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CO-INFECTION WITH *BABESIA CANIS* AND *BORRELIA BURGDORFERI* S.L. IN A DOG FROM NORTHEASTERN ROMANIA: A CASE REPORT

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ABSTRACT. This study describes a clinical case of a 9-year-old mixed-breed dog co-infected with *Babesia canis* and *Borrelia burgdorferi*. This dog was referred to a private clinic in northeastern Romania for a recurrent perianal tumour and a mild inflammation in the right elbow. The dog showed mild haemolytic anaemia, as well as increased alkaline phosphatase and glucose levels. Despite surgery and therapy, after four days, the patient had developed hyperthermia, severe anaemia and an inflammatory syndrome. The blood smear revealed the presence of piroplasm organisms identified as 'large' *Babesia* spp. On the 9th day of hospitalization the patient died during the blood transfusion, before applying the specific therapy for babesiosis. The blood collected before blood transfusion was tested for the following vector-borne diseases: *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Hepatozoon* spp. and *Borrelia* spp. using molecular analysis. The final outcome indicated a co-infection with *Babesia canis* and *Borrelia burgdorferi* s.l. In conclusion, the introduction of vector-borne disease screening approach prior any surgical procedure can prevent life-threatening events and improve diagnostic accuracy in dogs infected/co-infected simultaneously with different vector-borne diseases.

Keywords: canine babesiosis; canine borreliosis; co-infection; tick-borne diseases.

INTRODUCTION

Tick-borne diseases are an issue for humans and animals.

Lyme borreliosis, caused by spirochetes of the *Borrelia burgdorferi* group, is a tick-borne disease of humans and domestic animals and transmitted by ticks of the *Ixodes ricinus* complex. In the last decade, many studies regarding the serological prevalence and molecular identification of canine borreliosis have been performed in Europe (Namina *et al.,* 2019; Zanet *et al.,* 2020). Usually, the infected dogs do not display clinical signs, leading to an underestimation of the disease prevalence. Moreover, as

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dogs act like sentinels for Lyme disease, there is a high risk for disease spreading.

Ticks that belong to the genus *Ixodes* play an important role in the transmission and geographical spread of *Borrelia burgdorferi.* However, this bacterium is considered the most prevalent pathogen transmitted by ticks in the northern hemisphere (Hussain *et al.,* 2021). Also, bird migration and climatic changes have led to an increased spread in the distribution of infected ticks (Littmann *et al.,* 2018).

Borrelia burgdorferi is the primary Lyme agent. The clinical signs of canine borreliosis differ depending on the *Borrelia* species involved. Therefore, it is necessary to achieve an accurate identification of the species of the *B. burgdoferi* complex and to apply specific treatment as soon as possible.

Lyme borreliosis is considered one of the most important zoonoses, with more than 300 cases per 100,000 inhabitants reported each year in Europe (Lindgren *et al.,* 2006). In Romania, the first report of Lyme borreliosis was in 1988 (Crăcea *et al.,* 1988).

Other studies on Lyme borreliosis in Romania showed the identification of *Borrelia* species from ticks collected from humans (Briciu *et al.,* 2014), from the environment (Borșan *et al.,* 2021; Răileanu *et al.,* 2017) and from dogs. Serological (Cazan *et al.,* 2020; Kiss *et al.,* 2011; Mircean *et al.,* 2012*;* Răileanu *et al.,* 2015) or molecular studies of canine borreliosis have also been conducted.

Canine babesiosis is caused by several *Babesia* species. The transmission of the protozoan is performed by the bite of ixodid ticks. Thus, *Babesia canis* is transmitted by *Dermacentor reticulatus*, *Babesia vogeli* by *Rhipicephalus sanguineus* and *Babesia rossi* by *Haemaphysalis leachi* and *Haemaphysalis elliptica*. Also, *Rhipicephalus sanguineus* can transmit *Babesia gibsoni* (Ciucă *et al.,* 2021*;* Gray *et al.,* 2019).

Clinically, lethargy, anaemia, fever and haemoglobinuria are often associated with canine babesiosis (Gray *et al.,* 2019; Green, 2012; Leica *et al.,* 2017).

However, there is scarce information regarding the clinical picture displayed by dogs co-infected with tick-borne diseases (borreliosis and babesiosis) in Romania. The present study reports the clinical manifestation and the laboratory findings of a 9-year-old mixed-breed dog co-infected with *Babesia canis* and *Borrelia burgforferi* s.l.

MATERIALS AND METHODS

A 9-year-old mixed-breed male dog was admitted to the Margivet Veterinary Clinic, Suceava, Romania, for recurrent tumour excision in the perianal area. The first surgical removal of the tumour had been performed seven months before, in the same anatomical area. The dog lived exclusively outdoors, being periodically dewormed and protected with ectoparasiticides. The last treatment with ectoparasiticides (Fluralaner, Bravecto) had been performed six weeks before the first examination to the clinic. In addition, for several months, the owner had observed an intermittent lameness of the right limb.

Several blood analyses (haematology and biochemistry blood tests) were performed during the entire period of follow-up. Haematology blood tests were performed using the VetScan HM5 (Abaxis) analyser, and for the biochemistry blood tests, the VetScan VS2 (Abaxis) analyser was used following the manufacturer's instructions.

In addition, the dog was screened for the following tick-borne diseases: borreliosis, babesiosis, anaplasmosis and ehrlichiosis. For this, a blood sample on EDTA was collected and transferred to the Department of Parasitology of the Faculty of Veterinary Medicine of Naples, Italy, for molecular analysis, testing the presence of the following pathogens: *Babesia* spp. *Anaplasma* spp., *Ehrlichia* spp., *Hepatozoon* spp. and *Borrelia* spp.

Briefly, genomic DNA was extracted from 200 µL of blood using the DNeasy Blood and Tissue kit (Qiagen, Germany) according to the manufacturer's instructions.

The PCR test for the identification of *Babesia* spp. was performed according to the protocol described by Bajer *et al.* (2019). Briefly, the primers used were BabGF and BabGR primers to amplify the 18S rRNA gene fragment of *Babesia/Theileria* (559 bp), with the following thermal profile: 94°C for 3 minutes, followed by 45 cycles at 94°C for 30 seconds, 59°C for 30 seconds, 72°C for 60 seconds, with a final extension at 72°C for 7 minutes.

For *Borrelia* spp. detection, the PCR test was performed according to the protocol described by Cisak *et al.* (2012) and Sainz *et al.* (2015), using Fla1- and Fla2-specific primers. The thermal profile used was as follows: 95°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 54°C for 45 seconds, 72°C for 45 seconds, with a final extension step at 72°C for 3 minutes.

To identify *Hepatozoon* spp. DNA, a PCR test using the protocol described by Inokuma *et al.* (2002) was performed. Specific primers as HepF and HepR (666bp), and the following thermal profile 95° C for 12 minutes, followed by 34 cycles of 95°C for 30 seconds, 57°C for 30 seconds, 72°C for 60 seconds and 72°C for 7 minutes were used.

The PCR protocol used for the detection of *Anaplasma* spp./*Ehrlichia* spp. has been described by Goodman *et al.* (1996) and Massung *et al.* (2007), using PER1 and PER2 primers. The thermal profile used was 95°C for 10 minutes, followed by 40 cycles of 94°C for 60 seconds, 52.4°C for 45 seconds, 72°C for 60 seconds, with a final extension step at 72°C for 10 minutes.

Amplicons were introduced into the agarose gel (Bio-Rad) with ethidium bromide (2%). Sequencing was performed using forward and reverse primers (Eurofins, Germany), and the results obtained were compared using BLAST (Basic Local Alignment Search Tool).

RESULTS AND DISCUSSION

Clinical examination showed an inflammation located in the elbow of the right limb.

The haematological results showed only mild haemolytic anaemia (*Table 1*), and biochemical analysis revealed increased alkaline phosphatase and glucose levels (*Table 2*). Radiological evaluation of the lungs and abdominal radiographs were performed to exclude pulmonary or abdominal metastases. The diagnostic imaging of the lung and abdomen showed no obvious abnormalities.

General anaesthesia for the surgical removal of the perianal tumour included the following protocol: premedication with xylazine hydrochloride (1-2 mg/kg I.M.) and induction with ketamine (10-11 mg/kg I.M.) The surgical procedure ended without complications, and the patient remained hospitalised.

Therapy and follow-up

During the first 5 days of hospitalisation, the patient was treated with broad-spectrum antibiotics, anti-inflammatory drugs, analgesics and rehydration with 0.9% NaCl solution.

Table 1 - Haematology results

Parameter	Day 1	Day 6	Day 9	U.M.	Reference range
WBC	14.6	40.3	62.64	10^9/l	6-17
LYM	1.2	3.4	26.34	10^9/l	0.8-5.1
MON	0.2	0.5	0.37	10^9/l	0.0-1.8
GRAN	13.2	36.4	35.35	10^9/l	4-12.6
EOS	2.1	3.7	0.8	%	0
RBC	5.28	3.17	1.77	10^9/l	5.5-8.5
HGB	101	63	41	g/L	110-190
HCT	35.9	21.2	12.32	%	39-56.0
MCV	68	67.1	70	fl	62.0-72.0
MCH	19.1	19.8	22.9	Pg	20.0-25.0
PLT	305	364	186	10^9/l	117-460
PCT	0.301	0.345	0.26	%	
MPV	9.9	9.5	14.1	fl	7.0-12.9

Note: **WBC** - leukocyte; **LYM** - lymphocyte; **MON** - monocyte; **GRAN** - granulocyte; **EOS** - eosinophile; **RBC** - red blood cells; **HGB** - hemoglobin; **HCT** - hematocrit; **MCV** - mean corpuscular volume; **MCH** - mean corpuscular hemoglobin; **PLT** - platelet; **PCT** - procalcitonin; **MPV** - mean platelet volume

Table 2 - Biochemistry results

Parameter	Day 1	Day 6	Day 9	U.M.	Reference range
ALP	374	327	-	U/L	18-214
ALT	64	85	-	U/L	12.0-101.0
TBIL	0.18	0.17	-	mg/dL	0.0-1.0
BUN	12.1	+200.0	189	mg/dL	7.0-29.0
CRE	1	-	2.2	mg/dL	0.3-1.5
TP	6.2	5.4	-	g/dL	5.3-8.4
ALB	2.4	2.0	-	g/dL	2.2-3.9
GLOB	3.8	3.5	-	g/dL	2.1-4.9
GLU	535	120	-	mg/dL	74-146
Ca	8.8	-	-	mg/dL	9.0-13.4
PHOS	5.7	-	-	mg/dL	2.0-6.0
TRIG	47	-	-	mg/dL	8-100
CHOL	126	-	-	mg/dL	100-330
AMY	762	-	-	U/L	500-1,400
AST	-	42	-	U/L	2.0-43.0
GGT	-	7	-	U/L	0.0-7.0

Note: **ALP** - alkaline phosphatase; **ALT** - alanine aminotransferase; **TBIL** - total bilirubin; **BUN** - urea nitrogen; **CRE** - creatinine; **TP** - total protein; **ALB** - albumin; **GLOB** - globulin; **GLU** - serum glucose; **Ca** - serum calcium; **PHOS** - phosphate; **TRIG** - triglycerides; **CHOL** - cholesterol; **AMY** - amylase; **AST** - aspartate aminotransferase; **GGT** - Gamma-glutamyltransferase

On the 9th day of hospitalisation, the dog received blood transfusion due to progressive anaemia (Day 6: RBC - 3.17 (reference range: 5.5-8.5 10^9/l); HGB - 63 (reference range: 110-190 g/L) - Table 1; HTC - 21.2; Day 9: RBC - 1.77; HGB - 41; HTC - 12.32).

Haematology and cytology results (before the blood transfusion) showed normocytic, normochromic, hyper-

regenerative anaemia; leukocytosis; neutrophilia with left nuclear index deviation; eosinophilia; monocytosis; lymphocytosis. The values of renal markers showed a slight improvement. Despite the efforts, the dog died during blood transfusion.

Molecular analysis revealed *B. canis* and *Borrelia burgdorferi* co-infection. Moreover, the blood sample collected before the transfusion revealed the presence of piroplasm organisms, typical forms of 'large' *Babesia* spp. on the stained blood smear (Diff Quick stain), The PCR tests resulted negative for the other screened pathogens (*Anaplasma* spp., *Ehrlichia* spp., *Hepatozoon* spp.).

Worldwide, Lyme disease affects more than 250,000 people annually, with most cases reported in Europe as well as in North America and China (Rudenko *et al.,* 2011; Wu *et al.,* 2013).

Borreliosis has a different evolution in dogs than in humans. Although the occurrence of erythema migrans has frequently been reported as a common clinical sign in human borreliosis, in dogs, it was rarely reported until now (Inokuma *et al.,* 2013; Gerber, 2010; Little *et al.,* 2010; Skotarczak, *2014;* Parry, 2016; Liu *et al.,* 2019) or generally rarely reported (Bhide *et al.,* 2008; Romney *et al.,* 2021).

The exact time of transmission of *Borrelia burgdorferi* from ticks to dogs cannot be determined, and it is accepted that not all infected dogs have clinical manifestations (Krupka *et al.,* 2010). Studies involving animal models have shown that spirochete transmission can occur in less than 16 hours (Cook, 2015). In dogs, the clinical signs found in the acute form are nonspecific, often disappearing within a few days (Parry, 2016).

Symptomatic dogs can develop, after a few weeks to months, pain and joint inflammation, followed by lameness, due to local reactions caused by the spread of spirochetes (Krupka *et al.,* 2010; Parry, 2016). In the case described here, the therapy of the inflammation located in the right elbow was postponed because the main pathology was the tumour that had to be removed first. Also, the lameness that had been observed by the owner occasionally was not considered a threat at the moment of clinical examination. Krupka *et al.* (2010) described intermittent lameness in canine borreliosis, with the possibility of recurrence after a few weeks; the pain was more pronounced when the dog was moving (walking or when going up and down stairs).

During hospitalisation, the patient developed kidney failure, which was managed by continuous rate infusion (CRI) and supportive medication.

Kidney failure during the acute phase of canine borreliosis has also been reported in certain breeds of dogs naturally infected with *Borrelia* spp. However, the renal disease is often fatal and progressively evolving (Krupka *et al.,* 2010).

In the present case report, the severe anaemia after surgery was attributed to babesiosis. The treatment strategy was to stabilise the patient, perform the blood transfusion and continue the supportive treatment, and then to administer the specific treatment for babesiosis (imidocarb dipropionate), especially since the renal parameters

began to decrease, according to the last tests performed.

Rostami *et al.* (2011) described a case of a 1-month-old dog confirmed with borreliosis. Moreover, the blood tests showed neutrophilia, severe regenerative anaemia and thrombocytopenia. The latter two parameters have frequently been observed in canine borreliosis (Shaw *et al.,* 2005). Lymphopenia and eosinophilia have also been observed in experimental infections in dogs (Jackson *et al.,* 2007; Whitney *et al.,* 2007). Adaszek et *al.* (2020) reported a dog with borreliosis that had fever and dilated cardiomyopathy, and the only abnormality in blood tests was leucocytosis. The patient from the present study showed fever, severe regenerative anaemia, eosinophilia and leukocytosis. Indeed, these clinical signs have also been reported in other studies (Shaw *et al.,* 2005; Jackson *et al.,* 2007; Whitney *et al.,* 2007; Rostami *et al*., 2011).

Moreover, the decrease in plasma proteins displayed by the patient from the present study, was not in agreement with a previous study that reported an increased value of plasma proteins (Scorpio *et al.,* 2008). However, there are other studies that reported similar values of plasma proteins, in agreement with our results (Rostami *et al.,* 2011). For instance, Whitney *et al.* (2007) showed an albumin value of 2.5 mg/dl in dogs with borreliosis, similar to the values presented by the patient from the present study (albumin value on day 1 - 2.4 mg/dl; after 5 days, the albumin value had decreased to 2.0 mg/dl) (*Table 2*).

Unlike babesiosis, the diagnosis of borreliosis can be sometimes challenging for the practitioners due to the occurrence of various non-specific clinical signs and because the animals frequently do not develop the disease after infection. In addition, the detection of the antigen of *Borrelia*, using the immune-chromatographic tests, represents the most widely used serological technique for the diagnosis of canine borreliosis (Green, 2012).

In the study conducted by Schánilec *et al.* (2010), the diagnostic protocol for canine borreliosis was performed based on the following factors: 1) the presence of suggestive clinical signs; 2) differential diagnosis with other tick-borne diseases; 3) history of tick infestation; 4) positive response after administration of antibiotic therapy; 5) detection of antibodies in serum.

Fluralaner, the drug used in this study, belongs to the class of isoxazolines; it is rapidly absorbed and has a high bioavailability (Beugnet *et al.,* 2018). Being an adult dog of a large breed, the joint problems shown at clinical examination were justified as common problems due to his age and breed size. Moreover, because the dog was protected against ectoparasites, the exclusion of the co-infection with tick-borne diseases was delayed.

Co-infections with two or more vector-borne diseases are frequently reported in areas where there is an increased vector density. In fact, various studies have been reported co-infections in dogs, such as *Babesia* spp. and *Ehrlichia* spp. or *Anaplasma* spp. and *Borrelia* spp., *Dirofilaria* spp. and *Ehrlichia* spp. or with *Babesia* spp. (Otranto *et al.,* 2009a; 2009b).

Animal and human studies have shown the possibility of multiple tick-

borne diseases, and whilst the co-infections may be developing simultaneously, the clinical signs could be presented individually or with non-characteristic symptoms (Skarda, 2005). The dog presented here, was simultaneously affected by borreliosis and babesiosis infections, although the clinical signs were more appropriate for borreliosis. Because borreliosis is extremely rare in animals in this area, this tick-borne disease was not included in the differential diagnosis.

Giudice *et al.* (2003) and Remesar *et al.* (2022) considered that stress, gestation or immunosuppressive treatments could influence the immune system in patients with tick exposure. Hence, this could lead to the appearance of clinical signs or their worsening.

Canine babesiosis is an endemic vector-borne disease in Romania. Indeed, there are plenty studies regarding the diagnosis, pathogenicity or treatment used in canine babesiosis, including co-infections with other tick-borne pathogens that have been conducted worldwide in Romania and Europe as well (Ionita *et al.,* 2012; Imre *et al.*, 2013; Andersson *et al.,* 2017; Leica *et al.,* 2017; Baneth, 2018; Otranto, 2018; Ciucă *et al.,* 2021).

The typical clinical signs of canine babesiosis are fever, moderate to severe anaemia, thrombocytopenia and haemoglobinuria (Green, 2012).

The therapy used for canine babesiosis is based onimidocarb dipropionate. However, this drug only reduces the parasite load and improves the symptomatology (Baneth, 2018). Therefore, recovered dogs are considered carriers, specifically when they are immunosuppressed (Yang *et al.*, 2022). In fact, we therefore assume that prior to surgery, the dog could have had both pathogens (e.g., *Babesia canis* and *Borrelia burgdorferi*) in a latent stage, developing clinical signs after surgery due to the occurrence of immunosuppression and the postoperative stress factors.

CONCLUSIONS

The introduction of vector-borne disease screening approach, prior any surgical procedure can prevent life-threatening events and improve diagnostic accuracy in dogs infected/co-infected simultaneously with different vector-borne diseases.

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