CO-INFECTION BY CANINE TRANSMISSIBLE VENEREAL TUMOR, HEPATOZOONOSIS, AND DIROFILARIASIS – A CLINICAL CHALLENGE IN DOGS – CASE REPORT

COINFECÇÃO POR TUMOR VENÉREO TRANSMISSÍVEL CANINO, HEPATOZOONOSE E DIROFILARIOSE – UM DESAFIO CLÍNICO EM CÃES – RELATO DE CASO

Breno Sérgio Corona Dias[1], Nicolas Lamburghini Negrelli[1], João Henrique Binda Ilário[1], Rania Luiza De Angeli Della Valentina[1], Rafaela Lima Coppo[1], Karina de Mesquita Calmon[2], Mariana Caetano Teixeira[3], Clairton Marcolongo-Pereira[4]

[1]Graduando em Medicina Veterinária no Centro Universitário do Espírito Santo UNESC. [2]Médica Veterinária autônoma, Clínica Veterinária Casa do Criador, Vitória-ES.[3]Graduação em Medicina Veterinária pela Universidade Luterana do Brasil. Mestrado e Doutorado concluídos em Ciências Veterinárias com ênfase em Parasitologia Clínica na Universidade Federal do Rio Grande do Sul. Experiência em docência na UNOESC/Xanxerê entre 2011 e 2013. Desde 2013 é professora do Centro Universitário Ritter dos Reis (UNIRITTER). [4]Graduação em Administração pela Fundação de Assistência e Educação - FAESA (2001), Medicina Veterinária pela Universidade Vila Velha (2006), residência em Medicina Veterinária pela Universidade de Vila Velha (2007), mestrado em Sanidade Animal pela Universidade Federal de Pelotas (2010), doutorado em Sanidade Animal pela Universidade Federal de Pelotas (2014), estágio pós doutoral pela Universidade Federal de Pelotas (2015) e pela Universidade Federal do Rio Grande do Sul (2017). Atuou como apoio à coordenação do curso de Medicina Veterinária do Centro Universitário Ritter dos Reis - UniRitter. Atualmente é professor do Centro Universitário do Espírito Santo UNESC (ES). Tem experiência na área de Medicina Veterinária, com ênfase em patologia animal, fisiologia, histologia, doenças infecciosas, zoonoses, ornitopatologia, toxicologia e plantas tóxicas.

RESUMO

O tumor venéreo transmissível canino (TVTC) é uma neoplasia de células redondas de origem mesenquimal, transmissível principalmente por coito, que afeta a mucosa genital de cães. Este estudo descreve um caso de coinfecção envolvendo TVTC, hepatozoonose canina e dirofilariose em um cão macho, sem raça definida, atendido na região metropolitana da Grande Vitória, Espírito Santo. O paciente apresentava sangramento peniano persistente e, após exames complementares, foi diagnosticado com TVTC por citologia aspirativa, hepatozoonose por esfregaço sanguíneo e dirofilariose por meio do teste SNAP 4Dx® Plus, que também revelou coinfecção por *Ehrlichia canis* e *Anaplasma* spp. O manejo terapêutico incluiu quimioterapia com sulfato de vincristina para o TVTC, dipropionato de imidocarb para hepatozoonose, doxiciclina para erliquiose e anaplasmose, e ivermectina para dirofilariose. O caso evidenciou o desafio clínico de tratar coinfecções em pacientes imunossuprimidos devido à quimioterapia, o que favorece a manifestação de infecções oportunistas como a hepatozoonose. A utilização de métodos diagnósticos complementares e o acompanhamento hematológico contínuo foram cruciais para o sucesso do tratamento. Este relato reforça a importância de uma abordagem clínica integrada

Autor correspondente: cmpereira@unesc.br

para o diagnóstico e manejo de coinfecções, especialmente em regiões endêmicas para doenças transmitidas por vetores, visando otimizar o prognóstico e a qualidade de vida dos pacientes.

Palavras-chave: Tumor venéreo transmissível canino, hepatozoonose, coinfecção, dirofilariose, cães.

ABSTRACT

Canine transmissible venereal tumor (CTVT) is a round cell neoplasm of mesenchymal origin that is primarily transmitted through the coitus, affecting the genital mucosa of dogs. This study describes a case of co-infection with CTVT, canine hepatozoonosis, and dirofilariasis in a mixed-breed, intact male dog from the metropolitan region of Grande Vitória, Espírito Santo, Brazil. The patient presented with persistent penile bleeding and, after complementary examinations, was diagnosed with CTVT through fine-needle aspiration cytology, hepatozoonosis via blood smear, and dirofilariasis using the SNAP 4Dx® Plus test, which also revealed co-infection with Ehrlichia canis and Anaplasma spp. The therapeutic approaches included vincristine sulfate chemotherapy for CTVT, imidocarb dipropionate for hepatozoonosis, doxycycline for ehrlichiosis and anaplasmosis, and ivermectin for dirofilariasis. This case highlights the clinical challenge of managing co-infections in immunosuppressed patients due to chemotherapy, which facilitates the manifestation of opportunistic infections such as hepatozoonosis. The use of complementary diagnostic methods and continuous hematological monitoring is crucial for successful treatment. This report emphasizes the importance of an integrated clinical approach for diagnosing and managing co-infections, especially in regions endemic to vector-borne diseases, to optimize patient prognosis and quality of life.

Keywords: Canine transmissible venereal tumor, hepatozoonosis, co-infection, dirofilariosis, dogs.

1 INTRODUCTION

Canine transmissible venereal tumor (CTVT) is a round cell neoplasm of mesenchymal origin that predominantly affects the mucosa of the external genitalia in dogs regardless of sex (Strack *et al*., 2021). Transmission occurs most commonly during coitus through the mechanical implantation of neoplastic cells, although alternative routes, such as licking or direct contact with affected areas, have also been reported (Strack *et al*., 2021). Clinically, CTVT presents with signs such as serosanguineous discharge, foul odor, licking of the affected area, dysuria, ulceration, and tissue necrosis. The tumor mass typically appears friable with a cauliflower-like surface and may be solitary or multiple (Araújo *et al*., 2016).

A definitive diagnosis of CTVT is achieved through fine-needle aspiration or imprint cytology combined with clinical history and observed signs (Ferreira *et al*., 2017). Chemotherapy with vincristine sulfate remains the treatment of choice, with a recommended dose ranging from 0.50 mg/m² to 0.75 mg/m² administered weekly over four to six weeks, achieving high success rates. However, treatment may lead to adverse effects such as neurotoxicity and bone marrow suppression, which can predispose patients to opportunistic infections (Ferreira *et al*., 2017).

Canine hepatozoonosis is another significant parasitic disease characterized by an insidious and often subclinical course, typically marked by low parasitemia (O'Dwyer, 2011). Infection occurs when dogs ingest ticks, mainly from the *Rhipicephalus* genus, carrying protozoa of the *Hepatozoon* genus, usually while feeding on a parasitemic host (Baneth *et al*., 2007). The disease is more prevalent in rural areas owing to increased exposure to ticks and potential contact with wild animals (Miranda et al., 2014). Although subclinical, severe cases with high parasitemia can occur, particularly in puppies, the elderly, or immunosuppressed dogs, leading to life-threatening conditions. Clinical signs include weight loss, pale mucous membranes, anorexia, diarrhea, and gait abnormalities, whereas hematological findings often reveal anemia, thrombocytopenia, and leukocytosis with neutrophilia (Lasta, 2008; Borges *et al.*, 2016). Diagnosis is commonly made through blood smear examination, which is a practical but low-sensitivity method for detecting infected leukocytes (Marques *et al*., 2022).

Another significant vector-borne disease affecting dogs is dirofilariasis caused by *Dirofilaria immitis*, commonly known as canine heartworms. This parasitic filarial nematode primarily inhabits the right ventricle and pulmonary arteries, posing severe health risks to dogs, cats, wild canids, ferrets, and sea lions, as well as incidental hosts, such as horses, bears, and primates, including humans (McCall *et al*., 2008). The parasite is transmitted by mosquito vectors of the genera *Culex*, *Aedes*, *Anopheles*, and *Ochlerotatus*. In dogs, adult worms induce progressive vascular damage, potentially leading to right ventricular enlargement, right-sided heart failure, chronic cough, dyspnea, exercise intolerance, and weight loss (McCall *et al*., 2004). In humans, although the parasite fails to complete its life cycle, immature forms can lodge in the pulmonary arteries, leading to lesions that may be misdiagnosed as neoplastic growths (Duran-Struuck *et al*., 2005).

The prevalence of canine heartworm disease varies geographically and is influenced by vector density, climate, and the preventive care provided to dogs. Coastal regions with abundant mosquito populations show higher infection rates (Labarthe et al., 2014). In Brazil, heartworm disease has been reported in all regions, with hyperendemic areas being noted before the introduction of chemoprophylaxis. Although the use of preventive treatments has significantly reduced infection rates, resurgence has been observed since 2010 in certain regions, highlighting the need for ongoing control measures (Labarthe *et al*., 2014).

In Brazil, reports of canine hepatozoonosis remain limited and are primarily concentrated in the southern region (Lasta et al., 2009; Schneider et al., 2018). Given the clinical complexity and challenges posed by co-infections, this study aimed to describe a case of canine hepatozoonosis associated concurrently with CTVT and dirofilariasis. This report emphasizes the diagnostic and therapeutic challenges encountered in managing multiple parasitic and neoplastic diseases in the same patient, particularly in areas endemic to vector-borne diseases.

2 CASE REPORT

A mixed-breed, intact male dog presented to a veterinary clinic located in the metropolitan region of Grande Vitória, Espírito Santo, Brazil. According to the owner, the dog had a semi-domesticated lifestyle with frequent contact with other animals, and had been experiencing persistent penile bleeding for approximately six months. The owner also reported that the dog had been treated with antiparasitic tick control medication for approximately six months prior to the consultation.

During physical examination, the dog appeared normorexic and normothermic, with slightly pale mucous membranes and an active level of consciousness. A detailed evaluation of the genital area revealed hyperemic penile mucosa without any visible external tumors, although active bleeding from the preputial orifice was noted. On palpation, an internal mass measuring approximately 5 cm in length was detected at the base and along the shaft of the penis. The mass had friable consistency and a characteristic cauliflower-like appearance, raising the suspicion of a neoplastic lesion.

Complementary diagnostic tests were requested to further investigate this case, including complete blood count (CBC), SNAP 4Dx® Plus test, and fine-needle

aspiration cytology (FNAC) of the suspected tumor. The SNAP 4Dx® Plus test returned positive results for antibodies against *Ehrlichia canis* and/or *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, and/or *Anaplasma platys*, as well as for the *Dirofilaria immitis* antigen.

Cytological analysis from the FNAC was suggestive of canine transmissible venereal tumor (CTVT), confirming the initial clinical suspicion (Figure 1).

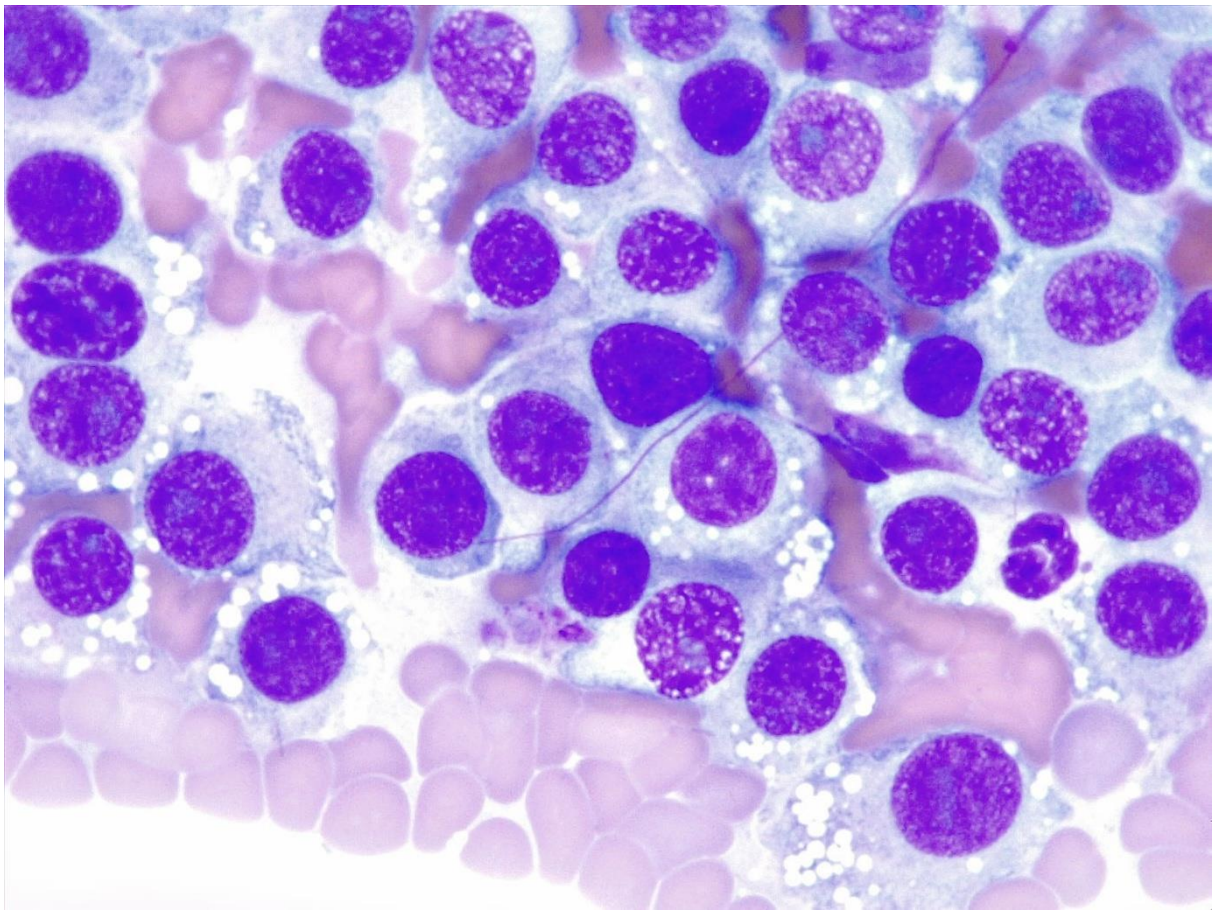

Figure 1. Fine-needle aspiration cytology (FNAC) of canine transmissible venereal tumors (CTVT) was performed using the panoptic method. The image shows round to oval-shaped cells and nuclei with finely granular blue cytoplasm containing distinct, well-defined, clear vacuoles. Source: Author, 2025.

A complete blood count (CBC) revealed mild leukocytosis with elevated levels of segmented neutrophils, eosinophils, and monocytes exceeding the reference limits. Additionally, a *Hepatozoon* spp. gamont was observed within the neutrophil (Figure 2A), confirming the diagnosis of hepatozoonosis. Microfilaria was also detected (Figure 2B), confirming the concurrent diagnosis of dirofilariasis.

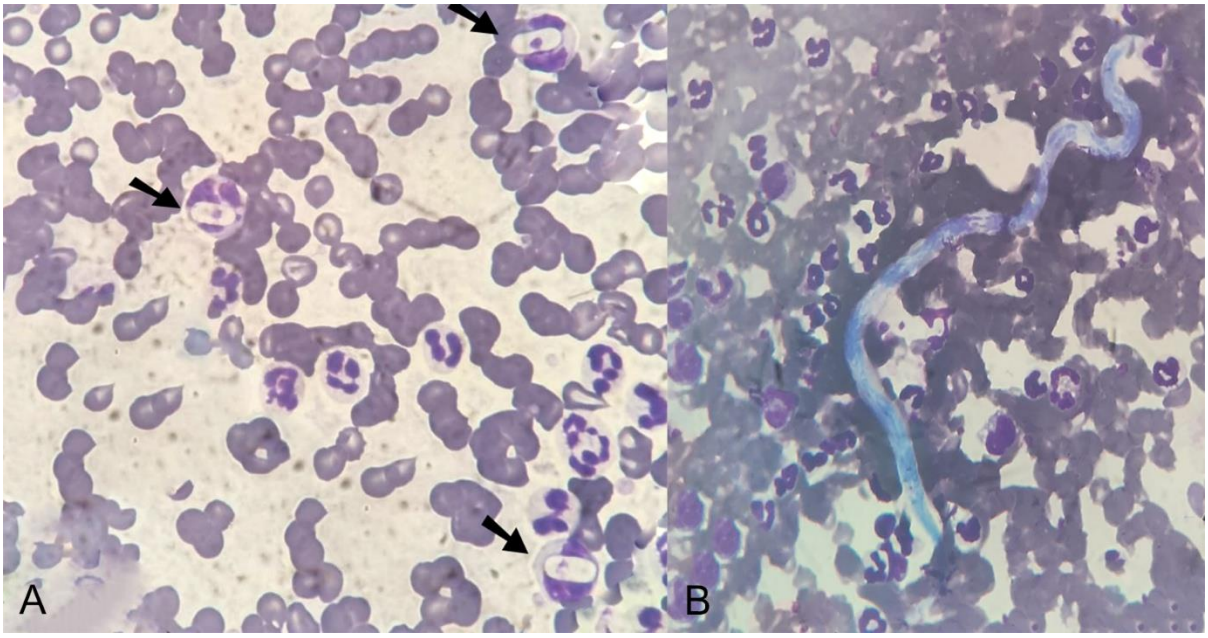

Figure 2. (A) Neutrophils containing a *Hepatozoon* spp. Gamont (arrows). (B) Presence of microfilaria identified in the blood smear.
Source*:* Author, 2025*.*

To manage ehrlichiosis and anaplasmosis, doxycycline was administered at a dose of 10 mg/kg twice daily for 28 days, given its proven efficacy against tick-borne bacterial infections.

To support overall health and boost the immune response, the dog was given Eritrós Dog Tabs (one tablet daily for 30 days) for nutritional supplementation and vitamin-mineral replenishment. Simultaneously, Munnomax was prescribed at a dose of two tablets daily for 30 days to strengthen the immune system.

Hepatozoonosis was treated with two doses of imidocarb dipropionate (IMIZOL®) at 5 mg/kg administered subcutaneously, with a 14-day interval between the doses. To mitigate potential side effects, particularly cholinergic reactions associated with imidocarb, atropine was administered at 0.04 mg/kg subcutaneously, 10 min before each imidocarb injection.

After three weeks of parasitic treatment, chemotherapy was initiated for the canine transmissible venous tumor. The protocol involved weekly intravenous administration of vincristine sulfate (0.03 mg/kg for six consecutive weeks, following established treatment guidelines for CTVT.

To treat dirofilariasis, ivermectin was administered at a dose of 5µg/kg subcutaneously every 21 days or monthly, with adjustments based on the owner’s availability and the dog's tolerance to the protocol.

3 DISCUSSION

Coinfection involving canine transmissible venereal tumor (CTVT), canine hepatozoonosis, and dirofilariosis, as reported in this study, presents a significant clinical challenge due to the complexity of therapeutic management and immunological implications for affected dogs.

Chemotherapy with vincristine sulfate remains the treatment of choice for CTVT given its high efficacy and complete remission rates in up to 90% of cases (Ferreira et al., 2017). However, it is essential to highlight the potential adverse effects associated with this drug, such as myelosuppression and neurotoxicity, which can predispose patients to opportunistic infections, such as hepatozoonosis (Strack et al., 2021). The case described here supports the existing literature by demonstrating patient immunosuppression, possibly exacerbated by the chemotherapy protocol, which facilitates the clinical manifestation of hepatozoonosis, a disease that often remains subclinical.

Canine hepatozoonosis, caused by protozoa of the *Hepatozoon* genus, has a complex life cycle that involves *Rhipicephalus* ticks as vectors. Infection occurs through the ingestion of infected ticks during blood-feeding (O'Dwyer, 2011). Generally, healthy dogs develop subclinical forms of the disease; however, in immunosuppressed, juvenile, or elderly animals, hepatozoonosis can manifest severely, leading to symptoms such as fever, weight loss, anemia, and gait abnormalities (Baneth et al., 2007; Borges et al., 2016). In this case, the identification of *Hepatozoon* spp. gamonts within neutrophils via a blood smear was crucial for diagnosis, despite the low sensitivity of the method (Marques *et al*., 2022).

In this study, the SNAP 4Dx® Plus test was employed as a complementary diagnostic method to detect co-infections. This test utilizes highly purified reagents in an ELISA platform, enabling the detection of antibodies against *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi*, and antigens of *Dirofilaria immitis*. According to IDEXX Laboratories, Inc., the test demonstrated a sensitivity of 97.1% and specificity of 95.3%, using IFA and ELISA as gold standards (SNAP 4DX PLUS TEST, [s.d.]).

Hepatozoonosis was treated with imidocarb dipropionate, preceded by atropine administration to minimize cholinergic side effects, as recommended in the literature (Strack et al., 2021). This protocol is widely accepted because of its efficacy against

protozoans, although relapses may occur in cases of high parasitemia or in immunosuppressed patients (O'Dwyer, 2011). Concurrently, doxycycline was administered to treat co-infections with *Ehrlichia canis* and *Anaplasma* spp., an appropriate choice given its documented efficacy against tick-borne bacterial infections (Baneth *et al*., 2007).

The main therapeutic challenge in cases of co-infection is balancing CTVT treatment, which requires chemotherapy-induced immunosuppression, while controlling opportunistic infections that exploit the patient's compromised immune system. Studies by Ferreira *et al*. (2017) emphasize the importance of hematological monitoring during chemotherapy to detect early signs of severe immunosuppression, such as leukopenia, anemia, and thrombocytopenia. In the present case, regular monitoring allowed for the necessary adjustments to the therapeutic protocol, contributing to the patient's recovery.

Ultimately, this case highlights the importance of an integrated clinical approach in dogs diagnosed with CTVT, particularly in regions endemic for tick-borne diseases, such as hepatozoonosis. Early recognition of co-infections is crucial for successful treatment and minimizing complications related to chemotherapy-induced immunosuppression. Effective clinical management should not only focus on neoplasm treatment, but also on controlling secondary infections, ensuring a safe and efficient therapeutic protocol for the patient.

4 CONCLUSION

This study highlights the complexity of clinical management in cases of co-infection involving CTVT, hepatozoonosis, and dirofilariasis, emphasizing the importance of early diagnosis and integrated treatment approaches. Continuous monitoring, along with a comprehensive and multidisciplinary clinical strategy, is essential for achieving successful therapeutic outcomes. This case report contributes to a deeper understanding of the interactions between neoplastic conditions and opportunistic infections in dogs, reinforcing the need for vigilant and holistic veterinary care in managing complex co-infections.

ACKNOWLEDGMENTS

This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES), Finance Code 001. Additional support was provided by the Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES).

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

We, the authors of the article entitled “Co-infection by Canine Transmissible Venereal Tumor, Hepatozoonosis, and Dirofilariasis – A Clinical Challenge in Dogs – Case Report” for all intents and purposes, declare that the project that gave rise to its data was not submitted to the Ethics Committee for evaluation. However, we are aware of the provisions of the resolutions of the Conselho Nacional de Controle de Experimentação Animal - CONCEA (http://www.mct.gov.br/index.php/content/view/310553.html) for projects involving animals. Therefore, the authors assume full responsibility for the data presented herein and are available to answer any questions should they be required to by the competent authorities.

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