A presumptive case of cerebral babesiosis in a dog in Poland caused by a virulent Babesia canis strain

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Key words
Babesia canis, cerebral babesiosis, dog, PCR, vector-borne diseases

Summary
The aim of this paper was to present the first case of cerebral canine babesiosis due to infection by Babesia canis in a dog in Poland. A 5-year-old American Staffordshire Terrier was presented with an unusual clinical manifestation of acute babesiosis that included neurological signs and pancytopenia. Despite treatment the dog died. Diagnosis was based on microscopic examination of Giemsa-stained blood smears (detection of piroplasms in red blood cells) and post mortem examination of the brain by histopathology and PCR method. The amplified segment of the Babesia 18S RNA gene was sequenced. This enabled to determine that the cause of the disease had been the strain 18S RNA-B EU622793. This is one of two B. canis strains found endemically in Poland, which reveals a greater virulence than the strain 18S RNA-A EU622792. The described case indicates that this form of canine babesiosis should be taken into account in differential diagnosis in dogs exhibiting neurological symptoms, especially in the tick activity season.

Introduction
Canine babesiosis is a common and clinically significant tick-borne disease caused by hematozoan parasites of the genus Babesia (1, 16). The classification of Babesia spp. places them in the order Piroplasmida within the phylum Apicomplexa. Two morphologically distinct forms of the erythrocytic stage in the canine host were recognized in early studies that led to the naming of the larger form, measuring approximately 3–5 μm as B. canis, and the smaller (1–3 μm) as B. gibboni. However, the development of molecular methods has demonstrated that other Babesia species such as Babesia conradae, Babesia microti-like piroplasm, Theileria spp. and a yet unnamed large form Babesia spp. infect dogs and cause distinct diseases (17). Babesia canis rossi, Babesia canis canis and Babesia canis vogeli, previously considered subspecies of Babesia canis, are morphologically identical but differ in the severity of clinical manifestations which they induce, their tick vectors, genetic characteristics, and geographic distributions, and are therefore currently considered as separate species (12, 33). So far, only Babesia canis canis, which is transmitted by Dermacentor reticulatus ticks, has been found in dogs in Poland (1, 6, 7, 34). These parasites are also the most common etiologic factor of babesiosis in dogs in Europe (11, 16, 20, 28), except its southern parts, where Rhipicephalus sanguineus, the vector of B. vogeli (23), is more prevalent than D. reticulatus (29).

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Clinically, these pathogens cause remittent fever, progressive anemia, hemoglobinuria, marked splenomegaly and hepatomegaly in dogs and, in some cases, the death of infected animals (2). In rare cases severe disease is accompanied by symptoms suggestive of involvement of the brain. Cerebral babesiosis is uncommon, but carries a poor prognosis (22, 26). The pathological mechanism has been suggested to be endothelial cell damage and necrosis, followed by segmental microvascular necrosis with perivascular edema and hemorrhage (19). In Poland cerebral babesiosis has never been diagnosed in dogs so far. To achieve a definitive diagnosis of this form of babesiosis the following parameters should be evaluated: identification of the parasite species involved, disturbance of brain function and absence of concomitant infections (26). In fatal cases, detection of parasite accumulations in brain capillaries could verify the diagnosis (26).

The aim of this paper was to present the first case of cerebral canine babesiosis due to infection by *Babesia canis* in a dog in Poland.

**Case description**

**Clinical findings**

A male, 5-year-old American Staffordshire Terrier dog weighing 35 kg was admitted to the Clinic of Infectious Diseases, Faculty of Veterinary Medicine in Lublin in a general poor condition. According to the history the animal had been apathic and had lost its appetite and thirst several days ago. The owners took the dog to a local veterinary clinic, where it was unsuccessfully treated for 3 days by means of penicillin-streptomycin, sodium metamizole, and Ringer’s fluid administered subcutaneously. Immediately prior to the visit in the Clinic of Infectious Diseases, the condition of the animal suddenly worsened.

![Fig. 1](image-url) A considerably thickened wall of the left cardiac ventricle with numerous extravasations under the endocardium.

**Abb. 1** Deutlich verdickte Wand des linken Ventrikels mit zahlreichen subendokardialen Extravasationen

![Tierärztliche Praxis Kleintiere 5/2012](image-url) © Schattauer 2012

On admission the dog displayed symptoms of dyspnea, abundant salivation, vomiting, ataxia and seizures occurring at hourly intervals. He had a body temperature of 40.5 °C. The bilaterally and symmetrically dilated pupils did not react to light. The pulse was weak and accelerated (93/min), breathing was shallow and rapid (41/min). Mucous membranes were cyanotic and dry.

The dog came from a breed located in western Poland and had never travelled outside the country. The animal was fed with a commercial pet food and was periodically vaccinated against basic infectious diseases (rabies, parovirus infection, canine distemper, CAV-1, parainfluenza) and regularly checked for endoparasites. The owners did not use any tick prevention and repeatedly removed ticks from the body surface of the dog. They did so for the last time a few days before development of the disease.

**Laboratory tests**

Blood was collected from the animal for hematological, biochemical and molecular testing. The hematological test (Boule Sweden) showed anemia (HCT: 28.3%, RBC: 5.63 × 10⁶/mm³, Hb: 9.8 g/dl), thrombocytopenia (56,000/mm³) and leukopenia (5,100/mm³). The microscopic examination (Olympus CH 20 Japan) of the blood smears stained by the Giemsa method revealed piroplasms caused by the strain 18S RNA-B (559 bp) enabled to determine that the disease had been caused by the strain 18S RNA-B *Babesia canis* EU622793. Simultaneously, PCR tests of the dog’s blood for *Anaplasma phagocytophilum* and *Ehrlichia canis* 16S RNA gene (4), faeces for canine parvovirus type 2 VP2 gene (3) and urine for canine adenovirus type 1 E1B 19K gene (13), as well as serological tests for the presence of anti-C6 *Borrelia burgdorferi* antibodies (IDEXX Snap-3Dx test) were performed. All these tests gave negative results.

Serum biochemical assay (Mindray BS-130) showed an increased activity of ALT (177 U/l), AST (155 U/l), an elevated concentration of bilirubin (1.21 mg/dl), urea (142.9 mg/dl) and creatinine (3.30 mg/dl) as well as a slightly reduced level of glucose (65 mg/dl). Additionally reduced levels of sodium (142.71 mmol/l) and chloride (106.1 mmol/l) and a decrease of serum pH (pH = 7.22) were observed (BM ISE, Biomaxima).

**Treatment**

As a causal treatment, imidocarb dipropionate (6 mg/kg bw, s. c., Imizol, Shering Plough Animal Health) was used. The supportive therapy included administration of oxygen, hydration of the dog by intravenous application of a mixture of 5% glucose and a multi-electrolyte liquid (60 ml/kg BW; Baxter Poland) with the addition of vitamin C (20 mg/kg BW, Vitaminum C, Biowet Pulawy, Poland), application of flumetasone (500 mg i. v., Vector, Polfa Warszawa S. A., Poland) and enrofloxacin (5 mg/kg BW, s. c., Enrobio-
flox 5% Injectio, Vetoquinol Biowet Sp. z o.o., Poland). Seizures were abolished with diazepam (0.5 mg/kg BW i. v., Relanium, Polfa Warszawa S.A., Poland).

Twenty-four hours after the application of imidocarb, the condition of the patient slightly improved. Fever, dyspnea and salivation receded and the frequency of seizures decreased to three per day. The dog also began to drink water unaided. However, ataxia and mydriasis still remained. Despite continued treatment the condition of the dog suddenly worsened on the third day. Therefore the owners made a decision about euthanasia and agreed on conducting a post mortem examination.

**Post mortem examination**

The post mortem examination was carried out according to a generally accepted scheme (15). It revealed an increased amount of a bloody, clear liquid in the abdominal and thoracic cavities. The tracheal mucous membrane was light pink in colour and covered with a small amount of foamy mucus. The lungs, dark red, showed a fluffy texture and rounded edges. Decreased pulmonary aeration and a considerable amount of a foamy, bloody fluid flowing from the sliced lungs were observed. The myocardium had a dark red colour, and the endocardium was covered with numerous extravasations (Fig. 1). The spleen was enlarged, dark red in colour, with the consistency of dough. The kidneys were enlarged, strongly padded and symmetrical. The renal capsule could easily be detached, the surface of the kidneys after the removal of the capsule was dark red and smooth. The ratio of the renal cortex to the renal medulla was preserved. The gastric wall was significantly thickened with the mucous membrane of red-brown colour, covered with mucus with the addition of blood. The mucosa of the small intestine and colon was dark red in colour and covered with an increased amount of mucus. In the lumen of the intestines, a small amount of the intestinal chyme was observed. Macroscopic examination of the brain did not reveal any lesions.

Sections of the liver, kidneys, myocardium and the brain were collected for histopathological examination and fixed in 10% neutral formalin for 24 hours. The specimens prepared by the paraffin technique were stained with hematoxylin and eosin. The microscopic image of the liver showed the presence of numerous erythrocytes in the sinusoidal spaces, resulting from the congestion of the liver. A similar histopathological image (hyperemia) was observed in the specimens from the kidneys and the myocardium. Within the liver, numerous focuses of necrosis, of necrosis coli- quativa character were observed. In the renal interstitial tissue, a massive infiltration mainly consisting of mononuclear cells, being a symptom of the interstitial, acute nephritis, were demonstrated. Arterioles and capillaries of the brain were swollen and filled with blood (blood circulation disorder). In addition, within the cerebral tissue, the endothelium of the small blood vessels was partially damaged (Fig. 2), and perivascular edema and hyperemia (caused by the damage of blood vessels) could be observed. In the convolution layer of the brain, around the meninges, an inflammatory infiltration mainly consisting of lymphocytes and neutrophils (meningitis) was seen (Fig. 3). Babesia parasites could not be detected in erythrocytes in brain sections, but Real-Time PCR confirmed the presence of genetic material of piroplasms in this material. The Ct values read from the amplification curve fluctuated around 17 cycles for the examined sample. The sequencing confirmed that the parasites found in the brain belonged to the same strain of *Babesia canis* 18S RNA-B, EU622793 as the protozoa detected in the blood of the sick dog.

Detection of *Babesia canis* DNA both in the blood and in the brain, together with the clinical picture of the disease and results...
of the hematological tests (pancytopenia) were the reason why we assumed that the dog’s disease had been caused by the infection with these protozoa. The case that we presented is the first description of cerebral babesiosis in dogs in Poland.

Discussion

Cerebral babesiosis (CB) has been described or suspected in infections with Babesia bovis and Babesia bigemina in cattle (8, 14), B. caballi in horses (21) and B. canis canis in dogs (22, 32). This form of infection develops only in a small proportion of infected animals. Exact figures are not available, but less than 0.1% of 1200 African dogs assessed for rabies over a period of 3 years (that had died with cerebral pathology) showed a massive accumulation of infected erythrocytes in the brain which is suggestive of a low incidence (24). In a study of cerebral pathology in cattle, only 8% of the animals had babesiosis, of which only 10% exhibited heavily parasitized capillaries (8, 26). Cerebral babesiosis can result from either sludging of parasitized erythrocytes in small vessels in the brain or a metabolic derangement. Tissue hypoxia, and hypoglycemia can lead to neurological signs (10). Immune complex deposition in blood vessels of the brain and vasculitis in the course of the disease (27), as well as DIC can also lead to the development of CB (22). Cerebral malaria may result from nitric oxide-induced changes of neurotransmission. A similar pathogenesis could also hold true for canine babesiosis (18, 26). Cerebral babesiosis is usually associated with high mortality (10, 30). Clinical signs such as seizures and altered consciousness, similar to those described for this dog, have been reported (10). Other neurological signs of CB can include ataxia, paresis, muscle tremor, anisocoria, and vestibular signs (10, 22, 30).

In Europe the cases of cerebral babesiosis were described in Hungary (two cases; [22]) and in Belgium (one case [32]). The first of these reports confirms the view about CB as a disease with poor prognosis. One of two dogs with cerebral babesiosis from Hungary showed epileptiform seizures. The other dog displayed ataxia and excitement, and finally it developed opisthotonus and coma. Both animals died despite treatment. In this report the course of the disease was fatal similar to the observed course in our patient.

It should be noted however, that the cerebral form of babesiosis does not always end with the death of the infected animal. Van de Maele et al. (32) described a case of this form of the disease in a 10-year-old Akita Inu dog, presented to a veterinary clinic with symptoms of apathy and fever. Hematological examination showed pancytopenia, and computed tomography revealed cortical atrophy and changes in the cerebellum. Nervous symptoms disappeared within 2 days after imidocarb application, but complete recovery lasted 5 months.

B. canis, the most common species of Babesia in dogs in Europe, has an intermediate pathogenicity (30). The severity of the disease depends on strain pathogenicity and the host’s immune response (26). Generally, early diagnosis and treatment of the disease may have contributed to a favorable outcome. Unfortunately in this case, the etiological factor of the disease was a virulent strain of B. canis, and the outcome of the infection was fatal. Cerebral babesiosis in dogs has not been diagnosed in Poland so far. This is the first report of canine cerebral babesiosis in our country. Diagnosis was based on the detection of piroplasms in Giemsa-stained blood smears and post mortem examination of the brain by histopathology and PCR method. Due to positive results of microscopic and molecular studies for Babesia canis the serology was not performed, although its results could have given more information about the time point of infection. The amplified segment of the 18S RNA gene was sequenced, what enabled to determine that the cause of the disease had been the strain 18S RNA-B EU622793. This is one of two B. canis strains found endemically in Poland, which reveals a greater virulence than 18S RNA-A EU622792 (1, 2, 5, 7). The detection of the protozoa in the brain of the dog and the fact that the blood concentration of glucose was only slightly below its reference value, indicate that the cause of the neurological symptoms experienced by the dog was not a metabolic disorder, but due to a hindered blood flow through the brain vessels. It could be caused by the accumulation of the parasites in the capillaries, sequestration of erythrocytes and stasis of blood flow. This diagnosis is also supported by the damaged endothelium of the small blood vessels, characteristic of the cerebral form of babesiosis, which was observed in the histopathological examination, as proposed by Schetters and Eling (26).

In humans, neuroimaging is routinely performed in patients with infectious and parasitic diseases such as cerebral malaria, neuroborreliosis, and Rocky Mountain spotted fever (9, 25, 31). Abnormalities seen on CT images, although mostly nonspecific, correlate well with illness severity and clinical outcome (9, 25, 26). The most frequent patterns encountered are no abnormalities, cerebral edema, thalamic and cerebellar hypopattenuation, and cerebral atrophy. According to the authors’ knowledge, brain CT findings for canine cerebral babesiosis have only been reported in one case report (32). This imaging technique was not available in the present case.

Since the hematological testing of the dog’s blood showed developing pancytopenia, other infectious disorders such as ehrlichiosis, parvovirus infection, canine hepatitis virus infection, septicaemia, and endotoxaemia had to be considered in the differential diagnostics. During chronic ehrlichiosis, pancytopenia is due to bone marrow hypoplasia (8). In this case, PCR test for Ehrlichia Anaplasmata was negative. Also the examinations of stool and urine (PCR) for the presence of genetic material of CPV-2 and CAV-1 gave negative results. History and diagnostic tests results did not support toxic or drug-related causes, or neoplasia, myelofibrosis, and myelophthisis as possible causes of pancytopenia. Additionally, based on the results of the IDEXX Snap-3Dx test, Lyme disease was excluded in this dog.

To complete the list of potential differentials of CB, canine distemper virus and Toxoplasma/Neospora infections should be considered. After obtaining positive results for piroplasmosis, given...
that the dog died, we abandoned the implementation of the tests for these infections.

Conflict of interest

The authors confirm that they do not have any conflict of interest.

References


Conclusion for practice

This case illustrates the fatal case of atypical babesiosis in a dog with neurological signs and pancytopenia. It suggests, that the symptoms from the central nervous system can be a new potential complication of canine babesiosis. This disease should be taken into account in dogs exhibiting neurological symptoms, especially during the tick activity season.