

# Clinic, macroscopic, cytomorphologic and histopathologic features of canine transmissible venereal tumor in two bitches: First report from Kyrgyzstan. Clinical case

## Características clínicas, macroscópicas, citomorfológicas e histopatológicas del tumor venéreo transmisible canino en dos perras: Primer informe de Kirguistán. Caso clínico.

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### ABSTRACT

Canine transmissible venereal tumour (CTVT) is a spontaneously arising, contagious neoplasm in dogs that propagates via the direct implantation of viable tumour cells between animals. Transmission predominantly occurs during mating, leading to the development of growths chiefly on the external genitalia of both males and females. In this case report, it is aimed to describe the clinical, cytological, macroscopic and histopathological findings of two bitches with CTVT. In the clinical examination of both bitches, an 8-month-old Husky and 2-years-old Alabai crossbred, protruding, irregular, ulcerated and bleeding structures were observed in the vulva. In cytological examinations, round to polyhedral-shaped cells with an eccentrically located nucleus, multiple mitosis and the characteristic distinct, cytoplasmic vacuolations with clear outlines were observed. Histopathological examinations of both cases revealed round or polyhedral neoplastic cells arranged in solid sheets or rows supported by fibrovascular connective tissue septa. Numerous mitotic figures were also observed together with tumor cells. Tumor cells with indistinct cell borders had eosinophilic and finely granular cytoplasmic structures. Necrotic changes in tumor cells and hemorrhage foci were also observed. A tumor cell embolism consisting of tumor cells adherent to the vessel wall was observed in the vessel lumen. It is think that these cases are important because they are being reported for the first time in Kyrgyzstan.

**Key words:** Canine transmissible venereal tumor; clinic; cytomorphologic; macroscopic, histopathologic.

### RESUMEN

El tumor venéreo transmisible canino (TVTC) es un cáncer transmisible de origen natural que se propaga entre perros mediante la transferencia alogénica de células cancerosas vivas. La enfermedad suele transmitirse durante el coito y da lugar a la aparición de tumores asociados con mayor frecuencia a los genitales externos de perros machos y hembras. En este informe de caso, se pretende describir los hallazgos clínicos, citológicos, macroscópicos e histopatológicos de dos perras con CTVT. En el examen clínico de ambas perras, una cruce de Husky de 8 meses y una Alabai de 2 años, se observaron estructuras protuberantes, irregulares, ulceradas y sangrantes en la vulva. En los exámenes citológicos, se observaron células de forma redonda a poliédrica con un núcleo situado excéntricamente, mitosis múltiples y las características vacuolaciones citoplasmáticas con contornos claros. Los exámenes histopatológicos de ambos casos revelaron células neoplásicas redondas o poliédricas dispuestas en sábanas o hileras sólidas sostenidas por septos de tejido conectivo fibrovascular. También se observaron numerosas figuras mitóticas junto con las células tumorales. Las células tumorales con bordes celulares indistintos presentaban estructuras citoplasmáticas eosinofílicas y finamente granulares. También se observaron cambios necróticos en las células tumorales y focos de hemorragia. En la luz del vaso se observó una embolia de células tumorales adheridas a la pared del vaso. Se cree que estos casos son importantes porque se notifican por primera vez en Kirguistán.

**Palabras clave:** Tumor venéreo transmisible canino; clínica; citomorfológico; macroscópico, histopatológico

## INTRODUCTION

Canine transmissible venereal tumour (CTVT) is the oldest documented contagious cancer in dogs, with the first descriptions dating back to the nineteenth century. It has been variously termed canine infectious sarcoma, venereal granuloma, transmissible lymphosarcoma, round-cell sarcoma, and Sticker's sarcoma [1, 2, 3]. Clinically, CTVT most often manifests on the mucosal surfaces of the external genitalia in both male and female dogs. As transmission typically occurs during coitus, the disease predominantly affects young, sexually mature animals. Although venereal spread is the primary route of infection, CTVT cells can also be transplanted experimentally to other body sites and may disseminate between dogs via licking, biting, or direct contact with existing tumours [4, 5, 6].

This tumor has also been observed in other canid species such as coyotes (*Canis latrans*), jackals (*Canis aureus*), wolves (*Canis lupus*), and foxes (*Vulpes vulpes*) [7]. It is prevalent worldwide and considered endemic in over 90 countries [8]. Both male and female dogs (*Canis lupus familiaris*) can be infected, with an incidence rate of 35.5% in males and 64.5% in females. The occurrence is typically highest during the peak sexual activity, especially in female dogs during their estrus period. Dogs of any breed, age, or sex can be susceptible to this tumor [9, 10].

Genomic investigations indicate that CTVT originated from the somatic tissues of a single canine ancestor approximately 11 000 years ago [11, 12]. Despite its ancient emergence, time-resolved phylogenies demonstrate that global CTVT populations only diverged within the past few hundred years [8, 11, 12]. Cytogenetic analyses consistently demonstrate that CTVT cells carry an anomalous chromosomal complement—typically 57 to 64 chromosomes, averaging 59—whereas normal canine somatic cells maintain 78 chromosomes [13].

The clinical presentation of CTVT can vary among dogs depending on the location of the tumors. Infected dogs exhibit symptoms such as bloody preputial and vaginal discharges, genital swelling, reduced penile exposure, skin with ulcerative nodules, and a tendency to sniff the infected lesions [9, 14]. Metastases have been reported in organs such as tonsils, liver, kidney, spleen, brain, eye, skin, peritoneum, skeletal muscle, tongue, mesenteric lymph nodes and maxillary bone [5, 10, 15, 16, 17, 18].

When studies on CTVT are examined, it is seen that this study is the first report presenting an evaluation of the occurrence of CTVT in Kyrgyzstan. The aim of this case report was to describe the clinical, cytological, macroscopic and histopathological findings of two dogs with CTVT in Kyrgyzstan.

## MATERIALS AND METHODS

### Cases descriptions

No experimental animals were used in this study. The cases studied were part of routine clinical practice and surgical resection of tumors were performed at the Faculty of Veterinary Medicine of Kyrgyz-Turkish Manas University, December 2023 and April 2024, where they were brought for treatment by treating veterinarians at the request of their owners and not for reasons related to the study.

The animal owners were informed before the operation and a consent form was obtained. In conducting this study, it is important to note that ethical clearance was not sought or required. This article represents a case report, not a formal research study involving experimentation or clinical trials on animals.

Two bitches were brought for examination to the clinics of the Kyrgyz-Turkish Manas University Faculty of Veterinary Medicine in Bishkek, Kyrgyzstan. The first case was an 8-month-old, bitch, Husky, the second one was (2-years-old), bitch, Alabai crossbred. In the first case, the owner reported that the bitch had a good appetite and had difficulty urination. The dog in the first case was adopted 6 days ago and was brought to the clinic because it was thought to be in heat. Therefore, there is no information about it before. In the second case, it was reported that despite antibiotic treatment for the wounds on the bitch's vulva in another clinic, there was no improvement and it was brought to the Veterinary Faculty clinics for treatment. In the second case, as part of a project within the scope of the rehabilitation of stray dogs, it was learned that one week ago antibiotic treatment was applied to wounds on the vulva in another clinic. For this reason, no information about the animal's previous life was obtained. As the result of this treatment was not obtained, the bitch was brought to the clinic of the Veterinary Faculty for treatment.

Initially, imprint cytology preparations were made from the cut surfaces of the excised masses by gently touching or scraping the tissue. These smears were air dried, stained using a Diff Quick kit (Fast Color Kit, DDK Italia, Italy), and examined under a light microscope (Euromex IS.1153.EPL, The Netherlands) for cytological assessment. Thereafter, representative tissue samples were fixed in 10 % neutral buffered formalin for 24 hours, processed routinely, and embedded in paraffin. Sections 5 µm thick were cut on a microtome (Leica RM2255, Germany) and stained with hematoxylin and eosin following standard protocols [19].

## RESULTS AND DISCUSSION

The CTVT is a contagious malignancy in dogs that propagates via the transfer of living tumor cells between animals. Spread most frequently during mating, it gives rise to mass lesions predominantly on the external genitalia of both males and females. CTVT has now been documented on every continent where dogs are present [8, 15, 20, 21, 22]. This disease poses a significant global canine health burden and is especially common in regions with large stray dog populations and unregulated breeding behaviors [15, 22]. Infection occurs chiefly in young, sexually active dogs and shows no clear preference for any particular breed or sex [8, 20, 23]. However, no reports have been found regarding the occurrence of CTVT in Alabai or Alabai crossbred dogs. When the studies on the subject are evaluated, it is noteworthy that this diagnosed CTVT case was reported for the first time in an Alabai crossbred.

CTVT is distinguished by its ability to spread through the direct implantation of viable tumor cells into previously unaffected tissues—most often during coitus but also via close social interactions such as sniffing, licking, or biting [15, 23]. When located on the genital mucosa, these tumors exhibit a characteristic clinical presentation. These are usually localized, hemorrhagic, possess a cauliflower-like growth pattern, and a friable texture. Tumors may be single or multiple, ranging from a small nodule to a large mass [8, 15, 22, 23].



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In the clinical examination of both dogs, protruding, irregular, ulcerated and bleeding structures were observed in the vulva (FIG. 1 A-B). After general checks, the masses along with other surrounding structures in the vulva of both dogs were surgically removed and sent to the pathology laboratory for diagnosis. In the first case (Husky), the mass removed by surgery was 4.5x2.5x2 cm in size, had a friable consistency, grayish-white in color, and occasional bleeding and ulcerated areas were noted. In the second case (Alabai crossbred), the mass was protruding from the vulva, cauliflower-like and had an ulcerated appearance. Nodular structures protruding from the vaginal mucosa were also noted. The mass was 19x12x10 cm in size, hard in consistency and the cut surface was yellowish-gray in color, and lobular and occasionally oval-round structures were also seen (FIG. 2 A-B). On thorax radiography (Poskom PXP-40HF, Korea), a small number of structures of different sizes in the lung that may be related to metastasis were observed (FIG. 3).

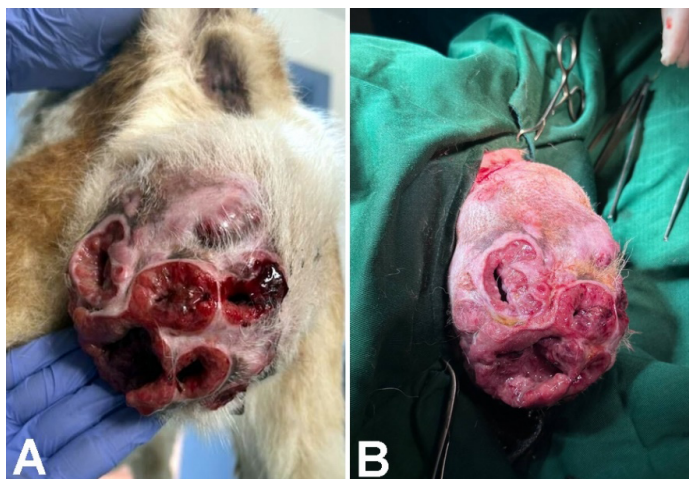


FIGURE 1. A. Clinical appearance of protruding, irregular, ulcerated and bleeding structures in the vulva, B. Preoperative appearance of the vulvar mass, (Alabai crossbred bitch).

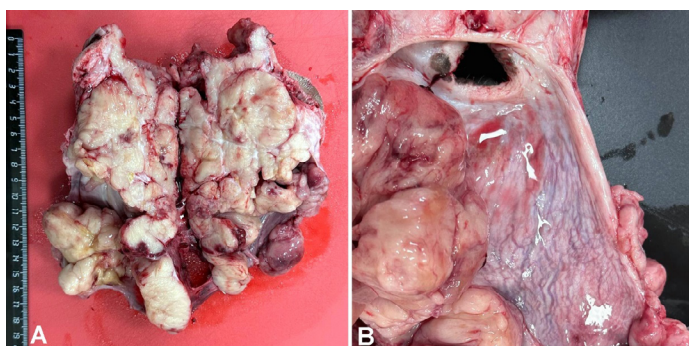


FIGURE 2. A. Cauliflower-like mass in the vaginal lumen with a hard consistency and a yellowish-gray color on the cut section, (second case), B. Lobular and occasionally oval-round nodular structures in the vaginal lumen, (second case).

In both cases, similar clinical signs were reported by the owners and the extragenital appearance and macroscopic structures of the masses were consistent with those reported previously [4, 5, 6, 8, 23].

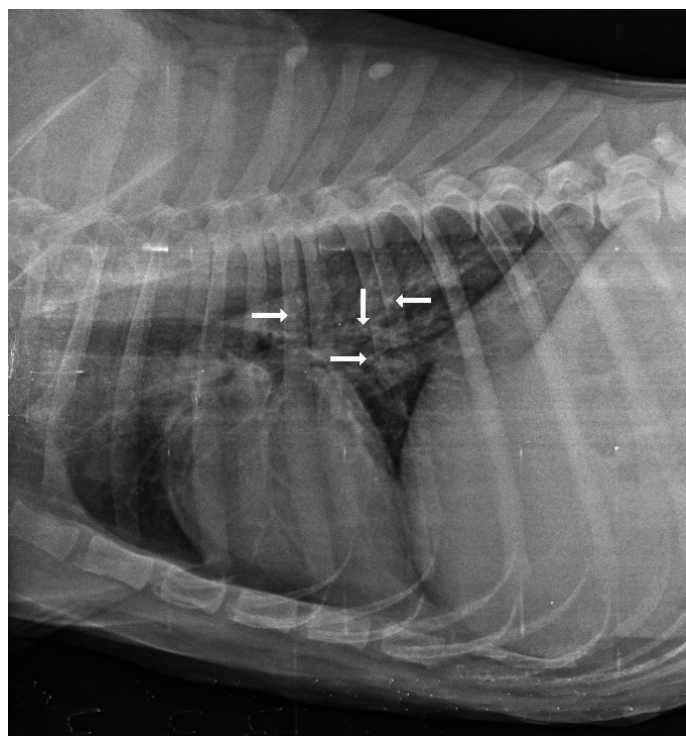


FIGURE 3. Thorax radiography. Foci of different sizes (arrows) in the lung that may be associated with metastasis (second case).

Similarly, to other neoplasms, CTVT requires meticulous anamnesis and clinical examination as the first steps in the diagnostic procedure. Additionally, this tumor exhibits characteristic cytological features; hence, cytology is often used to provide a prompt diagnosis of CTVT [15, 22, 23]. Although histopathology remains the gold standard for diagnosing CTVTs, cytological evaluation—a fast, low cost, and field applicable method—has often been overlooked [13]. In comparison to routine histopathological analysis, cytology provides equally accurate, if not superior, confirmation of CTVT [24]. This diagnostic benefit is particularly important given that the microscopic appearance of TVT can closely resemble other roundcell neoplasms—such as histiocytoma, lymphosarcoma, or mast cell tumor—especially when lesions occur at extragenital sites [15].

In the cytological examinations of the presented cases, round to polyhedral-shaped cells (FIG. 4 A-B-C), with an eccentrically located nucleus, multiple mitosis (FIG. 4 A-C) and the characteristic distinct, cytoplasmic vacuolations with clear outlines (FIG. 4 A-B-C) were observed. A marked difference in shape and size was observed in the nuclei of some tumor cells (anisonucleosis) (FIG. 4 B).



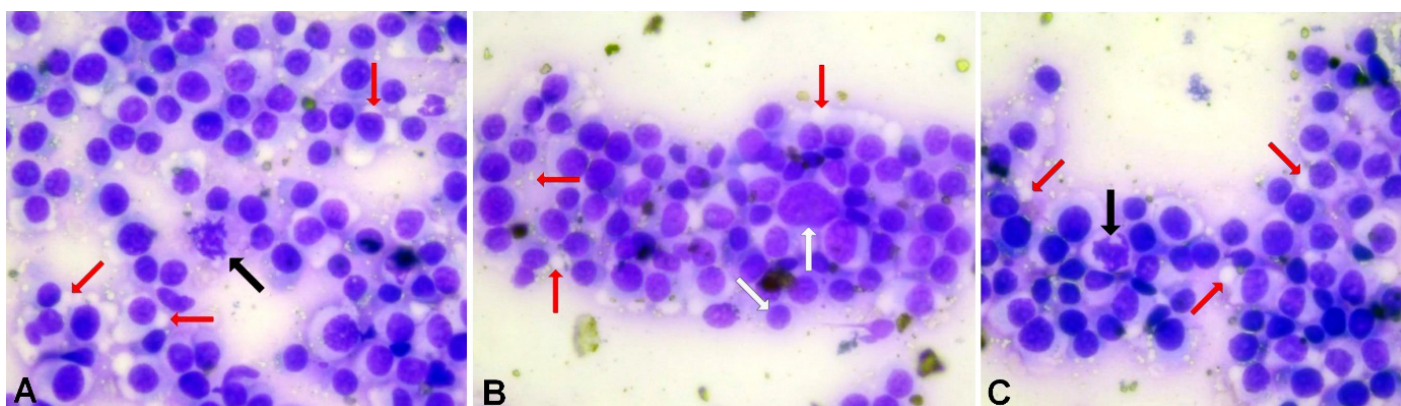


FIGURE 4. Cytologic findings of first case (A) and second case (B,C). Round to polyhedral-shaped tumor cells, with an eccentrically located nucleus, multiple mitosis (black arrows), the characteristic distinct, cytoplasmic vacuolations with clear outlines (red arrows), marked difference in shape and size in the nuclei of some tumor cells (anisoneucleosis) (white arrows), Diff-Quick, 40x

Distinct, sharply demarcated cytoplasmic vacuoles are the most characteristic finding in TVT cytology, with their size and number varying according to the individual tumor cell's morphology. In cases where these vacuoles are absent, TVT cells can closely resemble other round cell neoplasms, although the overall cellular shape and the tumor's anatomical location often provide critical diagnostic clues [13]. Additionally, TVT cells typically exhibit a relatively low nuclear to cytoplasmic ratio alongside the distinct punctate vacuoles—features that together help to distinguish this neoplasm on cytological smears [25]. These changes reported in the cytological examination of CTVT, which is recommended by the researchers as a rapid and reliable diagnostic method, were observed in both cases.

Histopathological examinations of both cases revealed round or polyhedral neoplastic cells arranged in solid sheets or rows supported by fibrovascular connective tissue septa. Numerous mitotic figures were also observed together with tumor cells (FIGS. 5 A-B and 6 A). Tumor cells with indistinct cell borders had eosinophilic and finely granular cytoplasmic structures. The nuclei of tumor cells were usually large and round, and size variations were noticeable (FIGS 5 A-B and 6 A). Tumor cell nuclei had centrally located nucleoli and some nuclei showed marginal hyperchromasia (FIG. 5 B).

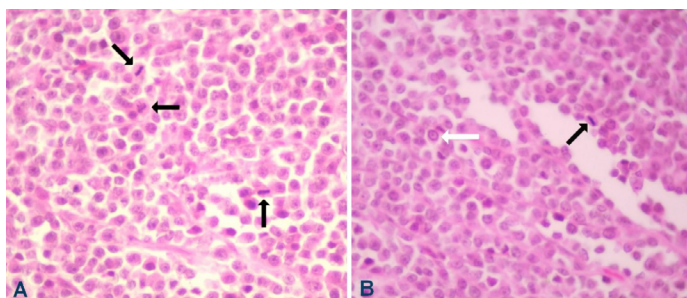


FIGURE 5. A-B. Round or polyhedral neoplastic cells arranged in solid sheets or rows supported by fibrovascular connective tissue septa. Numerous mitotic figures (black arrows), together with tumor cells, H&E40x, B. Marginal hyperchromasia (white arrow), H&E, 40x, (First Case).

Neutrophil granulocytes were also found along with tumor cells in ulcerated areas resulting from degeneration and desquamation of vaginal epithelium. Mononuclear cell

infiltration was observed in some areas along with tumor cells. In some sections, necrotic changes in tumor cells (FIG. 6 B) and hemorrhage foci (FIG. 7 B) were observed. In one section, a tumor cell embolism consisting of tumor cells adherent to the vessel wall was observed in the vessel lumen (FIG. 7 A-B).

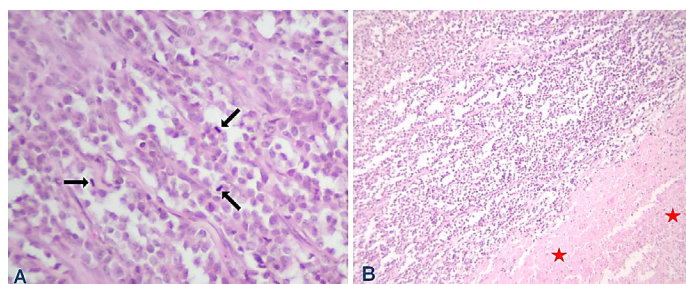


FIGURE 6. A. Round or polyhedral neoplastic cells with mitotic figures (black arrows) H&E, 40x, B. Necrotic changes in tumor cells (red stars) and neoplastic cells, H&E, 10x, (Second Case)

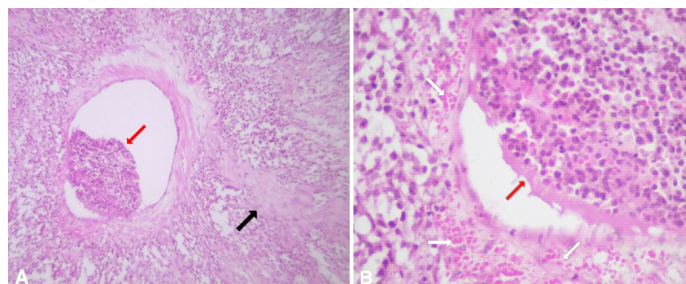


FIGURE 7. Tumor cell embolism consisting of tumor cells adherent to the vessel wall in the vessel lumen (red arrow) (A and B), fibrovascular connective tissue septa (black arrow) (A), H&E, 10x, B. Perivascular hemorrhage foci (white arrows), H&E, 40x, (Second Case)

Histopathological changes previously reported by the researchers [4, 5, 10, 15] in tumor tissue and tumor cells in CTVT cases were observed in both cases. In addition, inflammatory changes were detected in ulcerated areas in these cases. In second case, necrotic changes in tumor cells (FIG. 6 B) were noticed in some areas. Hendrick [26] stated that these tumors are often infiltrated by varying quantities of lymphocytes—occasionally clustering into aggregates—along with plasma cells, eosinophils, and macrophages. In lesions undergoing

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regression, there is typically marked inflammation accompanied by areas of necrosis and fibrous tissue formation. Foster [27] further reported that CTVT can develop multifocal necrotic foci and may regress spontaneously through lymphocyte mediated cytotoxic mechanisms. In the presented cases, the observation of lymphocyte infiltration in some areas together with necrotic tumor cells supports these views [26, 27]. However, it is thought that necrosis may develop as a result of inadequate vascularization as a result of rapid growth of tumor tissue.

Metastatic dissemination of CTVT has been reported in subcutaneous tissues, skin, regional lymph nodes, eyes, tonsils, liver, spleen, oral and nasal mucosa, brain, and bone marrow. Although fewer than 5 % of CTVT cases develop metastases, evidence indicates that such systemic spread markedly worsens clinical outcomes and is a documented cause of mortality. These rare but severe events highlight the critical need for prompt diagnosis and aggressive management when extragenital involvement is suspected [2, 5, 10, 15, 16, 17, 18]. In the second case (Alabai crossbred), structures related to lung metastasis were seen on thorax radiography (FIG. 3). In the same case, microscopic examinations showed tumor cell embolism adhered to the vessel wall and in the vessel lumen in a section (FIG. 7 A-B), which was evaluated as evidence that hematogenous metastasis had formed in this case. It should not be ignored that lung metastasis of CTVT, which has been reported by some investigators [28, 29], may lead to respiratory problems since it was also observed in this case.

CTVT is amenable to multiple treatment strategies. Traditionally, management has centered on surgical excision of the lesion, which is frequently followed by single agent or combination chemotherapy protocols, and occasionally by radiotherapy. Surgical removal is reserved for solitary, well localized masses and is generally avoided in cases with widespread or metastatic involvement. Because conventional excision carries a risk of tumor cell implantation at the wound site, techniques such as electrosurgical resection or cryosurgery are often employed as safer alternatives. Published recurrence rates after standard surgical excision vary widely, from approximately 12 % to 68 % [2, 7, 22, 30]. Vincristine is the most preferred and most reliable chemotherapeutic agent that can be used even in cases with metastases outside the genital organs. Vincristine is an agent that inhibits the cell division during the metaphase stage. For this reason, it should be administered slowly and ensure that it does not infiltrate to subcutaneous tissue during the intravenous administrations [22, 30]. Combination chemotherapy remains the cornerstone of CTVT treatment, with the classic regimen of vincristine, cyclophosphamide, and methotrexate demonstrating high efficacy and remission rates. Several alternative protocols have been evaluated—including cyclophosphamide with prednisolone, vinblastine combined with cyclophosphamide or methotrexate, and adjunctive vincristine–ivermectin therapy—to optimize outcomes and address drug resistance [2, 7]. In parallel, immunotherapy has evolved from early passive techniques (convalescent serum or whole blood transfusion, tumor cell homogenate vaccines with bacterial adjuvants) to sophisticated active strategies, such as cytokine gene delivery (interleukin 2, IL 6, IL 15 plasmids), dendritic cell vaccines loaded with tumor exosomes, and explorations of oncolytic viral vectors, all aimed at potentiating host cellular immunity against CTVT [15].

## CONCLUSIONS

In the presented case report, CTVT was diagnosed to the cases due to the appearance of round-oval shaped tumor cells, mitotic figures and pronounced vacuoles in the cytoplasm in the cytological examinations of both cases. Changes observed in tumor tissue and cells during histopathological examinations support this diagnosis.

When the studies conducted on the distribution and prevalence of CTVT in dogs in the world and the literature that can be examined were evaluated, it was deemed appropriate to share these cases due to the lack of any reports in Kyrgyzstan related to CTVT. For this reason, these case reports are important because they are being reported for the first time in Kyrgyzstan.

It was deemed appropriate to report the clinical, cytological, macroscopic and histopathological findings of CTVT that diagnosed in two different cases in order to contribute to the field of veterinary oncology and to be useful to clinicians.

## Conflict of interest

There is no conflict of interest between the authors

## Funding

This research received no external funding.

## Informed Consent Statement

The animal owners were informed before the operation and a consent form was obtained. In conducting this study, it is important to note that ethical clearance was not sought or required. This article represents a case report, not a formal research study involving experimentation or clinical trials on animals.

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