

## CASE REPORT

# Computer tomographic imaging in 4 dogs with primary nasal canine transmissible venereal tumor and differing cellular phenotype

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Primary nasal canine transmissible venereal tumor (CTVT) without genital affection is uncommon. The aim of this report was to describe the primary nasal CTVT findings and CT staging in 4 dogs with different cytological phenotypes. Three male dogs and 1 bitch were evaluated for their chronic histories of sneezing, snoring, mucopurulent nasal discharge and nasal deformation. Cytological examination of nasal secretions suggested CTVT, confirmed by histopathological examination and LINE-1/c-myc. Males had the plasmacytoid phenotype of CTVT, and the bitch had the lymphocytoid phenotype. CTVT were staged based on the CT findings using modified Adams staging system. The bitch was classified as stage 1, 2 males were classified as stage 3 and 1 male as stage 4. All dogs had a complete tumoral remission after chemotherapy. Plasmacytoid phenotype was identified in cases with most important damage of the nasal cavity. However, the cytological type did not affect the response to chemotherapy.

**KEYWORDS**

CTVT, cytology, dogs, nasal tumor

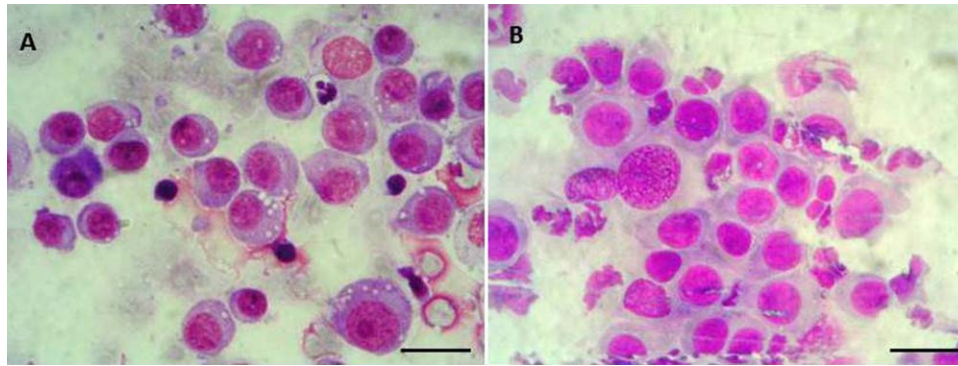
**CASE 1**

The first case was an 8-year-old entire male mixed breed Golden Retriever weighing 26 kg. He had been a stray dog until adoption 1 week before presentation for sneezing, snoring, mucopurulent nasal discharge, progressive nasal deformation, and dyspnea. Physical examination revealed snoring, serosanguinous nasal discharge, facial deformation, and pain upon palpation of the nasal area. Oral examination revealed a mass with the aspect of granulation tissue and mucohemorrhagic discharge at the level of the left maxillary premolar teeth as well as a similar circular mass at the caudal aspect of the hard palate. The left maxilla lacked part of some teeth. The mandibular lymph nodes were bilaterally enlarged. Open mouth radiographs of the nasal cavity

revealed increased soft tissue opacity and a loss of the ethmoid turbinates of the caudal left nasal cavity, destruction of the nasal septum, and lysis on the left side of the maxillary bone. There was no evidence of pulmonary disease on thoracic radiographs. Cytological examination of the nasal discharge suggested round cell neoplasia. Many ovoid cells with abundant cytoplasm, eccentrically located nuclei, and several clear vacuoles in the cytoplasm were observed. Many neutrophils and Gram-negative bacteria were also observed. In addition, neoplastic cells were detected in the left mandibular lymph node. The right mandibular lymph node and both prescapular and popliteal lymph nodes were also aspirated and did not reveal neoplastic infiltration. The cytological findings supported a diagnosis of plasmacytoid canine transmissible venereal tumor (CTVT; Figure 1A).<sup>1</sup> Hematologic assessment revealed leukocytosis ( $29.2 \times 10^3/\text{mL}$ , reference range [RI]  $5.3\text{--}19.8 \times 10^3/\text{mL}$ ) associated with neutrophilia ( $17.3 \times 10^3/\text{mL}$ , RI  $3.1\text{--}14.4 \times 10^3/\text{mL}$ ), eosinophilia ( $3.4 \text{ cells} \times 10^3/\text{mL}$ , RI  $0.0\text{--}1.6 \times 10^3/\text{mL}$ ), monocytosis

**Abbreviations:** CTVT, canine transmissible venereal tumor; CT, computed tomography; HU, Hounsfield Units; LINE, long interspersed nuclear elements; PCR, polymerase chain reaction; RI, reference interval.

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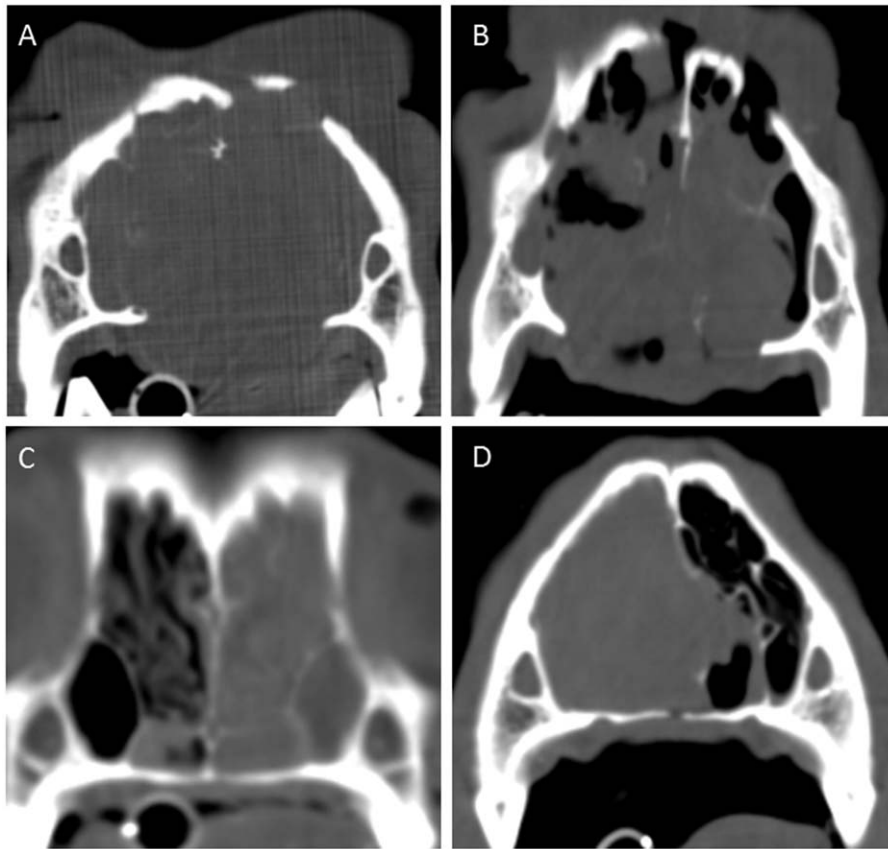
**FIGURE 1** Nasal cytology smears showing the plasmacytoid CTVT phenotype with ovoid cells, abundant cytoplasm, several vacuoles, and an eccentrically located nucleus (A). Nasal cytology smears showing the lymphocytoid CTVT phenotype with round cells, finely granular cytoplasm, few clear vacuoles, nuclei centrally located and a coarse chromatin pattern and 1–2 distinct nucleoli (B). Diff-Quick stain, Scale Bar = 20  $\mu$ m

( $2.1 \times 10^3$ /mL, RI  $0.1\text{--}1.4 \times 10^3$ /mL), and lymphocytosis ( $6.2 \times 10^3$ /mL, RI  $0.9\text{--}5.5 \times 10^3$ /mL). Abnormalities were not detected on serum biochemistry, except for a mild increase in alkaline phosphatase (291 U/L, RI 20–155 U/L), blood urea nitrogen (48 mg/dL, RI 5–30 mg/dL), and creatinine (2.5 mg/dL, RI 0.7–1.8 mg/dL); the renal parameters returned to reference values after 2 days of hospitalization with free access to water. A contrast-enhanced CT (PQ 600, Universal Systems, Solon, Ohio) and subsequent rhinoscopy (60003 VB1, Storz, Karl Storz GmbH & Co. KG, Tuttlingen, Germany) with biopsies were performed. CT revealed extensive soft tissue attenuation with heterogeneous enhancement involving both nasal cavities and frontal sinuses. Additionally, lysis of the nasal septum, palatine bone, rostral portion of the left maxillary bone, caudal portion of the nasal bone, and rostral portion of the frontal bone was also detected. Erosion of the cribriform plate was observed (Figure 2A). Bilateral mandibular lymph nodes were enlarged, but the left mandibular lymph node showed a moderate homogenous enhancement. Rigid endoscopy showed an abundant hemorrhagic exudate with a mass extending on both sides of the nasal cavity. The images were consistent with a nasal tumor that was categorized as stage 4 according to the modified Adams staging system (Supporting Information Table S1).<sup>2</sup> No genital affection was detected. Histopathological examination revealed oval and polyhedral cells organized in a compact mass infiltrated with thin bands of fibrovascular tissue with a trabecular appearance. The cells lacked a cytoplasm and had a prominent ovoid nucleus with granular and fine chromatin and 1 or 2 nucleoli. The marginal zone presented a diffuse hemorrhagic zone (Figure 3). The histopathological diagnosis was transmissible venereal tumor. This diagnosis was confirmed using PCR to detect LINE-1/c-myc (LINE-1/c-myc primers, IDT, Coralville, Iowa) as a specific genetic alteration of this tumor.<sup>3–5</sup> Antibiotic therapy with amoxicillin-clavulanic acid (Amoxicillin-Acid Clavulanic, Sinulox<sup>TM</sup>, Pfizer, New York) at 20 mg/kg q12h for 1 week and chemotherapy with doxorubicin (Doxorubicin, Adriamycin, Pfizer) was initiated. A complete blood count (CBC), ventricular fractional shortening, and electrocardiogram were obtained before each chemotherapy session. The use of doxorubicin was determined based on the local invasiveness of the tumor and evidence of dyspnea. After the first week of treatment, the

dog showed signs of improvement, with reduced nasal deformation, sneezing and nasal discharge. One month after the 4 chemotherapy sessions, absence of respiratory stridor, sneezing, and nasal discharge were observed. In addition, slight nasal deformation without pain and normal mandibular lymph nodes were detected at clinical examination. Rhinoscopy with cytological examination of the nasal cavity and mandibular lymph nodes showed normal findings. Contrast-enhanced CT images showed complete remission of CTVT without evidence of regional lymph node involvement. Only residual nasal fluid attenuation (<28 HU) was detected (Figure 4A). The dog was re-evaluated at 6 months after medical discharge, and no abnormal clinical signs were detected.

## CASE 2

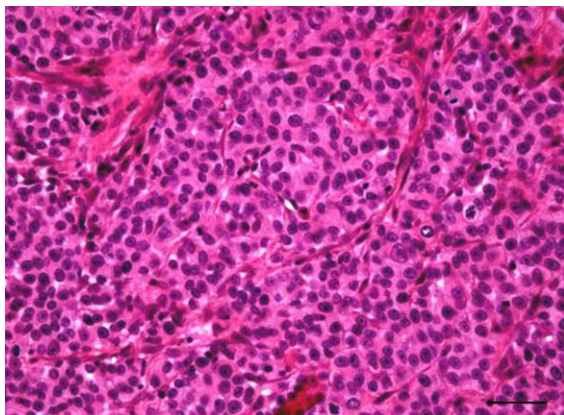
A 3-year-old, entire male mixed breed Dachshund (formerly a stray dog), weighing 20 kg, was evaluated for chronic snoring, frequent sneezing, and noisy breathing since adoption. The dog was referred when a facial deformation between the eyes was detected and right mandibular lymph node was enlarged at presentation. A CBC revealed mild leukocytosis ( $23.2 \times 10^3$ /mL, RI  $5.3\text{--}19.8 \times 10^3$ /mL) associated with neutrophilia ( $16.3 \times 10^3$ /mL, RI  $3.1\text{--}14.4 \times 10^3$ /mL) with a left shift ( $0.9 \times 10^3$ /mL, RI  $0.0\text{--}0.2 \times 10^3$ /mL) and monocytosis ( $2.3 \times 10^3$ /mL, RI  $0.1\text{--}1.4 \times 10^3$ /mL). The serum biochemistry revealed an increase in alkaline phosphatase (253 U/L, RI 20–155 U/L). Skull radiographs revealed a bilateral increased soft tissue opacity of the nasal cavity with ethmoid turbinate osteolysis, thinning of the nasal septum and soft tissue opacity of the left frontal sinus. Abnormalities were not detected on thoracic radiographs. Contrast-enhanced CT images showed enhancing soft tissue material extending in both sides of the nasal cavity and frontal sinuses as well as a bilateral destruction of the turbinates, maxillary hard palate, soft palate, septum, and nasal bone. The cribriform plate was unaltered (Figure 2B). Enlarged right mandibular lymph node with a moderate rim enhancement was detected. Left mandibular lymph node was normal. The CT findings were consistent with a nasal neoplasm that was categorized as stage 3 according to the modified Adams staging system (Supporting



**FIGURE 2** Transverse computed tomographic images of the nasal cavities and maxillary recesses of four dogs with nasal CTVT. CT showed an irregular, soft-tissue mass occupying nasal turbinates and paranasal bones destruction (A, B, D). The soft tissue attenuation was between 80 and 120 HU

Information Table S1).<sup>2</sup> Cytological analyses of the nasal cavity showed multiple round cells and a vacuolated cytoplasm with prominent and eccentric nuclei, suggesting plasmacytoid CTVT. The right mandibular lymph cytology revealed nonspecific reactive hyperplasia. No evidence

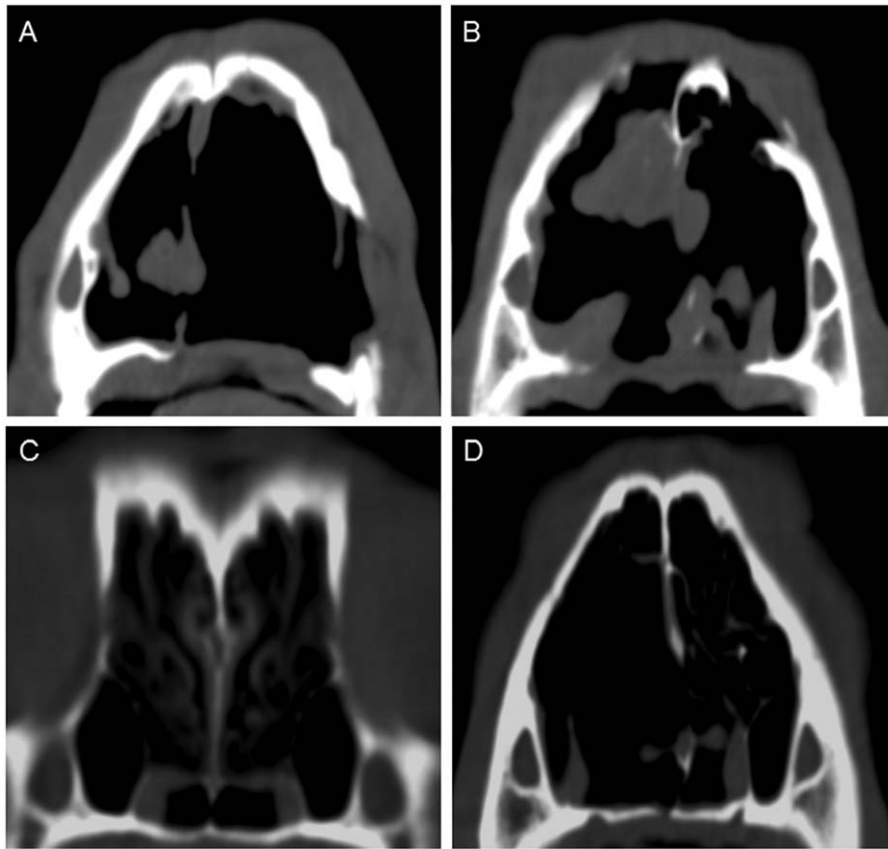
of a genital tumor was detected. Histopathological examination confirmed CTVT. The definitive confirmation was made with LINE-1/c-myc detection by PCR. Chemotherapy with vincristine (Vincristina, Pfizer, New York, New York) was started. The dog's clinical signs improved from the second session onwards. A follow-up contrast-enhanced CT was performed one month after the seventh chemotherapy session and revealed residual fluid attenuation (<22 HU), absence of enhancing soft tissue material in the nasal cavity and frontal sinus, and no contrast enhancement in the mandibular lymph nodes (Figure 4B). Without clinical signs of nasal CTVT, rhinoscopy, and nasal cytology were used to confirm the complete tumoral remission.



**FIGURE 3** Nasal primary CTVT histopathology showing the multinodular compact proliferation of pleomorphic, round cells separated by a delicate fibrovascular stroma. The cells have a large prominent nucleus and a moderate amount of cytoplasm, large ovoid nuclei with 1–2 prominent nucleoli and multiple mitotic figures. Hematoxylin and eosin stain, Scale Bar = 30  $\mu$ m

### CASE 3

A 3-year-old, entire female, Toy Poodle weighing 5 kg was presented for chronic nasal discharge and sneezing of 1-month duration. The clinical signs had progressed, despite treatment with enrofloxacin (Enrofloxacin, Baytril, Bayer, Leverkusen, Germany; 5 mg/kg PO q24h) for 14 days. The vaccinations and deworming were up to date. Nasal discharge from the left nostril was the only abnormality detected during the physical examination. Skull radiographs revealed soft tissue opacity in the region of the ethmoid turbinates. Abnormalities were not detected on thoracic radiographs. CT revealed soft tissue attenuating



**FIGURE 4** Transverse computed tomographic images of the nasal cavities and maxillary recesses of four dogs with nasal CTVT after completing chemotherapy. CT showed turbinate and paranasal bones destruction with fluid attenuation associated with mucus accumulation (A, B, D). The soft tissue mass completely disappeared without evidence of bone destruction (C)

material with moderate enhancement invading the left nasal cavity and left frontal sinus. There was no turbinates or paranasal bones destruction (Figure 2C). No enhancement of the cranial cervical lymph nodes was detected. CT images indicated modified Adams stage 1 disease (Supporting Information Table S1).<sup>2</sup> Rhinoscopy revealed a hemorrhagic mass in the left nasal cavity. Cytological examination identified round cells with a finely granular cytoplasm containing few clear vacuoles; round nuclei were centrally located with a coarse chromatin pattern and 1–2 nucleoli. The cytological diagnosis was lymphocytoid CTVT (Figure 1B).<sup>1</sup> A complete vaginal examination did not detect any tumor. Histological examination and PCR for LINE-1/c-myc confirmed CTVT. Four sessions with vincristine were necessary to eliminate the clinical signs. Contrast-enhanced CT images, rhinoscopy, and cytologic findings 1 month after the last chemotherapy session showed a complete remission of CTVT (Figure 4C).

#### CASE 4

A 2-year-old, entire male stray dog weighing 16 kg was presented with a facial deformation and hemorrhagic nasal discharge without previous medical information. Clinical examination revealed bilateral hemorrhagic nasal discharge. The mandibular lymph nodes were enlarged bilaterally. Cytologic examination of the nasal discharge detected

neoplastic cells compatible with plasmacytoid CTVT.<sup>1</sup> The mandibular lymph node aspirate revealed a nonspecific reactive hyperplasia. Abnormalities were not detected on thoracic radiographs. Skull radiographs revealed nasal septum destruction and unilateral soft tissue opacity in the caudal portion of the mass of the right nasal cavity. Contrast-enhanced CT revealed an enhancing soft tissue mass invading the right nasal cavity, destruction of the ethmoid turbinates and lysis of the nasal bone (Figure 2D). Enlarged mandibular lymph nodes showed a mild rim enhancement. The CT findings indicated a modified Adams stage 3 (Supporting Information Table S1).<sup>2</sup> Histopathological examination and PCR with LINE-1/c-myc confirmed CTVT. Penis examination did not reveal any tumor. Six sessions with vincristine were sufficient to attain complete clinical remission, which was confirmed by CT images, rhinoscopy, and cytology at 1 month after the last chemotherapy session (Figure 4D).

#### DISCUSSION

Our study describes 4 cases of primary nasal CTVT with different cytological phenotypes and CT staging of primary nasal CTVT. CTVT is a cancer that spreads by allograft transmission. CTVT primarily affects the genital external epithelium of male dogs and bitches.<sup>6,7</sup> CTVT is transmitted when malignant cells are transferred directly from 1 dog to

another dog via coitus, licking, biting or sniffing tumor areas, such as the external genitalia or skin.<sup>6,8,9</sup> CTVT presents a low metastatic potential<sup>7,10</sup>; however, metastases to the skin, lungs, abdominal organs, and central nervous system have been described.<sup>8,11-14</sup> The biological behavior and pathogenesis of the primary extragenital forms of CTVT are still not well established because the number of cases is small in relevant reports.<sup>15,16</sup> Damage to the external genital mucosa facilitates cutaneous inoculation of CTVT cells via cell transplantation by biting or scratching<sup>7</sup>; however, no reasonable explanation has been given for the intranasal form of the disease. A possible explanation is that TVT cell inoculation and growth on the nasal mucosa are initiated through sniffing of another dog's infected genital organs.<sup>16,17</sup> Although previous cases of intranasal CTVT have been reported, few have been treated,<sup>16-18</sup> and the consequent information related to the chemotherapy response or tumor invasion is scarce. The nasal form of CTVT represents 5%-13% of all CTVT cases according to previous reports; most of these cases occur in adult male dogs.<sup>6,19</sup> The 3 dogs reported here were males older than 1 year of age. However, some studies have reported a higher prevalence in females,<sup>4,15</sup> likely associated with areas with no birth control or many females and fewer males. However, other studies have shown a higher prevalence in males,<sup>6,16-19</sup> suggesting a strong relationship between CTVT and promiscuous reproduction behavior in dogs that directly sniff and lick the vaginal tumor.

One of the most important features of CTVT is the development of immune evasion strategies via down-regulation of cell surface major histocompatibility complex class I and class II proteins.<sup>7</sup> These strategies could be enhanced in stray dogs because of their immune-incompetent condition.<sup>15</sup> However, the 4 cases reported here did not show any evidence of immunosuppressive conditions, despite the fact that 3 of these animals had a history of being stray dogs.

Nasal discharge is a common finding that is not exclusively because of nasal CTVT and may be observed in persistent nasal diseases, such as nonspecific rhinitis, nasal neoplasia, fungal infection, cleft palate, periodontal disease, parasites, foreign bodies, and primary bacterial disease.<sup>20</sup>

In spite of routine cytological diagnosis for CTVT, the use of histopathological examination and molecular techniques, such as PCR for LINE-1/c-myc, ensure a definitive diagnosis. Unlike previously reported nasal CTVT cases in which diagnostic imaging principally consisted of radiography,<sup>16,21,22</sup> these cases were evaluated with CT images. CT findings were evaluated according to the modified Adams staging system, which associates the CT findings with the treatment outcome of canine nasal tumors.

Case 1 had a mild affection of the cribriform plate and therefore was categorized as stage 4.<sup>2</sup> Eventually, progressive tumoral growth could have invaded the central nervous system. In cases 2 and 4, CT revealed that CTVT invaded the nasal cavity and subcutaneous tissue up to the cribriform plate. These cases were categorized as stage 3 diseases. Case 3 had a unilateral mass with no bone affection beyond turbinate and therefore was categorized as a stage 1 disease. Despite total or partial nasal bone destruction, particularly in cases 1, 2, and 4, follow-up with contrast-enhanced CT images; nasal and lymph node cytology after chemotherapy showed complete remission. Case 3

showed preserved ethmoid turbinates after treatment. The modified Adams CT-based staging system for canine intranasal neoplasia could not be used to obtain a prognosis for CTVT because the treatment showed complete tumoral remission in all 4 cases. However, future studies could determine the prevalence of chronic rhinitis and osteomyelitis in a major population of dogs with nasal CTVT after chemotherapy. These findings represent common complications in dogs<sup>23</sup> and humans<sup>24</sup> after resection or destruction of turbinates. Previous studies have described CTVT in nasal cavities with a low metastatic rate.<sup>7,16,22</sup> Despite the paranasal bones destruction detected in cases 1, 2, and 4, the only metastasis evidence was detected in the left mandibular lymph node of case 1. This situation is particularly controversial regarding CTVT behavior because despite the nasal destruction detected, the low metastatic potential is similar to described for genital presentation.<sup>7,10</sup> Some authors have reported that CTVT metastasis typically occurs in malnourished, young dogs or those with immunosuppression.<sup>8,15</sup> Favorably, none of the dogs reported here had any of the above conditions.

On the basis that CTVT shows a different biological behavior, response to chemotherapy and aggressiveness, 3 cytological phenotypes were determined: lymphocytoid, plasmacytoid and mixed. Lymphocytoid or plasmacytoid phenotypes are determined when 1 of these types represents more than 60% of total CTVT cells. A mixed-phenotype is classified when neither type exceeds 59% of total cells.<sup>1,25</sup> The plasmacytoid CTVT cell type could be related to major tumoral invasiveness, chemotherapy resistance or extragenital location.<sup>5,11,25</sup> In our study, 3 dogs with severe nasal bone destruction showed plasmacytoid CTVT. The dog with the lymphocytic CTVT type did not show any bone destruction of the nasal cavity, despite a marked invasion in the nasal cavity. Complete tumoral remission was reached with chemotherapy in all 4 cases. Information regarding the effectiveness of chemotherapy or evolution of nasal CTVT under treatment is extremely limited because the prognosis of nasal CTVT is poor in many cases, and therefore, the dogs are euthanized.<sup>8,16,26</sup>

In this series, cases 2, 3, and 4 were treated with vincristine, whereas case 1 was treated with doxorubicin instead of vincristine with the expectation of a faster response considering the severe respiratory signs and plasmacytoid cytological phenotype. Solely on the basis of these reports, it is difficult to make a recommendation for the treatment of CTVT with nasal damage. Vincristine is the chemotherapy of choice and has been used to attain complete resolution, even in disseminated cases.<sup>11</sup> However, P-glycoprotein, which is associated with multidrug resistance, has stronger expression in plasmacytoid CTVT cells than in lymphocytoid phenotypes.<sup>27</sup> Despite the limited number of cases described here, the findings described in our study suggest that the plasmacytoid phenotype could be more closely associated with local aggressiveness than chemoresistance.<sup>25,27,28</sup>

In case 3, which was basically an indoor dog, there was early detection of the clinical signs by the owner. This difference from the other 3 dogs could explain the absence of paranasal bones destruction. Therefore, the plasmacytoid phenotype could be a progressive stage of the lymphocytoid pattern. In these cases, chemotherapy with vincristine or doxorubicin was effective for primary nasal CTVT. The

outcomes were irrespective of the cytological CTVT type and CT-based tumor staging.

#### CONFLICT OF INTEREST DECLARATION

The authors declare that they have no conflict of interest with the contents of this article.

#### OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

#### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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#### SUPPORTING INFORMATION

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