Genetic diversity in *Babesia canis* and associated comorbidities can be fatal in dogs` babesiosis – a case study

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Abstract
The aim of this paper is to briefly present some aspects of Babesia spp. taxonomy, incidence, clinical signs of their infection, and possibilities of prevention, as an introduction to a case study of canine babesiosis presentation. A 7-year-old Malinois dog was presented in August 2020 with signs of generalized icterus, high body temperature and mustard urine, all of them indicating babesiosis. Cytological examination confirmed the large Babesia canis spp., and the biochemical investigations revealed renal and hepatic failure. Although the therapeutic protocol included the specific antidote, imidocarb dipropionate – Imizol® (0.5 ml/10 kg body weight, in a single dose), fluid therapy, vitamin therapy, an antiemetic drug, and supplements for renal and hepatic functions sustaining, the investigated dog died. The post-mortem investigation revealed generalized icterus. We consider the delaying of dog presentation at vet an important factor of this outcome; although an infection with various subspecies of Babesia canis was not excluded, the therapeutic intervention would have been the same.
Keywords: *Intra erythrocyte parasites, jaundice, anemia, antidote*

Introduction

Babesiosis is a tick-borne disease caused by various species of *Babesia* genus parasite. This disease is particularly common in dogs, its worldwide distribution drawing a special attention (Solano-Gallego and Baneth, 2011; Sudhakara Reddy et al., 2014). Infecting *Babesia* species in dogs were classified in two main groups, large forms being a part of *Babesia canis* group while small species were generally considered as *Babesia gibsoni* [Fabisiak et al., 2010; Solano-Gallego and Baneth, 2011; Zahler et al., 2000(a)].

The infection with *Babesia canis* is attributed to several species of ticks, such as *Dermacentor reticulatus* (Fabisiak et al., 2010; Födvári et al., 2005), while an additional transmission path via blood transfusion was reported in dogs for *Babesia gibsoni* (Stegeman et al., 2003) or from dog to dog during their fights (Solano-Gallego and Baneth, 2011).

The diagnosis of babesiosis in dogs is basically done by clinical examination and parasite` identification on May-Grünwald Giemsa stained peripheral blood smears (Fabisiak et al., 2010). Clinical signs of *B. canis* infection often include dehydratation, apathy, anorexia or decrease appetite, fever (Furlanello et al., 2005; Solano-Gallego et al., 2008); associated anemia has a multifactorial component based on plasma volume increasing, erythrocyte retention in the spleen, erythrocyte destruction partly due to parasite proliferation (Schetters et al., 1997). Other clinical signs may include jaundice or icterus, congested conjunctiva, tachycardia, tachypnea, lymphadenopathy, haemoglobinuria, bilirubinuria, with a general condition of lethargy or dullness (Fabisiak et al., 2010; Sudhakara Reddy et al., 2014). Splenomegaly, hemolytic anemia, and thrombocytopenia were reported in infections with *B. gibsoni* (Stegeman et al., 2003).

The diagnosis in peripheral blood smears take into account the size and morphological appearance of the intraerythrocytic parasites (Födvári et al., 2005; Muhlnickel et al., 2008). Unfortunately, such an examination only provides a presumptive diagnosis of the two groups of *Babesia,* the large and small ones. Different subspecies, although morphologically identical, are important to be independently diagnosed due to the difference in their clinical signs (Solano-Gallego and Baneth, 2011).

B. gibsoni can be difficult to be detected in red blood cells (RBCs) on peripheral blood smear examination, and serologic investigations using fluorescent antibody tests give unsatisfactory results in young dogs (1-3 months old) and due to serologic cross-reactivity among *Babesia spp.* (Farwell et al., 1982; Stegeman et al., 2003). Therefore, molecular investigations using Polymerase Chain Reaction (PCR) represent the best method to distinct various subspecies of this parasite (Cacciò et al., 2002). For example, the results obtained on pairwise identities, distance, parsimony, and maximum likelihood analyses of the 18S rRNA gene provided valuable information about genetic phylogeny of various types of *Babesia* [Zahler et al., 2000(a), (b)]. Subsequent sequencing of PCR products allows identity to be established by comparison with nucleotide sequences already known and recorded in GenBank®, as in the case of *B. canis canis,* accession numbers AY611731.1; AY611732.1; AY611733.1 (Adaszek and Winiarczyck, 2008; Födvári et al., 2005).

For this purpose of taxonomic identification, Cacciò et al. (2002) and Fabisiak et al. (2010) reviewed three distinct but morphologically identical subspecies of *Babesia canis*, such as *B. canis canis, B. canis rossi,* and *B. canis vogeli,* isolated from dogs in Europe, Africa, and US, respectively. The presence of *B. canis canis* in European isolates and of *B. canis rossi* in South-African isolates was confirmed by Schetters et al. (1997b), while *B. canis vogeli* was detected in dogs from Croatia, along with other infectious species such as *B. canis canis, B. gibsoni, Theileria annae* and, surprisingly, with two parasites usually found in horses, *Babesia caballi* and *Theileria equi* (Beck et al., 2009). In 2004, Matjila et al. confirmed for the first time *B. canis vogeli* in domestic dogs in South Africa; they also reported the presence of *B. canis rossi* but none of the investigated dogs was a carrier of both subspecies together. Solano-Gallego et al. (2008) reported the vast majority of *B. canis canis* infections in Northern Italy, while *B. canis vogeli* was mainly detected in Central and Southern Italy. In 2012, the presence of *Babesia vogeli* in a clinically normal dog in Romania was confirmed for the first time. It was part of a group of five asymptomatic dogs whose blood was comparatively tested by PCR technique with that of 11 other dogs with symptoms of babesiosis. Investigations of the 18S rRNA segment showed that all dogs with symptoms of babesiois were infected with *Babesia canis* (Ionita et al., 2012). Although the authors did not explicitly specify the identified subspecies of *Babesia canis*, it is well-understood the presence of *Babesia canis canis,* considering the type of vector predominantly found on the Romanian territory *(Dermacentor reticulatus).* They revised the transmission of *Babesia vogeli* by *Rhipicephalus sanguineus,* commonly called the brown dog tick, while the other *Babesia canis* subspecies, *Babesia rossi* is transmitted by *Haemaphysalis elliptica*, with eastern and southern Africa location (Beugnet et al., 2019; Fourie et al., 2019).

Various isolates from infected dogs demonstrated close phylogenetic relationships with other *Babesia* species, such as *B. microti, B. rodhaini, B. conradae, B. bigemina, B. divergens,* and *B. odocoilei* [Birkenheuer et al., 2004; Camacho et al., 2001; Solano-Gallego and Baneth, 2011; Zahler et al., 2000(a), (b)]. In 2008, Muhlnickel et al. firstly identified *B. gibsoni* in dogs from Australia, and in 2009, Beck et al. reviewed that some of *Babesia* small species appear to be closely related to *Theileria* genus. However, several classical differences, such as the absence of extra-erythrocytic multiplication (schizogony) in *Babesia,* or the forming of two daughter cells (merozoites) in *Babesia* comparing to four in *Theileria,* may contribute to their individualization; however, recent opinions do not consider *Babesia microti* as a *Babesia, Babesia equi* being already designated as *Theileria equi* (Uilenberg, 2006).

In conclusion to this introductive review, there is a need for molecular characterization of each isolated *Babesia* parasite in order to perform an appropriate treatment. Large *Babesia* species are commonly treated with imidocarb dipropionate at 5 mg/kg body weight, given intramuscularly

as a single dose, associated with fluid therapy and even blood transfusion for a good clinical response (Irwin and Hutchinson, 1991; Solano-Gallego and Baneth, 2011). Vercammen et al. (1995) reported the use of imidocarb dipropionate at 6 mg/kg b.w. They also reported a dog who became subclinical chronic carrier of *B. canis* as a result of a treatment with long acting oxytetracycline (20 mg/kg b.w.). Sudhakara Reddy et al. (2014) reported the use of diminazene aceturate, 5 mg/kg b.w., single dose, along with supportive and symptomatic therapy in *Babesia* infection of dogs. Both diminazene aceturate and imidocarb dipropionate drugs were reported safe to use for haemoprotozoan diseases in the same dosage (Olukunle et al., 2018).

Small *Babesia spp.* are considered more resistant to anti parasitic drugs, an incomplete elimination of *B. gibsoni* being translated in chronic carrier survivors (Solano-Gallego and Baneth, 2011; Stegeman et al., 2003). A treatment with clindamycin (25 mg/kg b.w., per os, at 12 hours, for 14 days) of 10 experimentally infected dogs with *B. gibsoni* was reported to reduce parasitemia levels as a result of parasite degeneration, with the improvement of clinical signs of the disease (Wulansari et al., 2003).

Vaccination of dogs against *B. canis* using soluble parasite antigens (SPA) has been reported as a limiting factor in splenomegaly development and an improvement in the immune response at 6 days post-vaccination (Schetters et al., 1997).

The aim of this paper is to review some taxonomic and incidence aspects of *Babesia spp.* and to present a case study of *Babesia* in dogs whose unfortunate ending shows the importance of fast therapeutic intervention until various comorbidities develop.

Material and methods

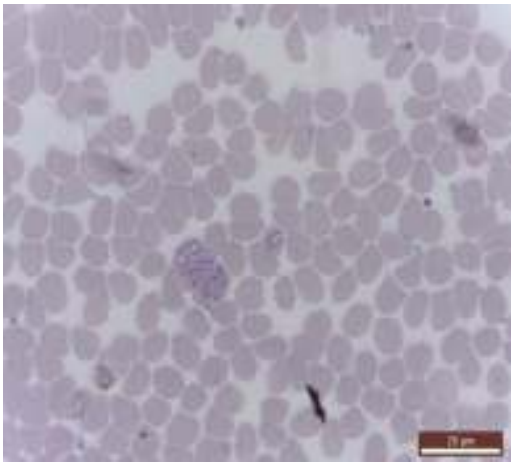
Some aspects reported in medical science about *Babesia spp.* infection, taxonomic including, clinical evolution, treatment and prevention were debated basing on 27 scientific papers studying. All these were used to provide an overview of a case study of babesiosis in dog, the diagnosis of which was established on the basis of usual clinical examination together with cytological investigations of May-Grünwald Giemsa stained peripheral blood smear. Blood samples were biochemically investigated for the following parameters: serum creatinine, gamma glutamyl transferase (GGT), aspartate aminotransferase (ASAT) or (serum) glutamic-oxaloacetic transaminase (GOT), alanine aminotransferase (ALAT) or (serum) glutamic-pyruvic transaminase (GPT), serum urea [all of these by the method of spectrophotometry], serum potassium and serum sodium [both by indirect Ion Selective Electrode (ISE) method]. Post-mortem, a necropsy examination was performed in order to observe changes in the organs.

Results and discussions

In august 2020, a 7-year-old Malinois dog with signs of babesiosis was presented for investigations at the Medical Clinic of Veterinary Medicine Faculty of Iași, Romania. Increased body temperature (40.6^0C) and general jaundice, at which mustard urine was associated, firstly drew the attention at the clinical examination (Fig. 1).

Fig. 1. Various clinical aspects in a 7-years-old Malinois dog babesiosis

As a result of cytological examination, *Babesia canis* was confirmed as parasiting some of RBCs (Fig. 2).



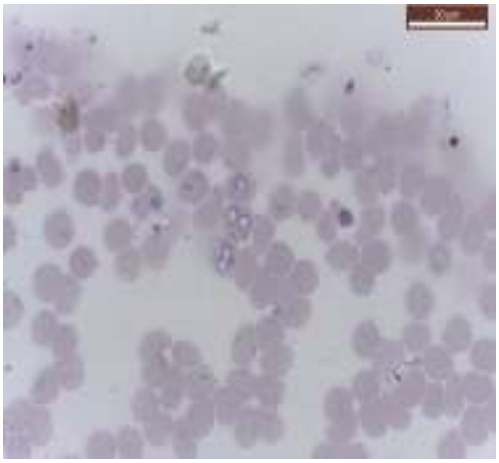


Fig. 2. Intra erythrocyte *Babesia canis.* May-Grünwald Giemsa

The therapeutic intervention was prompt, with imidocarb dipropionate - Imizol® specific antidote, in a single dose (5 mg/kg b.w. or 0.5ml/10 kg solution 8.5%), together with fluid and vitamin therapy. Two days after specific antidote administration, the dog showed polydipsia and polyuria.

Meanwhile, biochemical analyses were performed and their results showed normal values but at the lower limit only for serum potassium and serum sodium (3.7 mmol/L and 140 mmol/L, respectively). The other investigated parameters exceeded the upper reference limit, such as: serum creatinine 2.52 mg/dL vs. 1.8; GGT 147 U/L vs. 10; ASAT 286 U/L vs. 50; ALAT 714 U/L vs. 40; serum urea 151.9 mg/dL vs. 26.

After 5 days, although the clinical evolution was better and the dog started to eat, he died.

On necropsy, jaundice was generalized, including important organs, such as kidneys, heart, and liver (Figure 3).

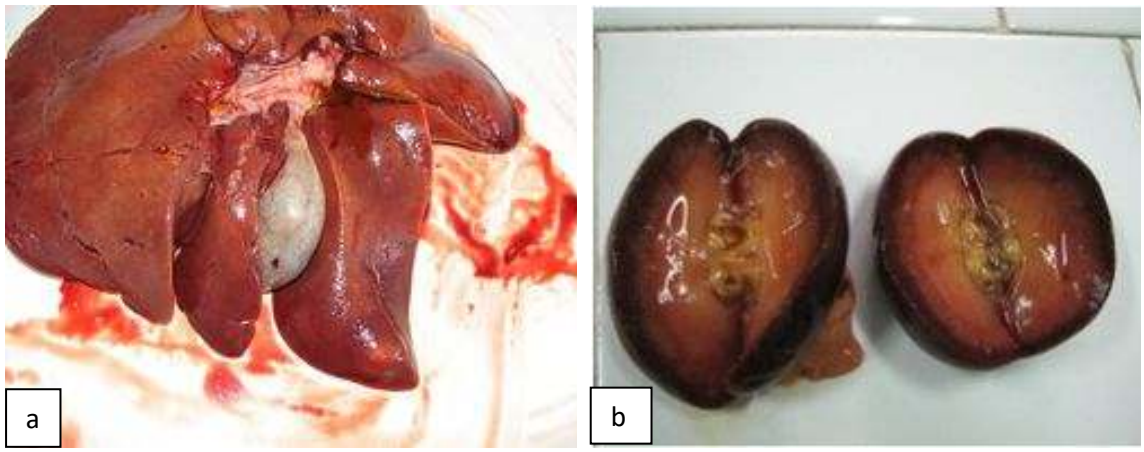


Fig. 3. Aspect of liver (a) and kidneys (b) on necropsy

From our point of view, the established therapeutic intervention was medically correct. The specific antidote for *B. canis* was chosen, which was confirmed as safe by medical studies. The first signs of polydipsia and polyuria led us to think of renal failure, along with that of liver, both confirmed by the results of laboratory tests. In order to dilute urea, a sustained fluid therapy was performed. A probiotic supplement with *S. thermophilus, L. acidophilus,* and *B. longum* was administered to support the renal function, as well as a dietary supplement in food, containing chitosan. Liver function was sustained with a phospholipid-based supplement, and a maropitant-based drug (Cerenia®) was administered as antiemetic, the dose of which was correlated with the animal`s weight. Therefore, the therapeutic intervention was correctly performed and in a sustained way for the healing of the dog. Its dead may be firstly justified by comorbidities developed (such as liver and renal failure) in the context of its delayed presentation to the doctor. Although we were not able to diagnose subspecies of *B. canis,* the established treatment is considered effective for all large *Babesia spp.* However, a precise taxonomic classification would have been helpful in justifying the clinical evolution.

Conclusions

Babesia genus includes two main groups, of large and small species, worldwide distributed and with a variety of clinical signs in their infection. A 7-year-old dog infected with large *Babesia canis* died although its clinical evolution after a specific antidote improved. Although therapeutic protocol was right instituted, comorbidities installed (kidney and liver failure) as a result of its delaying to vet consult decided this outcome.

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