Prevalence and distribution of transmissible venereal tumor in dogs from a rural community of Mexico

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ABSTRACT

Objective. The aim of this work was to determine the rates of prevalence and recurrence of TVT infection in owned dogs, as well as the distribution of the disease, in Cuajinicuilapa, Guerrero, Mexico. Materials and methods. This work was an observational, descriptive, longitudinal study. A TVT diagnosis was performed in all dogs in the community based on clinical records, a physical examination, and a cytological evaluation, and further confirmed by histopathology. Tissue samples were surgically obtained for histopathological analysis and, when required, to remove tumors and give treatment with vincristine; a GPS system was used to identify possible spatial groupings. All cases were re-evaluated one year after. Results. A prevalence of 5.15% was found in 1047 dogs (512 females and 535 males; 25 positive females and 29 positive males). TVT infection was more frequent in 2–3-years-old dogs. A spatial aggregation pattern was observed in the local cases. One year after, no lesions were found in the 29 animals treated. Conclusions. TVT is a common disease, and its distribution suggests its aggregation in certain areas of the locality. Further studies on the dynamics of dog populations in small cities are required.

Keywords: Canines; Neoplasia; Transmissible venereal tumor (Source: CAB).
Resultados. Se encontró una prevalencia de 5.15% en 1047 perros (512 hembras y 535 machos; 25 hembras positivas y 29 machos positivos). La infección por TVT fue más frecuente en perros de 2-3 años. Se observó un patrón de agregación espacial en los casos locales. No hubo lesiones en los 29 animales tratados un año después. Conclusiones. El TVT es una enfermedad común, y su distribución sugiere su agregación en ciertas áreas de la comunidad. Se requieren más estudios sobre la dinámica de la población de perros en ciudades pequeñas.

Palabras clave: Caninos; Neoplasia; Tumor venéreo transmisible (Fuente CAB).

INTRODUCTION

Malignancies are among the most common pathological conditions affecting dogs (1), with a high prevalence in the reproductive organs (82%) (1,2). The transmissible venereal tumor (TVT) is one of the most frequent malignancies in tropical and subtropical regions, affecting mostly stray and malnourished dogs (3); similarly, it is common in marginal areas of big cities in developing countries, where no plan to control dog population exists (4). TVT cases have been detected in all five continents, with the highest prevalence rates found in Latin America; a prevalence of about 20% has been reported in Mexico (5). TVT, also known as infectious sarcoma, venereal lymphosarcoma, venereal granuloma, canine condyloma, transmissible reticulal cell tumor, and Sticker’s sarcoma, affects both male and female dogs. The tumor physical appearance can vary from a small, 5-mm nodule to a 10-cm (or larger) cauliflower-like mass (1,6) with bloody and/or purulent secretion (7).

Histologically, TVT is characterized by round, ovoid, or polyhedral-shaped cells arranged in compact masses, intertwined by a delicate vascular stroma, with a single, centric, big, and hyperchromatic nucleus (8). Tumor cells exhibit a very rapid growth, leading to a significant tumor mass and a great destruction of local tissues (9). TVT is transmitted as an allograft, mainly during coitus (10,11), although it can also be transmitted by biting, sniffing, and laceration licking; thus, it can also be found on the face skin, nasal passages, oral cavity, and inside/around the eyes; occasionally, it has been found in the spleen, liver, brain, and lungs of infected dogs, which indicates its capacity for metastasis (9,12).

A TVT presumptive diagnosis is based on the macroscopic morphology of the tumor, its location, secretions, and anamnesis. A definitive diagnosis requires cytological,
poverty and social marginalization, 67.4% of population live in poverty; 33.5% suffer from educational deficit, and 65.2% lack access to basic housing services (Figure 1). Based on a previous rabies vaccination campaign, a population of 1828 dogs was estimated in the locality.

Figure 1. Geographical location of Cuajinicuilapa, Guerrero, Mexico.

TVT prevalence. The complete locality was covered, on a house-to-house basis, to determine dog ownership. In those houses with one or more dogs and when dog owners allowed it, a genital exploration was performed in the animals to identify tumor masses or any other abnormality similar to TVT lesions. Information on the reproductive status of the animals and whether they were allowed to roam freely was also obtained.

All suspicious cases were referred to the Facultad de Medicina Veterinaria y Zootecnia, Unidad Académica No. 2, Universidad Autónoma de Guerrero, where a follow-up examination was performed as described below. While treatment was free of charge, only those dog-owners who wanted their animals to be treated took them to the follow-up.

Clinical diagnosis. It was established by anamnesis, determining the age and sex of each animal and measuring physiological constants like body temperature, respiratory rate (RR), heart rate (HR), capillary refill time (CRT), and body condition (BC) (14).

Cytological analysis. Tissue samples were obtained from dogs showing lesions suggestive of TVT for imprint cytology. The lesion area was washed with physiological saline and dried with a paper towel. A clean, degreased glass slide was placed on the zone, without pressing, trying to obtain various imprints in the same slide. Imprints were fixed with methanol, left to dry, and then stained with the Wright technique, to be observed under a microscope.

Biopsy. Tissue samples were surgically obtained for histopathological study and as a part of tumor treatment. The animals were tranquilized with 2% xylazine (Xilasyn® 2, Virbac, Mexico), intramuscularly (i.m.) administered at a dose of 1.1 mg/kg and anesthetized with intravenous (i.v.) pentobarbital (Pisabental®, Virbac, Mexico), at a dose of 10–30 mg/kg. Malignant lesions smaller than 7 cm in diameter were completely excised; in larger lesions, as much tissue as possible was removed. Tumors were kept in 10% buffered formalin until processed as described below.

Treatment. After surgery, the animals were administered with broad-spectrum antibiotic, 5% enrofloxacin (Enroxil 5%, Senosiain, Mexico) i.m. at a dose of 5 mg/kg/day for 3 days, along with i.m. flunixin meglumine (Megludyne®, Virbac, Mexico) as an analgesic, at a dose of 0.5 mg/kg for 3 days. Additionally, the dogs were administered with vincristine sulfate (Crivosin® Vet, PiSA, Mexico) by slow i.v. injection, at a dose of 0.025 mg/kg (1, 13); this dose was given weekly until malignancy regression.

Histopathological studies. Tissue samples were placed in plastic jars containing 10% buffered formalin and processed for histopathological analysis following a standard method and staining with H&E for microscopical observation.

Evaluating the spatial distribution of cases. The houses of those dog owners who allowed their animals to be diagnosed and treated were visited and georeferenced in an urban area map of the locality (Google Inc., Mountain View, CA, USA). The map was divided into 100 m² quadrants (see Figure 5) using the software Qgis v.3.10 (www.qgis.org). The location of cases was analyzed to determine the homogeneity of case dispersion, assuming a Poisson's distribution, and testing the homogeneity in each quadrant with a goodness-of-fit test for a Poisson distribution, using the following equation.

\[ X^2 = \sum_{i=1}^{m} \frac{(x_i - \bar{x})^2}{\bar{x}} = (m - 1) \frac{\hat{\sigma}^2}{\bar{x}} \]
$S^2/x$ times $(m-1)$ has an approximate chi-square distribution when the sample has a Poisson distribution; $m$ = total number of subdivisions, where each one contains $x_i$ values. The degrees of freedom are the number of observed subdivisions minus one, $m-1$.

**Recurrence determination.** Treated animals were followed up one year after treatment to determine their health status and evaluate possible relapses. The houses initially recorded were visited again, and the surviving animals were diagnosed by exploration of genital, nasal, and ocular mucous membranes.

**Result analysis.** Data were analyzed by descriptive statistics. Case frequency by age and sex was assessed by a chi-square test, and any difference of constants with respect to the parameters established was assessed by a Tukey’s t-test.

**Ethical considerations.** All dog owners provided a written consent for the inclusion of their pet animals in this study. Given its observational nature, no permit from the Ethics Committee was required for this work. All diagnostic and therapeutic interventions were free of charge for the dog owners.

**RESULTS**

**Population.** In total, 446 houses were visited, and 1047 dogs were examined, about 57% of the estimated population. From that universe, 271 houses (60.7%) had one or more dogs, while up to 12 animals were counted in a house. The mean number of dogs per house was 2.5.

With respect to sex, male dogs were slightly predominant, with 535 (51%) and 512 females (49%) (Table 1). All included animals were owned, and all of them were allowed in some degree to roam freely. All included animals were not spayed nor neutered.

**Prevalence.** Cytological analysis. In total, 63 dogs were suspicious by showing a lesion suggestive of TVT; however, only 54 dogs (5.15%) were determined as positive for TVT by cytology. The number of positive males (29.53%) was higher than that of females (25.47%), but this difference was not significant (OR: 1.11; CI: 0.64–1.93; p=0.69) (Table 1, Figure 2).

<table>
<thead>
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<th>Sex*</th>
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<td>29</td>
<td>5.4</td>
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<table>
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<tr>
<td>Total</td>
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</tr>
</tbody>
</table>

*OR: 1.11; CI: 0.64–1.93; p=0.69.

All physiological constants were within normal limits: body temperature, 38.5±0.40; RR 29.3±7.6; HR 83.3±17; CRT, 2; BC, 2.3±0.65.

With respect to age, the highest positivity frequency was found in 3-years-old dogs, with 16 cases (29.6%), but TVT infection cases were found in animals whose age ranged from 1 to 6 years (Table 1). The mean age of infected dogs was $3.14 \pm 1.35$ years.

![Figure 2. TVT in dogs. 1a) In a female, before, and 1b) after surgery and treatment. 2a) In a male before, and 2b) after surgery and treatment.](image-url)
The owners of 29 TVT-positive dogs (53.7%) allowed the animals to be treated and followed up, and a surgical excision of tumors was performed in 25 dogs.

**Cytology.** Lesion imprints showed neoplastic cells, with predominance of the lymphocytoid cytologic subtype, round cells with poorly defined cell borders, scanty and finely granular cytoplasm, with presence of vacuoles in the cell periphery. Nuclei are round, eccentric and with finely granular achromatic chromatin, with no obvious nucleoli. In other areas, neoplastic cells showed a plasmocytic, ovoid cells, poorly defined borders, abundant cytoplasm with presence of vacuole, and eccentric nuclei without evident nucleoli (Figure 3).

**Histopathological studies.** Histopathological analysis showed a group of round or slightly oval cells of mesenchymal origin, with scanty solid conjunctive support tissue and well-defined borders; moderate to abundant basophilic, finely granular cytoplasm; eccentric nucleus with broadly granular, hyperchromatic chromatin; other cells showed various small, round, basophilic, hyperchromatic nucleoli. Slight anisocytosis, karyorrhexis, karyolysis, and scanty mitotic cells were also observed (Figure 4).

**Treatment.** Treated dogs were given 1–8 doses of vincristine sulfate. Tumor involution was observed in all animals.

**Case distribution.** From the 54 TVT-positive dogs, 48 domiciles could be georeferenced. Case distribution and their frequency by quadrant is shown in Figure 5. According to a Poisson’s distribution, cases are not homogeneously distributed in the urban area (p<0.01).
Figure 5. Location of TVT-infected dogs in Cuajinicuilapa, Guerrero, Mexico. Total number of quadrants, 361:326 with no dogs, 26 with one dog; 7 with two dogs, and 2 with three dogs.

**Case follow-up.** When evaluated one year after treatment, no relapsing animal was observed.

**DISCUSSION**

To date, no other studies have been published on the prevalence of TVT in dogs from Guerrero, Mexico, particularly in the Costa Chica region. The wide variation in prevalence rates reported in various studies makes it difficult to compare data, especially because some works were conducted on stray dogs, while other studies focused on animals in shelters or dog pounds, and yet some others examined owned dogs, which changes the risk factors for the infection (5). Considering this, our prevalence was lower than that reported in Mexico, which has been about 20%. These results indicate that Mexico and other countries in Latin America have higher TVT prevalence rates with respect to Europe, Asia, and Oceania (5). It is noteworthy that, while the dogs under study are owned, they spend most of the day in the streets. Starkova and Murchison (5) found that Mexico is the most affected country in North America; they also observed a weak negative correlation between TVT prevalence and the socio-economic status of affected countries, along with a higher prevalence in places where dogs are allowed to roam freely; this latter situation was clearly observed in the locality, since most local dogs are allowed to roam the streets. This is relevant considering that the locality under study has an index of severe poverty of 23%, and an index of moderate poverty of 44% (15); thus, it is likely that pet welfare is not a priority for the inhabitants. Thus, actions that promote a better life for companion animals should be taken.

With respect to the role of sex in TVT prevalence, there are conflicting reports in the literature. Some studies showed a higher risk in females; for instance, de la Cruz *et al.* reported that 58% of infected animals were females (16). This is in contrast with our results, that show a slightly higher infection frequency in males, a similar trend to that described by Zerpa and Rojas, who found that 3 out of 5 positive TVT cases were males (17).

The proportion of dogs with respect to humans in Cuajinicuilapa, Guerrero, is similar to those found in other Mexican localities. The 1828 dogs recorded in a rabies vaccine campaign in a locality with approximately 9392 inhabitants yield a human:dog ratio of 5.13:1; this value is within the range of 6:1–4:1 found in the Cuahtémoc locality in Mexico City (18). The finding that virtually all dogs in the locality were not neutered or spayed increases the risk of TVT transmission; thus, we strongly suggest implementing sustained animal birth control and canine sterilization programs (19).

Although most dog owners in Cuajinicuilapa do not control the reproduction of their animals, the high mortality in the canine population, especially due to causes like going missing or being hit by cars, results in a constant replacement of the dog population and denotes a lack of care for the animals.

No precise information on the dynamics of the canine population in Cuajinicuilapa is available. This information is key to estimate the frequency of various conditions, including zoonotic diseases. Thus, it is necessary to make dog owners aware of the advantages of dog neutering and spaying and of preventing an uncontrolled reproduction, which only increase the population of street dogs and favors the dissemination of sexually-transmitted diseases like TVT.

The distribution of cases accumulated in certain areas of the locality can be explained considering that TVT is transmitted by direct contact, which poses a higher risk for animals living near a case, as well as the higher density of dogs in a given zone (20). Thus, the location of cases is relevant, since it would allow us to identify risk areas, especially in larger urban areas.
Further studies on the dynamics of dog populations are required, and these studies should not only include the animals in a group under study, but all dogs in this and other rural localities, to know the frequency of free roaming, the health services that the dogs receive, and their mortality rates, to set up effective strategies to prevent and control various diseases, including TVT.

**Conflict of interest**

The authors have declared no conflict of interest.

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**REFERENCES**


