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## Cytological and histopathological evaluation of canine transmissible venereal tumour (CTVT)

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**Abstract**

Canine Transmissible Venereal Tumour (CTVT) is a unique clonally transmissible round cell neoplasm of dogs, predominantly affecting the external genitalia. Accurate diagnosis is essential for confirmation and clinical decision-making. The present study was undertaken to evaluate the cytological and histopathological characteristics of CTVT using fine needle aspiration cytology (FNAC), impression smear, and histopathological examination. Dogs clinically suspected for CTVT were subjected to cytological and histological evaluation prior to therapeutic intervention. FNAC and impression smear revealed characteristic round to polyhedral neoplastic cells with vacuolated cytoplasm, high nuclear-to-cytoplasmic ratio, hyperchromatic nuclei and prominent nucleoli. Histopathological examination confirmed the diagnosis by demonstrating sheets and cords of tumour cells supported by delicate connective tissue stroma with high mitotic activity. The study highlights the diagnostic significance of FNAC and impression smear as rapid preliminary tools, while histopathology remains the gold standard for definitive diagnosis of CTVT.

**Keywords:** Canine transmissible venereal tumour, FNAC, impression smear, histopathology, diagnosis

**Introduction**

Transmissible venereal tumour (TVT) is one of the most uncontrolled tumours in dogs (Ucar, 2016) [15]. It is one of the most common benign tumours in dogs that can be seen in both genders (Kucukberkir *et al.*, 2021) [6]. Canine Transmissible Venereal Tumour (CTVT) is a naturally occurring, clonally transmissible round cell neoplasm of dogs, transmitted primarily through direct contact during coitus and occasionally by social behaviours such as licking or sniffing of affected areas. It is one of the few known naturally transmissible cancers and is widely prevalent in tropical and subtropical regions, particularly among free-roaming and sexually active dogs (Nowinsky, 1876; Murgia *et al.*, 2006) [12, 11].

Clinically, CTVT commonly affects the external genitalia of both male and female dogs and presents as a friable, haemorrhagic, cauliflower-like mass associated with vaginal or preputial bleeding (Das and Das, 2000; Purohit, 2009) [1, 13]. Although gross appearance often suggests the diagnosis, confirmation is essential to differentiate CTVT from other genital neoplasms and inflammatory conditions.

CTVT, macroscopically, can be solitary or multiple tumour masses with cauliflower-like ulceration, haemorrhagic, friable and irregular appearance can be seen. Lesions are fragile and mostly haemorrhagic as a result of low cohesion amongst neoplastic cells. Tumour size varies from millimeters (mm) to several centimeters (less than 1 to 3 mm in diameter) with a dark red to greyish pink colour (Das and Das, 2000; Purohit, 2009; Lopes *et al.*, 2015) [1, 13, 15]. As microscopically diagnosis is made by histopathological examination of the biopsy specimen, large cells with round or oval vague contours are observed on histological examination. CTVT is morphologically categorised as a spherical (round) cell tumour, however, although the precise origin of the cells is unknown, immunohistochemical investigations revealed histiocytic and mesenchymal origin (Do Amaral *et al.*, 2007) [2]. Cytologically, CTVT cells are round or oval, 14 to 30 µm in diameter, with pale blue cytoplasm which is particularly vacuolated, with a relatively elevated nuclear: cytoplasmic ratio. The cells also comprise dense chromatin, visible nucleoli and numerous mitotic structures, as well as anisocytosis and anisokaryosis (Erüinal-Maral *et al.*, 2000) [3].

Cytological techniques such as fine needle aspiration cytology (FNAC) and impression smear are widely used as rapid, minimally invasive, and cost-effective diagnostic tools for CTVT. These techniques allow early identification of characteristic tumour cells with high diagnostic accuracy (Erüinal-Maral *et al.*, 2000; Ganguly *et al.*, 2016) [3, 4]. However, histopathological examination remains the gold standard for definitive diagnosis, as it provides detailed information regarding tissue architecture, cellular arrangement, mitotic activity, and stromal response (Mukaratirwa and Gruys, 2003) [9]. Therefore, combined cytological and histopathological evaluation is recommended for accurate diagnosis of CTVT.

### Materials and Methods

Dogs presented with clinical signs suggestive of CTVT, such as genital bleeding and visible tumour masses, were included in the study. All cases were subjected to confirmatory diagnostic evaluation prior to initiation of therapy.

Dogs under Group 1 (6 animals) were treated with vincristine sulphate at the dose rate of 0.025 mg/kg body weight intravenously, once a week, for 3 to 5 weeks depending upon the regression of tumour mass. Dogs under Group 2 (6 animals) were treated with bacillus Calmette-Guerin (BCG) at the dose of  $2-8 \times 10^6$  CFU dissolved in 5.0 ml of normal saline. The solution was administered intratumorally and also applied as a local spray once a week for 3 to 5 weeks. Dogs under Group 3 (6 animals) were treated with combination therapy of vincristine sulphate and BCG using the same dose and protocol as described for Groups 1 and 2.

Diagnosis of TVT was confirmed by impression smear, fine needle aspiration cytology (FNAC) and histopathological examination. FNAC was performed by aspirating tumour tissue using a fine gauge needle under aseptic conditions. The aspirated material was smeared onto clean glass slides, air-dried, and stained using routine cytological stains. The smears were examined under light microscopy for cellular morphology and arrangement. Impression Smear Impression smears were prepared by gently pressing the freshly cut surface of the tumour mass onto clean glass slides. The smears were air-dried, stained, and examined microscopically to evaluate cytological features. Histopathological Examination Tumour biopsy samples were collected from representative areas of the mass. The tissues were fixed in 10% neutral buffered formalin, processed by routine paraffin embedding, sectioned at 4-5  $\mu$ m thickness, and stained with Haematoxylin and Eosin (H&E). Histological evaluation focused on tumour cell morphology, mitotic activity, stromal response, and inflammatory cell infiltration.

### Results

FNAC smears revealed high cellularity with numerous round to polyhedral neoplastic cells arranged in sheets and rows. The tumour cells showed moderate to abundant pale basophilic cytoplasm with distinct cytoplasmic vacuolation. Nuclei were round to oval, hyperchromatic, and exhibited prominent nucleoli with a high nuclear-to-cytoplasmic ratio. Frequent mitotic figures and cellular pleomorphism were observed. Inflammatory cells, mainly lymphocytes and neutrophils, were also present in the background.

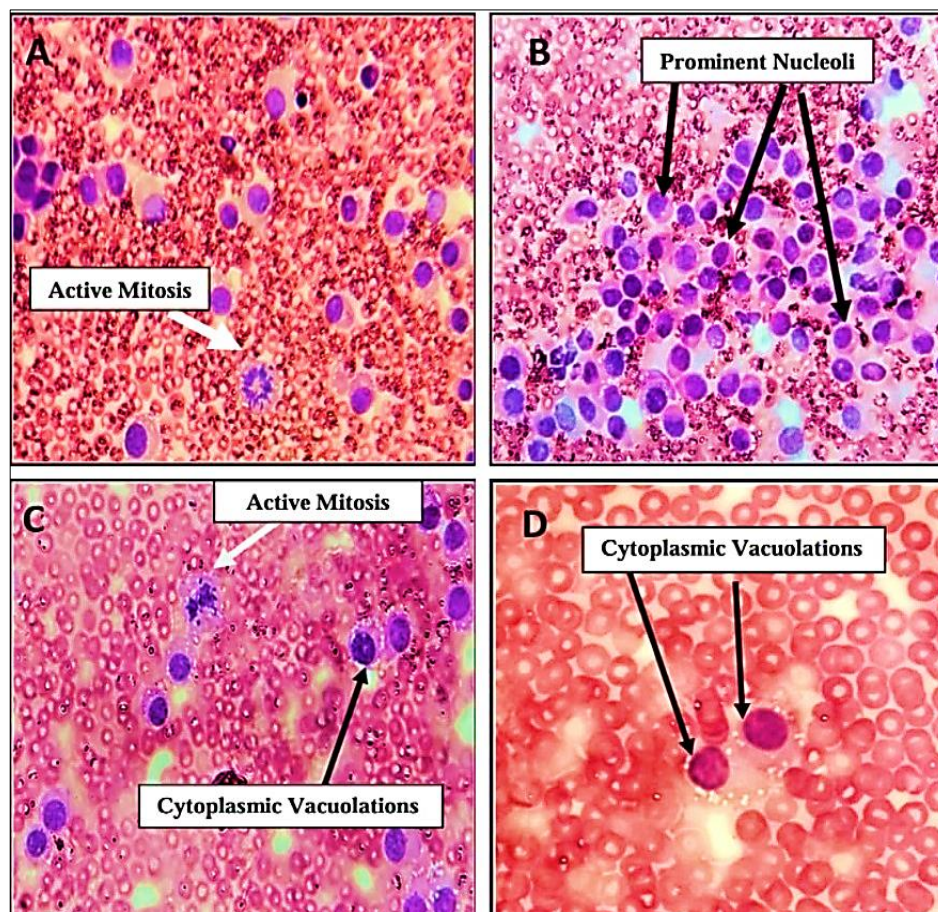


**Fig 1:** Cytology feature of nodular masses by FNAC of TVT in canine

**Impression Smear Findings** Impression smears demonstrated cytological features similar to FNAC. The smears showed round tumour cells with vacuolated cytoplasm, centrally placed nuclei, coarse chromatin, and

prominent nucleoli. The background commonly showed haemorrhage and inflammatory cells. Impression smears provided rapid confirmation of tumour cell morphology but lacked architectural details.

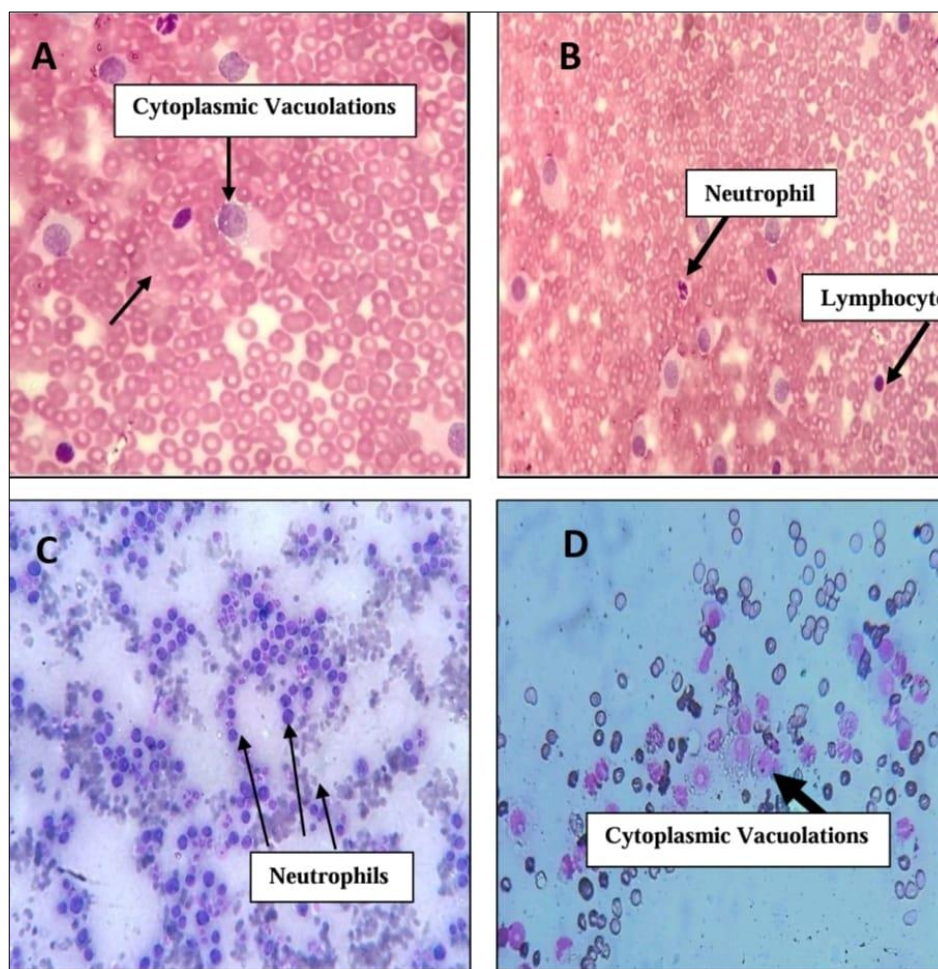


**Fig 2:** Cytology feature of impression smear of TVT in canine

Histopathological examination of pre-treatment biopsy samples revealed classical features of CTVT. The tumour was composed of round to polyhedral cells arranged in sheets, cords, and clusters, separated by delicate connective tissue stroma. The neoplastic cells exhibited a high nuclear-to-cytoplasmic ratio, round nuclei with coarse to delicate chromatin, prominent nucleoli, and lightly acidophilic

cytoplasm with cytoplasmic vacuoles. Numerous mitotic figures indicated high proliferative activity. Minimal connective tissue proliferation was observed, along with infiltration of inflammatory cells such as lymphocytes, plasma cells, and occasional neutrophils within the stromal framework.

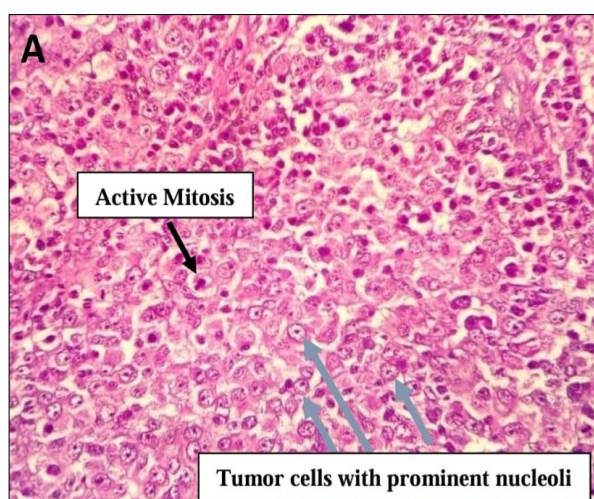


**Fig 3:** Pre treatment histopathology in which tumour cells with

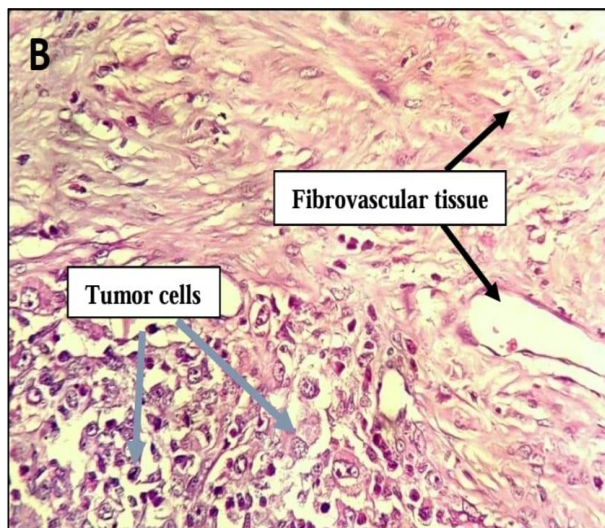


**Fig 4:** Post treatment histopathology in which tumour cells and prominent nucleoli fibrovascular tissue

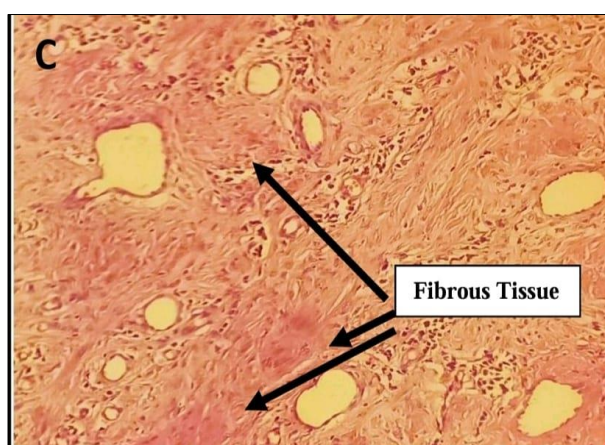


**Fig 5:** Replacement of tumour parenchymal with fibrovascular tissue

Post-treatment histopathological examination revealed a decrease in oedema, appearance of macrophages and an increase in the number of tumour infiltrating lymphocytes (TILs).. During the subsequent weeks there was a gradual increase in the number of tumour infiltrating lymphocytes

(TILs) followed by tumour cell apoptosis and necrosis, reduction in mitotic figures and gradual replacement of the tumour parenchyma by fibrovascular tissue. At the end of treatment, most of the tumours were completely replaced by the fibrovascular tissue.

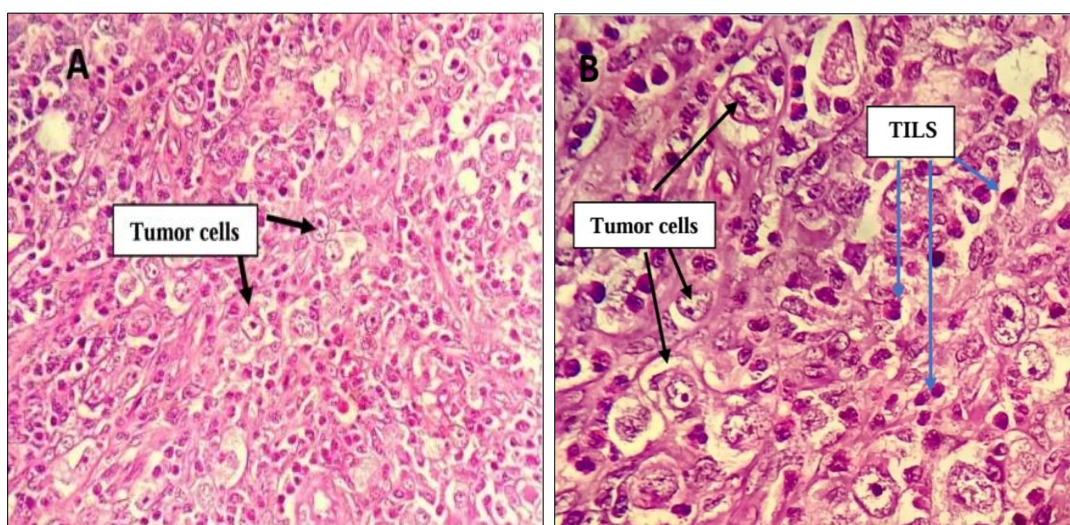


**Fig 6:** Pre treatment histopathology indicate prominent nucleoli Post treatment histopathology indicate tumour cells and tumour infiltrating lymphocytes increased

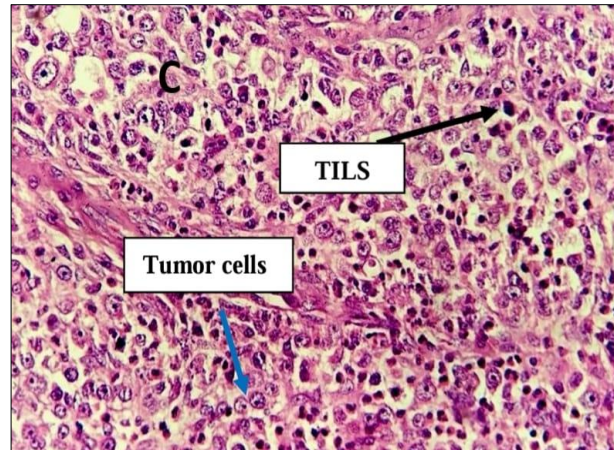


**Fig 7:** Post treatment histopathology indicate no reduction in tumour cells, minimal fibrovascular tissue and increase in TILs

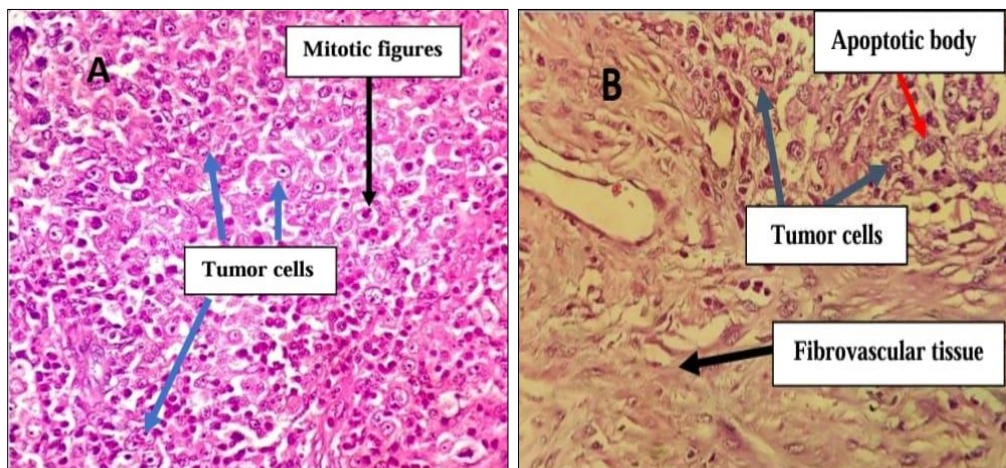


**Fig 8:** Pre treatment histopathology indicate prominent nucleoli Post treatment histopathology indicate tumour cells, fibrovascular tissue and apoptosis of tumour cells

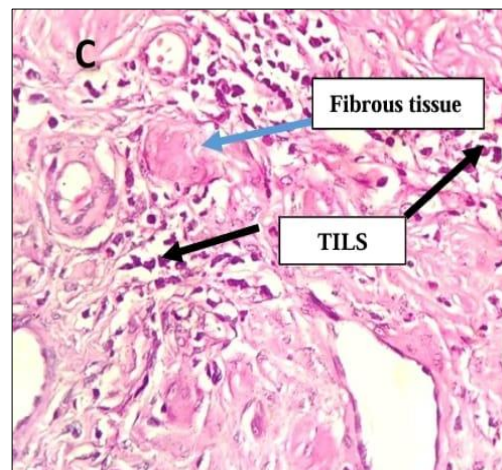


**Fig 9:** Post treatment histopathology indicate tumour infiltrating tissues and fibrovascular tissue

## Discussion

The present study demonstrates that FNAC, impression smear, and histopathological examination are valuable diagnostic modalities for the diagnosis of canine transmissible venereal tumour. FNAC and impression smear provided rapid and reliable preliminary diagnosis by revealing characteristic round to polyhedral tumour cells with vacuolated cytoplasm, high nuclear-to-cytoplasmic ratio, hyperchromatic nuclei, and prominent nucleoli. Similar cytological features have been reported as diagnostic hallmarks of CTVT by Erünal-Maral *et al.* (2000)

[3] and Ganguly *et al.* (2016) [4].

FNAC yielded better cellularity and clearer nuclear details compared to impression smear, making it a preferred cytological technique, which is in agreement with earlier reports (Gurel *et al.*, 2002; Lather *et al.*, 2017) [5, 7]. Impression smear, although simple and quick, lacked consistency in cellular yield and did not provide information on tissue architecture.

Histopathological examination confirmed the diagnosis by demonstrating sheets and cords of round to polyhedral tumour cells supported by delicate connective tissue stroma,

along with high mitotic activity and inflammatory cell infiltration. These findings are consistent with the classical histological description of CTVT reported by Mukaratirwa and Gruys (2003) <sup>[9]</sup> and Stockmann *et al.* (2011) <sup>[14]</sup>. Histopathology also allows differentiation of CTVT from other round cell tumours and provides essential baseline data for assessment of tumour behaviour and therapeutic response.

Thus, while cytological techniques are useful for rapid screening and field diagnosis, histopathology remains indispensable for confirmatory diagnosis. A combined diagnostic approach enhances accuracy and reliability, as supported by previous studies (Ganguly *et al.*, 2016; Mukaratirwa *et al.*, 2009) <sup>[4]</sup>.

## Conclusion

FNAC and impression smear are effective, rapid, and minimally invasive diagnostic techniques for the preliminary diagnosis of canine transmissible venereal tumour. Histopathological examination remains the gold standard for definitive diagnosis due to its ability to assess tissue architecture and cellular characteristics. A combined diagnostic approach using cytology and histopathology enhances diagnostic accuracy and supports effective clinical management of CTVT in dogs.

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