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CANINE BABESIOSIS AND THERAPY OPTIONS – A REVIEW

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ABSTRACT

Babesiosis is a disease caused by intraerythrocytic protozoal parasites, which occurs in animals and humans. In dogs, babesiosis can be caused by eight species of *Babesia* gene: i.e., *B. canis*, *B. rossi*, *B. vogeli*, *B. coco*, *B. gibsoni*, *B. conradae*, *B.*, and *B. negevi*, which are bound to certain geographical areas. The disease has a focal nature and its transmission depends mainly on vectors, which are ticks of various species. Due to transstadial, and transovarial transmission, babesiosis is able to persist in natural foci in several generations of ticks, even without the presence of a susceptible host. Typical clinical signs associated with canine babesiosis are: fever, apathy, weakness, pale mucous membranes, icterus and hemoglobinuria. The disease can have an acute or peracute course, and subclinical and subacute infections have also been described. The clinical manifestations of babesiosis may vary depending on the particular species and strains, and their specific virulence, but also depending on factors that determine the host's response to infection, such as age, individual immune status, and the presence of concurrent infections or other diseases. Medicines, from the group of antiprotozoans, a selected group of antibiotics, or their combinations are used for therapy. There are differences in the therapy of babesiosis depending on the *Babesia* species, the animal is often cured of the acute phase, but the parasite remains in the organism.

Key words: antiprotozoan; *Babesia;* dog; occurrence; therapy

INTRODUCTION

Babesiosis is a focal transmissible parasitosis with stationary occurrence. Canine babesiosis is a tick-borne disease caused by protozoan intraerythrocyte parasites of the genus *Babesia*. During the life cycle of the pathogen, ticks are the definitive hosts (zygote formation takes place in the tick's intestine), and dogs are intermediate hosts, (erythrocytes contain mainly pear-shaped, less round, oval or amoebic babesia, and asexual division of red blood cells takes place inside) [1, 31].

The incubation period of the disease is 8–20 days. In the blood – in erytrocytes, *Babesia* reproduce asexually. After division, the individuals remain in a typical position at an acute or obtuse angle, the host cell bursts, and the released parasites attack other erythrocytes. After sucking infected blood, sexual reproduction of the parasite takes place in the intestine of the tick. Then schizogony occurs with the formation of mobile vermiculae that reach various organs, such as especially the ovaries, where division

is repeated to form thousands of vermiculae. The whole process takes an average of 7 days. In the tick's ovaries, vermicelli penetrate the tick's eggs and further develop into a new generation of ticks (transovarial transmission). Further reproduction takes place in newly hatched tick larvae. Vermicelli collect in the salivary glands and are inoculated into the blood of a susceptible host during sucking by the tick larvae. After sucking blood, the tick larvae are released from the host, undergo a transformation in the soil to a higher stage, called a nymph, while the transstadial transfer of *Babesia* occurs, which is also repeated during the transformation into adults [1, 8].

Currently, eight species of the genus *Babesia* are recognized, which cause infection in dogs. *Babesia* occurring in dogs are divided into two groups, namely large species. *B. canis, B. rossi, B. vogeli,* and *B. coco* – their developmental stages, such as trophozoites and merozoites, are larger than these stages in the second group, i.e., the so-called small species, which include *B. gibsoni, B. conradae* and *B. vulpes* [19]. The developmental stages of *B. negevi*, which were described in 2020, are smaller than large babesia but larger than small [2].

Until recently, *B. canis, B. rossi* and *B. vogeli* were considered one species, *B. canis*. Based on DNA sequencing, they were further specified and divided into *B. canis, B. vogeli* and *B. rossi*. Large canine species of *Babesia* cannot be distinguished by light microscopy through blood smear examination. To accurately identify the species, molecular-biological examinations are necessary, e.g. polymerase chain reaction (PCR). Serological testing is also available, but this can sometimes be misleading, e.g. previous infection [2].

MANIFESTATION OF THE DISEASE

Babesiosis is considered an immune-mediated disease characterized by high cytokine production and tissue hypoxia in various organs. The main clinical symptoms: are fever, apathy, weakness, anorexia, pale mucous membranes and general poor health of the patient. All *Babesia* species can cause: enlarged lymph nodes and spleen, anemia, thrombocytopenia, jaundice and hemoglobinuria. The disease can lead to: coagulopathy, kidney damage, hepatopathy, pancreatitis, cardiac disorders, pulmonary edema, cerebral complications, endocrine disorders, systemic inflammatory response syndrome, multiple organ dysfunction syndrome, and septic shock in infected dogs. In all *Babesia* species, anemia is caused by a combination of intravascular , and extravascular hemolysis resulting from parasite damage and red blood cell breakdown, increased osmotic cell fragility, and the activity of secondary immune-mediated processes. Depending on the *Babesia* species, and the course of the infection, the anemia can be regenerative or non-regenerative, which is more typical of *B. canis* infection. Thrombocytopenia can occur to varying degrees, but the presence of petechiae or ecchymoses is less common. Thrombocytopenia, when present, varies from mild to severe, as does anemia. Other abnormalities that can be detected include hypoalbuminemia and hyperbilirubinemia [7, 10, 20, 21, 27, 28, 37, 43, 44, 45].

Depending on the type of parasite and the immune status of the host, the infection can lead to a mild, moderate, or severe form of the disease. The species *B. rossi* causes the most severe form of the disease in domestic dogs. Infections caused by *B. canis* are usually milder than those caused by *B. rossi*, but both pathogens cause acute babesiosis. *B. vogeli* infection in adult dogs can be mild, or even subclinical, but can cause severe anemia in young dogs [19]. The pathogenicity of *B.coco* is unknown, mainly due to the low number of detected cases and published studies. Disease caused by this species has been recognized in dogs after splenectomy, or in dogs after chemotherapy. It is believed that infection leads to the development of the disease mainly in immunosuppressed dogs, but the possibility of the existence of subclinical asymptomatic cases is not excluded [4, 6].

CANINE *BABESIA* SPECIES

B. canis is typically found mainly in Europe. The main vector of *B. canis* is *Dermacentor reticulatus*, which also occurs in Slovakia. *B. canis* infection rates in *D. reticulatus* ticks collected from vegetation or animals have been reported to range up to more than 82 %, depending on the site [35]. The role of another species of tick, *Ixodes ricinus*, is not yet entirely clear. *B. canis* DNA was detected in larvae, nymphs, and adult male and female *I. ricinus* ticks, demonstrating that *B. canis* can be transmitted transovarially and maintained transstadially in this tick species. *I. ricinus* is thought to play a role in the transmission of *B.*

canis to dogs, but the role of *I. ricinus* as a vector of *B. canis* has not yet been proven. *B. canis* DNA was also detected in other species of ticks, e.g. *Ripicephalus sanguineus*, *Ixodes hexagonus* and *Dermacentor marginatus* [24].

B. rossi is a typical species for the African continent transmitted by ticks of the genus *Haemaphysalis*. South Africa and Nigeria (West Africa) are countries where *B. rossi* is endemic in dogs. However, infections have also been detected in dogs in other sub-Saharan African countries (Uganda, Angola, Malawi, Zambia, Kenya and Sudan). Isolated cases have been reported in North America (USA), South America (Brazil), Asia (Singapore), and Europe (France, Germany, Switzerland, Romania, and Russia) [22, 34, 43].

B. vogeli is mainly transmitted by ticks of *R. sanguineus*, whose various varieties (including at least 17 species designated as *R. sanguineus sensu lato*) are distributed throughout the world. *R. sanguineus* sl also occurs in the southern parts of Europe, but it has already been identified in the United Kingdom, Iceland, Poland and northern Germany (Berlin), which means the risk that *B. vogeli* will gradually spread throughout Europe. *B. vogeli* is also transmitted by other ticks, e.g. *R. linnaei* and DNA has been captured in the tick genera *Dermacentor*, *Haemaphysalis* and *Ixodes* [16, 43].

The first case of babesiosis in dogs caused by an unnamed large species of *Babesia* sometimes referred to as *Babesia* sp. "*Coco*" was discovered in the United States (North Carolina) in May 2002, the name was derived from the dog's name, "*Coco*", and some authors use it as an official designation. *B. coco* infection primarily occurs in the eastern and southeastern United States [4, 6].

In addition to ticks, *B. gibsoni* can also be transmitted to a dog by being bitten by another dog or canine animal, by direct contact between dogs through wounds, by ingestion of saliva or blood. Most of the dogs infected by bites have been reported in the US, Australia and Europe and have been pit bull terriers. The small species *B. conradae* or *B. vulpes* are also transmitted by bites between individuals [36]. *B. gibsoni* is considered quite resistant to available active substances, its complete eradication from the body is very complicated and relapses are frequent [23, 26].

B. conradae is the most common species in domestic dogs in California and presents as severe hemolytic anemia and thrombocytopenia, which can be fatal without treatment. The parasite was classified in 2006 based on the analysis of the 18 S rRNA gene. Coyotes (*Canis latrans*) are believed to be reservoirs of *B. conradae*, and in a survey in Los Angeles County, 3 of 29 coyotes were seropositive for antibodies to this pathogen [6, 16].

The species *B. vulpes* is associated with babesiosis in European dogs. The tick vector or other vectors of *B. vulpes* transmission have not been definitively identified. In the USA, a high prevalence of *B. vulpes* was found in American Staffordshire Terrier and Pit Bull Terrier dogs rescued from dog fighting. This is similar to transmission of *B. gibsoni* in pit bull terriers, where the primary routes of infection are thought to be direct dog-to-dog transmission via bite wounds and potentially transplacental transmission [3].

Babesiosis caused by *B. negevi* was first described in 2020 in five dogs [2]. Few cases of *B. negevi* have been detected so far, which may be due to inaccurate diagnosis of the specific species. In 2021, they confirmed two more cases in Jordan [9], and later three cases, in a female and her two puppies in Israel [33].

Babesiosis caused by large species of *Babesia* does not occur after a bite from an infected dog and is dependent on the geographic distribution of the main vectors. With gradual warming, tick vectors also reach colder areas, where they did not occur before. However, the occurrence of certain species of *Babesia* outside their foci within continents, even intercontinentally, is increasingly recorded, also due to the transport of dogs [6, 36, 43].

Infections in dogs caused by both small and large species can also be transmitted vertically (from mother to offspring), or by blood transfusion (merozoites circulating in the blood). *Babesia* are capable of transstadial and transovarial transmission, thanks to which several generations of ticks are infected, and babesiosis is maintained in natural outbreaks even in the absence of a susceptible host [6, 36, 43, 44].

Canine *Babesia* species are not considered to be anthropozoonoses and do not pose a health risk to humans. In humans, babesiosis caused by animal *Babesia* species is a rare disease and is primarily caused by variants of four species: *B. divergens*, which causes babesiosis in cattle in Europe; *B. microti*, which is a pathogen of babesiosis in small rodents in the United States; *B. duncani* most often caught in Canada and in the United States and B. vena-

torum causing bovine babesiosis [12, 38, 42]. Infections may have a milder course but may lead to severe clinical disease, especially in immunocompromised patients (undergoing chemotherapy, splenectomy or human immunodeficiency virus positive) [38, 39, 42].

TREATMENT OF BABESIOSIS

Antiprotozoal treatment

A limited number of drugs and their combinations are effective against babesiosis in dogs. The mechanisms of their action against babesiosis are largely unknown or not studied in detail. Some drug combinations have an apparent positive effect in clinical trials, but the mechanism of their action is not clear. A very important factor in treatment is the dog itself, because infected dogs can react differently to medication. During treatment, it is necessary to take into account the individual clinical condition of the affected dog, its age and immune status [36]. Despite several options for antiprotozoal treatment of babesiosis in dogs in the world, suitable drugs are often not registered or approved for the specific indication of use against babesiosis in individual countries. Between large and small *Babesia* spp. there are clear differences in drug sensitivity both within groups, and between species. The treatment of small species of babesiosis is complicated, as they often cannot be eliminated despite treatment, so treated dogs can remain chronic carriers capable of further transmitting the infection and relapsing with the clinical disease. Treated dogs should be followed and monitored for persistent infection and parasitemia by blood smear and PCR. Symptomatic supportive treatment is a matter of course in the treatment of babesiosis. It is ideal to avoid the risk of transmission of babesiosis by prevention in the form of antiparasitic protection of dogs against ticks [31].

The following were evaluated as effective against babesiosis: imidocarb dipropionate, diminazene aceturate, the combination of atovaquone, and azithromycin, and the combination of buparvachon and azithromycin. Among other drugs, phenamidine, pentamidine, parvaquone, artemisinin derivatives and antibiotics with certain antiprotozoal activity, such as e.g. doxycycline, minocycline, clindamycin, enrofloxacin and metronidazole, which have an uncertain therapeutic effect [6, 36, 43].

Imidocarb dipropionate

Antiprotozoan imidocarb dipropionate is most often used in the therapy of parasitemia caused by the species *B. canis*, but, also by other large species of *Babesia.* In the treatment of small species of *Babesia* spp. there are frequent cases of complete elimination of parasites from the body, or relapse of babesiosis is possible. Imidocarb dipropionate is an aromatic diamidine (substituted carbanilide) for which several mechanisms of action against babesiosis have been proposed. Most likely, it is damage to nucleic acids and inhibition of cell repair and replication, or the effect is directed directly at the parasite, at the cell nucleus, the composition of the cytoplasm (vacuolization), or it induces hypoglycemia in the protozoa and inhibits the division of its single cell [31].

In Slovakia, the therapeutic dose is approved as a single dose of 2.125–4.25 mg imidocarb.kg^{-1} subcutaneously, in practice, a second dose is usually used after two weeks. In the USA, the recommended dose for babesiosis is 6.6 mg.kg^{-1} intramuscularly or subcutaneously with a repeat dose in 2 weeks. During treatment, parasympathetic symptoms may appear in some animals: salivation, vomiting, general weakness, tremors, muscle spasms. The effects can be mitigated by premedication with atropine at a dose of 0.05 mg.kg^{-1}. Other less common effects are: dyspnoea, restlessness, diarrhea, renal tubular or hepatic necrosis, and inflammation at the injection site, less commonly ulceration, which usually heals within days to weeks. Death due to anaphylactic reaction has also been reported. Excretion of the drug is ensured by the kidneys and liver, and it is excreted in the urine and feces [14, 31].

Diminazene aceturate

Diminazene aceturate is also an aromatic diamidine. The mechanism of its action against *Babesia* is not well understood and is thought to interfere with parasite DNA synthesis and aerobic glycolysis [31]. Diminazene aceturate is most often used for the treatment of babesiosis caused by *B. rossi* in dogs in South Africa [30]. The dose for babesiosis in dogs is 3.5 mg.kg^{-1} intramuscularly once. The effectiveness of the treatment is variable and it often happens that the drug has a greater effect against large forms of *Babesia* spp. A major disadvantage is the possibility of toxic side effects with severe neurological symptoms that can be unpredictable even at therapeutic doses. Diminazene is excreted in the urine and faeces.

Undesirable effects of diminazene aceturate treatment in dogs include: gastrointestinal disturbances (vomiting and diarrhea), pain and inflammation at the injection site and a transient decrease in blood pressure. Sometimes there are neurological symptoms in the form of ataxia and seizures. The death was also recorded [31, 40]. There have been described cases of the development of resistance to diminazene aceturate and therefore a combination treatment of diminazene with imidocarb dipropionate has been tried. The obtained results indicate the effectiveness of drug combinations to inhibit *B. microti* in mice, future studies are required to determine the clearance of this combination from products of the treated animals [13, 32, 40].

Atovaquone and azithromycin

The combination of atovaquone and azithromycin is used in the treatment of small species of *Babesia*: *B. gibsoni, B. conradae, B. vulpes* and *B. negevi.* Atovaquone (hydroxy-1,4-naphthoquinone) selectively blocks protozoal mitochondrial electron transport causing inhibition of pyrimidine and ATP (adenosine triphosphate) synthesis. In Japan and Taiwan, resistance to atovaquone has been detected in dogs with *B. gibsoni* parasitemia. The resistance was found to be caused by a mutation in the cytochrome b-M121I gene. In addition to inhibiting bacterial protein synthesis, azithromycin (macrolide antibiotic) also acts on apicoplasts (non-photosynthetic plastid organelles in which important metabolic processes take place) in *Babesia* [4, 5, 15, 31].

The dose of atovaquone is 13.3 $mg.kg^{-1}$ *per os* every 8 hours in combination with azithromycin at a dose of 10 $mg.kg^{-1}$ *per os* once a day for 10 days for the treatment of the mentioned three species of *Babesia: B. gibsoni, B. conradae* and *B. vulpes* [36]. In the case of babesiosis caused by *B. negevi*, animals treated with imidocarb dipropionate were indeed cured of the acute phase of the disease, but to completely eliminate the parasite from the blood (*B. negevi* remained present for seven months during treatment with imidocarb dipropionate) it is necessary to use atovaquone and azithromycin [33]. It is recommended to check the success of the treatment using PCR after 60 and 90 days. It is advisable to find out whether the treatment led to a permanent suppression or elimination of the organisms, and whether there was only a temporary suppression of parasitemia [18].

There are currently no known adverse effects of atovaquone in dogs. A combination of atovaquone and azithromycin is also used to treat *Cytauxzoon felis* piroplasma infection in cats. A tablet containing atovaquone and proguanil HCl is available on the pharmaceutical market for the treatment of human malaria, however, this combination has been reported to cause gastrointestinal side effects in dogs due to the proguanil component and should be avoided [5, 14, 29]. Some authors prefer a three-drug combination such as clindamycin, imidocarb dipropionate, and diminazene acetate to treat *B. gibsoni* instead of atovaquone and azithromycin [25].

Buparvachon and azithromycin

Buparvaquone is a lipophilic hydroxynaphthoquinone compound, a drug similar to atovaquone. It is used for the treatment of *B. vulpes* infection in dogs at an off-label dose (prescribed for an indication other than that approved by the competent authorities) of 5 $mg.kg^{-1}$ intramuscularly twice 48 hours apart in combination with azithromycin in a dose of 10 $mg.kg^{-1}$ per os once a day for 10 days [14]. In a study comparing the success rate of babesiosis treatment, 96 naturally infected dogs were monitored. Imidocarb dipropionate, atovaquone with azithromycin and buparvaquone with azithromycin were used for treatment. All three treatment regimens were well tolerated and safe and buparvaquone and azithromycin protocol was the only one not showing any adverse events in treated dogs [14].

NEW THERAPEUTICAL OPTIONS TESTED

DMQDAs

Due to the complicated treatment of babesiosis not only in dogs, but also in other animals and especially in humans, new therapeutic options are still being sought against *Babesia* spp. Anti-babesial activity of a series of 6,7-dimethoxyquinazoline-2,4-diamines (DMQDAs) were tested against the growth of *B. gibsoni,* to in vitro screening were included 20 compounds, 14 of them exhibited over 90 % inhibitory effects. *In vivo* testing of selected six compounds against the non-lethal *B. microti* revealed that one promising candidate - SHG02 had superior anti-babesial activity. Also evaluation in a mouse model indicated the excellent effectivity of SHG02 on the lethal *B. rodhaini* infection [17].

Essential oils

Due to the problematic treatment of babesiosis, it is necessary to search for new therapeutic options. Available antiprotozoals and some antibiotics have limited effect in some cases or complete resistance of the parasite is present. The testing of plant-based substances, which appear to be very promising for the future in treatment, is coming to the fore. Many plant essential oils and their ingredients are a source of biologically active molecules with various mechanisms of action against bacteria, viruses, fungi and parasites, which could also be used in the case of *Babesia.* A 2020 study investigated the *in vitro* anti-*Babesia canis* activity of nine essential oils. Essential oils from *Achillea millefolium, Eugenia caryophyllus* and *Citrus grandis* were evaluated as the most active (IC_{50} values 51.0, 60.3 and 61.3 $\mu g.ml^{-1}$) [11]. Zhang et al. monitored the inhibitory activity against *B. duncani* in the hamster red blood cell culture model of up to 97 essential oils, of which they noted 10 essential oils (garlic, black pepper, tarragon, palo santo, coconut, pine, meditation, cajeput, moringa, and stress relief) that showed a good effect. Available results suggest that essential oils may be helpful in the treatment of babesiosis [41].

CONCLUSIONS

Babesiosis is a protozoan disease that can be caused by up to 8 *Babesia* species in dogs. Currently, in dogs are recognized large species: *B. canis*, *B. rossi*, *B. vogeli* and *B. coco*; small species :*B. gibsoni*, *B. conradae*, *B. vulpes* and *B. negevi*, which is dimensionally at the interface of both groups. In the clinic, the diagnosis of babesiosis is possible using a blood smear, but the determination of the exact species is possible only in reference laboratories that use molecular-biological methods. The disease can have an acute or peracute course, and subclinical and subacute infections have also been described. The clinical manifestations of babesiosis may vary depending on the particular species and strains and their specific virulence, but also depending on factors that determine the host's response to infection, such as age, individual immune status, and the presence of concurrent infections or other diseases. The disease takes the form of hemolytic anaemia with destruction of erythrocytes and a systemic inflammatory response that can lead to organ dysfunction. One of the pathognomic signs of babesiosis: is high fever and lethargy, dogs show clinical signs of anemia, liver, lung, kidney or brain dysfunction, and hemostatic abnormalities including coagulation and electrolyte imbalance. Medicines from the group of antiprotozoans, a selected group of antibiotics or their combinations are used for therapy. There are differences in the therapy of babesiosis depending on the *Babesia* species, the animal is often cured of the acute phase, but the parasite remains in the organism. The prevention of babesiosis consists only in the prevention of being attacked by the tick that transmits this disease to dogs. Antiparasitics are used most often in the form of spot on (e.g. fipronil, selamectin, imidacloprid with moxidectin), collars (e.g. deltamethrinum, imidacloprid with flumethrin) and pills (fluralaner, sarolaner, afoxolaner).

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