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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 842-845

www.thepharmajournal.com
Received: 04-10-2021
Accepted: 06-11-2021

Dr. A Arulmozhi
M.V.Sc., Ph.D., Assistant Professor, Department of Veterinary Pathology Veterinary College and Research Institute, Salem, Tamil Nadu, India

Dr. P Shanumugavadivu
M.V.Sc., Scholar, Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Dr. M Selvaraju
Ph.D., Dean, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Dr. K Elamurugan
M.V.Sc., Scholar, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Dr. GA Balasubramaniam
Professor and Head, Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Corresponding Author
Dr. A Arulmozhi
M.V.Sc., Ph.D., Assistant Professor, Department of Veterinary Pathology Veterinary College and Research Institute, Salem, Tamil Nadu, India

Pathomorphological studies on canine transmissible venereal tumour (CTVT) in 320 cases

Dr. A Arulmozhi, Dr. P Shanumugavadivu, Dr. MSelvaraju, Dr. K Elamurugan and Dr. GA Balasubramaniam

Abstract
The aim of the present study is to explore the incidence of genital tumours in dogs. The dogs with tumour mass at the external genitalia brought to Veterinary Clinical Complex (VCC) were recorded from 2015 to 2019. The gross lesions were noted and impression smears from the tumour mass were collected for cytological studies. Biopsy samples / surgically removed masses were collected in 10 per cent neutral buffered formalin for histopatholgical examination. The higher incidence occurred in bitches than male dogs especially during 4 to 5 years of age. Breed-wise incidence showed that the Non-Descriptive dogs are mostly affected followed by chippiparai. Grossly, there was small papule to cauliflower like growth noticed in external genitalia of male and female dogs. The cytological picture exhibited sheets of discrete round to oval cells with punctate cytoplasmic vacuoles and were confirmed as Transmissible Venereal Tumour (TVT). Histopathology of TVT tissue sections showed sheets of round cells with vesicular nucleus, thin fibrovascular stroma and numerous mitotic figures. Interestingly, two cases of extra genital form of TVT also recorded during this period.

Keywords: cytology, dog, histopathology and TVT

Introduction
Canine transmissible venereal tumour (CTVT), also known as Sticker's sarcoma is a round cell transmissible cancer that affects dogs. CTVT causes tumours usually associated with the external genitalia of both male and female dogs. It is spread by the transfer of living cancer cells between dogs, usually during mating (Das and Das, 2000; Tella *et al.*, 2004) [1, 2] but also be transferred by licking, sniffing or biting of tumour affected areas (Murchison, 2008) [3]. It also affects skin via direct implantation of tumour cells during contact between skin and tumour cells (Stockmann *et al.*, 2011) [4]. The TVT cells contain abnormal number of chromosome 59 ranging from 57 to 64 instead of normal number of 78 found in other cells of the dog (Purohit, 2009) [5]. Its global distribution is associated with the presence of free-roaming dogs.

The CTVT is a foreign graft within its host and is able to evade host immune mechanism, allowing its worldwide spread in dogs (Belov, 2012) [6]. The mechanism of CTVT to escape from the immune system may be due to down-regulation of major histocompatibility complex (MHC) molecules and recruitment of an immunosuppressive microenvironment. CTVT is usually treated with chemotherapy and most dogs respond well to three intravenous injections of vincristine sulfate at weekly intervals. Regression is the result of a humoral immune response (IgG) that makes the dog highly resistant to subsequent tumor implantation (Meuten, 2002) [7].

The incidence of CTVT cases was getting increased during recent years. Hence, a detailed study was carried out to assess the age, sex and breed susceptibility, cytomorphological features and histopathology pattern of TVT.

Materials and Methods
Data collection
Male and female dogs with tumour growth in the external genitalia were regularly brought to Veterinary Clinical Complex for treatment. They were treated with chemotherapeutic drugs. Based on the location and response to treatment, surgery was performed to remove the growth. Detailed clinical data was recorded viz. age, sex, breed, duration of the mass, breeding history, location of the mass / masses, respond to treatment, surgical requirement and incidence of extra genital form.

Gross pathology
The sizes, shape, location of the mass in the genital area were recorded. In the extra genital form of TVT, the number and location of the variable growth were also noted.

Cytology
Impression method, exfoliative cytology and fine needle aspiration cytology were employed to collect the cytological smears depends on size and location of the tumour. Impression cytology was taken by applying gentle pressure on a slide against the neoplastic tissue and allowing the smear to dry at room temperature. Cotton swab was used to collect the exfoliated TVT cells in the genital tract. Fine needle aspiration cytology (FNAC) was taken using 23-25 Gauge needle and 5 ml syringe. After collection, the slides were fixed in methanol for 5 minutes and were subjected to Giemsa and Leishman stain to study the cytomorphological features of TVT (Cowell and Meinkoth, 2008) [8]. Based on the microscopic observation of the cytological picture, they were categorized into three groups (Ajayi *et al.,* 2018*;* Amaral *et al.,* 2007) [9, 10].

Histopathology
The biopsy specimen from intact growth, tissue from surgically removed masses and the neoplastic growth from different locations in the extra genital form were collected in 10 per cent neutral-buffered formalin and sent to Department of Veterinary pathology for histopathology examination. The paraffin embedded tissues were sectioned to 5 µm thickness and stained by haematoxylin and eosin (H&E) for microscopic examination (Bancroft and Gamble, 2006) [11].

Results and Discussion
Canine transmissible venereal tumors are histiocytic tumors that may be transmitted among dogs through coitus, licking, biting and sniffing of tumor affected areas (Cingi *et al.,* 2020) [12]. The similarity of the karyotype observed in CTVT samples from distant geographical areas suggested that this tumor originated from a common ancestor (Fujinaga *et al.,* 1989) [13].

Sex, Age and Breed susceptibility of CTVT
Totally 320 cases were presented to Veterinary Clinical Complex with tumour like growth in the genital areas. Out of them, 221 cases were female and 99 were of male dogs (Table 1). Females are infected more often (64.5%) than males (35.5%) because one infected male often mates with numerous females in free range or stray group and also in kennels (Singh *et al.,* 1996) [14].
The susceptibility of dogs to this venereal tumour can occur at any age throughout its life time. It mainly occurs in young, sexually mature animals (Rogers, 1997) [15]. In the present study, the incidence was recorded from 1 to 10 years of age (Table 2). But the highest prevalence rate (28%) was noticed in between 4 and 5 years of old followed by 3 to 4 years (20%).
There was wide variation in the vulnerability of CTVT among various breeds of dogs (Table 3). The frequency of this tumour was highest in non-descriptive dogs (36%) followed by Chippiparai (31%), Combai (17%) and Rajapalayam (10%). The lowest incidence was recorded in Spitz (6%) when compared to other breeds of dogs. Canine transmissible venereal tumors are most commonly seen in sexually active dogs in tropical and subtropical climates where there are large populations of stray dogs (Mello *et al.,* 2005) [16].

Gross morphology
Grossly, there were different types of lesion viz., small nodule to proliferative multiple nodules, papillary type, multilobulated mass and cauliflower like growth (Eze *et al.* 2007) [17]. Initially it usually appeared as a small papule and then progressed into cauliflower like pedunculated mass. The tumours were visible and protruding from base of the penis (Figure 1) in male and vagina or /and vulva (Figure 2) in female dogs. The tumors size may vary from 2 cm to 15 cm in diameter and were soft in consistency and had tendency to bleed. Its surface is usually ulcerated and haemorrhagic. Interestingly, extra genital form of TVT was also recorded in two dogs. One at the corneal surface of eye in a male dog and another one occurred as cutaneous nodules in a female dog. In the eye, it appeared as soft pinkish progressive papillary growth over the corneal surface and occupied the 3/4th of eye. This ocular form of TVT might be due to the absence of local humoral immune response against TVT (Ferreira *et al.,* 2000) [18]. In the present study, metastatic form of TVT was not observed as it is uncommon and occurs only in puppies and immuno-compromised dogs (Purohit, 2009) [5].

Cytology
Cytological diagnosis is a rapid and simple field diagnostic technique for confirmation of TVT. Cytology gives better evidence than histopathology (Ganguly *et al.,* 2016) [19] and provides a better characterization of the cell type, malignancy criteria, less cellular distortion when compared to histological samples (Lima *et al.,* 2013) [20]. Based on the cell type and location of the nucleus, it was categorized into lymphocytoid form, plasmacytoid form and mixed form. In the lymphocytoid form, the cells were round and centrally placed round nuclei with coarse chromatin. The cytoplasm was finely granular and vacuoles were present at periphery of the cell (Figure 3). In the plamacytoid form, round to oval shaped cells with abundant cytoplasm and eccentric placed nuclei were noticed. The cytoplasm was slightly blue, finely granular and may or may not contained vacuoles. In some cases, there were mixed cellularity picture was noticed. But TVT cells did not show anisocytosis, anisokaryosis, hyperchromasia and magakaryosis in most of the cases (Denicola *et al.,* 2007) [21]. The interesting cytological feature in the extra- genital form was absence of cytoplasmic vacuolations in TVT cells.

Histopathology
Histopathology of TVT tissue sections from genital areas and extra genital forms revealed similar changes. The neoplastic tissue were consisted of diffuse loose sheets of round cells arranged in rows and cords attached with thin connective tissue stroma (Figure 4). In some cases, neoplastic cells had pseudoalveloar pattern with delicate fibrovascular stroma and numerous mitotic figures (Meuten, 2002) [7]. Individual neoplastic cells were round or oval shape with indistinct cytoplasmic margins. It had a large round nucleus with a single centrally placed nucleolus surrounded by coarsely stippled chromatin. There is a moderate amount of light pink to clear cytoplasm. The mitotic index was very high ranging from 4 to 6 mitotic figures per high power field. There were variable numbers of lymphocytes, plasma cells and macrophages infiltrated into the tumor (Park *et al.,* 2006) [22].

Table 1: Sex-wise incidence of CTVT

Year	Total No of Animals	Sex	
		Female	Male
2015	47	28	19
2016	50	33	17
2017	61	47	14
2018	61	35	26
2019	101	78	23
Total	320	221	99

Table 2: Age-wise incidence of CTVT

Year	Age of the dogs (in years)							Total
	1-2	2-3	3-4	4-5	5-6	6-7	> 8	
2015	4	6	10	15	7	3	2	47
2016	6	8	9	17	5	4	1	50
2017	2	11	9	20	8	6	5	61
2018	5	13	17	9	4	6	7	61
2019	8	16	18	29	14	12	4	101
Total	25	54	63	90	38	31	19	320

Table 3: Breed-wise incidence of CTVT

Year	Breed					Total
	Non-descript	Chippiparai	Combai	Rajapalayam	spitz	
2015	12	18	5	7	5	47
2016	18	15	8	5	4	50
2017	24	16	11	6	4	61
2018	24	18	9	7	3	61
2019	38	29	22	8	4	101
Total	116	96	55	33	20	320

Fig 1: Male dog with cauliflower like growth at the base of the penis

Fig 2: Female dog with reddish pink TVT mass in the vagina

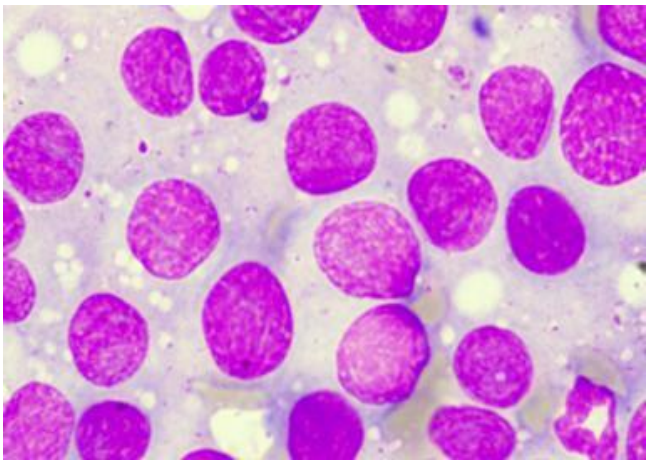

Fig 3: Cytology - Lymphocytoid type of TVT: Round cells with a centrally placed round nuclei and cytoplasmic vacuoles (Leishman stain, 1000)

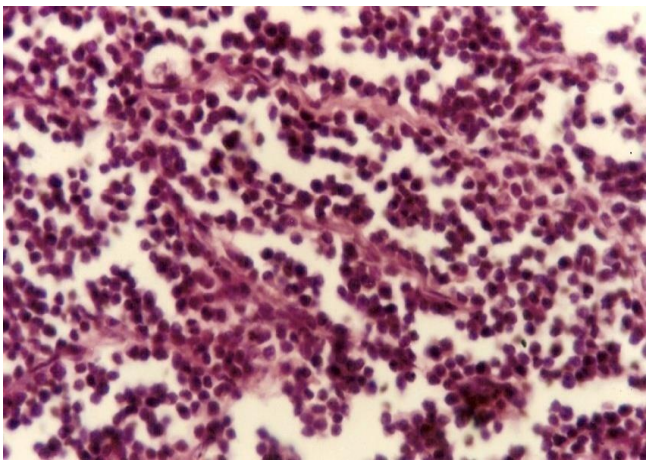

Fig 4: Histopathology - Sheets of round cells attached with thin fibrous stroma (H&E, 100)

Conclusion

The cytomorphological classification and their correlation with gross morphology and malignant potential are highly useful to decide chemotherapy schedule. The role of stray and wild dogs makes the disease difficult to control and necessitates sustained animal birth control in stray dogs along with prompt therapy of the affected dogs. In addition, strict measures should be undertaken to prevent mingling of pet dogs with stray dogs.

Acknowledgment

The authors are highly thankful to Tamil Nadu Veterinary and Animal Sciences University for providing necessary facilities to conduct the research.

References

1. Das U, Das AK. Review of canine transmissible venereal sarcoma. Vet. Res. Commun 2000;24:545-556.
2. Tella MA, Ajala OO, Taiwo VO. Complete regression of transmissible venereal tumour (TVT) in Nigerian mongrel dogs with vincristine sulphate chemotherapy. Afr. J Biomed. Res 2004;7(3):133-138.
3. Murchison EP. Clonally transmissible cancers in dogs and Tasmanian devils. Oncogene 2008;27(2):19-30.
4. Stockmann D, Heitor FF, Alexandre LA, Rodrigo AL, Tereza CC, Maria CRL. Canine Transmissible Venereal Tumors: Aspects Related to Programmed Cell Death. Braz J Vet. Pathol 2011;4:67-75.
5. Purohit G. Canine transmissible venereal tumor: A Review. The Internet J Vet. Med 2009;6(1):1-6.
6. Belov K. Contagious cancer: lessons from the devil and the dog. Bioessays 2012;34:285-292.
7. Meuten DJ. Tumours in domestic animals. Fourth Edition. Iowa State Press, 2002, 115-117
8. Cowell RL, Meinkoth JH. Sample Collection and Preparation. In Cytology and Hematology of Dog and Cat. 3rd edn Mosby Elsevier, 2008, 1-9.
9. Ajayi OL, Oluwabi M, Ajadi RA, Antia RE, Omotainse, SO, Jubril AJ *et al*. Cytomorphological, histopathological and immunohistochemical observations on the histiocytic origin of canine transmissible venereal tumour. Sokoto J Vet. Sci 2018;16(2):10-20.
10. Amaral AS, Sandra BS, Isabelle F. Cytomorphological characterization of transmissible canine venereal tumor. Revista Portuguesa Ciências Veterinárias 2007;102:253-260.
11. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 6th Edn. Churchill Livingstone, Philadelphia, 2006, 99-112.
12. Cingi C, Yeni D, Yazici E, Cine TA and Mehmet. Investigation of Transmissible Venereal Tumor in Male Dogs by Cytological Examination. Indian J Anim. Res 2020;54:1544-1548.
13. Fujinaga T, Yamashita M, Yoshida MC, Mizuno S, Okamoto Y, Tajima M *et al*. Chromosome analysis of canine transmissible sarcoma cells. Zentralbl. Veterinarmed A 1989;36:481-489.
14. Singh J, Rama JS, Sood N, Pangawkar G, Gupta PP. Clinico-pathological studies on the effect of different antineoplastic chemotherapy regimens on transmissible venereal tumours in dogs. Vet. Res. Commun 1996;20:71-81.
15. Rogers KS. Transmissible venereal tumour. Comp. Contin. Educ. Pract. Vet 1997;19:1036-1045.
16. Mello MM, Souza F, Gobello C. The Canine Transmissible Venereal Tumor: Etiology, Pathology, Diagnosis and Treatment. In: Recent Advances in Small Animal Reproduction (Concannon PW, England G, Verstegen, J. and Linde-Forsberg C.). International Veterinary Information Service, Ithaca, New York, 2005, 1-7.
17. Eze CA, Anyanwu HC, Kene RO. Review of Canine Transmissible Venereal Tumour in dogs. Nigeria Vet. J 2007;28(1):54-70.
18. Ferreira AJ, Jaggy A, Varejao AP, Ferreira MLP, Correia JMJ, Mullas JM *et al*. Brain and ocular metastases from a transmissible venereal tumour in a dog. J Small Anim. Pract 2000;41:165-168.
19. Ganguly B, Das U, Das AK. Canine transmissible venereal tumour: a review. Vet Comp Oncol 2016;14(1):1-12.
20. Lima CRO, Rabelo RE, Vulcani VAS, Furtado AP, Helrigel PA, Brito LAB *et al*. Morphological patterns and malignancy criteria of transmissible venereal tumor in cytopathological and histopathological exams. Braz. J. Vet. Res. Anim. Sci 2013;50(3):238-246.
21. Denicola D, Cowell RL, Tyler RD, Meinkoth JH, Denicola DB. Diagnostic cytology and hematology of the dog and cat. 3rd ed. St. Louis, Mosby, 2007.
22. Park MS, Kim Y, Kang MS, Oh SY, Cho DY, Shin NS *et al*. Disseminated transmissible venereal tumour in a dog. J Vet. Diagn. Investig 2006;18(1):130-133.