Clinicopathological abnormalities, treatment, and outcome in in a 45-day-old puppy with acute *Babesia canis* infection

Klinische und labordiagnostische Befunde, Therapie und Outcome bei einem 45-Tage-alten Welpe mit akuter *Babesia canis*-Infektion

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ABSTRACT

The knowledge regarding *Babesia canis* infections in puppies is limited to 3 dogs aged 43–46 days. A 45-day-old, male intact German Shepherd dog was presented due to lethargy, inappetence, and gait disorders. An acute *Babesia canis* infection was diagnosed by PCR. Marked thrombocytopenia, anemia, and hyperbilirubinemia were noted. A subcutaneous injection of imidocarb dipropionate (ID, 2.0 mg/kg bodyweight) was applied. The puppy's general condition significantly improved, but vomitus was noted. A negative PCR and mild hematological abnormalities were seen 14 days later. A second subcutaneous injection of ID (4.2 mg/kg bodyweight) was applied. *Babesia canis* infections should be considered in puppies with fever, marked thrombocytopenia, and marked anemia in the immunological gap. Next to vectorial transmission, transplacental *Babesia canis* infections are reported but considered unlikely in the presented puppy. The puppy responded well to a lower-ranged dose of ID, but side effects were noted (vomiting). In adult dogs, treatment of acute *Babesia canis* infections using ID dosed 6.6 mg/kg bodyweight is recommended. No safety regulations are published by the manufacturer regarding the use of ID in puppies, but vomitus is reported as a potential side effect after the use in adult dogs. A second ID injection (4.2 mg/kg bodyweight) was applied to avoid a potential disease relapse. The case report highlights the significance of the immunological gap in puppies with *Babesia canis* infections and the need for ectoparasite prophylaxis in puppies, if vector contact is possible.

ZUSAMMENFASSUNG

Der Kenntnisstand bei Welpen mit akuten *Babesia canis*-Infektionen ist auf 3 Fälle im Alter von 43–46 Tagen beschränkt. Im vorliegenden Fallbericht wurde ein männlicher Deutscher Schäferhund-Welpe im Alter von 45 Tagen aufgrund von Lethargie, Inappetenz und Koordinationsstörungen vorgestellt. Eine akute *Babesia canis*-Infektion wurde mittels positiver PCR diagnostiziert. In der Blutuntersuchung waren eine hochgradige Thrombozytopenie, Anämie und Hyperbilirubinämie nachweisbar. Der Welpe wurde mit einer subkutanen Injektion von 2,0 mg/kg Körpergewicht Imidocarb Dipropionat (ID) behan-

delt, worauf sich der Allgemeinzustand besserte. Jedoch wurde von Vomitus berichtet nach der Injektion. Zwei Wochen später wurde eine negative PCR und geringgradige hämatologische Befunde festgestellt. Eine zweite subkutane ID-Injektion in der Dosierung 4,2 mg/kg Körpergewicht wurde verabreicht. *Babesia canis*-Infektionen sollten bei Welpen mit Zeckenbefall, Fieber, hochgradiger Thrombozytopenie und hochgradiger Anämie differentialdiagnostisch berücksichtigt werden. Auch transplazentare *Babesia canis*-Infektionen sind möglich, in diesem Fall jedoch unwahrscheinlich. Der Welpe reagierte gut auf die erste ID-Injektion in niedriger Dosierung, jedoch wurde Vomitus als mögliche Nebenwirkung berichtet. Allgemein wird bei akuten *Babesia canis*-Infektionen bei erwachsenen Hunden eine hohe Dosierung von 6,6 mg/kg Körpergewicht empfohlen. Vomitus ist als mögliche Nebenwirkung bei erwachsenen Hunden beschrieben, jedoch liegen keine Angaben zur Sicherheit und Verträglichkeit des ID bei Welpen vor. Die zweite ID-Injektion wurde vorgenommen, um einen klinischen Krankheitsrückfall zu vermeiden. Dieser Fallbericht unterstreicht die Bedeutung der immunologischen Lücke bei Welpen mit *Babesia canis*-Infektionen und die Bedeutung der Ektoparasitenprophylaxe bei Welpen mit möglichem Vektorkontakt.

Background

Babesia canis is a haemoprotozoal pathogen transmitted by *Dermacentor reticulatus*-ticks. *Dermacentor reticulatus*-ticks are active throughout the whole year, including the winter months [1–5]. Therefore, infections with *B. canis* must be considered in dogs with corresponding clinicopathological abnormalities year-round. Thrombocytopenia is the most frequent hematological abnormality in dogs with acute *B. canis* infections and is often severe [6, 7]. The federal states of Berlin and Brandenburg, Saxony, Saxony-Anhalt, Saarland as well as the Ruhr area and the Rhine-Main area were recently classified as high-risk areas for infections with *B. canis* in dogs in Germany [3, 6, 8].

In adult dogs, acute infections with *B. canis* may vary from subclinical infections to severe life-threatening disease [7]. The knowledge in puppies in Europe is limited to 3 Asian Shepherd puppies in Poland tested positive for *B. canis* by PCR, which were presented with decreased appetite and activity in autumn 2011 [9]. Only the mother dog had outdoor access and was infested with *D. reticulatus*-ticks. Transplacental infection with *B. canis* was considered most likely. Pale mucous membranes, elevated rectal temperatures, marked thrombocytopenia, marked anemia, mild to moderate leukopenia, and mild to moderate hypoproteinemia were noted in all 3 puppies. Mild elevations in aspartate aminotransferase (AST) were present in 2 of 3 dogs, and mild elevation of urea in one of the 3 puppies. One puppy was treated with imidocarb dipropionate (5 mg/kg BW s.c., Imizol, Intervet International BV, Boxmeer, Holland). The remaining 2 puppies were also treated with imidocarb dipropionate, but the dosage was not reported [9].

Diagnosis of an acute infection with *B. canis* in dogs is primarily based on positive polymerase chain reaction (PCR) results from venous and/or capillary blood, which shows high sensitivity and specificity. Microscopic detection of merozoites in erythrocytes is less sensitive and specific, but, especially with smears from capillary blood, useful as a rapid and cost-effective in-house method [10–12]. However, negative results should be confirmed by PCR. Additionally, positive PCR results allow differentiation of the various *Babesia* spp. by sequencing. Positive *Babesia* spp. antibody serology demonstrates past pathogen contact but does not prove an acute infection with *B. canis* [10, 13]. High *Babesia* spp. antibody levels at the time of infection correspond with less severe clinicopathological abnormalities and lower infection intensity, indicating protective antibodies [7].

In general, 2 subcutaneous or intramuscular injections of 6.6 mg/kg bodyweight (BW) imidocarb dipropionate 14 days apart are approved by the Food and Drug Administration (FDA) in the USA under NADA 141–071 for treatment of acute *B. canis* infections in adult dogs [14]. Imidocarb dipropionate is labelled with a therapeutic dosage of 0.25 ml/10 kg BW (3.0 mg/kg BW) in Europe. For prophylaxis 0.5 ml/10 kg BW (6.0 mg/kg BW) are recommended by the manufacturer. Safety and effectiveness of imidocarb dipropionate have not been determined in puppies or in breeding, lactating, or pregnant animals [14], and no safety instructions are published by the manufacturer Merck Sharp & Dohme Corporation and the distributor Intervet Inc. (both Raway, USA).

Currently, there are no guidelines or safety instructions available regarding the dosage of imidocarb dipropionate for puppies and the knowledge regarding the management of acute *B. canis* infections in puppies remains limited. Therefore, the aim of this case report was to describe the management of an acute *B. canis* infection in a 45-day-old German Shepherd puppy in Germany in December 2025.

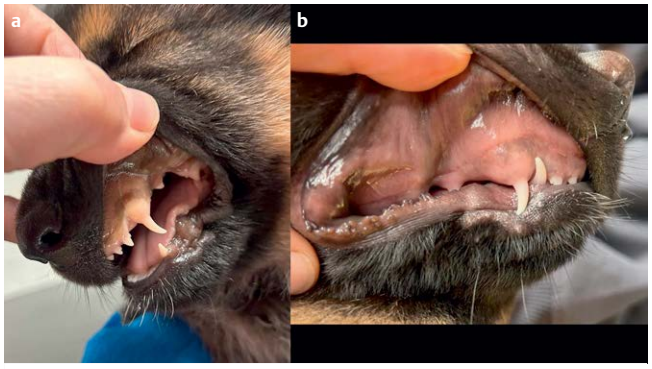
Case presentation

A 45-day-old, male intact German Shepherd dog living in the federal state of Saxony-Anhalt, Germany, was presented to a small animal veterinary practice on December 29th, 2025, (day 0) with lethargy, inappetence, and problems with standing and walking since the day before this first presentation. The puppy had never left Germany, and tick infestation was reported by the owners in the mother dog as well as the described puppy (▶ **Fig. 1**). No ectoparasite prophylaxis was applied in the mother dog nor the puppy. The diseased puppy was not vaccinated, and fenbendazole (Panacur Pet-Paste Wurmkur, Intervet Deutschland GmbH, Unterschleissheim, Germany) was applied once. The whole dog family had been outdoors in the meadows several times.

At general examination, the puppy was lethargic with difficulties in standing and walking. Pale and icteric, dry mucous membranes (▶ **Fig. 2**), fever (rectal temperature 40.3 °C), and panting were recognized. The capillary refill time was not possible to evaluate due to the pale mucous membranes. Both testicles had descended. The peripheral lymph nodes were not enlarged. The abdomen was tender and not painful at palpation. The puppy's body weight (BW) was 5.5 kg.

▶ **Fig. 1** *Dermacentor reticulatus* ticks attached to a 45-day-old German Shepherd dog with acute *Babesia canis* infection in December in Germany. Quelle: K. Fürst, I. Schäfer.

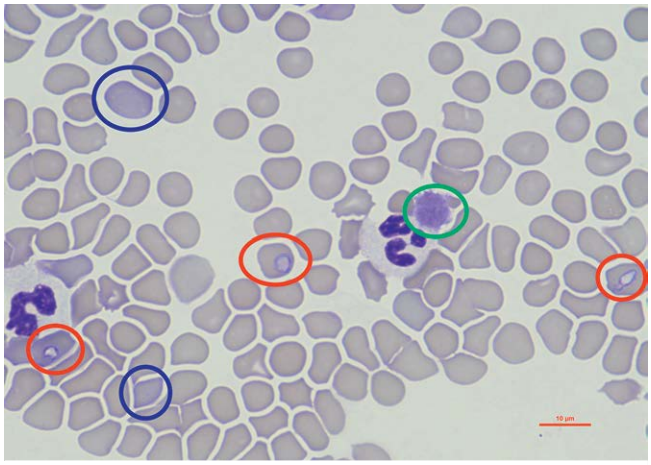
▶ **Abb. 1** Nachweis von *Dermacentor reticulatus*-Zecken bei einem 45-Tage alten Deutschen Schäferhund-Welpen mit einer akuten *Babesia canis*-Infektion im Dezember in Deutschland. Source: K. Fürst, I. Schäfer.



▶ **Fig. 2** Pale, icteric, and dry mucous membranes on day 0 (**a**) and pale pink mucous membranes on day 14 after treatment with imidocarb dipropionate (**b**) in a 45-day-old German Shepherd dog with acute *Babesia canis* infection. Source: K. Fürst, I. Schäfer.

▶ **Abb. 2** Blasse, ikterische und trockene Schleimhäute an Tag 0 (**a**) sowie blass-rosa und feuchte Schleimhäute an Tag 14 (**b**) nach Start der Behandlung mit Imidocarb Dipropionat bei einem 45-Tage alten Deutschen Schäferhund-Welpe mit akuter *Babesia canis*-Infektion. Quelle: K. Fürst, I. Schäfer.

An acute infection with *B. canis* was diagnosed due to microscopic detection of large merozoites in the peripheral blood smear (▶ **Fig. 3**) and confirmed by a positive piroplasm PCR on venous EDTA-blood (forward primer: 5′-AAT ACC CAA TCC TGA CAC AGG G-3’; reverse primer: 5′-TTA AAT ACG AAT GCC CCC AAC-3′, based on Olmeda et al. [15] followed by a species differentiation by Sanger sequencing).



▶ **Fig. 3** Venous blood smear of a 45-day-old German Shepherd puppy with acute *Babesia canis* infection with merozoites of large *Babesia* spp. in erythrocytes (red circles), moderate anisocytosis and polychromasia (blue circles), and activated macro-platelets (green circle) (1000 x magnification, diff-quik-staining). Source: I. Schäfer.

▶ **Abb. 3** Blutausstrich mit Nachweis von Merozoiten großer Babesien in Erythrozyten (rote Kreise), mittelgradiger Anisozytose und Polychromasie (blaue Kreise) und aktivierten Makrothrombozyten (grüne Kreise) bei einem 45-Tage alten Deutschen Schäferhund-Welpen mit akuter *Babesia canis*-Infektion (1000-fache Vergrößerung, Diff-Quik-Färbung). Quelle: I. Schäfer.

Hematology (Sysmex XN-V analyzer, Sysmex Deutschland GmbH, Norderstedt, Germany) and biochemistry (Cobas 8000, Roche Deutschland Holding GmbH, Mannheim, Germany) was done in the Laboklin laboratory (Bad Kissingen, Germany), including a CRP analysis (Gentian Canine CRP Immunoassay on Cobas 8000). Marked thrombocytopenia, marked anemia, marked hyperbilirubinemia, marked elevation of CRP, moderate hypoproteinemia, mild hyponatremia, and mild elevations of AST, urea, and phosphorus were noted (▶ **Table 1**). Age-dependent reference intervals were not provided by the laboratory. Reference intervals for hematology and biochemistry in 46 to 60-day-old dogs by Rortveit et al. [16] were added, but these ranges should be interpreted with caution due to different methodologies and breeds (▶ **Table 1**). *Babesia* spp. immunoglobulin G (IgG) antibody levels were negative in the Laboklin laboratory (< 0.1 technical units [TE], Babesia ELISA Dog, Afosa, Blankenfelde-Mahlow, Germany; > 19.0 TE positive) (▶ **Table 1**).

Immediately after diagnosis, 2.0 mg/kg BW imidocarb dipropionate (Imizol 85 mg/ml Solution for Injection, MSD Animal Health, Unterschleissheim. Germany) was administered subcutaneously. Additionally, an intravenous infusion with Glucose 5 % (Deltajonin G5, Deltamedica GmbH, Reutlingen, Germany) was applied. Non-hospital care with daily presentations of the puppy was discussed with the owners. The owners were explicitly advised to closely monitor the puppy and to seek immediate emergency veterinary care if the general condition worsens. Additionally, blood transfusion was discussed but the owners decided to monitor the dog’s condition closely at home.

On day 1, the general condition of the puppy improved, and food intake was considered normal. Vomiting had been reported. The puppy was able to stand and walk, no lethargy was noted any-

▶ **Table 1** Complete blood count, biochemical parameters, *Babesia* spp. antibody levels, and microscopic detection of merozoites in a 45-day-old, male intact German Shepherd puppy with an acute *Babesia canis* infection.

▶ **Tab. 1** Blutbild, Blutchemie und Babesien-Antikörperspiegel sowie Nachweis von Merozoiten bei einem 45-Tage alten männlichen Deutschen Schäferhund-Welpe mit akuter *Babesia canis*-Infektion.

Parameter	Reference interval Laboklin laboratory[1]	Reference interval for puppies aged 46–60 days[2]	Day 0	Day 14
Complete blood count[3]				
RBC (x 10^{12}/l)	5.5–8.5	3.9–5.7	2.01	4.53
HGB (g/dl)	15.0–19.0	8.2–11.8	4.6	10.1
HCT(l/l)	0.44–0.52	0.26–0.38	0.17	0.35
MCV (µm^3)	60–77	61–75	85.6	76.6
MCH (pg)	17–23	–	22.9	22.3
MCHC (g/dl)	31–34	29–34	26.7	29.1
Ret (x 10^9/l)	<110.0	–	103.7	115.1
WBC (x 10^{12}/l)	6.0–12.0	8.4–25.5	6.7	10.8
Seg (x 10^9/l)	3.0–9.0	3.6–14.2	–	5.1
Lymphs (x 10^9/l)	1.0–3.6	2.4–12.4	–	4.0
Eos (x 10^9/l)	0.04–0.6	0.0–2.2	–	0.3
Monos (x 10^9/l)	0.04–0.5	0.5–2.1	–	1.3
THR (x 10^9/l)	150–500	173–714	23	207
Biochemistry[4]				
Total protein (g/dl)	5.4–7.5	3.5–4.8	3.4	4.5
Albumin (g/dl)	2.5–4.4	0.9–1.8	2.6	3.2
Globulins (g/dl)	<4.5	1.5–2.2	0.8	1.3
Urea (mmol/l)	3.3–8.3	2.0–5.6	8.9	7.7
Creatinine (µmol/l)	<125.0	34.0–63.0	33.0	45.0
Phosphorus (mmol/l)	0.7–1.6	2.1–3.5	2.2	3.2
Calcium (mmol/l)	2.3–3.0	2.6–3.1	2.6	2.9
Potassium (mmol/l)	3.5–5.1	4.4–7.7	4.1	5.3
Sodium (mmol/l)	140–155	139–149	135	143
Magnesium (mmol/l)	0.6–1.3	–	0.7	0.7
Bilirubin (µmol/l)	<3.4	–	94.9	2.6
ALT (U/l)	<88	18–34	77	24
AP (U/l)	<147	90–249	142	169
AST (U/l)	<51	19–43	80	31
CK (U/l)	<200	165–702	115	331
GLDH (U/l)	<8.0	–	6.8	4.0
G-GT (U/l)	<10.0	–	<0.1	2.4
Glucose (mmol/l)	3.05–6.1	3.3–8.1	5.5	7.1
Cholesterol (mmol/l)	3.1–10.1	3.7–11.2	7.7	8.4
Triglycerides (mmol/l)	<3.9	–	2.3	1.84
Iron (µmol/l)	15–45	–	36.4	46.1
DGGR-Lipase (U/l)	<120.0	–	51.0	29.5
CRP (mg/l)	<15.0	–	73.0	6.5
Parasitology				
Merozoites	Negative	–	Positive	No
Piroplasm PCR[5]	Negative	–	Positive[6]	Negative
Babesia spp. antibody ELISA (TE)[7]	<19.0	–	<0.1	<0.1

▶ **Table 1** Continued.

▶ **Tab. 1** Fortsetzung.

[1]No age-dependent reference intervals were available in the laboratory; [2]Reference intervals in 46 to 60-day-old dogs according to Rortveit et al. [16]; [3]Sysmex XN-V analyzer, Sysmex Deutschland GmbH, Norderstedt, Germany; [4]Cobas 8000, Roche Deutschland Holding GmbH, Mannheim, Germany; [5]based on Olmeda et al. [15]; [6]identified as *Babesia canis* via Sanger sequencing; [7]Babesia ELISA Dog, Afosa, Blankenfelde-Mahlow, Germany.

ALT = alanine transaminase; *AP* = alkaline phosphatase; *AST* = aspartate aminotransferase; *CK* = creatine kinase; *CRP* = C-reactive protein; *DGGR-lipase*: 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester lipase; *EDTA* = ethylenediaminetetraacetic acid; *ELISA* = enzyme-linked immunosorbent assay; *Eos* = eosinophilic granulocytes; *G-GT* = gamma-glutamyl transferase; *GLDH* = glutamate dehydrogenase; *HCT* = hematocrit; *HGB* = hemoglobin; *Lymphs* = lymphocytes; *MCH* = mean corpuscular hemoglobin; *MCHC* = mean corpuscular hemoglobin concentration; *MCV* = mean corpuscular volume; *Monos* = monocytes; *RBCs* = red blood cells; *Seg* = segmented neutrophilic granulocytes; *Ret* = reticulocytes; *TE* = technical units; *THR* = platelets; *WBCs* = white blood cells. bolded values = parameters outside the reference intervals

more, the mucous membranes were slightly pale with a capillary refill time < 1 second, and the rectal temperature had decreased to 38.5 °C. Another intravenous infusion with Glucose 5 % (Deltajonin G5, Deltamedica GmbH, Reutlingen, Germany) was applied. Due to the gastrointestinal losses by vomiting, glucose was added to the infusion protocol.

On day 2, the behavior was classified as normal by the owners, the puppy was active and playful. No further vomiting was noted, and the food intake was considered normal. The general examination was unremarkable with pale to pale pink mucous membranes, a capillary refill time < 1 second, and a rectal temperature of 38.4 °C. The BW was noted with 5.5 kg. Another intravenous infusion with a balanced electrolyte solution (Ringer-Lactat-Lösung DELTAMEDICA, Deltamedica GmbH, Reutlingen, Germany) was applied.

On day 14, the BW had increased to 7.7 kg, and the general condition was still unremarkable. The mucous membranes were slightly reddish with a capillary refill time below one second (▶ **Fig. 2**), and a rectal temperature of 38.3 °C. Mild regenerative anemia, mild lymphocytosis, and mild to moderate monocytosis were noted in the complete blood count (CBC). In biochemical analysis, moderate hyperphosphatemia, mild hyperkalemia and hyperglycemia, as well as mild elevations in creatine kinase (CK), alkaline phosphatase (AP), and iron were detected. The piroplasm PCR was negative, and the *Babesia* spp. antibody level was still negative with < 0.1 TE (▶ **Table 1**). A second subcutaneous injection with 4.2 mg/kg BW imidocarb dipropionate (Imizol 85 mg/ml Solution for Injection, MSD Animal Health, Unterschleisheim, Germany) was administered. Until submission of this report three months later, the dog stayed clinically healthy.

On day 2, the mother dog, a 5-year-old German Shepherd, was presented for a general health check. The mentioned puppy was the only one born in the litter. Behavior and food intake was reported as normal by the owners. No stays abroad from Germany were reported. The mother dog was also infested by *D. reticulatus*-ticks, and no ectoparasite prophylaxis was applied. The EDTA-blood submitted for hematological analysis was coagulated and therefore no CBC was possible. The biochemical analysis was unremarkable, PCR-testing for piroplasms was negative, but the *Babesia* spp. antibody level was positive with 96.8 TE (▶ **Table 2**).

Discussion and conclusions

Acute infections with *B. canis* should be considered as a differential diagnosis in dogs presented with fever and most often marked thrombocytopenia, even in puppies and in winter months, especially in areas endemic for canine babesiosis. Fever, lethargy, inappetence, most often marked thrombocytopenia, hemolytic anemia with prehepatic hyperbilirubinemia, and CRP elevations indicating an acute-phase response are considered typical for acute *B. canis* infections [6–8], in accordance with the clinicopathological abnormalities noted in the 45-day-old German Shepherd. The puppy had a history of *D. reticulatus*-infestation but, in contrast to the puppy, the mother dog did not show a positive PCR. Thus, transmission of *B. canis* by the vector tick is most likely rather than transplacental infection.

Imidocarb dipropionate is considered as the drug of choice to treat acute infections with *B. canis* in dogs. In Germany, imidocarb dipropionate is used off label. Safety and effectiveness of imidocarb dipropionate have not been determined in puppies yet [14], which makes the dosage in puppies as in the 45-day-old German Shepherd worth discussing. In adult dogs, the package leaflets indicate a single therapeutic dose of 0.47–0.58 ml/10 kg BW (corresponding to 4.0–5.0 mg/kg BW) up to 0.25 ml/10 kg BW (corresponding to 2.125 mg/kg BW). For prophylactic use 0.5 ml/10 kg BW corresponding to 4.25 mg/kg BW are stated for subcutaneous or intramuscular injection in adult dogs. The FDA approved a subcutaneous or intramuscular administration of imidocarb dipropionate twice at a dosage of 6.6 mg/kg BW 2 times with a 14 day interval [14]. The dosage in the literature to treat *B. canis* infections in adult dogs ranges from 5.0–7.5 mg/kg BW [17, 18]. Safety and effectiveness of imidocarb dipropionate have not been determined in puppies nor in breeding, lactating, and pregnant dogs [14].

Since no manufacturer safety data are currently available regarding the use of imidocarb dipropionate in puppies, the 45-day-old German Shepherd Dog puppy received an initial dose of 2.0 mg/kg BW imidocarb dipropionate, adapted from the lower-ranged recommended dosage in adult dogs of 2.125 mg/kg BW. Vomiting was reported the day after and is in accordance with reported side effects in adult dogs. Imidocarb dipropionate injections may cause parasympathomimetic manifestations such as salivation, nasal dis-

▶ **Table 2** Biochemical parameters, and *Babesia* spp. antibody levels, in a 5-year-old German Shepherd mother dog (note that hematological analysis was not possible due to coagulation of the submitted EDTA-blood).

▶ **Tab. 2** Biochemische Parameter und Babesien-Antikörperspiegel bei einer 5-Jahre-alten Deutschen Schäferhund-Mutterhündin (aufgrund von koaguliertem EDTA-Blut war kein Blutbild möglich).

Parameter	Reference interval	Day 0
Biochemistry[1]		
Total protein (g/dl)	5.4–7.5	6.4
Albumin (g/dl)	2.5–4.4	3.8
Globulins (g/dl)	<4.5	2.6
Urea (mmol/l)	3.3–8.3	5.1
Creatinine (µmol/l)	<125.0	79.0
Phosphorus (mmol/l)	0.7–1.6	1.1
Calcium (mmol/l)	2.3–3.0	2.6
Potassium (mmol/l)	3.5–5.1	5.0
Sodium (mmol/l)	140–155	144
Magnesium (mmol/l)	0.6–1.3	0.8
Bilirubin (µmol/l)	<3.4	1.2
ALT (U/l)	<88	21
AP (U/l)	<147	25
AST (U/l)	<51	19
CK (U/l)	<200	60
GLDH (U/l)	<8.0	2.6
G–GT (U/l)	<10.0	2.2
Glucose (mmol/l)	3.05–6.1	4.7
Cholesterol (mmol/l)	3.1–10.1	5.8
Triglycerides (mmol/l)	<3.9	0.6
Iron (µmol/l)	15–45	18.7
DGGR-Lipase (U/l)	<120.0	44.9
CRP (mg/l)	<15.0	4.0
Parasitology		
Merozoites	Negative	Negative
Piroplasm PCR[2]	Negative	Negative
Babesia spp. antibody ELISA (TE)[3]	<19.0	**96.8**

[1]Cobas 8000, Roche Deutschland Holding GmbH, Mannheim, Germany; [2]based on Olmeda et al. [15]; [3]Babesia ELISA Dog, Afosa, Blankenfelde-Mahlow, Germany

ALT = alanine transaminase; *AP* = alkaline phosphatase; *AST* = aspartate aminotransferase; *CK* = creatine kinase; *CRP* = C-reactive protein; *DGGR-lipase*: 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester lipase; *EDTA* = ethylenediaminetetraacetic acid; *ELISA* = enzyme-linked immunosorbent assay; *Eos* = eosinophilic granulocytes; *G-GT* = gamma-glutamyl transferase; *GLDH* = glutamate dehydrogenase; *HCT* = hematocrit; *HGB* = hemoglobin; *Lymphs* = lymphocytes; *MCH* = mean corpuscular hemoglobin; *MCHC* = mean corpuscular hemoglobin concentration; *MCV* = mean corpuscular volume; *Monos* = monocytes; *RBCs* = red blood cells; *Seg* = segmented neutrophilic granulocytes; *Ret* = reticulocytes; *TE* = technical units; *THR* = platelets; *WBCs* = white blood cells. bolded values = parameters outside the reference intervals

charge, epiphora, and vomiting [14]. In another case report in puppies, a dosage of 5.0 mg/kg was applied to a 43-day-old Central Asian Shepherd with acute *B. canis* infection [9], a higher dosage compared to the German Shepherd puppy. No follow-up was provided, and therefore information on the tolerance of the dosage is missing. In the 45-day-old puppy, the clinical signs improved significantly within a short period of time. Mild anemia, mild lymphocytosis, hyperphosphatemia, and mild elevation in AP may all be explained by the early age of the puppy and the fact that no age-dependent reference ranges were applied. The monocytosis is most likely linked to the *B. canis* infection, showing an inflammatory condition. To avoid parasympathomimetic side effects as e. g., vomiting especially in puppies, an application of atropine accompanied by the imidocarb dipropionate injection may be advisable, and, based on the very limited experience, a lower ranged dosage of imidocarb dipropionate may be used (2.0–5.0 mg/kg BW).

Interestingly, all 4 puppies with acute *B. canis* infections in Europe mentioned in the literature so far were presented at an age of 43 to 46 days after birth. The period of 6th to 8th week of life is crucial in the development of immunity in dogs. Maternally derived immunity starts to decrease fast, and individual immunity starts to establish [19]. Infections in general may remain subclinical until the time at which maternally derived immunity starts to decrease. The mother dog in our case report had a high level of *Babesia* spp antibodies, and antibodies are protective against severe clinicopathological abnormalities in acute *B. canis* infections in adult dogs [7]. As the mother dog had no stays abroad from Germany, *B. canis* pathogen contact in the highly endemic federal state of Saxony-Anhalt in Germany seems most likely.

It can be presumed that the 45-day-old German Shepherd developed marked clinicopathological abnormalities due to an acute infection with *B. canis* via tick bites at the shift from maternally derived to individual immunity. Additionally, no elevation in the *Babesia* spp. antibody level was seen on day 14. This may be explained by the immunological status of the 45-day-old German Shepherd, which did not result in antibody production. Therefore, and to avoid a potential disease relapse due to the low dose of 2.0 mg/kg imidocarb dipropionate, a second injection dosed 4.2 mg/kg BW was applied to the puppy, and no further parasympathomimetic side effects were noted. The dog stayed clinically well till the publication of this case report.

In puppies, treatment recommendations should implicate a more careful approach due to parasympathomimetic side effects of imidocarb dipropionate. Based on the very limited data on puppies in literature, a first injection of 2.0 mg/kg BW up to 5.0 mg/kg BW may be advisable. In the 45-day-old German Shepherd, 2.0 mg/kg BW imidocarb dipropionate resulted in a negative PCR result 14 days later. The second imidocarb dipropionate injection should be critically discussed, as the PCR was negative and the puppy did not show any significant clinicopathological abnormalities on day 14. The first imidocarb dipropionate injection might have decreased the *B. canis* parasite load in the peripheral venous blood below the detection limit of the PCR assay, but merozoites in the capillary blood might still be present and therefore the pathogen might not have been fully eliminated. The second injection was therefore applied to prevent a disease relapse as discussed above. It should be considered that puppies may not build up protective *Babesia* spp.

antibody levels due to their immunological status. Additionally, the mother dog had high *Babesia* spp. antibody levels, which might have been transferred transplacental to the puppy as maternally derived antibodies. In the immunological gap of the 45-day-old puppy, there might have already been a lack of maternally derived antibodies, supported by the negative *Babesia* spp. antibody levels. This might have predisposed the puppy to develop severe clinicopathological abnormalities after an acute *B. canis* infection, as discussed above. To the authors' knowledge, there are no studies on the persistence of maternally derived antibodies in case of *B. canis* infections in puppies available. However, the clinicopathological findings in 4 puppies with an acute *B. canis* infection and especially the narrow timeframe of clinical presentation at an age from 43 to 46 days after birth makes an impact of an immunological gap likely.

CONCLUSION FOR PRACTICE

In conclusion, transmission of *B. canis* by *D. reticulatus* ticks should be considered next to transplacental infections in puppies presented with fever, inappetence, anemia, and thrombocytopenia. *Dermacentor reticulatus*-ticks are meanwhile active year-round in Central European countries as e. g. Germany, and year-round ectoparasite prophylaxis with licensed acaricides, even in the winter months, is important to prevent canine babesiosis [1–5]. However, acaricides are currently licensed for use in dogs only from 7–8 weeks of age onwards, as no safety data are available for younger puppies. Nevertheless, as demonstrated by the present case report, the risk–benefit assessment supports the use of acaricidal treatment even in younger dogs when exposed to *D. reticulatus*. Puppies may be presented with severe clinicopathological abnormalities in acute *B. canis* infections and should be treated with imidocarb dipropionate in a lower-ranged dosage, referring to the limited experience in puppies and the still unknown safety status of imidocarb dipropionate in this age group. A second injection may be advisable in puppies due to the immunological status, especially to avoid a relapse of disease.

Ethics approval and consent to participate

The owner of the dog agreed in publishing the case report including the figures.

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Conflict of interest

Ingo Schäfer and Torsten J. Naucke are employees, and Elisabeth Müller is the CEO of the Laboklin GmbH & Co. KG; Andreas Moritz, Christina Strube, and Ingo Schäfer have repeatedly lectured for and/or acted as consultants for diagnostic and (veterinary) pharmaceutical companies; Andreas Moritz and Christina Strube have previous and ongoing research collaborations with various diagnostic and (veterinary) pharmaceutical companies. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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