Songklanakarin J. Sci. Technol.
42 (3), 608-614, May - Jun. 2020

http://www.sjst.psu.ac.th

Original Article

Intraocular transmissible venereal tumors in dogs: A retrospective review of 21 cases

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Received: 31 December 2018; Revised: 20 February 2019; Accepted: 5 March 2019

Abstract

Twenty-nine canine intraocular transmissible venereal tumor (TVT) tissue samples from 21 dogs diagnosed between 2008 and 2014 were reviewed retrospectively. The following clinical data were compared: patient signalment; onset of clinical signs; ophthalmic signs; location of intraocular lesion; duration of unilateral intraocular TVT to bilateral intraocular TVT during the two-year follow-up period; and treatment. Thirty-eight percent (8/21) of the dogs with intraocular TVT eventually had bilateral intraocular TVT within two years. Seventy-five percent (6/8) of the dogs with bilateral intraocular TVT had initially presented with a unilateral lesion. Two dogs that presented with intraocular TVT had no history of genital or extragenital TVT. Chemotherapy did not lead to complete remission of intraocular TVT in contrast to genital and extragenital TVT. Enucleation was performed for all affected eyes. The spread of genital/extragenital TVT to unilateral or bilateral intraocular TVT should be considered and monitored for at least 24 months or longer.

Keywords: dog, eye, intraocular, transmissible venereal tumor

1. Introduction

Transmissible venereal tumors (TVT) commonly affect the genitalia. TVT is a histolytic tumor of the external genitalia and is transmitted during mating. It represents one-third of known transmissible cancers in mammals. This tumor can occur at any age and affects all dog breeds and no sex predilection. TVT is a benign tumor and is shown to regress in dogs with good body condition (Santos *et al.*, 2008). In dogs with reduced immune response due to conditions such as stress, young age, old age, immunosuppression or in general poor health, the tumor tends to grow aggressively and metastasize (Albanese, Salerni, Giodarno, & Marconato, 2006; Boscos, Ververidis, Tondis, Stamou, & Samartzi, 1998; Ferreira *et al.*, 2000; Mukaratirwa & Gruys, 2003). TVT cells are transmitted by contact of genital mucous membranes (Papazoglou, Koutinas, Plevraki, & Tontis, 2001), and extra-genital TVT can occur in the nasal or oral cavity via licking or

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sniffing the genital TVT (Siddle & Kaufman, 2014). Metastatic TVT spreads via hematogenous (Albanese *et al.,* 2006; Ferreira *et al.,* 2000) and lymphatic routes (Miller, Albert, & Boosinger, 1990; Rodrigues, Alessi, & Laus, 2001). The metastatic rate of TVT is 1.5–6% (Das & Das, 2000; Dass & Sahay, 1989; Pigatto, Hunning, Bercht, & de Albuquerque, 2011; Rodrigues *et al.,* 2001). Ocular involvement may be primary extragenital tumors via mucous membrane contact (Abbott, 1966; Komnenou, Thomas, Kyriazis, Poutahidis, & Papazoglou, 2015; Milo & Snead, 2014; Pigatto *et al.,* 2011) or presents as metastatic tumors (Almeida *et al.,* 2003; Boscos *et al.,* 1998; Ferreira *et al.,* 2000; Komnenou *et al.,* 2015; Pereira, Silva, Martins, Ferreira, & Brooks, 2000; Rodrigues *et al.,* 2001). Intraocular TVT tends to present more often as metastatic tumors than primary tumors (Das & Das, 2000; Ferreira *et al.,* 2000; Pereira *et al.,* 2000; Rodrigues *et al.,* 2001). Metastasis of TVT to the eye has been reported at the conjunctiva (Boscos *et al.,* 1998), nictitating membrane (Almeida *et al.,* 2003), orbit (Dass & Sahay, 1989), and intraocular tissue (Ferreira *et al.,* 2000; Miller *et al.,* 1990; Pereira *et al.,* 2000; Rodrigues *et al.,* 2001). The common presenting clinical signs of intraocular TVT are uveitis and elevated intraocular pressure (Pereira *et al.,* 2000).

The objective of this study was to review the signalment, clinical signs, the timing link with other sites and the second eye being affected, and the results of chemotherapeutic protocols.

2. Materials and Methods

Clinical data of 21 dogs with 29 intraocular TVT tissue samples were retrieved from the out-patient clinic and ophthalmology clinic of Kasetsart University Veterinary Teaching Hospital, Bangkok, Thailand between June 2008 and October 2014. Clinical data included sex, breed, age, onset of ophthalmic signs of intraocular TVT noticed by the owners after genital or extragenital TVT diagnosis, ophthalmic signs examined with slit-lamp biomicroscopy and tonometry, duration of unilateral intraocular TVT to bilateral intraocular TVT over a two-year follow-up period, treatment, and affected intraocular structure from the histopathological examination. In the case of unilateral intraocular TVT, the second eye was monitored during the two-year follow-up period.

Diagnosis of intraocular TVT was made by cytological (5 eyes) and histopathological examination (24 eyes). Initially, cytology was done in cases of visual field defect. Sector iridectomy was performed in one eye for histopathology due to its unidentified cytology. Twenty-nine eyes were enucleated and twenty-four eyeballs were submitted for pathological examination. The eyeballs were fixed in 10% (w/v) formalin, embedded in paraffin, sectioned at 4 µm and then stained with hematoxylin and eosin (H&E). Histopathological diagnosis of TVT was based on the histopathological criteria (Agnew & MacLachlan, 2017; Komnenou *et al.,* 2015). Intraocular TVT was characterized by an unencapsulated mass of round cells with large round nuclei, prominent single nucleolus to binucleoli and abundant cytoplasm. Cells were moderately anisocytosis and contained a high mitotic index.

Immunohistochemistry was performed on two eyes from two dogs which had no history of genital or extragenital TVT. The specimens were sectioned at 4 µm, placed on positively charged slides for labeling with S-100 protein (4C4.9, Cell Marque™, CA, USA), melanosome (HMB45, Dako Denmark A/S, Glostrup, Denmark), vimentin (V9, Cell Marque™, California, USA), cytokeratin (AE1/AE3, Novocastra™, Newcastle upon Tyne, UK), Melan-A (A103, Novocastra™, Newcastle upon Tyne, UK), CD3 (Dako Denmark A/S, Glostrup, Denmark), and Pax5 (1EW, Novocastra™, Newcastle upon Tyne, UK). Immunohistochemical examinations for S-100 protein, melanosome, vimentin, and cytokeratin were performed on an auto-immunohistochemistry stainer (Leica Microsystems Bond maX System, Leica Microsystems, Bannockburn, IL, USA) according to the manufacturer's recommendation at the Institute of Pathology, Department of Medical Services Ministry of Public Health, Bangkok, Thailand. Immunohistochemistry for Melan-A, CD3, and Pax5 was performed using the standard protocol (Pereira *et al.,* 2000). Briefly, all sections were performed by an antigen retrieval method in citrate buffer (pH 6.0) at 720 W in a microwave oven for 16 min before immunohistochemical staining. The specimens were incubated with primary antibody for 60 min in a humidified chamber at 37 °C. The antibodies were made visible with an indirect immunoperoxidase method. The secon-dary HRP-conjugated anti-rabbit antibody (DAKO EnVision™+/HRP kit) was applied. Diaminobenzidine chromogen was added to the specimens. The sections were counterstained with Mayer's hematoxylin.

Chemotherapy with vincristine sulfate (0.7 mg/m^2 IV weekly) for 4–6 weeks was administered for intraocular TVT in four dogs, with doxorubicin (30 mg/m^2 IV three weeks interval) for 2–3 cycles in two intraocular dogs, and with vincristine followed by vinblastine (2 mg/kg IV two weeks interval) combined with methotrexate (0.1 mg/kg PO every other day) in one intraocular dog. Enucleation without chemotherapy was performed due to severe uveitis and glaucoma in 14 dogs. The follow-up for the second eye was done for two years post-operatively.

3. Results

Of the 21 dogs with intraocular TVT, 19 were males and only two were females. The mean age was 5.52±2.23 years (range 2–10 years). Sixteen were mixed breed and the others were Golden Retriever (2), Shih Tzu (1), Chihuahua (1), and Poodle (1). Only two dogs presented with bilateral intraocular TVT. Seven dogs had a previous history of genital TVT, two dogs had a previous history of genital TVT concurrent with extragenital TVT, and one dog had a history of extragenital TVT at the subcutaneous tissue of the dorsal thorax. Three dogs had concurrent intraocular TVT with genital TVT. One dog had intraocular TVT with extragenital TVT and one had intraocular TVT with genital and extragenital TVT at the time of presentation. Two dogs did not have any history of TVT. At the two-year follow-up, unilateral intraocular TVT was diagnosed in 13 dogs (six in the right eye and seven in the left eye) and bilateral intraocular TVT was diagnosed in eight dogs (Table 1).

The most common presenting signs of intraocular TVT were conjunctivitis (28/29, 96.55%), vision loss (26/29, 89.66%), corneal edema (25/29, 86.21%), abnormal mass within the eye (22/29, 75.86%), aqueous flare (22/29,

Table 1. Signalment and duration of intraocular transmissible venereal tumor (TVT) after presenting with external genitalia or extragenital TVT.

Case No.	Breed	Age (y)	Sex	Eye	Location	Genital/extragenital TVT	Duration genital/extragenital TVT to eye (months)	Duration between both eyes (months)
1	Chihuahua	5	M	OD	Iris, ciliary body	Penis, inguinal, popliteal lymph node, skin, sublumbar mass	5	-*
2	Poodle	7	M	OD OS	Iris, ciliary body n/a	Penis, both popliteal lymph node, Lf. axillary lymph node, skin	1.5	0.5
3	Cross	4	M	OS OD	Iris, ciliary body Iris, ciliary body	Prepuce, skin	0	6
4	Cross	4	M	OD	Iris, ciliary body	Prepuce	1	-*
5	Cross	6	M	OS OD	n/a Iris, ciliary body	Penis	0	24
6	Cross	7	F	OS	Iris, ciliary body	Penis	24	-*
7	Cross	5	M	OS OD	Iris, ciliary body Iris, ciliary body, choroid	Penis	4	2
8	Cross	9	M	OD	Iris, ciliary body	Back/dorsal thorax	0	-*
9	Golden Retriever	9	M	OD	Iris, ciliary body	None	-	-*
10	Cross	8	M	OS	Iris, ciliary body	Penis	9	-*
11	Cross	7	M	OD	Iris, ciliary body	Penis	13	OS: enucleation from other cause
12	Golden Retriever	4	F	OD	Iris, ciliary body, choroid	None	-	-*
13	Cross	2	M	OS OD	Iris, ciliary body, subretinal choroid n/a	Penis	0	8
14	Cross	4	M	OD OS	Iris, ciliary body, subretinal choroid n/a	Skin form	2	0
15	Cross	2	M	OS OD	n/a Iris, ciliary body	Penis	8	4
16	Cross	5	M	OS	Iris, ciliary body, choroid	Prepuce	0	-*
17	Cross	10	M	OS	Iris, ciliary body	n/a	n/a	n/a
18	Cross	5	M	OD OS	Iris, ciliary body Iris, ciliary body	n/a	n/a	0
19	Shih Tzu	5	M	OS	Iris, ciliary body	n/a	n/a	n/a
20	Cross	3	M	OS	Iris, ciliary body	n/a	n/a	-*
21	Cross	5	M	OS	Iris, ciliary body	Prepuce	n/a	-*

n/a = not available because of diagnosis by cytology
-* = Intraocular TVT was present only in one eye

75.86%), secondary glaucoma (18/29, 62.07%), buphthalmos (16/29, 55.17%), hyphema (14/29, 48.28%) (Figure 1), and ocular perforation (2/29, 6.90%).

Cytological results showed numerous round cells. These cells have round, concentric nuclei with 1–2 prominent nucleoli. The chromatin was coarse and sometimes mitosed. The cytoplasm was abundant with some refractile vacuoles. The histopathological findings of intraocular masses after enucleation revealed typical TVT with an unencapsulated mass of loosely packed round cells separated by delicated fibrovascular stromal tissue. The cells were hyperchromatic with round to oval nuclei, coarse chromatin, and prominent nuclei as was found in the cytology. The cells were moderately anisocytotic. Mitotic figures were frequently seen (Figure 3a). Intraocular TVT was found in the iris and ciliary

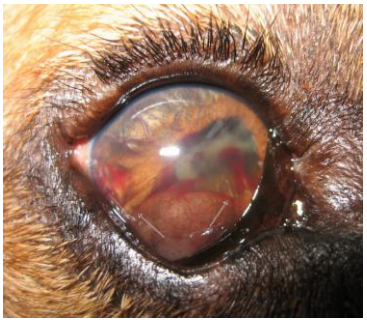

Figure 1. Clinical presentation of intraocular TVT of the right eye, showing the hyphema, hypopyon, posterior synechia, and iris mass at 5–7 o'clock (arrow).

body in 19 eyes and involved the iris, ciliary body, and choroid in five eyes (Figure 2). Immunohistochemical studies of the ocular mass in two dogs with no history of genital or extragenital TVT revealed tumor cells that were immune-positive for vimentin (Figure 3b) and negative for the S-100 protein, melanosome, Melan-A, cytokeratin, CD3, and Pax5 (Figures 3c–h).

The mean duration of intraocular TVT signs after presentation of genital or extragenital TVT of 14 dogs observed by the owner was 6.5±8.4 months (range 0–24 months), while the mean duration of intraocular TVT spreading from one eye to the other eye in 8 dogs was 5.6±8.0 months (range 0–24 months).

Weekly single agent (vincristine) chemotherapy was used for treatment of genital or extragenital TVT in nine dogs (case nos. 1, 2, 3, 7, 10, 11, 14, 15, and 16), and led to complete remission after 4–8 weeks of treatment in eight dogs. One dog (case no. 1) did not respond to treatment, and the genital and subcutaneous skin forms of TVT spread to the lymph nodes. This dog was subsequently administered vinblastine with doxorubicin (Calvert, Leifer, & MacEwen, 1982) and the TVT masses at the subcutaneous tissue and lymph nodes went into partial remission. Surgery was performed to remove inguinal lymph nodes and a subcutaneous mass together with biopsy of the inguinal lymph nodes. The owner of one dog (case no. 4) declined genital TVT chemotherapy treatment. Surgical mass removal at the back and dorsal thorax was done in one dog (case no. 8) without chemotherapy. The information regarding treatment of genital or extragenital TVT could not be retrieved in eight dogs (case nos. 5, 6, 13, 17, 18, 19, 20, and 21). Two dogs (case nos. 9, and 12) did not have a history of genital or extragenital TVT. Chemotherapy was subsequently performed in seven dogs with intraocular TVT. Four dogs (case nos. 2, 3, 5, and 14) received vincristine (Pigatto *et al.*, 2011; Ucar, 2016) (0.7 mg/m^2 IV weekly), two dogs (case nos. 1 and 7) received doxorubicin (Calvert *et al.*, 1982) (30 mg/m^2 IV three weeks interval), and one dog (case no. 11) received vincristine followed by vinblastine (Singh, Rana, Sood, Pangawkar, & Gupta, 1996) (0.1 mg/kg IV two weeks interval) combined with methotrexate (Boscos, 1988) (0.1 mg/kg PO every other day). Enucleation without chemo-therapy was performed in 14 dogs due to severe intraocular damage.

Chemotherapy was unsuccessful in all dogs in treating the intraocular masses. Initially, the masses showed partial remission and the ocular signs improved, but then the tumors recurred and progressed. Hence, enucleation was recommended due to the uncontrolled severe uveitis and secondary glaucoma.

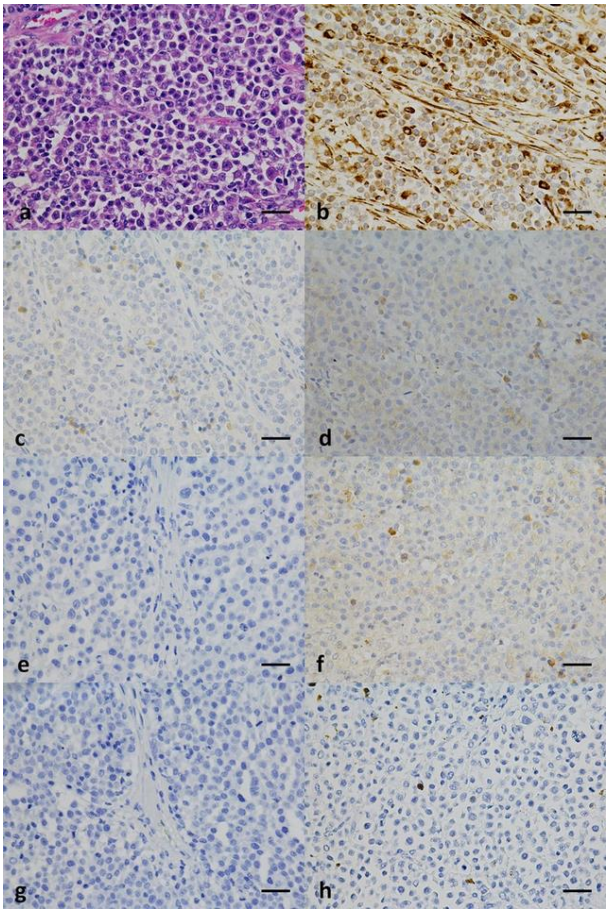

Figure 3. (a) Histopathological findings of a poorly circumscribed unencapsulated mass of neoplastic discrete cells display hypercellularity and moderately anisocytosis. Cells contain hyperchromatic nuclei, prominent single nucleolus to sometimes binucleoli, and abundant cytoplasm. The mitotic index was high. H&E, positive immunoreactivity for (b) vimentin, and negative immunoreactivity for (c) S-100 protein, (d) melanosome, (e) Melan-A, (f) cytokeratin, (g) CD3, and (h) Pax5. (Bar = 20 µm).

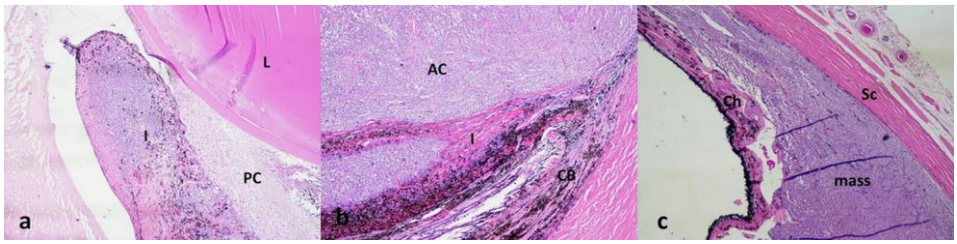

Figure 2. (a) The iris shows massive infiltration of discrete cells that displayed hyperchromatic nuclei, prominent nucleoli, and abundant cytoplasm. Vascular congestion and multifocal necrosis are also seen. Posterior chamber accumulated infiltrating inflammatory cells, necrotic debris, and red blood cells. (b) Iris and ciliary body are massively infiltrated by neoplastic cells. (c) An unencapsulated mass of neoplastic discrete cells over the choroid causing retinal separation and distortion. (I: Iris, PC: Posterior chamber, L: Lens, CB: Ciliary body, AC: Anterior chamber, Ch: Choroid, Sc: Sclera) (H&E, 4x).

4. Discussion

In this study, intraocular TVT presented more frequently in male than female dogs, consistent with previous reports (Boscos, 1988; Boscos *et al.,* 1998; Komnenou *et al.,* 2015; Papazoglou *et al.,* 2001; Srivastava, Singh, Srivastava, Sharama, & Sinha, 2013). In addition, metastatic cases in previous reports were observed mostly in adult male dogs (Boscos, 1988; Pandey, Chandpuria, Bhargava, & Tiwari, 1989). This result contrasted with reports which stated that the incidence of TVT was more common in female than male dogs (Santiago-Flores, Jaro, Recuenco, Reyes, & Amparo, 2012; Singh *et al.,* 1996). That can possibly be explained by the male behavior of mating with several females (Das & Das, 2000; Komnenou *et al.,* 2015). A recent report suggested no gender difference in canine TVT (Strakova & Murchison, 2014). However, intraocular involvement has not been reported.

Canine TVT is commonly reported in dogs 2–5 years of age (Pigatto *et al.,* 2011; Das & Das, 2000). That is consistent with the average age of intraocular TVT in this report. Increased sexual activity at a young age is a possible explanation for the incidence of canine TVT in this age group (Pigatto *et al.,* 2011; Santiago-Flores *et al.,* 2012).

Mixed breed was the most common type of dog in this state, which was consistent with some previous reports (Calvert *et al.,* 1982; Komnenou *et al.,* 2015; Papazoglou *et al.,* 2001; Rogers, Walker, & Dillon, 1998; Srivastava *et al.,* 2013) and in contrast with one previous report (Das & Das, 2000). This might simply reflect that mixed breeds form the major proportion of the dog population in Thailand, rather than their greater susceptibility. Similar to previous reports, uveitis and glaucoma were the most common presenting signs (Pereira *et al.,* 2000).

In the two-year follow-up period, the development of intraocular TVT after genital or extragenital TVT ranged from 0 to 24 months. The longest time observed for the spread from one site to the other site in this study was 24 months. Therefore, at least a 24-month if not longer follow-up period is recommended. In general, sector iridectomy in an early-detected iridal tumor may be beneficial for saving the eye (Diters, Dubielzig, Aguirre, & Acland, 1983; Gelatt, Johnson, & Peiffer, 1979), but it seemed to be an ineffective treatment for intraocular TVT because of the high risk of recurrence (Dass &Sahay, 1989; Komnenou *et al.,* 2015; Ucar, 2016). This was consistent with our finding in dog no. 11. Close monitoring of ocular abnormalities after an initial TVT diagnosis might be useful for early detection but ultimately unlikely to change the outcome.

Intraocular TVT was found mostly at the anterior or posterior uvea because they are highly vascularized and that is where metastatic cells enter the eye (Ferreira *et al.,* 2000). Local invasion from masses next to the eye was rare because of the tough fibrous coat being the barrier (Gould, 2003). It was surprising that intraocular TVT possibly occurred even without pre-existing genital or other extragenital TVT as in case no. 9 and case no. 12. Those two dogs had only one owner since the dogs were young and had no history of genital and extragenital TVT. Extragenital TVT in the absence of pre-existing genital involvement was typically the result of primary auto- or hetero-implantation (Milo & Snead, 2014). The mechanism of intraocular TVT without pre-existing genital or extragenital TVT is still elusive.

Biopsy was the most reliable method for definitive diagnosis of TVT (Das & Das, 2000; Nak, Nak, Cangul, & Tuna, 2005). However, the diagnosis of intraocular TVT from two dogs with no history of preexisting TVT was confirmed by negative immunohistochemical staining to S-100, melanosome, Melan-A, cytokeratin, CD3, and Pax5, in addition to the positive staining for vimentin (Mozos, Mendez, Gomez-Villamandos, Martin de las Mulas, & Perez, 1996; Pereira *et al.,* 2000). Their expression was used for a differential diagnosis to exclude lymphoma, histiocytic tumor, amelanotic melonoma, poorly differentiated carcinoma, and poorly differentiated mast cell tumor (Mascarenhas *et al.,* 2014; Mozos *et al.,* 1996; Pereira *et al.,* 2000). Vimentin is a specific marker of mesenchymal derivation or differentiation (Leader, Collins, Patel, & Henry, 1987; Mascarenhas *et al.,* 2014; Mukaratirwa & Gruys, 2003) and stains positive in TVT, fibrosarcoma, melanoma, hemangiosarcoma, mastocytoma, leiomyosarcoma, and liposarcoma (Pereira *et al.,* 2000). However, S-100 was used to differentiate TVT from neurogenic sarcoma, melanoma, hemangiosarcoma, liposarcoma, and leiomyosarcoma (Ferreira *et al.,* 2000; Pereira *et al.,* 2000), while melanosome and Melan-A were used to rule out melanoma (Pereira *et al.,* 2000). Cytokeratin was used to differentiate TVT from poorly differentiated carcinomas, such as squamous cell carcinoma and apocrine adenocarcinoma/carcinoma (Ferreira *et al.,* 2000; Mozos *et al.,* 1996; Pereira *et al.,* 2000), while CD3, Pax5, and vimentin were used to differentiate TVT from lymphoma (Ferreira *et al.,* 2000; Pereira *et al.,* 2000). Previous immunohistochemical studies for canine TVT were lysozyme, ACM1, and alpha-1-antitrypsin (ATT) (Mozos *et al.,* 1996; Mukaratirwa and Gruys, 2003), which were specific for histiocytic cell origin and canine TVT. Immunoreactivity of lysozyme was variable ranging from 0% (Mascarenhas *et al.,* 2014) to 40% (Mozos *et al.,* 1996), and 100% (Marchal, Chabanne, Kaplanski, Rigal, & Magnol, 1997), whereas ACM1 was 79% (Marchal *et al.,* 1997), and ATT was 56% (Mozos *et al.,* 1996). The differential diagnosis between TVT and histiocytoma should be based on the clinical and histiopathologic characteristics (Mozos *et al.,* 1996; Mukaratirwa & Gruys, 2003) because there is no significant difference between the immunophenotype of histiocytomas and TVT. These results confirmed that intraocular TVT should also be considered in dogs with no history of genital or extragenital TVT. According to previous reports, metastasis can occur in dogs that had no primary genital or extragenital TVT, if the primary lesion had completely regressed before examination (Boscos *et al.,* 1998; Das & Das, 2000; Mozos *et al.,* 1996).

The standard treatment for genital TVT is chemotherapy with vincristine. Remission has ranged from 82 to 100% (Amber, Henderson, Adeyanju, & Gyang, 1990; Calvert *et al.,* 1982). Complete remission of TVT of the adnexa, such as conjunctiva and nictitating membrane, tended to be achieved after chemotherapy with vincristine (Almeida *et al.,* 2003; Boscos *et al.,* 1998; Komnenou *et al.,* 2015; Milo & Snead, 2014; Pigatto *et al.,* 2011). In contrast, intraocular TVT was not responsive to treatment with vincristine (Ferreira *et al.,* 2000; Miller *et al.,* 1990; Pereira *et al.,* 2000). Doxorubicin was usually effective for genital or extragenital

TVT remission (Calvert *et al.*, 1982; Nak *et al.*, 2005). However, vincristine or doxorubicin failed to provide complete intraocular TVT remission in this study. A combination of vinblastine and methotrexate, which had a variable response on genital TVT, was also ineffective for complete intraocular TVT remission. Regardless of chemotherapy and response, intraocular TVT usually causes severe uveitis and secondary glaucoma resulting in enucleation (Boscos, 1988; Pereira *et al.*, 2000; Rodrigues *et al.*, 2001; Willis & Wilkie, 2001).

5. Conclusions

This report presented the characteristics of intraocular TVT including signalment, common clinical signs, duration of genital TVT or extragenital TVT spreading to intraocular TVT, and duration of intraocular TVT spreading from one eye to the other eye. In addition, intraocular TVT with no previous history of genital or extragenital TVT may be present and should be considered. All eyes with intraocular TVT required enucleation. Unfortunately effective chemotherapy or treatment techniques for intraocular TVT are not yet available.

References

Abbott, P. K. (1966). Venereal transmissible tumour on eyelid of dog. *Australian Veterinary Journal*, *42*, 29. doi:10.1111/j.1751-0813.1966.tb04609.x

Agnew, D.W., & MacLachlan, N. J. (2017). Tumors of the genital systems. In D. J. Meuten (Ed.), *Tumors in domestic animals* (5th ed.) (pp. 689-722). Iowa, IA: John Wiley and Sons.

Albanese, F., Salerni, F. L., Giodarno, S., & Marconato, L. (2006). Extragenital transmissible venereal tumour associated with circulating neoplastic cells in an immunologically compromised dog. *Veterinary and Comparative Oncology*, *4*, 57-62. doi:10.1111/j.1476-5810.2006.00092.x

Almeida, O. C., Pimenta, P. A., Lopes, A. P., Costa, M. M., Oliveira, J. P., & Sousa, A. P. (2003). Joint meeting of the British Association of Veterinary Ophthalmologists, European College of Veterinary Ophthalmologists, European Society of Veterinary Ophthalmology, and the International Society of Veterinary Ophthalmology, Cambridge, UK. June 26–29, 2003: Abstract No. 13: Ocular localization of canine transmissible venereal tumor: A case report and literature review. *Veterinary Ophthalmology*, *6*, 343-350. doi:10.1111/j.1463-5224.2003.00324.x

Amber, E. I., Henderson, R. A., Adeyanju, J. B., & Gyang, E. O. (1990). Single-drug chemotherapy of canine transmissible venereal tumor with cyclophosphamide, methotrexate, or vincristine. *Journal of Veterinary Internal Medicine*, *4*, 144-147. doi:10.1111/j.1939-1676.1990.tb00887.x

Boscos, C. (1988). Transmissible venereal tumour in the dog: Clinical observations and treatment. *Animalis Familiaris*, *3*, 10-15.

Boscos, C. M., Ververidis, H. N., Tondis, D. K., Stamou, A. I., & Samartzi, F. C. (1998). Case report: Ocular involvement of transmissible venereal tumor in a dog. *Veterinary Ophthalmology*, *1*, 167-170. doi:10.1046/j.1463-5224.1998.00012.x

Calvert, C. A., Leifer, C. E., & MacEwen, E. G. (1982). Vincristine for treatment of transmissible venereal tumours in the dog. *Journal of the American Veterinary Medical Association*, *181*, 163-164.

Das, U., & Das, K. (2000). Review of canine transmissible venereal sarcoma. *Veterinary Research Communications*, *24*(8), 545-556. doi:10.1023/A:1006491918910

Dass, L. L., & Sahay, P. N. (1989). Surgical treatment of canine transmissible venereal tumour – a retrospective study. *Indian Veterinary Journal*, *66*, 255-258.

Diters, R. W., Dubielzig, R. R., Aguirre, G. D., & Acland, G. M. (1983). Primary ocular melanoma in dogs. *Veterinary Pathology*, *20*, 379-395. doi:10.1177/030098588302000401

Ferreira, A. J. A., Jaggy, A., Varejao, A. P., Ferreira, M. L. P., Correia, J. M. J., Mulas, J. M., . . . Prada, J. (2000). Brain and ocular metastases from a transmissible venereal tumour in a dog. *Journal of Small Animal Practice*, *41*, 165-168. doi:10.1111/j.1748-5827.2000.tb03187.x

Gelatt, K. N., Johnson, K. A., & Peiffer, R. L. (1979). Primary iridal pigmented masses in three dogs. *Journal of the American Animal Hospital Association*, *15*, 339-344.

Gould, D. (2003). Ocular tumours. In J. M. Dobson & B. D. Lascelles (Eds.), *BSAVA Manual of canine and feline oncology* (2nd ed.) (pp. 329-337). Gloucester, England: British Small Animal Veterinary Association.

Komnenou, A. T., Thomas, A. L. N., Kyriazis, A. P., Poutahidis, T., & Papazoglou, L. G. (2015). Ocular manifestations of canine transmissible venereal tumour: A retrospective study of 25 cases in Greece. *Veterinary Record*, *176*, 523-527. doi:10.1136/vr.102968

Leader, M., Collins, M., Patel, J., & Henry, K. (1987). Vimentin: An evaluation of its role as a tumour marker. *Histopathology*, *11*(1), 63-72. doi:10.1111/j.1365-2559.1987.tb02609.x

Marchal, T., Chabanne, L., Kaplanski, C., Rigal, D., & Magnol, J. P. (1997). Immunophenotype of the canine transmissible venereal tumour. *Veterinary Immunology and Immunopathology*, *57*, 1-11. doi:10.1016/S0165-2427(96)05757-1

Mascarenhas, M. B., Peixoto, P. V., Ramadinha, R. R., Yamasaki, E. M., Costa, S. Z. R., Driemeier, D., . . . Franca, T. N. (2014). Immnunohistochemical study of genital and extragenital forms of canine transmissible venereal tumor in Brazil. *Pesquisa Veterinária Brasileira*, *34*, 250-254. doi:10.1590/S0100-736X2014000300009

Miller, W. W., Albert, R. A., & Boosinger, T. R. (1990). Ocular metastasis of a transmissible venereal tumor. *Canine Practice*, *15*, 19-21.

Milo, J., & Snead, E. (2014). Case report: A case of ocular canine transmissible venereal tumor. *Canadian Veterinary Journal*, *55*, 1245-1249.

Mozos, E., Mendez, A., Gomez-Villamandos, J. C., Martin de las Mulas, J., & Perez, J. (1996). Immuno-histochemical characterization of canine transmissible venereal tumor. *Veterinary Pathology*, *33*, 257-263. doi:10.1177/030098589603300301

Mukaratirwa, S., & Gruys, E. (2003). Canine transmissible venereal tumour: Cytogenetic origin, immune-phenotype, and immunobiology. A review. *Veterinary Quarterly*, *25*, 101-111. doi:10.1080/01652176.2003.9695151

Nak, D., Nak, Y., Cangul, I. T., & Tuna, B. (2005). A clinico-pathological study on the effect of vincristine on transmissible venereal tumour in dogs. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine*, *52*, 366-370. doi:10.1111/j.1439-0442.2005.00743.x

Pandey, S. K., Chandpuria, V. P., Bhargava, M. K., & Tiwari, S. K. (1989). Incidence, treatment, approach and metastasis of canine transmissible venereal sarcoma. *Indian Journal of Animal Sciences*, *59*, 510-513.

Papazoglou, L. G., Koutinas, A. F., Plevraki, A. G., & Tontis, D. (2001). Primary intranasal transmissible venereal tumour in the dog: A retrospective study of six spontaneous cases. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine*, *48*, 391-400. doi:10.1046/j.1439-0442.2001.00361.x

Pereira, J. S., Silva, A. B. F., Martins, A. L. B., Ferreira, A. M. R., & Brooks, D. E. (2000). Case report: Immunohistochemical characterization of intraocular metastasis of a canine transmissible venereal tumor. *Veterinary Ophthalmology*, *3*, 43-47. doi:10.1046/j.1463-5224.2000.00097.x

Pigatto, J. A. T., Hunning, P. S., Bercht, B. S., & de Albuquerque, L. (2011). Transmissible venereal tumor in the palpebral conjunctiva of a dog: Case report. *Semina: Ciências Agrárias*, *32*, 1139-1144. doi: 10.5433/1679-0359.2011v32n3p1139

Rodrigues, G. N., Alessi, A. C., & Laus, J. L. (2001). Intraocular transmissible venereal tumor in a dog. *Ciência Rural*, *31*, 141-143. doi:10.1590/S0103-84782001000100023

Rogers, K. S., Walker, M. A., & Dillon, H. B. (1998). Transmissible venereal tumor: A retrospective study of 29 cases. *Journal of the American Animal Hospital Association*, *34*, 463-470. doi:10.5326/15473317-34-6-463

Santiago-Flores, M. L., Jaro, M. C., Recuenco, F. C., Reyes, M. F., & Amparo, M. R. G. (2012). Clinical profile of canine transmissible venereal tumor cases. *Philippine Journal of Veterinary and Animal Sciences*, *38*, 63-72.

Santos, F. G. A., Vasconcelos, A. C., Nunes, J. E. S., Cassali, G. D., Paixão, T. A., Martins, A. S., . . . Moro, L. (2008). Apoptosis in the transplanted canine transmissible venereal tumor during growth and regression phases. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *60*, 607-612. doi: 10.1590/S0102-09352008000300013

Siddle, H. V., & Kaufman, J. (2014). Immunology of naturally transmissible tumours. *Immunology*, *144*, 11-20. doi: 10.1111/imm.12377

Singh, J., Rana, J. S., Sood, N., Pangawkar, G. R., & Gupta, P. P. (1996). Clinico-pathological studies on the effect of different anti-neoplastic chemotherapy regimens on transmissible venereal tumours in dogs. *Veterinary Research Communications*, *20*, 71-81. doi:10.1007/BF00346579

Srivastava, A. K., Singh, B., Srivastava, A. K., Sharama. A. K., & Sinha, N. (2013). Canine transmissible venereal tumours (CTVT): A study on occurrence and distribution pattern. *Indian Journal of Canine Practice*, *5*, 65-71.

Strakova, A., & Murchison, E. P. (2014). The changing global distribution and prevalence of canine transmissible venereal tumour. *BMC Veterinary Research*, *10*, 168. doi:10.1186/s12917-014-0168-9

Ucar. M. (2016). Transmissible venereal tumor: A review. *Kocatepe Veterinary Journal*, *9*, 230-235. doi:10.5578/kvj.26524

Willis, A. M., & Wilkie, D. A. (2001). Ocular oncology. *Clinical Techniques in Small Animal Practice*, *16*, 77-85. doi:10.1053/svms.2001.22810