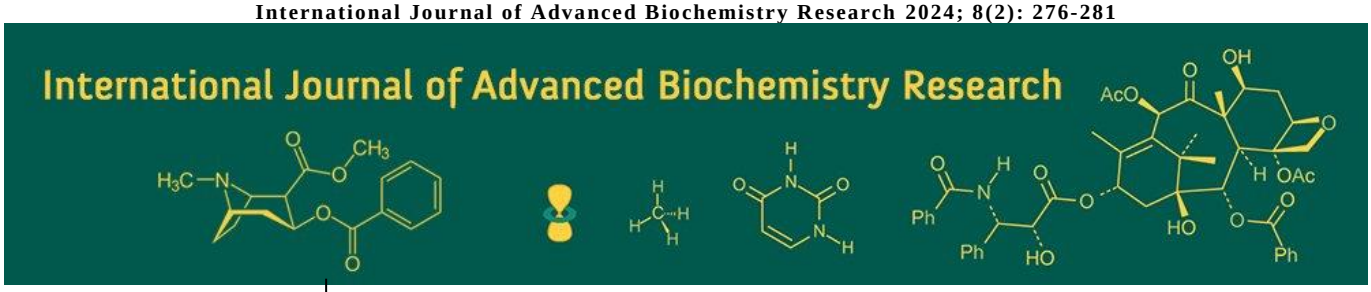

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An epidemiological study and Cytomorphological characterization of canine transmissible venereal tumor (CTVT)

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Abstract
The contagious venereal tumour also known as canine transmissible venereal tumor (CTVT) is most often found in dogs that live in close proximity to one another or in stray and wild canines who engage in unrestricted sexual behavior. This study aimed to investigate the epidemiological aspects of CTVT and its characterisation based on the cytomorphology. A total of 118 tumorous samples from dogs were collected at the Veterinary Clinical Complex (VCC), DUVASU, Mathura, and studied in the Department of Veterinary Pathology, DUVASU, and Mathura. The distribution of CTVT in terms of age, sex, and breed was evaluated. CTVT accounted for 38.13% of all tumours in dogs, with predominant occurrences in females (68.9%), non-descript breeds (44.45%), and dogs aged between 2-8 years (73.34%). Genital presentations comprised 77.77% of cases, with extragenital regions also affected. Cytological examination revealed various forms of CTVT, including lymphocytoid, plasmacytoid, and mixed forms, with lymphocytoid forms primarily localized in genital locations. Plasmacytoid forms were prevalent in extragenital sites, particularly in dogs over 7 years of age. Histopathological examination exhibited sheets of round to ovoid tumorous cells, mitotic figures, and hyperchromatic nuclei with sparse fibrous stroma. This study underscores the significance of epidemiology and cytology in understanding the diagnosis of Canine Transmissible Venereal Tumor (CTVT).

Keywords: CTVT, FNAC, cytology, lymphocytoid, plasmacytoid

1. Introduction
The global burden of cancers is subsequently increasing day by day that resulted in the leading cause of death in canines (Pang and Argyle, 2016) [32]. CTVT (Canine Transmissible Venereal Tumour) is one of the most important round-cell tumours in dogs. Other names for CTVT include sticker tumour, infectious sarcoma, transmissible lymphosarcoma, and venereal granuloma (Thangathurai *et al*., 2008) [41]. Using this tumor, Novinsky reported the first successful experimental tumour transplant (Murgia *et al*., 2006) [30].
Transmission of the tumour is favoured by the implantation of viable neoplastic cells mainly in the abraded mucous membranes (Do Amaral *et al*., 2007) [15]. Tumour may occur at the genital and extra-genital regions of mainly sexually active dogs. These are most common in tropical and subtropical regions (Birhan and Chanie, 2012) [5] Metastasis has been reported in different regions of body mainly including skin (Santos *et al*., 2008) [38].
Cytological examination, particularly through fine needle aspiration biopsy, plays a crucial role in diagnosing CTVT accurately, aiding in distinguishing it from other round cell tumors (Duncan and Prasse., 1979) [16]. This non-invasive method offers rapid results (Mathur *et al*., 2018) [27]. Without the need for surgery, facilitating early differentiation between benign and malignant tumours (Thompson *et al*., 1980) [42], thus enabling timely clinical intervention for improved prognosis (Ramos-Vara *et al*., 2011) [36]. FNAC emerges as a cost-effective, versatile diagnostic approach, offering advantages over traditional histological biopsies where the histological patterns of CTVT is often similar to other round cell tumors (Pagliuca *et al*., 2020) [31].

It is essential to understand the epidemiological aspects of this tumor, including its various cytomorphological forms, to achieve precise diagnosis and reduce the risk of underdiagnosing extragenital and atypical cases. Our study aims to enhance our understanding of CTVT by investigating epidemiological factors in conjunction with variations in cytomorphology.

2. Materials and Methods

The present study was performed at the Department of Veterinary Pathology (DUVASU), Mathura, Uttar Pradesh, India. The Institutional Animal Ethics Committee accepted the study under application number IAEC/22/19. 118 dogs were presented to the Veterinary Clinical Complex with a history of tumorous mass. The patient data including breed, age, sex, anatomical location of the tumor, size, and ulcerations in tumor was noted. FNAC was conducted for the non-ulcerated suspected masses as per Cowell *et al.* (2007) [12] with the help of a fine needle (22G) and the impression smears of the superficial ulcerated mass were made following staining with Giemsa stain (Thangathurai *et al.*, 2008) [41].

Cytologically, smears were analyzed under optical microscope and CTVT cells are characterized on the basis of predominant cell type as described by Amaral *et al.* (2007) [15] as lymphocytoid, plasmacytoid, mixed forms.

Lymphocytoid type is characterised by the presence of 60% or more of TVT cells viewed at 40X magnification with round morphology, presence of vacuoles tracking the cell periphery, round nucleus with rough chromatin, and the presence of one or two salient nucleoli with scarce cytoplasm. Plasmacytoid type is characterised by the predominance of 60% or more of TVT cells at 40X magnification, with ovoid morphology and the eccentric nucleus. Presence of a large number i.e., 1 per 2-3 high power fields of mitotic figures with abundant cytoplasm and lower nucleus to cytoplasmic ratio. Mixed type is characterised as mixed cellularity between lymphocytoid and plasmacytoid cell types, in which none surpassed 59% of the total at the 40X magnification.

For histopathology, representative tissue pieces were also collected from the tumour mass and were fixed in 10 percent buffered formalin, processed through alcohol and xylene, and embedded in paraffin. Sections were cut at 3-5 m thickness and stained by the Haematoxylin and Eosin (Luna, 1968) [26].

3. Results

3.1 Occurrence of CTVT and sex-wise, breed-wise and age-wise distribution

Forty five cases of CTVT were recorded during the present study. CTVT accounted for 38.13% of all canine neoplasms. CTVT was more recorded in females 31/45(68.9%) than males 14/45 (31.1%). Breed wise occurrence of CTVT was recorded more in non-descript breeds of dogs 20/45 (44.45%) followed by GSD 13/45 (28.9%), Pomeranian 6/45 (13.33%), Rottweiler 1/45 (0.22%), Chihuahua 1/45 (0.22%), Labrador 1/45 (0.22%), Maltese 1/45 (0.22%), Mongrel 1/45 (0.22%), and Doberman 1/45 (0.22%) (Fig. 1).

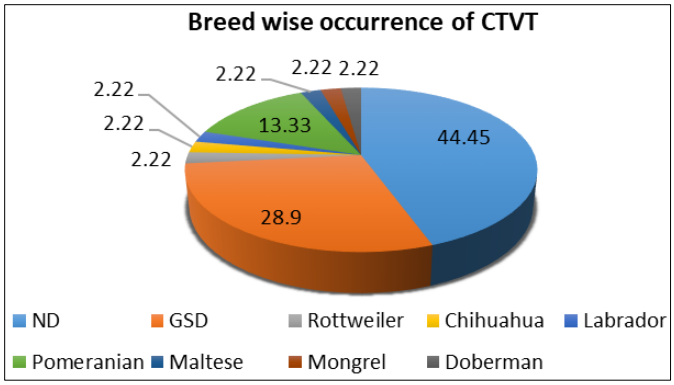

Fig 1: Pie-chart depicting the percentage of different breeds of dogs presented with CTVT, (ND- non-descript, GSD- German Shepherd)

In the study, the most commonly affected age group from CTVT was 2-8 years old age group with a total of 33 animals out of 45 (73.34%) followed by more than 8 year old age group 10/45 (22.22%). The least number of CTVT cases were seen in the age group below 2 years i.e., 2/45 (4.44%).

3.2 Gross findings

Genital regions were most commonly affected (77.77%), primarily the junction of the vestibule and vagina 11/45 (24.45%) (Fig. 2) followed by the posterior part of the vagina 7/45 (15.56%) (Fig. 3), and vestibules 5/45 (11.12%) (Fig. 4) in females. In males, the base of the penis exhibited the highest occurrence (20%) followed by the tip of penis 2/45 (4.44%), and scrotum 1/45 (2.22%). Extragenital CTVT accounted for 22.22% of cases (Fig. 5) predominantly affecting hind limbs (6.67%), followed by back, chest, and neck regions with two cases each (4.44%), and forelimb 1/45 (2.22%). Extragenital CTVT was recorded more in females (80%) than males (20%). CTVT sizes ranged from 2cm to 13.8 cm, with variations based on anatomical location. CTVT recorded at genital sites showed various size ranges from 2.1 to 8.2 cm in females, 2 to 4.5 cm in males. In extragenital sites the size of CTVT ranged from 3.2 to 13.8 cm.

Fig 2: CTVT in a female dog showing the irregular, cauliflower like reddish colour tumour mass of 7.2 cm in diameter on the vestibule and vaginal junction with highly inflamed and ulcerated surface.

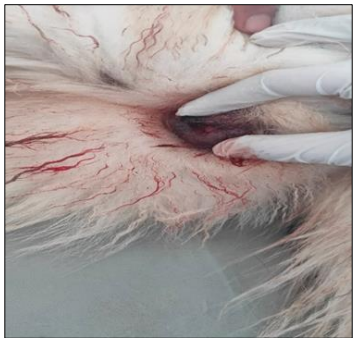

Fig 3: CTVT in a female dog showing an ulcerated nodular growth of 2.2 cm in diameter at the posterior part of vagina.

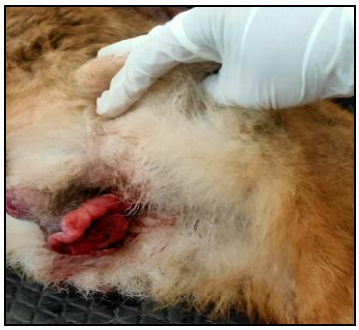

Fig 4: CTVT in a female dog showing an ulcerated cauliflower-like pink coloured growth of about 7 cm in diameter affecting the vestibule

Fig 5: Extragenital form of CTVT at the back of a dog presented as reddish ulcerated mass of about 13.8 cm in diameter with a well demarcated boundary

Most of the CTVT i.e., 38/45 (85%) were ulcerated (Fig. 2). Whereas, 7/45cases (15%) of CTVT cases were non-ulcerated. Most of the extragenital forms of CTVT were non- ulcerated. Grossly, CTVT cases were presented as a nodular (Fig. 3), pedunculated, cauliflower-like (Fig. 2) mass having a firm and friable consistency. They were pinkish (Fig. 4) to bright red (Fig. 2) in color.

3.3 Microscopic findings

The cytological examination of CTVT cases revealed the presence of a large number of round to ovoid cells with well-demarcated cytoplasmic borders, with increased nucleus to cytoplasmic ratio and basophilic cytoplasm containing clear cytoplasmic vacuoles. The nucleus contained coarse chromatin material with one or more nucleoli (Fig. 6) The CTVT showed different types of cytomorphological details, i.e., plasmacytoid, lymphocytoid, and mixed forms of tumor cells. Cytological smears revealed the presence of lymphocytoid forms in 19/45 cases (42.22%). In this form, more than 60% of tumorous cells were round with scarce cytoplasm, few cytoplasmic vacuoles and coarse chromatin material, with a prominent nucleus to cytoplasmic ratio (Fig. 6). The other cytomorphology recorded was the plasmacytoid form in 17/45 cases (37.78%). In this form, more than 60% of total tumorous cells were ovoid with ample cytoplasm, an eccentric nucleus, and a relatively smaller nucleus to cytoplasm ratio (Fig. 7). The third form is mixed forms of CTVT cells, that were observed in 9/45 (20%) cases with both lymphocytoid and plasmacytoid cells with neither type exceeding 59% of total cells (Fig. 8). Out of 10 extragenital CTVT cases, the plasmacytoid form was seen in eight cases, whereas two cases showed mixed form.

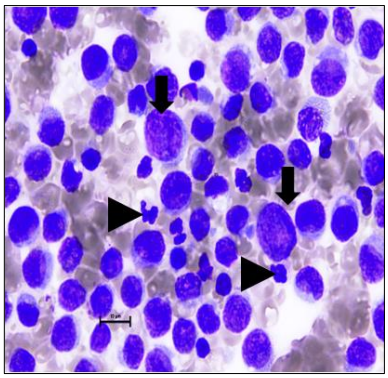

Fig 6: The Cytological smear of lymphocytoid form of CTVT showing numerous cells with round morphology (arrow) having scarce cytoplasm and nucleus with coarse chromatin material. The presence of neutrophils is also seen (arrowhead). Giemsa stain, 1000X

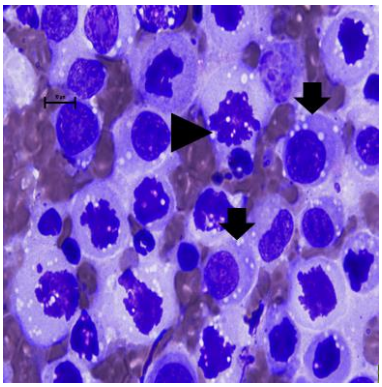

Fig 7: The cytological smear of plasmacytoid form of CTVT showing numerous cells with ovoid morphology (arrow), abundant basophilic cytoplasm and nucleus with coarse chromatin material. The presence of several mitotic Figures (arrowhead) are also noted. Giemsa stain, 1000X.

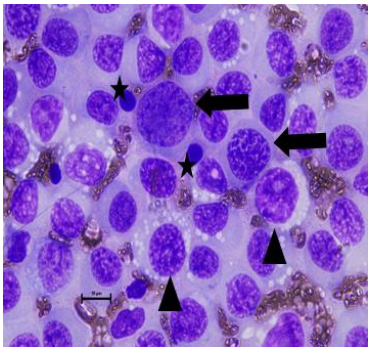

Fig 8: The cytological smear showing mixed form of CTVT with both lymphocytoid cells (arrow) and plasmacytoid form of cells (arrow head) along with lymphocytes (star). Giemsa stain, 1000X.

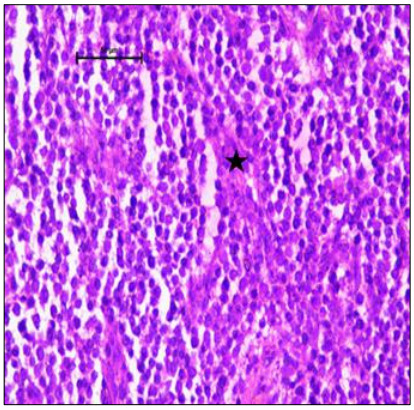

Fig 9: Histopathological section of CTVT showing the presence of the large number of round to polyhedral CTVT cells arranged in sheet like pattern with scarce fibrous tissue (star). The nuclei have coarse chromatin and increased nucleus cytoplasm ratio. H & E, 400X.

Histopathology of CTVT samples revealed the presence of sheets of round to ovoid tumorous cells with scarce fibrous stroma. The tumor cells showed mitotic figures, karyomegaly, hyperchromasia, coarse chromatin material, and prominent nucleoli (Fig. 9).

4. Discussion

Transmissible venereal tumour is one of the most commonly occurring round cell tumours and poses great difficulties in differentiation because this tumour may have similarities with its close entity, such as a mast cell tumour, histiocytoma, plasmacytoma etc. In the study, the CTVT was accounted for about 38.13% which is in accordance with the earlier findings of Chaudary and Rao (1982) [8]. The ranges of incidence may vary as per differences in environmental and host factors.

More females (68.9%) were affected with CTVT than males (31.1%). Similar results were observed by earlier workers (Sousa *et al*., 2000; Pimentel *et al*., 2021) [39, 35]. Females were found to be more susceptible to CTVT than males. One probable reason being a single affected male often mates with more females and thus spreading the tumor (Das and Das, 2000) [14].

The percentage of CTVT was more in non-descript breeds of dogs about 44.45% followed by GSD 28.9%, Pomeranian 3.33%, and others. An increased prevalence of CTVT in non-descript breeds could be due to its uncontrolled breeding in these animals (Khan *et al*., 2009) [24]. Animals though client-owned, may live outdoors and interact with stray dogs. Inadequate effective treatments and an uncontrolled population of stray dogs were found to cause local propagation of CTVT (Abeka, 2019) [1]. In the study, GSD was the second most affected breed from CTVT, probably because it comes in estrus three times a year and its aggressive nature leads to mating with stray dogs and being unable to control during the estrus period (Lakde *et al*., 2020) [25]. However, dogs of any breed, age, or sex are susceptible to CTVT (Das and Das, 2000) [14].

The average age of dogs affected with CTVT was around 5.6 years. The maximum number of cases (73.34%) were recorded between the age group two to eight years. This finding was in accordance with the earlier study done by Boscos and Ververidis (2004) [6]. In the present study, CTVT was also observed in dogs above 1 year of age which was similar to the previous findings (Rogers *et al*., 1998; Das & Das, 2000; Murchison, 2008) [37, 14, 29] as the dogs become sexually mature above one year of age (Gonzalez *et al*., 1997) [19].

The maximum 77.77% number of cases of CTVT were manifested as the genital form. This could be due to the unique nature of transmission of CTVT that potentiates the transplantation of these neoplastic cells onto genital mucosa during mating (Cohen, 1985; Johnston, 1991; Mukaratirwa and Gruys, 2003) [10, 22, 28]. Maximum cases were recorded from the junction of vestibule and vagina (24.45%) in females. This might be due to the maximum pressure in this region during the mating act (Boscos and Ververidis, 2004) [6]. The other regions recorded in the study were the posterior part of the vagina and vestibules. In males, the areas of maximum occurrence included the base of the penis, the tip of the penis, and testes. These findings matched with that of earlier studies (Boscos and Ververidis, 2004; Kabuusu *et al*., 2010; Sreekumar *et al*., 2015) [6, 23, 40].

The extragenital CTVT was seen in about 22.22% of the cases. Sniffing, biting, scratching and licking acts as a source in dogs for the transmission of CTVT in skin regions (Higgins, 1966; Amaral *et al*., 2007) [20, 15]. Extragenital CTVT was recorded in cutaneous regions of the hindlimbs, back, chest, neck regions and forelimbs. Similar findings were recorded by earlier workers (Parent *et al*., 1983; Das and Das, 2000) [33, 14]. In this study, the extragenital CTVT was more in the females as compared to males, which was in accordance with a previous study (Coskan *et al*., 2011) [11]. However, in another study by Bastan *et al*. (2008) [3] extragenital CTVT was recorded more in males. The development of extragenital CTVT depends on its growth and metastasis, which in turn depends on other factors like age, immune status, etc. of the animal.

The size of CTVT ranged from 2cm to 13.8 cm, which was in accordance with earlier studies (Brown *et al*., 1980; Park *et al*., 2006) [7, 34]. The gross appearance of the CTVT was nodular, pedunculated, and cauliflower-like with a firm and friable consistency. Tumorous masses were bright red, ulcerated, and inflamed this could be due to rapid growth and extensive vascularization (Das and Das, 2000; Hoque, 2002) [14, 21] while most of the extragenital cases were presented with non-ulcerated surfaces.

Cytology reveals distinct cytoplasmic and nuclear features and plays a great role in diagnosing CTVT. Therefore, it is more preferred over histopathology in the diagnosis of CTVT (Ganguly *et al*., 2016) [18]. The probable reason behind this could be the difficulty to differentiate CTVT from other round cell tumors in histopathological slides (Das *et al*., 1990; Ganguly *et al*., 2016) [13, 18]. Cytological examination of CTVT showed the presence of a large number of round to ovoid cells with well-demarcated cytoplasmic borders with an increased nucleus cytoplasmic ratio and basophilic cytoplasm containing clear cytoplasmic vacuoles and nucleus with coarse chromatin material with one or more nucleoli. These findings were similar to the earlier studies (Cowell *et al*., 2007; Flórez *et al*., 2012) [12, 17]. The cytomorphological classification of CTVT has prognostic importance (Amaral *et al*., 2007) [15]. CTVT showed different cytomorphological details represented as three different forms namely lymphocytoid form with 42.22%, plasmacytoid form with 37.78%, and mixed form with 20%. The earlier studies have shown that plasmacytoid forms of TVTs were found to be more aggressive than lymphocytoid TVTs. This has been correlated to their

metastatic potential manifested by the occurrence of plasmacytoid TVTs in extragenital locations (CH *et al*., 2016) [9]. This is in agreement with the present study showing most of the extragenital CTVT cases manifesting as the plasmacytoid form. The plasmacytoid forms were recorded mainly in dogs above 7 years of age.

Histopathology of CTVT samples revealed the presence of sheets of round to ovoid tumorous cells with scarce fibrous stroma. There was a presence of mitotic figures. The nucleus was large, hyperchromatic, and recorded with coarse chromatin material and prominent nucleoli. All these findings are in accordance with previous studies (Behera *et al*., 2012; Ajayi *et al*., 2018) [4, 2].

5. Conclusion

In conclusion, Canine Transmissible Venereal Tumor (CTVT) emerges as the predominant neoplasia of the external genitalia in dogs, particularly prevalent in tropical and sub-tropical regions. This study offers a comprehensive insight into CTVT epidemiology and cytology, highlighting its significant representation among canine neoplasms. Affected dogs, predominantly females, non-descript breeds, and those aged 2-8 years, showcase varied cytological forms of CTVT cells, aiding in accurate diagnosis and prognostication. Notably, plasmacytoid forms are found to be more aggresive in nature, are prevalent in older dogs and extragenital sites. The study emphasises the importance of cytological techniques, particularly fine needle aspiration biopsy for precise diagnosis and crucial in distinguishing CTVT from other round cell tumours. These findings enrich our understanding of CTVT, offering valuable insights for improved clinical intervention and prognosis in affected dogs. Moreover, the study underscores the utility of cytology as a rapid, field diagnostic tool, complementing histopathological examination in the diagnosis of CTVT.

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