattern of 28.6% was found in the state of Andhra Pradesh, while in Assam, it reached the highest prevalence at 42.8%. Clinical cases have also been reported in the deserts of Rajasthan, arid zones of Haryana, Madhya Pradesh, the Ganges basin in Uttar Pradesh, and in the Himalayan cities of Nainital (Abedin 2020) [1]. This distribution pattern suggests a consistent occurrence of the neoplasm across diverse altitudes and climates.

Factors affecting the prevalence and incidence of TVT

The prevalence of this acquired disease varies depending on the geographical location, particularly between urban and rural areas. It has been documented worldwide, and in India, the incidence of this disease in dogs is reported to range from 23% to 43%. It is most frequently found in subtropical to tropical urban areas. The occurrence of this disease is more closely associated with the age of highest sexual activity and in countries where the canine population is not subject to strict epidemiological control. Females are more commonly affected than males (Gonzalez *et al*., 1997) [13].

Seasonal factors also play a role, with a correlation to the estrus cycle in canines. Dogs typically exhibit estrus during rainy and summer seasons. When the infection is transmitted during the rainy season, the granulomatous growth becomes noticeable after 2-3 months, with a comparatively higher incidence of venereal granuloma in the winter season. Canines also go into estrus in the months of February and March, with infection transmission and subsequent growth being reported during the rainy season after 2-3 months. Consequently, the occurrences of venereal granuloma are more frequent during the rainy season (Das and Das 2000) [10].

In terms of sex and age, there is a higher incidence of the disease in females compared to males. Male dogs, due to their constant sexual receptivity, may have a greater opportunity to spread the disease compared to females, who only become sexually receptive once every 6-7 months. In fact, it has been observed that a single male dog with TVT can transmit the disease to 11 out of 12 females, and in some regions, it is naturally more prevalent in females than in males. As a result, the incidence of venereal granuloma is higher in females than in males (Martins *et al*., 2005) [22].

CTVT is most commonly found in young to middle-aged adult dogs. This could be attributed to the fact that young and middle-aged adult dogs are sexually more active, and since this disease is sexually transmissible, these dogs are more susceptible to exposure. It is most common in the age group of 1 to 5 years. Therefore, the incidence is higher in young and middle-aged adult dogs (Scarpelli 2008) [30].

Transmission

TVT is a histiocytic tumor that can be transmitted between dogs and other canids through various means, including sexual contact, licking, biting and contact with tumor-affected areas (Mac-Ewen 2001) [20]. The transmission occurs when viable tumor cells are implanted into mucous membranes, particularly in the presence of abrasions or compromised surface integrity. The notion that this tumor is naturally transmissible as an allograft is supported by three key observations. First, it can only be induced experimentally by transplanting living tumor cells, not by using killed cells or cell filtrates. Second, the tumor exhibits aneuploidy but consistently features characteristic marker chromosomes in tumors collected from different geographic regions. Third, a LINE insertion near the c-myc oncogene has been identified in all examined tumors, serving as a diagnostic marker to confirm the presence of CTVT (Murgia *et al*. 2006) [24].

Typically, it is transmitted to the genital organs during sexual intercourse, but it can also affect the skin through the direct implantation of tumor cells during contact with tumor masses. Transplantation occurs when host tumor cells, with intact MHC class I and II molecules, lose their expression, allowing the transfer of the tissue to a healthy animal through contact between the skin and damaged mucosa (Liu *et al*. 2008) [19]. Tumor cells can only be transferred between healthy animals that share the same MHC or into immuno-compromised recipients, as these cells trigger an immune response in healthy recipients. These cells can originate from mutations induced by viruses, chemicals, or radiation in lymphohistiocytic cells, and then these tumor cell clones can be disseminated through allogeneic transplantation (Abeka 2019) [2].

Clonal transmission is related to dogs having 78 chromosomes, of which 76 are acrocentric. In cells isolated from animals in different geographic regions, the chromosome number can vary from 57 to 59, with 15 to 17 chromosomes being metacentric or submetacentric. In addition to this unique feature, consistent and specific chromosomal abnormalities, such as the insertion of a LINE-1 near the c-myc oncogene in the CTVT genome, are present in most samples collected from various parts of the world. This

genomic rearrangement has not been identified in any other normal tissue of dogs and can be used for diagnosing this disease (Rebbeck *et al*. 2009) [27].

Pathogenesis

The tumor could be transferred from one susceptible host to another by introducing tumoral cells. Some authors initially attributed the cytoplasmic inclusions in the tumoral cells, which cause neoplasia, to the presence of a virus. However, the prevailing belief today is that TVT originates from allogenic cellular transplants, and the abnormal cells within the neoplasm act as carriers for its transmission. Physical contact increases the likelihood of transplanting these neoplastic cells onto genital mucosa during mating and can also result in their transmission to nasal or oral mucosa when the affected genitalia are licked (Johnston, 1991) [17]. The presence of mucosal lesions or breaches in mucosal integrity facilitates implantation. Tumor growth becomes evident within 15 to 60 days after implantation, and this growth can progress slowly and unpredictably for years or become invasive, eventually leading to malignancy and metastasis. TVTs are recognized as immunogenic tumors, and the host's immune system plays a pivotal role in restraining tumor growth and metastasis. Immune-compromised young dogs are at a higher potential risk for metastasis (Yang, 1988) [36]. Metastases have been notably more common in male dogs than in females. Metastatic sites include subcutaneous tissue, skin, lymph nodes, eyes, tonsils, liver, spleen, oral mucosa, peritoneum, brain and bone marrow. Extragenital lesions can occur in conjunction with or independently of genital lesions. Immunological studies have shown that the tumor can be transplanted across barriers of major histocompatibility complexes (MHC). Serum samples from dogs with TVT have tested positive for the presence of immune complexes. During the rapid growth phase, the tumor cells do not express Type I and II MHC antigens, but in the initial regression phase, around 30% to 40% of the cells express both antigens. This difference in MHC antigen expression is believed to contribute to an additional immune response in the host, which accelerates tumor regression (Abedin 2020) [1]. Different stages of tumor progression are associated with distinct cell types. In the progressive growth phase, tumor cells appear round and feature microvilli, whereas regressing tumors present transitional cells rather than fusiform ones. Regressing tumors tend to have an abundance of T lymphocytic cells. Investigations have revealed that substances secreted by the lymphocyte infiltrate induce cellular differentiation, ultimately driving tumor regression (Abedin 2020) [1].

Clinical presentation and findings

The clinical presentation of TVT varies depending on the location of the tumor within the specific tissue or organ. Hemorrhagic discharge is frequently associated with TVTs located on mucosal or membrane-based surfaces such as the genital, oral, and nasal cavities. These tumors tend to bleed easily due to extensive ulceration of the epithelial lining. In female dogs, the protrusion of tumor tissue from the genital area, depending on the tumor's size, is most noticeable. Male dogs with penile tumors often exhibit enlarged inguinal lymph nodes. The size of the tumor can range from 3 to 12 cm in diameter (Park *et al*. 2006) [26].

Clinical signs of genital TVT include bloody vaginal or preputial discharge, intermittent or persistent ulcerative skin lesions, genital swelling, and excessive licking of the genital area (Nak *et al*. 2005) [25]. An unpleasant odor or the appearance of visible neoplastic masses may also be present (Santos *et al*. 2005) [29].

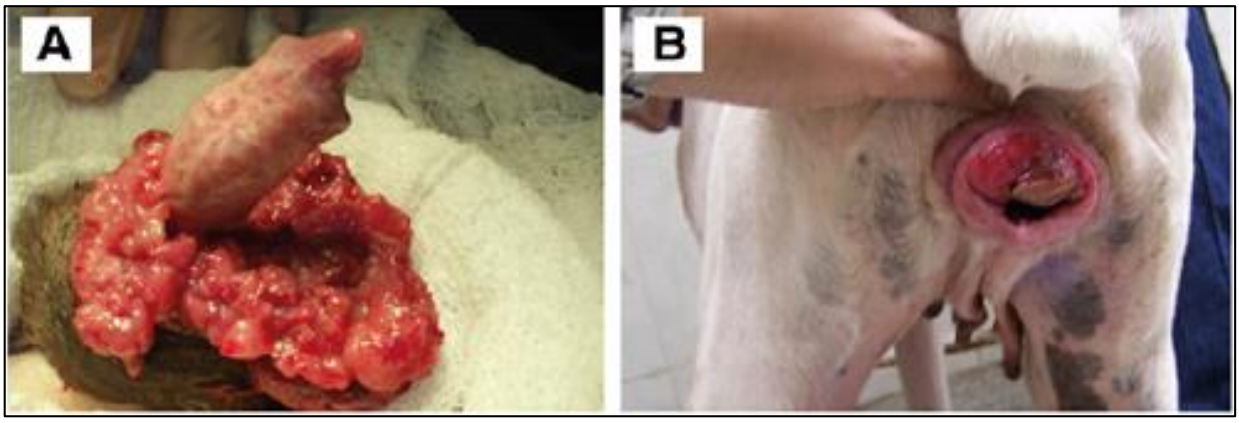

Fig 1: Canine Transmissible Venereal Tumor affecting (A) a male dog, (B) a female dog

CTVT predominantly affects the external genitalia in dogs of both sexes. In males, it's commonly found in the caudal part of the penis, the glans, and occasionally in the foreskin. In females, it's often located at the junction of the vestibule and the posterior region of the vagina, occasionally affecting the urethral opening (Made-well 2001) [21]. Dogs with this condition may experience pain, hemorrhaging, and exhibit a serosanguineous discharge from their external genitalia. These tumors typically have a cauliflower-like appearance, are friable, and appear red to flesh-colored. Generally, the overall health of affected dogs remains unaffected unless the tumor becomes necrotic and infected, obstructs the urethral orifice, or metastasizes (Gandotra 2014) [12].

TVT can also develop in extra-genital sites such as the skin, subcutaneous tissues, and within the oral and nasal cavities. Extra-genital tumors are usually well-defined and can measure 2-5 cm in size (Das and Das 2000) [10]. While metastases are infrequent, they can occur, particularly in puppies and dogs with compromised immune systems. These metastases are often considered as mechanical extensions of the primary tumor. However, they have been reported in inguinal lymph nodes and can also affect the skin, brain, eyes, liver, spleen, testicles and muscles (Park *et al*. 2006) [26].

Common signs of cancer in dogs include persistent or growing abnormal swellings, non-healing sores, weight loss, loss of appetite, bleeding or discharge from body openings, an

offensive odor, difficulty eating or swallowing, reduced exercise tolerance, persistent lameness or stiffness and difficulties with urination, defecation or breathing (Douglas 2003) [11].

Diagnosis

The clinical signs and symptoms of TVT vary depending on the tumor's location. Dogs with tumor localization in the genital area often exhibit hemorrhagic discharges. In males, the lesion is typically found at the cranial part of the glans penis, the preputial mucosa, or the bulbus glandis. If tumoral masses protrude from the prepuce, it can lead to phimosis. It's important not to mistake this discharge for conditions like urethritis, cystitis, or prostatitis (Rogers, 1997) [28]. Larger tumors in males frequently lead to regional lymph node involvement. In female dogs, the tumors appear grossly similar to those in males, with localization in the vestibule and/or caudal vagina. Tumors protruding from the vulva can cause deformities in the perineal region. Persistent hemorrhagic vulvar discharge may result in anemia. This discharge can attract male dogs, and owners might mistakenly interpret this as estrus. Tumor localization in the uterus is uncommon (Aprea *et al*., 1994) [6].

For cases with extra-genital tumor localization, diagnosis can be challenging since clinical signs depend on the tumor's anatomical location. These signs may include epistaxis, sneezing, excessive tearing, bad breath, tooth loss, skin lumps, protruding eyes, facial or oral deformities, as well as regional lymph node enlargement (Rogers, 1997) [28]. A definitive diagnosis is based on a physical examination and cytological findings typical of TVT, which can be obtained from exfoliated cells at the tumor site through swabs, fine needle aspirations or imprints (Abedin 2020) [1]. Large number of pleomorphic cells with uneven nuclei, moderate anisocytosis, increase N:C ratio and multiple vacoulation in cytoplasm (Ahuja *et al.* 2017) [3]. In histopathological examination, neoplastic cells arranged in solid sheets or in cords interlaced by connective tissue stroma, round cells with prominent nucleoli, clear or finely granular cytoplasm, high N:C ratio and coarse chromatin were observed in TVT (Gupta and Sood 2012; Ajayi *et al*., 2018) [14, 4].

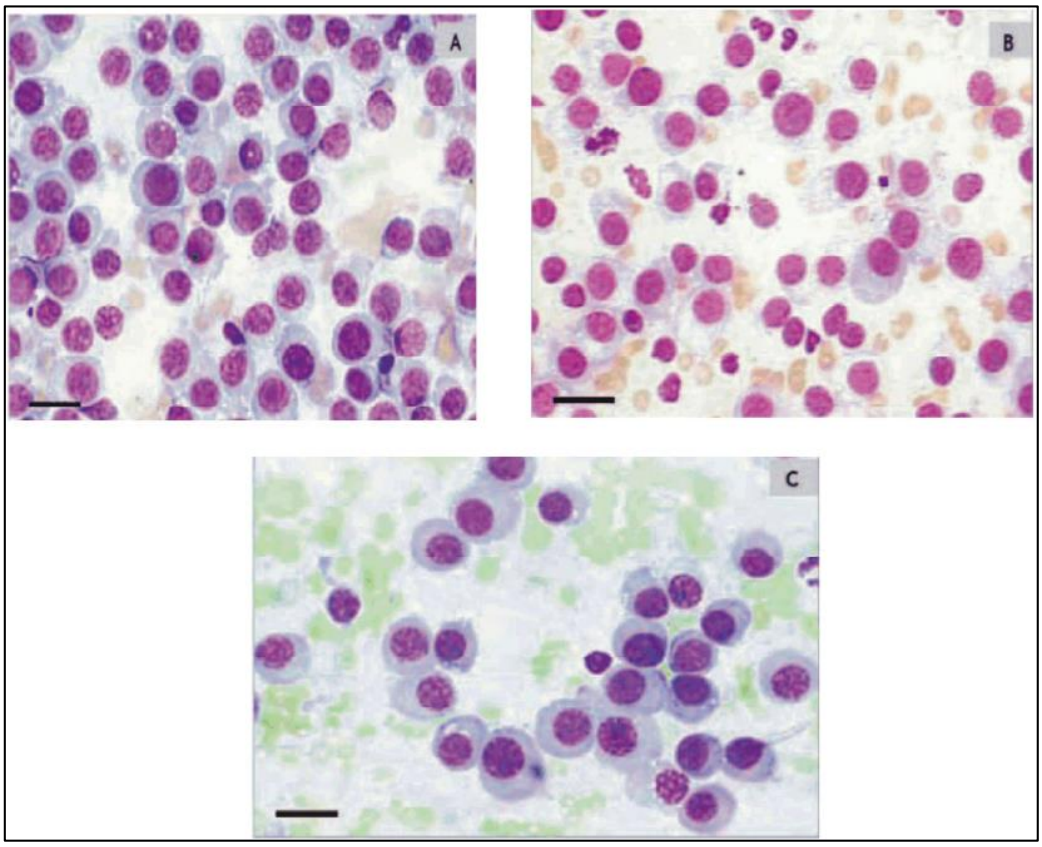

Fig 2: Cytological samples of transmissible venereal tumor of different cytomorphological types: (A) lymphocytic pattern (Predominance of round cells, scarce cytoplasm and high nucleus: cytoplasm ratio); (B) plasmacytic pattern (Predominance of ovoid cells, ample cytoplasm and eccentric nucleus); (C) mixed pattern (Presence of both morphological types without predominance of either. Giemsa, bar = 20µm.(Source: Amaral *et al*. 2007) [5].

Treatment

Various treatment approaches, both invasive and non-invasive, have been employed to address TVT. Surgical excision is an option for small, localized TVTs, but in cases involving large invasive tumors, the recurrence rate can be as high as 50 - 68% (Rogers, 1997) [28]. It has been noted that contamination of the surgical site with TVT cells could serve as a source of recurrence (Boscos and Ververidis, 2004) [8].

Transmissible venereal tumors have demonstrated sensitivity to radiation, with X-ray tubes operating at voltages ranging from 100 to 500 kV and cobalt being used for treatment (Rogers, 1997) [28]. Biotherapy studies have been conducted for treatment purposes, but they have shown limited success. Intratumoral application of Calmette-Guérin's bacillus (BCG) was utilized for three weeks, but the recurrence rate was found to be higher. Case recurrences have been observed after immunotherapy using Staphylococcus protein A or BCG (Rogers, 1997) [28].

Chemotherapy has proven to be the most effective and practical treatment, with vincristine sulfate being the most

commonly administered drug. Vincristine is typically given weekly at a dose of 0.025 mg/kg intravenously (IV) (Abedin 2020) [1]. The lesions gradually regress, and complete remission of the tumor usually requires 2 to 8 injections. When cases are treated in the early stages of progression, a cure rate of nearly 100% can be achieved. In chronic cases, longer periods of therapy are necessary, and the cure rate is much lower (Boscos and Ververidis, 2004) [8]. Vincristine is a cytostatic drug that can lead to myelosuppression and gastrointestinal effects, resulting in leukopenia and vomiting in 5 to 7% of cases. Paresis is also a potential side effect due to peripheral neuropathy (Withrow and McEwen, 1996) [35]. Therefore, it is advisable to conduct a complete white blood cell count before administering the drug. If the WBC count is below 4000 mm3, the administration of the drug should be delayed for 3 to 4 days, and the dose rate can be reduced to 25% of the initial dose.

Other chemotherapeutic agents that have been used and validated for treatment include cyclophosphamide (5 mg/kg, orally, for 10 days as a single drug therapy or in combination with prednisolone at 3 mg/kg for 5 days), weekly vinblastine (0.1 mg/kg, IV for 4 to 6 weeks), methotrexate (0.1 mg/kg, orally, every other day), or a combination of these three drugs. However, case reports have indicated that there is no significant advantage in using a combination of chemotherapy over vincristine injection alone. For cases that are resistant to treatment, the treatment regimen may include doxorubicin injection at 30 mg/m2, IV, with three applications every 21 days (Souza *et al*., 1998) [33].

In cases where the tumoral mass does not disappear with chemotherapy, electro-cauterization or cryocauterization can be useful (Rogers, 1997 and Abedin 2020) [28, 1]. Small remaining lesions may spontaneously disappear within 1 or 2 weeks after therapy. For patients not responding to chemotherapy, radiotherapy has been reported to yield positive results.

Conclusion and Recommendation

In summary, TVT in dogs is a naturally occurring tumor that is transmitted from one animal to another during mating through viable tumor cells. It primarily affects the external genitalia but can also occasionally involve the internal genital organs. Generally, it responds well to treatment, and dogs that experience spontaneous regression become immune to future tumor challenges. This type of neoplasm is most commonly found in the external genitalia of dogs living in tropical and sub-tropical urban areas. Its regional prevalence is notable among stray dog populations in these regions, owing to its highly contagious nature among dogs and the potential to transfer to other wild canids such as foxes, jackals and coyotes. A significant stray dog population and uncontrolled sexual behavior contribute to its high incidence. This tumor is most frequently observed in sexually active male and female dogs, typically between 2 to 8 years of age that are allowed to roam freely. Managing this condition, especially in developing countries, can be challenging as many owners cannot afford the cost of surgical intervention and/or radiotherapy.

In light of the above conclusions, the following recommendations should be considered:

- Dog owners and breeders should ensure that their dogs undergo annual examinations by veterinarians, which should include screening for TVT. This should involve checking for any signs of the disease, such as genital growths, through procedures like extrusion of the penis and digital palpation of the vagina.
- Suspected stray dogs with TVT should be captured for treatment to prevent the disease's spread, and neutering these dogs may be a beneficial step to reduce sexual activity among local dogs, thereby further preventing disease transmission.
- Owners whose dogs are diagnosed with TVT should receive thorough client education to enhance compliance and encourage return visits for treatment, ultimately contributing to the eradication of the tumor in the area.
- Government policies should be established to implement strict control measures for stray dog populations, effectively reducing disease transmission.

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