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Case report

NEOSPOROSIS AND VISCERAL LEISHMANIOSIS IN A DOG IN CENTRAL–WEST BRAZIL

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Dogs act as definitive hosts for several diseases caused by protozoa, some of which are zoonotic. Due to their close contact with humans and other animals, they play a crucial role in the transmission of these diseases. Although infection with *Neospora caninum* or *Leishmania infantum* is not a determining factor for another, co-infection with these protozoa can aggravate clinical signs and increase the mortality rate. Though, there are reports of success in the treatment of neosporosis, the prognosis is generally considered unfavorable, especially in young dogs. The objective of this study is to report a case of infection by both protozoa in a dog treated at a university veterinary hospital, highlighting the clinical remission of nervous signs of neosporosis after treatment with the combination of sulfadoxine and clindamycin, followed by reduction of clinical signs of canine leishmaniasis (CanL) with treatment with miltefosine and allopurinol. Despite the worse prognosis for co-infection with *N. caninum* and *L. infantum*, the dog presented remission of neurological signs and a reduction in parasite load and clinicopathological signs associated with CanL.

Keywords: *Neospora caninum, Leishmania infantum,* co-infection, miltefosine.

INTRODUCTION

Dogs can contract infections from various protozoa that directly harm their health and have a zoonotic potential, representing a significant health challenge [1]. Among these protozoa, *Neospora caninum* gained worldwide recognition about 30 years ago, initially as a neuromuscular disease of dogs and later as an important cause of abortions in cattle [2]. Dogs, definitive hosts of *N. caninum*, become infected when they ingest food or water contaminated with oocysts, or when they consume cysts present in raw or undercooked meat. Furthermore, vertical transmission is also a possibility [3]. Many

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dogs with neosporosis may be asymptomatic, but especially young dogs may present clinical signs associated with polyradiculoneuritis, polymyositis or meningoencephalitis [4]. Early recognition of the disease is crucial to quickly initiate treatment, aiming for an effective recovery from canine neosporosis, although *ante-mortem* diagnosis presents challenges [5]. According to Garosi et al. [6] there are reports of success in the treatment of neosporosis, however the prognosis is generally considered unfavorable. Treatment is more likely to be successful in young dogs when instituted early in the course of the disease. Usually, the focus of treatment is to control the active inflammation and prevent progression of clinical signs, and it involves a combination of anti-inflammatory therapy with steroids and antibiotics, trimethoprim sulfadiazine and/or clindamycin [3].

Another endemic protozoosis in Brazil that has dogs as the main domestic reservoir for the etiological agent *Leishmania infantum* is visceral leishmaniasis [7]. According to the World Health Organization, 50 to 90 thousand new cases occur each year and Brazil is among the countries with the highest incidence [8]. Regarding the control of Canine Leishmaniasis (CanL) in Brazil, euthanasia is still a recommended measure for reactive dogs [9], but dog culling is not an effective measure [10]. However, since 2016, the Ministry of Agriculture, Livestock and Supply has authorized the use of Miltefosine for the treatment of CVL [11].

In Brazil, infection of dogs by *L. infantum* and *N. caninum* is endemic in several states [1,9,12]. In Mato Grosso, one state located in the Central-West region of Brazil, the prevalence of CanL is variable, from 4.2% to 48.4% [13-17]. Regarding neosporosis, there are only prevalence studies in the state capital, Cuiabá. According to Benetti et al. [18], the prevalence was 45% in dogs treated at a veterinary hospital and from farms. Melo et al. [19] found a lower prevalence of 6.6%.

Studies have shown that co-infection with these two protozoa in dogs can result in worsening of the disease and increased mortality, even if one infection is not a risk factor for the other [20,21]. Given the relevance of these two pathogens to dogs, since both use the dog as a definitive host and can cause a variety of clinical signs, ranging from the absence of clinical signs to severe neurological disorders [22] and the lack of description of the concomitant clinical and therapeutic monitoring of these diseases, this report aims to describe a case of neosporosis and CanL. In this case, there was remission of both neurological and systemic signs after treatment with the combination of sulfadoxine and clindamycin, followed by miltefosine plus allopurinol.

CASE PRESENTATION

A male mixed-breed dog, 1.5 years old, not neutered, weighing 6.8 kg, was treated at the Veterinary Hospital of the Federal University of Mato Grosso (HOVET-UFMT), Cuiabá, Mato Grosso, Brazil, presenting with ataxia for one day, weakness, abdominalgia and kyphosis, progressing to tetraparesis. During the anamnesis, the owners reported that they had adopted the dog 9 days ago from the rural area with

tick infestation. On physical examination, the dog presented paresis of the thoracic and pelvic limbs, lateral decubitus, dyspnoea, aerophagia, temperature 36.9ºC, mild dehydration, heart rate of 80 beats per minute and abdominal pain but was alert and conscious.

Then, the dog was hospitalized, and the following blood count and serum biochemistry were performed, such as alanine amino transferase, albumin, creatinine, and urea (Table 1), immunochromatographic rapid test (SensPERT Dechra®) for *Morbillivirus*, Polymerase Chain Reaction (PCR) for *Ehrlichia canis, Babesia* sp. and *Morbillivirus* (*in – house*), chest X-ray and abdominal ultrasound. Chest X-ray showed no changes in the lung parenchyma, only gastric distension due to aerophagia. Abdominal ultrasound showed marked hepatomegaly, bile sediment and moderate splenomegaly. In the first three days, the dog received supportive therapy, Ringer's lactate solution for hydration, tramadol 2mg/kg BID intravenous (IV) for analgesia, in addition to nursing care, such as alternating lateral decubitus every 2 hours. After exclusion of infection by *Ehrlichia canis, Babesia* sp. and *Morbillivirus*, Indirect Immunofluorescence Assay (IFAT) was performed for toxoplasmosis and neosporosis, being reactive for *N. caninum* with a titer of 1:3200 (*in-house*). In addition, on the fourth day, the administration of sulfadoxine and trimethoprim 15mg/kg BID IV and clindamycin 15mg/kg TID IV [4] were added. On the eighth day, the animal was discharged to continue with home treatment, totaling 28 days.

Table 1. Hematological and biochemical findings of the dog with neosporosis and visceral leishmaniasis.

Variables (reference range)	Days				
	1	**35**	**56**	**83**	**205**
Erythrocytes (5.5 – 8.5 x 106/µl)	3.54	3.41	3.90	4.70	6.61
Hemoglobin (12.0 – 18.0 g/dl)	7.50	7.80	9.20	10.80	15.30
Hematocrit (37.0 – 55.0 %)	24.00	24.00	28.00	33.00	47.00
Leukocytes (6.0 – 17.0 x 103/µl)	11.00	6.00	7.40	18.00	13.40
Platelets (200 – 500 x 103/µl)	216	54	270	312	258
TPP (6.0 – 8.0 g/dl)	7.40	11.00	11.00	12.00	8.00
SP (5.4 – 7.1 g/dL)	-	-	11.80	12.90	7.60
Albumin (2.6 – 3.3 g/dl)	1.50	-	1.40	1.20	3.20
Globulin (2.7 – 4.4g/dL)	-	-	10.40	11.70	4.40
ALT (21 – 102 UI/L)	28	45	77	80	83
ALP (20 – 156 UI/L)	-	-	29	64	61
Creatinine (0.5 – 1.5 mg/dL)	1.10	0.80	0.50	0.80	1.00
Urea (21 – 59.9 mg/dL)	108	-	-	35	42
Parasite load (parasites/µl)			132.7		19.06

* **TPP** total plasma proteins, **SP** serum proteins, **ALT** alanine amino transferase, **ALP** alkaline phosphatase.

At the end of treatment for neosporosis (day 35), mild apathy, weight loss, hypotrichosis located on the tail, pelvic and thoracic limbs, exfoliative dermatitis, lymphadenopathy, as well as anemia, thrombocytopenia and hyperproteinemia were observed. For confirmation of CanL, Enzyme Immunosorbent Assay (ELISA) and IFAT, reagent in both tests, with a titer of 1:320 were done. Then, hematological, and biochemical evaluation was performed, in addition to a skin biopsy with a 3 mm punch from the scapular region to determine the parasite load by qPCR for *L. infantum* [23]. Treatment began with a combination of miltefosine 2mg/kg SID for 28 days per os and allopurinol 10mg/kg BID for five months per os [24], and collar impregnated with deltramethrin. After this period, the dog showed no clinical changes and new blood, and skin samples were collected to monitor the treatment. These new findings demonstrated resolution of anemia, thrombocytopenia, hypoalbuminemia, and reduction in the skin parasite load.

DISCUSSION

In regions endemic for CanL, parasitic co-infections can result in clinical complications due to the immunosuppressive nature of CanL [21], as in the case described. In the study conducted by Ratzlaff et al. [12] co-infection was found in 50.9% (27/53) of dogs. However, infection by one protozoan did not predispose to infection by the other [12]. In contrast, a survey conducted by Cringoli et al. [25] in southern Italy, revealed that seropositivity for *N. caninum* was the main risk factor for seropositivity for *L. infantum* and vice versa, especially in an area endemic for CanL in asymptomatic dogs. Sharifdini et al. [21] suggest that co-infection is common in dogs and that CVL can compromise the T cell-mediated immune response to *N. caninum* antigens.

Another risk factor to be considered in this case is access to rural areas, reported by owners, especially in cattle farming regions [3]. In the study conducted by Benetti et al. [18] a high prevalence and significant association with positivity for *N. caninum* was found in dogs that had access to the street, especially close to rural areas. The infection can be acquired either through the ingestion of sporulated oocysts, through the ingestion of tissues infected with cysts, which are more common in dogs, or through vertical transmission, which is the most common cause in cattle [2,3]. Therefore, it is difficult to determine whether the *N. caninum* infection was recent or was already chronic and worsened due to immunosuppression caused by CanL.

Infection by the protozoan *N. caninum* can be asymptomatic or cause clinical signs that can be easily confused with other diseases, especially neurological disorders, which makes clinical diagnosis difficult and highlights the importance of laboratory confirmation [26]. This case describes neurological clinical signs like those associated with neosporosis, which include ataxia, progressive paraparesis, muscular atrophy and loss of patellar reflexes, progressing to paralysis of the pelvic limbs with rigid hyperextension [6].

The most common serological tests for neosporosis include IFAT and ELISA, with IFAT considered the gold standard for diagnosis. In IFAT, a titer equal to or greater than ≥1:50 indicates the dog's exposure to the agent. A titer equal to or greater than ≥1:800, as observed in this case (1:3200), especially in dogs with clinical signs, strongly suggests neosporosis [27,28]. After the diagnosis of the disease, the treatment indicated for the reported case was that recommended for neosporosis, that is, trimethoprim associated with sulfadoxine (15-20mg/kg, orally, twice a day for 4-8 weeks) and clindamycin (7.5-15mg/kg, orally, three times a day for 4-8 weeks), which can be administered alone or in combination with pyrimethamine. In situations where dogs present neurological signs, the recommendation is to opt for treatment with trimethoprim associated with sulfadoxine or pyrimethamine (1mg/kg, orally, once a day for 2-4 weeks) associated with sulfadoxine, due to better penetration into the central nervous system [29]. It is worth noting that, although clindamycin is effective in suppressing the replication and dissemination of tachyzoites, it apparently does not demonstrate efficacy against encysted bradyzoites [4,29].

In a retrospective study conducted by Fisher et al. [30], it was observed that complete clinical recovery of dogs after treatment was uncommon (5.6%), and relapses were frequent (27.8% of followed cases). This suggests that neosporosis usually has a poor prognosis, with a significant rate of relapses [30]. However, it is important to note that, in the case described, the dog showed signs of clinical improvement with only four days of treatment with sulfadoxine and trimethoprim in association with clindamycin, which was performed only for 4 weeks and there was no relapse during the 205 days of clinical follow-up. The favorable outcome of this case may be associated with rapid treatment with the combination of clindamycin and sulfonamide, which is more effective in the early stages, before muscle contracture has occurred [4].

Regarding to CVL, subclinical infection can progress to clinical disease, due to factors such as immunosuppression and intercurrent infections, which can alter the immune response [9,21]. In this case, the previous history of the dog was not known, but it is believed that the infection occurred before the dog was rescued, in the rural area where it previously resided. Since the incubation period of CanL could be from three months to several years, averaging three to seven months [9].

As in other studies [7,31,32], the association of miltefosine with allopurinol reduced the clinicopathological findings, as well as the parasite load after five months of treatment of the dog reported. Although therapy with antileishmanial drugs may induce clinical cure, treated dogs may remain infective to sand flies and require a longer period of therapy [24]. According to Miró et al. [33], miltefosine has become an important drug in the treatment of CVL, due to its leishmanicidal effect, in addition to its ease of administration, oral and low toxicity. Alopurinol, on the other hand, being a leishmaniostatic drug, has a more effective action when combined with other drugs [33]. Although the WHO recommends allopurinol as the only drug for the treatment of CVL in association with pentavalent antimonials, being the therapy of choice in Europe, it is not yet authorized in Brazil, and is only used for research purposes [31]. A

limitation of this report was the follow-up for only five months, since the duration of treatment with allopurinol can be prolonged, depending on the severity of the disease, the clinical and parasitological response to treatment and the individual tolerance to this medication [24].

In conclusion, the present report evidences a case of co-infection by *N. caninum* and *L. infantum*, with remission of neurological signs and reduction of parasite load and clinicopathological signs associated with CanL. Furthermore, these comorbidities must be investigated, especially in endemic areas, as well as the early institution of treatment.

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Author's contributions

BR clinical evaluation and writing. MSF and MNF clinical monitoring and laboratory diagnosis. VRFS and ABPFA supervision and review of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting of interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agreed that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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NEOSPOROZA I VISCERALNA LAJŠMANIOZA KOD PSA U CENTRALNO-ZAPADNOM BRAZILU: PRIKAZ SLUČAJA

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Psi su konačni domaćini za nekoliko bolesti uzrokovanih protozoama, od kojih su neke zoonoze. Zbog bliskog kontakta sa ljudima i drugim životinjama, oni igraju ključnu ulogu u prenošenju ovih bolesti. Iako infekcija *Neospora caninum* ili *Leishmania infantum* nije odlučujući faktor za drugu, istovremena infekcija ovim protozoama može pogoršati kliničke znake i povećati stopu mortaliteta. Iako postoje izveštaji o uspehu u lečenju neosporoze, prognoza se generalno smatra nepovoljnom, posebno kod mladih pasa. Cilj ove studije je da se prijavi slučaj infekcije obe protozoe kod psa lečenog u univerzitetskoj veterinarskoj bolnici, naglašavajući kliničku remisiju nervnih znakova neosporoze nakon tretmana kombinacijom sulfadoksina i klindamicina, praćenog smanjenjem kliničkih znakova. lajšmanijaze pasa (CanL) uz lečenje miltefozinom i alopurinolom. Uprkos lošijoj prognozi za koinfekciju sa *N. caninum* i *L. infantum*, pas je pokazao remisiju neuroloških znakova i smanjenje opterećenja parazitima i kliničko-patoloških znakova povezanih sa CanL.