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CASE STUDY

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UNDESIRABLE IMMUNE REACTIONS IN THE COURSE OF BABESIOSIS IN A 10-YEAR-OLD BERNESE MOUNTAIN DOG

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ABSTRACT

Canine Babesiosis is a common tick-borne disease that occurs throughout the world and is a threat to dogs throughout the year. The current piece of work is a description of the aggressive course of tick-borne disease in an 11-year-old male Bernese Mountain Dog, which led to the death of the animal. Based on the clinical case analysis, possible consequences of this disease for the health and life of dogs were presented. Particular attention was paid to the risk of occurrence of autoimmune anemia and thrombocytopenia. This paper describes disease symptoms, diagnostic methods, course and results of the treatment used. In the case described above, the protozoan disease *B. canis* led to advanced changes in the blood system which disrupting hemostasis of the whole organism.

Key words: tick, canine, anemia, autoagression, transfusion, treatment

INTRODUCTION

Canine babesiosis is a parasitic disease caused by protozoa like *Babesia* spp. Invasion of this parasite occurs endemically all over the world. The most common species is *B. canis* and *B. gibsoni*. Parasites of this genus are primarily transmitted through tick bites and as such can infect variety of domestic and wild animals, as well as humans, however this disease is particularly dangerous for dogs. The infection occurs through the contact of the tick's saliva (final host) with the dog's blood (intermediate host). Intensive proliferation of protozoan occurs in red blood cells [Boozer and Macintire 2003]. The host's immune system recognizes the infected red blood cells as a foreign antigen and destroys them to eliminate the parasite. This process leads to the development of anemia. As a result of the infection an inflammatory reaction of the whole organism occurs whichin the first place manifests itself by high temperature, apathy, loss of appetite, pale mucous membranes and brown urine (hemoglobinuria).

When looking at the defensive reaction of the immune system, attention should be paid to both cellular and humoral responses. The first to react are G-type immunoglobulins. They opsonize infected erythrocytes which leads to cell disintegration and elimination of the parasite. Moreover, NK cells and splenic macrophages limit the intensity of invasion which in consequence reduces the severity of parasitemia [Homer et al. 2000]. Th1 lymphocites which synthesise IFN-γ also play an important role in the immune system response to the invasion of the parasite. They participates in the activation of macrophages and the induction of IgG antibody synthesis [Brandao et al. 2003]. As a result of cytotoxic reactions and as a consequence of the breakdown of red blood cells, an type II autoimmune hypersensitivity reaction occurs. It is characterized by the termination of the intermediate host's auto tolerance to its own antigens. Because of this the immune system develops an aggressive reaction which is directed against its own blood cells. Autoantigens recognized by G and M class immunoglobulins are glycophorins that are membrane proteins of erythrocytes. The sequence of autoaggressive actions leads to the development of extrinsic or intravascular hemolytic anemia [Lobetti 2000]. Spleen as an organ that receives damaged erythrocytes is enlarged, which is often one of the characteristic symptoms in the ultrasound

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examination of a patient with babesiosis. The prognosis for a patient with autoimmune haemolysis depends on the state of the immune system.

CASE REPORT

The dog described in the article is a Bernese Mountain Dog, an unneutered male aged 10 years and 7 months. So far the dog was healthy, untreated for any chronic diseases, regularly vaccinated and dewormed. Despite the regular use of repellents, the episode of infection with protozoan *Babesia canis* occurred in the past. For some time the dog became less vigorous and did not perform as much activity as before. He reluctantly got up from his seat and slept more. Appetite gradually decreased and the dog did not finish his food portions. There were no problems with gums or teeth. He drank a lot, he urinated and defecated properly. When outside, he showed an eagerness to play. All the above behavioral changes were interpreted by the owners as part of the natural aging process.

At some point, the reduced appetite was replaced by a complete reluctance to eat. The dog started to flee and hide when it was time for feeding. When outside, he was lying on the cool ground at all times and refused to return home (it is probable that the dog had higher temperature and was doing this to cool down). The dog stopped showing any interest in playing. He slept a lot and no loud noises or provocations from the owners and their children disturbed his sleep. The dog's urine became dark. He was being intensely sniffed by the other dog in the house. The above-mentioned situation lasted less than two days. The owners of the dog became worried and decided to bring the dog to the veterinary clinic.

RESULTS AND DISCUSSION

Day 1. When the dog was brought to the clinic he was very apathetic and in a rather serious condition. Physical examination was performed and paleness of mucous membranes as well as increased body temperature (39.6°C) was noticed. Body weight of the dog was 40 kg. Morphology and blood biochemistry tests were performed. There were significant deviations from the norm of values of all morphological parameters (Table 1) and the presence of *B. canis* protozoan in blood smear was discovered (Fig. 1). The result of biochemical test: ALT (Alanine transaminase) 0.6 μkat · l^{-1}, AST (aspartate transaminase) 0.8667 μkat · l^{-1}, urea 33 mg · dl^{-1}, creatinine 0.67 mg · dl^{-1}. The following treatment was applied: Dexametasone 0.15 mg · kg^{-1} bw s.c., Ascorbic acid 20 mg · kg^{-1} bw i.v., Amoxicilinum + Acidumclavulanicum (7 mg + 1.75 mg · kg^{-1} bw) s.c., Metamizolum 50 mg · kg^{-1} bw, Imidocarb 3.5 mg · kg^{-1} bw s.c., 1500 ml Solution Ringeri i.v. After receiving the medication, the dog returned home with the owners. A follow-up visit was recommended on the following day for further treatment. Having returned home the dog was sleepy, refused to eat his food, drank a lot and his urine changed to a brighter colour. The dog was encouraged by the owner to eat a treat, however he vomited afterwards.

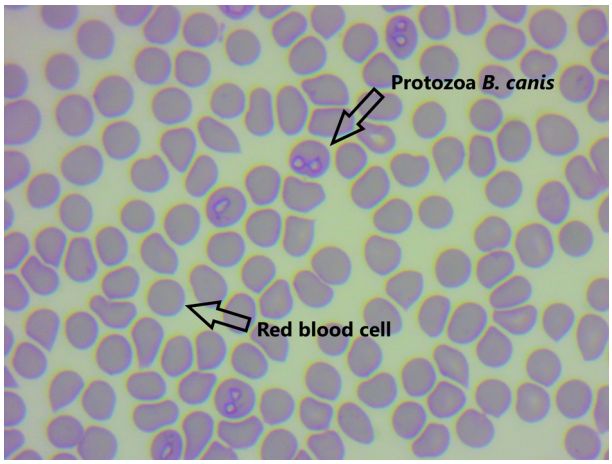

Fig.1. Blood smear on sick dog. Protozoa *B.canis* are visible inside the red blood cells (photo A. Garbiec)

Rys.1. Rozmaz krwi chorego psa. Pierwotniak *B. canis* widoczny wewnątrz krwinek czerwonych (fot. A. Garbiec)

Day 2. On the second day of the treatment the dog was in a slightly better condition than the previous day. During interactions he was wagging is tail. His body temperature was 38.5°C. The dog received the same treatment as on the previous day, however the dose of Solutio Ringeri was reduced to 1000 ml and an iron preparation with vitamins in a dose suitable for body weight was administered. After the treatment the dog returned home. In the evening the dog's condition deteriorated. The dog started having difficulties moving, he became to breath faster. The owners decided to take the dog for a consultation to another veterinary clinic. The condition of the animal was assessed as very severe, it was a careful prognosis. Morphology and blood biochemistry tests were performed (Table 1). Laboratory tests showed progressive deviations in the values of morphological parameters. No *Babesia canis* was found in the blood smear. Biochemical examination indicated increased ALT, 2.3 μkat · l^{-1}. An ultrasound examination of the abdomen was performed for the purpose of further diagnosis. A small amount of fluid was observed from the bladder. During the ultrasound examination a visible focal lesion (size 3.5×2.5 cm) which made the spleen more convex was discovered. The bottom part of the stomach wall had disturbed layering, mucous membranes up to 1 cm wide. Numerous consolidations and single oval changes of diameter up to 0.5 cm were found subpleural. No unusual fluid was found in the chest area. Treatment: Dexametasone 0.2 mg · kg^{-1}

Table 1. Results of laboratory tests – morphology

Tabela 1. Wyniki badań laboratoryjnych – morfologia

	1st day 1. dzień	2nd day 2. dzień	3rd day 3. dzień	4th day 4. dzień		5th day 5. dzień	6th day 6. dzień
RBC, $10^6 \cdot \mu l^{-1}$	1.76	1.25	2.18	1.47	1.32	2.13	1.65
HGB, mmol $\cdot$ l^{-1}	3.23	3.9	6.5	4.4	3.9	6	4.8
HCT, %	16.1	12.2	18.2	13.3	12.3	17.3	13.6
WBC, $10^3 \cdot \mu l^{-1}$	19.6	32.8	37.1	25	27.4	25.6	26.1
PLT, $10^3 \cdot \mu l^{-1}$	29	21	30	20	20	30	23

RBC – red blood cells, HGB – hemoglobin, HCT – hematocrit, WBC – white blood cells, PLT – thrombocytes.
RBC – czerwone krwinki, HGB – hemoglobina, HCT – hematokryt, WBC – białe krwinki, PLT – trombocyty

bw i.v., Cobalamin 0.15 mg $\cdot$ kg^{-1} bw i.v., hepatoprotective drug. Full blood transfusion was performed (Fig. 2). The donor was the second dog living in the home, less than eight-year-old female Labrador retriever. The blood transfusion was performed without any complications.

Day 3. The dog was in a better condition than the previous day. He came into the clinic independently and his mucous membranes were slightly pink. He still hadn't eaten, however he had been drinking a lot. A control morphology test was performed which showed that after the transfusion the parameters improved (Table 1). Treatment was continued as on the previous day, the dog was supplemented intravenously with the amino-acid preparation and another, increased dose of Amoxicilinum + Acidum clavulanicum antibiotic (10 mg + 2.5 mg $\cdot$ kg^{-1} bw s.c.) was administered. After returning home, the dog ate a handful of cooked chicken and rice. He did not vomit.

Day 4. The dog was brought to the clinic in the morning in order to continue the treatment and carry out control tests. Unfortunately, despite the treatment used so far, the levels of red blood cells and hemoglobin as well as hematocrit have dropped again (Table 1). As part of further diagnostics, the Vetexpert Caniv-4 test was performed and infection were excluded. Due to the lack of vomiting after eating the meal on the previous day, the dog received an oral steroid (Prednisone 0.5 mg $\cdot$ kg^{-1} bw). The antibiotic was changed to Doxycylinum at a dose of 2.5 mg $\cdot$ kg^{-1} bw p.o. An attempt of treatment with the oral antibiotic was unsuccessful due to vomiting. In connection with the above, an anti-emetic drug Ondansetron was administered at a dose of 0.2 mg $\cdot$ kg^{-1} bw s.c. and Dexametasone 0.2 mg $\cdot$ kg^{-1} bw s.c. The whole day the dog was sleepy, reluctant to stand up or go outside.

In the evening, the condition of the dog worsened significantly. There was a slight shortness of breath which was intensified when the dog was lying on the side. Furthermore, neurological symptoms occurred, which were slight left-sided paresis, slight problems with consciousness and great difficulty moving. The dog was transported to the clinic and the control morphology test showed a progressive decrease in the value of erythrocytes, hemoglobin and hematocrit. Dexametasonum was administered at a dose of 1 mg/kg bw i.v. Due to the condition of the dog a decision was made to perform another full blood transfusion- the donor was a male Great Dane dog. One unit of fresh blood was transfused without complications. The dog returned home at night, he vomited in the morning.

Day 5. Control visit at the veterinary clinic. Dog weakened, showed reluctance and difficulty in moving. Neurological symptoms and dyspnea have subsided. Control morphology test showed the improvement of parameters in response to the transfusion. The medication that was administered were: Dexametasonum (dose of 1 mg $\cdot$ kg^{-1} bw i.v.), amino-acid preparation with glucose i.v. Due to the failure of oral antibiotic therapy, it was decided to use Amoxicilinum + Acidumclavulanicum (10 mg + 2.5 mg $\cdot$ kg^{-1} bw) s.c again. Antiemetic therapy with Ondansetron 0.2 mg $\cdot$ kg^{-1} bw s.c. was continued. Due to the use of high doses of steroid drugs, Ranitidine was introduced to the treatment at a dose of 1.25 mg $\cdot$ kg^{-1} bw s.c. An interview was conducted with the owners regarding the patient's state of health, the possibility of continuing treatment and indications for discontinuing the treatment.

Day 6. The condition of the dog worsened again. The laboratory tests showed another drop in red blood cell parameters and hematocrit. Due to poor prognosis, the owners decided to stop the persistent treatment. Euthanasia was carried out for medical and humanitarian reasons. Xylazinum 2.5 mg $\cdot$ kg^{-1} bw and Pentobarbitalum 120 mg $\cdot$ kg^{-1} bw i.v. were used. The dog's body was subjected to individual cremation.

DISCUSSION

Described tick-borne disease causes a number of changes in homestasis of the whole organism, the first abnormalities can be seen in blood counts where a number of

pathological changes occur, i.e. anemia or thrombocytopenia [Fabisiak et al. 2010]. Repeated illness of Canine babesiosis carries an increased risk of damage to internal organs, especially of the liver and kidneys [Petra et al. 2018]. The disease progression may be more severe and have more extensive consequences in older dogs, due to lower regenerative capacity of organs [Köster et al. 2015]. In young animals, the first symptoms of the disease, such as apathy, decline in physical activity or lack of appetite are quickly noticed by the owners. On the other hand, in older animals, the same symptoms are often mistaken for decline in health related to the old age [Matijatko et al. 2010].

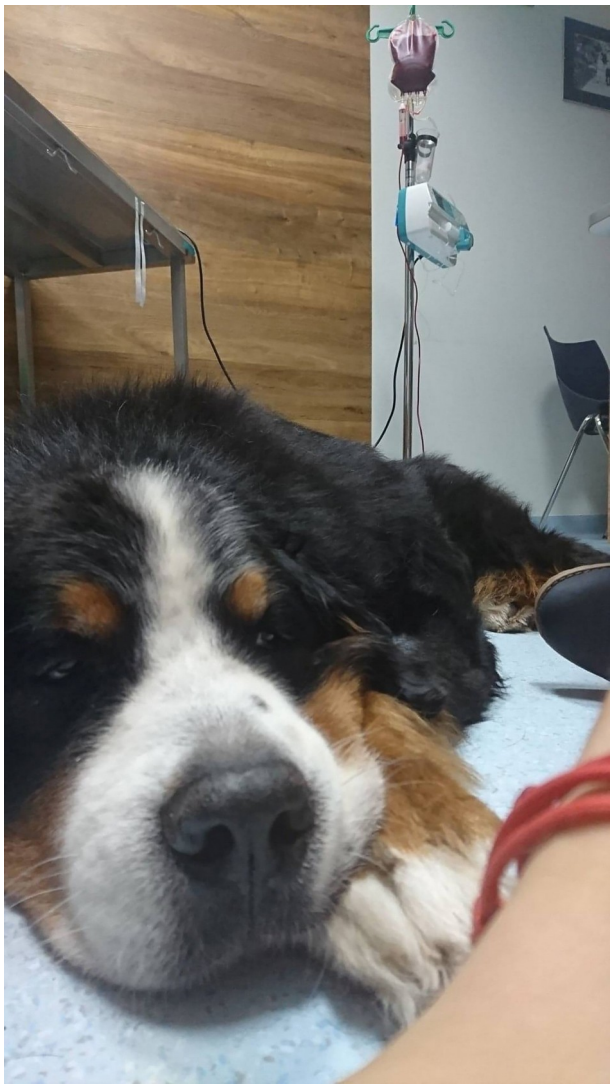

Fig. 2. The dog just before blood transfusion (photo J. Wojtaś)

Rys.1. Pies przed zabiegiem transfuzji krwi (fot. J. Wojtaś)

The conducted research shows that severe, irreversible damage to the parenchymal organs in the course of babesiosis occurs in older animals or those who have been re-infected with protozoan B. canis [Defauw et al. 2012]. It should be taken into account that the dog described above was regularly protected against ticks, and yet it was his second episode of developing this disease. According to scientific sources, they may be associated with climate change, immunization of protozoa against available preventive measures or individual animal propensities [Adaszek and Winiarczyk 2008]. Protozoan resistance to commonly used treatment has also been described, this resistance leads to difficulties in treating tick-borne disease and creates a need to use available alternative methods [Adaszek et al. 2017]. The clinical form of the disease is more common in dogs of this breed, and the level of antibodies in the Bernese serum is higher than in other breeds, which may suggest their hereditary tendency and increased sensitivity to tick-borne diseases [Nielsen et al. 2010]. Growing disorders in the blood system associated with the development of an adverse immune response are the most common cause of failure in the treatment of babesiosis in dogs [Zamokas et al. 2014]. Therefore most likely advanced anemia caused by autoimmunity of the immune system and advanced age of the animal were the main cause of therapeutic failure of the described case.

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NIEPOŻĄDANE REAKCJE IMMUNOLOGICZNE W PRZEBIEGU BABESZJOZY U 10-LETNIEGO BERNEŃSKIEGO PSA PASTERSKIEGO

STRESZCZENIE

Babeszjoza psów jest powszechną chorobą przenoszoną przez kleszcze, która występuje na całym świecie i stanowi zagrożenie dla psów przez cały rok. Niniejsza praca jest opisem agresywnego przebiegu choroby przenoszonej przez kleszcze u 11-letniego samca berneńskiego psa pasterskiego, co doprowadziło do śmierci zwierzęcia. Na podstawie analizy przypadku przedstawiono możliwe konsekwencje tej choroby dla zdrowia i życia psów. Szczególną uwagę zwrócono na ryzyko wystąpienia niedokrwistości autoimmunologicznej i małopłytkowości. W pracy opisano objawy choroby, metody diagnostyczne, przebieg i wyniki zastosowanego leczenia. W opisywanym powyżej przypadku choroba przenoszona przez pierwotniaka *B. canis* doprowadziła do zaawansowanych zmian w układzie krwionośnym, zaburzając hemostazę całego organizmu.

Słowa kluczowe: kleszcz, pies, anemia, autoagresja, transfuzja, leczenie

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