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*Case report*

# BRAIN METASTASIS IN A CASE OF CANINE TRANSMISSIBLE VENEREAL TUMOR AFTER A SUPPOSED SUCCESSFUL TREATMENT WITH VINCRISTINE SULFATE

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A ten year old mongrel bitch was diagnosed with a primary vulvar transmissible venereal tumor (TVT) causing subcutaneous, mammary and splenic metastasis and it was successfully treated with vincristine. Four months later the animal presented neurological disturbances, brain metastases were suspected and the animal was euthanized. A TVT metastatic mass was found in the brain and confirmed with immunohistochemical results, being positive for vimentin and lysozyme and negative for S-100, CD3 and cytokeratin. TVT metastases in the brain are a rare event and cannot be treated with usual chemotherapy as vincristine does not cross the blood-brain barrier, thus allowing the re-emergence of the tumor after a period of time.

**Key words:** Blood-brain barrier, brain metastases, dog, immunohistochemistry, TVT, vincristine.

## INTRODUCTION

Canine transmissible venereal tumor (TVT) is a round cell neoplasia that can be transmitted by allotransplantation of tumor cells from affected dogs during copulation or through licking, biting, or scratching, especially in the presence of wounds or loss of surface integrity [1]. Recent studies demonstrate that TVT is the oldest known somatic cell lineage, and its cells first arose in a dog with low genomic heterozygosity that may have lived about 11,000 years ago. The cancer spawned by this individual dispersed across continents about 500 years ago [2].

The most frequent site for TVT is the mucous membrane of the external genitalia of dogs but extragenital locations such as the oral cavity, skin and internal organs are also possible [1,3]. Primary masses develop after implantation from another dog but secondary masses can be a result of auto-implantation starting from the original mass [4]. Metastases are estimated between 1.5 and 17% and are found in multiple locations

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such as subcutaneous tissue, lung, liver, spleen, kidneys, lymph nodes and the central nervous system (CNS) [5]. TVT presents an irregular global distribution being more common in tropical and subtropical regions where it is considered endemic [6] while it has not been documented in other areas. In general its prevalence and incidence are decreasing in developed countries [7].

Cytology is the method of choice for diagnosis since it is rapid, simple and presents little invasiveness [3]. Histopathology demonstrate sheets, rows and cords of relatively uniform round to ovoid cells with large nuclei, and a moderate amount of clear cytoplasm with a high mitotic index [7]. Immunohistochemical studies are required for differentiation with other round cell tumours, TVT showing constant immunoreactivity to vimentin, variable positivity to lysozyme and alpha-1-antitrypsine and negative results for cytokeratin, S-100 protein, lambda light chain immunoglobulin, IgG, IgM, CD3 and CD79a antigen [8,9]. TVT is singular in its responsiveness to a variety of treatments [10], chemotherapy with vincristine sulphate being the most effective [11]. In tumours resistant to vincristine, doxorubicin is the drug of choice [12].

## CASE PRESENTATION

A ten year old mongrel bitch was presented at the Veterinary Hospital with an 8 cm smooth-surface vulvar mass. There was also a 4x2 cm subcutaneous mass in the lateral thorax and several nodules with different sizes in both mammary chains. The dog also presented mild lethargy, decreased appetite and mild dehydration. Alkaline phosphatase was increased. Nodules in liver and spleen, hepatomegaly and enlargement of sub iliac lymph nodes were also observed. Fine needle cytological samples were taken from the vulvar and subcutaneous thoracic masses, from the mammary mass and from a splenic nodule, all of them resulting in a round cell tumour indicative of metastatic TVT with round to oval cells with well-defined cytoplasmic borders and an oval, variable sized, eccentric nucleus, showing 1 or 2 prominent nucleoli. Chemotherapy treatment with vincristine sulphate (0.7 mg/m$^2$ I.V. once a week) was initiated, leading to total recession of all masses after six weeks of treatment with the exception of the mammary masses, which only reduced in size. Cytological samples from the remaining mammary masses were taken demonstrating the presence of a mammary fibrosarcoma, but full mastectomy was rejected by the owners.

Four months after ending the treatment the animal presented neurological disturbances, with generalized seizures. The neurological exam performed showed moderated obtundation, tetraparesis with counter clockwise circles and reduced proprioception on the right limbs. During clinical examination the animal had a tonic-clonic seizure with several minutes' loss of conscience. A cerebral metastasis of the mammary fibrosarcoma was suspected and the animal euthanized. The brain showed a 2 cm mass on the grey matter of the left mid cerebral cortex (Fig. 1). Microscopically, the not encapsulated cerebral mass was infiltrated and highly cellular. It was formed by diffusely-distributed round cells sometimes forming rows with eosinophilic cytoplasm, round to oval nuclei, prominent nucleoli and a moderate number of mitotic figures.

There was a fine stroma formed by connective tissue and rare vessels. A moderate lymphocytic inflammatory infiltrate was present among the tumor cells (Fig. 2). In the adjacent cerebral tissue there were several cuffings of neoplastic cells and a moderate astrogliosis and satellitosis. Immunohistochemical (IHC) results demonstrated marked immunostaining for vimentin (Fig. 2), low cellular percentage positive for lysozyme and negative results for S-100 and cytokeratin (AE1/AE3). Immunoreaction to CD3 protein was only seen in lymphocytes infiltrating the tumour.

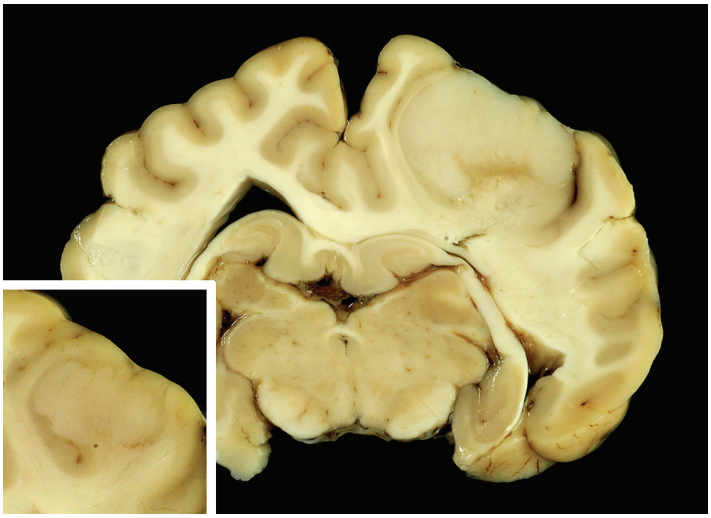

**Figure 1.** Mid cerebral cortex. Two centimeters mass in the gray matter, protruding into the white matter and causing pressure atrophy

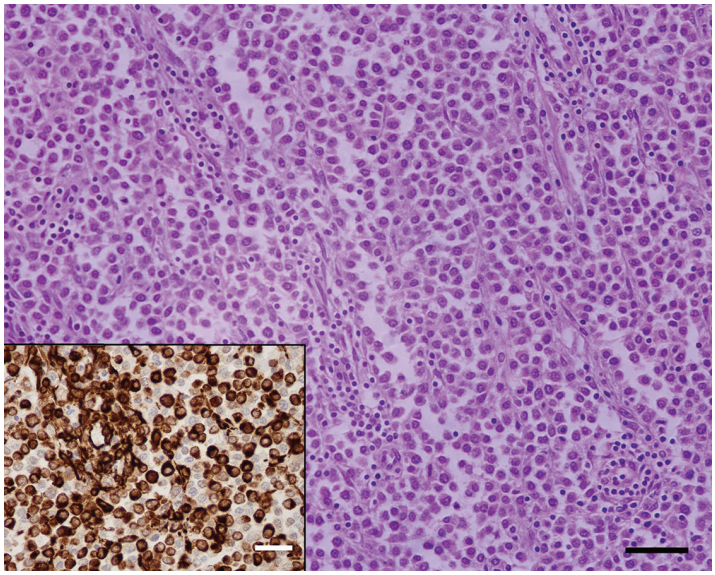

**Figure 2.** Section of the cerebral mass. Diagnostic dense round cell proliferation arranged in rows, infiltrated with lymphocytes and scarce connective tissue. HE 10x (scale bar 100 μm). Insert: Strongly positive cell immunostaining for vimentin. IHC 40x (scale bar 60 μm)

In addition, the animal exhibited a 10 cm, round mammary mass, with malignant spindle cell proliferation, associated with an intense, multifocal neutrophilic inflammatory infiltrate and large foci of necrosis, surrounded by macrophages, the mass being diagnosed as a mammary fibrosarcoma.

TVT seems to be a neoplasia of decreasing prevalence in Europe as only a few references have been published in the recent years [13,14]. However Murchison and co-authors [2] demonstrated the robustness of mammalian somatic cells to survive for millennia despite a massive mutation burden, which contributes for its perpetuity in the canine population. Therefore TVT can never be disregarded as a threat for dogs.

TVT chemotherapeutic treatment is well known and clinically reliable. The tumor rarely metastasizes and metastatic masses are susceptible to successful treatment. The importance of the present TVT case is that it showed widespread body metastases that included brain colonization and the later appearance of a metastatic TVT tumour in the CNS after a period of time of presumptive success after treatment. The treatment of choice for the TVT is chemotherapy with vincristine sulphate [12], having a high rate of success. However, vincristine sulphate does not cross the blood-brain barrier and if the TVT cells are able to colonize the CNS before the treatment, metastases will not be stopped in a later stage. In the present case, the animal presented several TVT masses on the first examination but all of them disappeared after six weeks of treatment. Apparently, TVT cells had crossed the blood-brain barrier and were able to develop a metastatic mass, only detected at necropsy. Ferreira and co-authors [5] also presented a case where some time after TVT treatment the animal presented neurological signs, therefore evidencing brain metastases. In some adult dogs the tumor regresses spontaneously after a period of logarithmic growth, due to the development of tumour immunity that also prevents successive recurrences [3]. Immunodeficiency states are related to the incapacity of spontaneous regression of the tumor and also a higher probability of metastasis. However, complete clinicopathological investigation in this case showed no evidence of immunodeficiency.

The immunohistochemical profile found was compatible with TVT, showing positive results for vimentin and lysozyme, being negative for cytokeratin, S-100 and CD3. Expression of lysozyme is normally seen, but in some cases it can be variable or even negative, evidencing a variability of its expression [9].

## CONCLUSION

In conclusion, this case is a reminder of the presence and the aggressive potential of canine TVT even after a supposedly successful treatment with appropriate chemotherapy. These findings are of great importance in the European context, where TVT seems to be reducing its prevalence and the awareness against it may be also decreasing.

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# METASTAZA TRANSMISIVNOG VENERIČNOG TUMORA U MOZGU PSA POSLE TRETMANA SA VINKRISTIN SULFATOM

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Kod kuje rase mešanac, stare 10 godina, dijagnostikovan je primarni transmisivni venerični timor (TVT) na vulvi, koji je uzrokovao stvaranje metastaza u supkutisu, mlečnoj žlezdi i slezini, a koji je uspešno tretiran vinikristinom. Četiri meseca kasnije, kod iste životinje su uočene nervne smetnje. Postavljena je sumnja da se radi o metastazama u mozgu, pa je životinja eutanazirana. Tumorske mase, kao posledica metastaze TVT, uočene su u mozgu. Nalaz je potvrđen imunohistohemijski, pri čemu je dobijena pozitivna reakcija na vimentin i lizozim, a negativna na S-100, CD3 i citokeratin. TVT metastaze u mozgu su retke i ne mogu da se tretiraju uobičajenom hemoterapijom pošto vinikristin ne prolazi krvno-moždanu barijeru. Iz tog razloga, omogućeno je ponovno pojavljivanje tumora posle izvesnog vremenskog perioda.