

ANTIBABESIAL TREATMENT PROTOCOLS AGAINST CANINE BABESIOSIS

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SUMMARY: Canine babesiosis is an important tick-borne disease caused by hemoprotozoan parasites of the genus *Babesia*, and the predominant species infecting dogs is *Babesia gibsoni*. The disease has been identified worldwide and is now recognized as a serious emergent disease in dogs. Although the incidence of canine babesiosis has been increasing, effective and crucial therapeutic modality is unavailable. The conventional antibabesial drugs including diminazene aceturate, pentamidine, and imidocarb dipropionate could not eliminate the parasites from infected dogs. Newer therapeutic modality for canine babesiosis using drugs such as clindamycin, atovaquone and multiple drug combinations has been suggested. Although these therapeutic modalities are beneficial and seem to partially provide a permanent cure, the treatments failures often occur. Thus, broad understanding of treatment protocols are required when implementing an appropriate treatment plan against canine babesiosis. The goal of this review is to provide veterinary practitioners with guidelines for successful treatment against canine babesiosis. It is expected that the review will answer the most frequently asked questions posed by veterinary practitioners.

BACKGROUND

Canine babesiosis is an important tick-borne disease caused by hemoprotozoan parasites of the genus *Babesia*, and the predominant species infecting dogs is *Babesia gibsoni* (Bandula *et al.*, 2012a, 2012b, Yamasaki *et al.*, 2002, 2003, 2007, 2011). The disease has been identified worldwide and is now recognized as a serious emergent disease in dogs (Birkenheuer *et al.*, 1999, Matsuu *et al.*, 2006). *B. gibsoni* in particular, has been reported to occur endemically in Asia, Africa, Europe, North America and Australia (Collett, 2000, Matsuu *et al.*, 2008). Many drugs have been utilized for the treatment of canine babesiosis, such as diminazene aceturate (Berenil), imidocarb dipropionate (Imizol), phenamidine isethionate, pentamidine isethionate, trypan blue, primaquone and quinuronium sulfate (Bandula *et al.*, 2012a, 2012b, Birkenheuer *et al.*, 2003, Matsuu *et al.*, 2008, Sakuma *et al.*, 2009); however, none of these drugs have been proven to be effective for the elimination of *B. gibsoni* organisms from infected dogs (Bandula *et al.*, 2012a, 2012b, Bielawski *et al.*, 2000). Some antibabesial drugs can reduce the severity of clinical signs and the mortality associated with the disease. Treatment or spontaneous recovery from an acute infection frequently fails to clear the organism from the host, resulting in a carrier stage (Wulansari *et al.*, 2003).

CONVENTIONAL ANTIBABESIAL DRUGS USED AGAINST CANINE BABESIOSIS

Diminazene aceturate and aromatic pentamidine analogues, such as DB75 ([2, 5-bis (4-amidinophenyl) furan]) and DB820 (6-[5-(4-amidinophenyl)-furan-2-yl] nicotinamidine) have been used as therapeutic agents for many parasite diseases for several decades. Currently, the most common conventional antibabesial drug used in *B. gibsoni* infection is diminazene aceturate. A single intramuscular injection of diminazene aceturate at a dose of 3.5 mg/kg (Breitschwerdt *et al.*, 1990) to 5 mg/kg (Birkenheuer *et al.*, 1999) has demonstrated efficacy against *B. gibsoni* infection (Wulansari *et al.*, 2003). In certain cases, diminazene aceturate needs to be repeated at 5 day intervals until clinical signs subside and parasites disappear from peripheral blood. However, it has been recommended that diminazene at 4.2 mg/kg intramuscular dose should not be repeated within a 21-days period as plasma concentrations remained even at 21 days (Miller *et al.* 2005). In general, diamidine analogues interact with the minor groove of the DNA double helix containing high adenine (A) + thiamine (T) content (Basselin *et al.*, 1998). This interaction would interfere with the transcription, replication and stabilization of DNA, leading to death of the parasites (Basselin *et al.*, 1998, Bell *et al.*, 1991, Coates *et al.*, 2002, Lane *et al.*, 1991, Mathis *et al.*, 2007, Purfield *et al.*, 2009). On the other hand, diminazene aceturate has a low therapeutic index, thus potentially dangerous and shows a propensity to develop

severe cerebral toxicity with classic cerebellar sulci haemorrhages particularly in Alaskan malamute (Donghyun *et al.*,2014). Furthermore, Dachshund, Golden Retriever, Labrador, Doberman and Rottweiler are more susceptible to diminazene aceturate toxicity. The clinical signs associated with diminazene toxicity are depression or stupor, continuous vocalization, ataxia, opisthotonos, extensor rigidity, nystagmus and seizures. It often causes fatal nervous complication after 24 - 48 hours of overdose (Milner *et al.*,1997). Although higher dose, repeated doses and intravenous administration of diminazene can induce toxicity more easily, toxicity can occur by lower dose, single dose and intravenous administration in dogs (Donghyun *et al.*,2014).

Although diminazene aceturate has been used as a first-line agent for the treatment of *B. gibsoni* infection in dogs (Sakuma *et al.*,2009), it cannot completely eliminate *B. gibsoni* from infected dogs, and relapses often occur (Farwell *et al.*,1982, Hwang *et al.*,2010a, Matsuu *et al.*,2008, Wulansari *et al.*,2003). It is reported that one of the reasons for the relapse of canine babesiosis might be the development of diminazene aceturate resistance in *Babesia* parasites (Collett, 2000, Matsuu *et al.*,2006, Sakuma *et al.*,2009). Diamidine resistance has been well described in *Trypanosoma* (Teka *et al.*,2011) and *Leishmania* species (Serenio *et al.*,1997). It is reported that diamidine is a substrate of P2 nucleoside transporters operative in *T. brucei* (Carter *et al.*,1995, Teka *et al.*,2011). The loss of that transporter and high-affinity pentamidine transporter functions in African trypanosomes has been partially implicated in resistance to diamidine (Carter *et al.*,1995, Teka *et al.*,2011). In addition, numerous dehydrogenases and F1F0-ATPase in mitochondria are decreased in diamidine-resistant parasites (Basselin *et al.*,2002, Mukherjee *et al.*,2006, Soto *et al.*,2009). However, the mechanisms of diamidine resistance in *Babesia* are less known and thus need to be investigated.

Imidocarb dipropionate (Imizol) is another conventional therapy for canine babesiosis. It is given at 5 - 6 mg/ kg by subcutaneous or intramuscular injection (Abdullah *et al.*,1984, Uilenberg *et al.*,1981). Very often two doses of imidocarb are given 2 weeks apart. The deep muscular injection is much safer because the drug causes severe inflammation (Abdullah *et al.*,1984). Imidocarb reduces morbidity and mortality of the parasite but ineffective for clearance of *B. gibsoni*. Some dogs treated by this drug recover very rapidly but others do not. Those that do recover may become chronic carriers that transmit the disease to other dogs. There are adverse effects of imidocarb such as difficulty in breathing, tachycardia, weakness and profuse diarrhea, pulmonary congestion, oedema, splenomegaly,

hepatomegaly and renomegaly (Abdullah *et al.*,1984). They suggested that these adverse effects of imidocarb may be due to excessive acetylcholine action (Abdullah *et al.*,1984). Nevertheless, imidocarb is generally considered as a safe drug for canine babesiosis especially for puppies and geriatric patients. In addition, when dogs are simultaneously infected with a second parasite, *Ehrlichia canis*, imidocarb can affect both parasites (Pasa *et al.*,2011).

USE OF MACROLIDE ANTIBIOTICS AND ANTIFUNGAL COMPOUNDS AS ANTIBABESIAL DRUGS

Yamasaki *et al.*,(2011b) have reported that nystatin; a membrane-active polyene macrolide antibiotic and an antifungal compound could destroy *B. gibsoni* by its ionophorous activity. The drug operates as a channel-forming ionophore and is membranolytic due to its lipid binding activity (Wiehart *et al.*,2006, Yamasaki *et al.*,2011b). Nystatin is not given intravenously to dogs because of its toxicity, attributed to the ability of polyene macrolides to bind cholesterol in mammalian cell membranes, albeit with lower affinity (Wiehart *et al.*,2006). Ionophorous antibiotics valinomycin and salinomycin-Na, have exhibited a strong *in vitro* effect against *B. gibsoni* (Yamasaki *et al.*,2005) by modifying the intracellular concentrations of monovalent cations. This action may activate transporters of monovalent cations, such as Na-K-ATPase, and increase the consumption of adenosine triphosphate, resulting in the depletion of intracellular ATP (Yamasaki *et al.*,2005). However, valinomycin and salinomycin-Na have no specificity, as they affect both the parasites and the host cells in which intracellular concentrations of monovalent cations are regulated by the function of an active transporter, and therefore, they are ineffective as therapeutics against *B. gibsoni* infection.

Amphotericin B is a membrane-active polyene macrolide antibiotic which has antibabesial effect. The interaction of such compounds with sterols in bilayer cell membranes can lead to membrane damage and ultimately cell lysis (Yamasaki *et al.*,2014). Although experimentally 0.5 and 1 mg/kg amphotericin B administered by the intravenous route to *B. gibsoni*-infected dogs has reduced parasitemia, recurrence of parasitemia had been observed, indicating that amphotericin B did not eliminate parasites completely (Yamasaki *et al.*,2014). Furthermore, blood urea nitrogen and creatinine levels of dogs were markedly elevated after the administration of 1 mg/kg amphotericin B (Yamasaki *et al.*,2014). Accordingly, amphotericin B has *in vivo* activity against *B. gibsoni*; however, it does not eliminate parasites from infected dogs and affects kidney function at a high dose. Thus,

amphotericin B needs to be used with care especially in patients with impaired renal functions.

Clotrimazole and ketoconazole are imidazole derivatives and antifungal agents that are also reported to inhibit the *in vitro* growth of *Babesia* species, including *B. equi*, *B. caballi* (Bork *et al.*,2003a), *B. bigemina*, and *B. bovis* (Bork *et al.*,2003b). However, the sensitivity of *B. gibsoni* to clotrimazole and ketoconazole was intermediate compared to other *Babesia* species (Matsuu *et al.*,2008). It has been suggested that their antiprotozoal activity might be due to their ability to cause major changes in calcium ion fluxes (Matsuu *et al.*,2008).

ATOVAQUONE AND ITS COMBINATION AS TREATMENTS FOR CANINE BABESIOSIS

Atovaquone is a novel antiprotozoal compound that has broad-spectrum activity against protozoan pathogens including *Babesia* species (Birkenheuer *et al.*,2004, Matsuu *et al.*,2006, 2008, Sakuma *et al.*,2009). Atovaquone is commercially available in 2 formulations: a single drug formulation and a 2.5: 1 combination with proguanil hydrochloride (Birkenheuer *et al.*,2004). Atovaquone blocks mitochondrial electron transfer and thereby the production of energy by the parasites (Matsuu *et al.*,2006). Proguanil is metabolized into its active metabolite, cycloguanil, which blocks dihydrofolate reductase and enzymes required for making pyrimidine which is needed for DNA synthesis (Birkenheuer *et al.*,2004). However, the therapeutic protocol has several problems in canine babesiosis, such as a relatively longer time to show clinical effectiveness and adverse effects such as vomiting and diarrhea. Moreover, atovaquone is expensive and not commonly available in many countries including Sri Lanka. Recurrence of disease and decreased sensitivity of *Babesia* to therapy have been reported when atovaquone alone was used (Matsuu *et al.*,2006, Birkenheuer *et al.*,2004). Mutation of the *cytochrome b* gene, which is located in the mitochondrial genome, has been described as a cause for less susceptibility of *B. gibsoni* to atovaquone (Matsuu *et al.*,2006). Meanwhile, it has been demonstrated that an atovaquone-azithromycin drug combination is an effective treatment for dogs that are chronically infected with *B. gibsoni* (Matsuu *et al.*,2006, 2008, Sakuma *et al.*,2009). Azithromycin is a macrolide antibiotic, which has also been confirmed to exhibit activity against *Plasmodium* species *in vitro* and *in vivo* (Nakornchai *et al.*,2006). The lack of adverse clinical, hematological and biochemical abnormalities reported with the combination treatment is promising, but many studies are underway to fully assess the safety of the

atovaquone-azithromycin drug combination in dogs.

MULTIPLE DRUG COMBINATIONS FOR CANINE BABESIOSIS

An alternative therapy of additive and synergistic strategy is combination therapies with clindamycin-metronidazole-doxycycline at dose rates of clindamycin at 25 mg/kg, metronidazole at 15 mg/kg and doxycycline at 5 mg/kg orally with frequency of 12 hours for 10 days (Nandini *et al.*,2016). This combination boosts the innate immunity and is known as the Marshall Protocol. That is probably the reason for lack of adverse reactions and the dogs show clinical and haematological improvement from few days of treatment onwards and recover uneventfully by the end of therapeutic protocol (Nandini *et al.*,2016, Wulansari *et al.*,2003) Clindamycin is a semisynthetic derivative of lincosamin. The *in vitro* activities of clindamycin against *B. divergens* were demonstrated with an IC₅₀ value of 2200 - 3400 mg/l, which corresponds to 4.36 - 6.73 mM (Brasseur *et al.*,1998). However, Matsuu *et al.*,(2008) have observed that the activity of clindamycin against *B. gibsoni* was 16 - 24 times lower than that against *B. divergens*. Thus, it may explain that treatment with clindamycin alone is not sufficient to eliminate the clinical symptoms and *Babesia* organisms from the peripheral blood of *B. gibsoni* infected dogs (Wulansari *et al.*,2003). Nevertheless, it is also been suggested that clindamycin stimulate humoral and cellular immunity against *Babesia* infection and results in improvement in clinical condition (Hwang *et al.*,2010, Nandini *et al.*,2016, Wulansari *et al.*,2003). For a long time, tetracycline antibiotics; doxycycline hydrochloride and minocycline hydrochloride have been known to exhibit activity against *Babesia* parasites such as *B. divergens* (Losson *et al.*,1989) and *B. canis* has been recognized only *in vivo* (Vercammen *et al.*,1996). Tetracycline, macrolide and lincosamin exhibit inhibitory effects against the apicomplex protozoa by probably targeting the apicoplast (Dahl *et al.*,2008). Metronidazole is a nitroimidazole compound used commonly as an antitrichomonal. Although metronidazole was reported to have been used as part of the combination therapy (Suzuki *et al.*,2007), no activity was observed in *in vitro* studies of *B. gibsoni* (Hwang *et al.*,2010a, Matsuu *et al.*,2008). Nevertheless, the combined therapy of clindamycin, metronidazole and doxycycline is an efficacious alternative treatment strategy for chronic clinical babesiosis with less adverse effects that has been observed in the limited research conducted so far. Meanwhile, there are many reports of development of antibiotic resistance for those antibiotics, for example,

emergence of clindamycin-resistant in methicillin-resistant *Staphylococcus aureus* (Rich *et al.*,2005), metronidazole-resistant *Trichomonas vaginalis* (Cudmore *et al.*,2004) and doxycycline-resistant strains of *Streptococcus* (Chalker, *et al.*,2012) in animals. Thus, combination treatments of these antibiotics against babesiosis warrant further studies due to possible emergence of not only antibiotic-resistant bacteria but also *Babesia*.

Another drug combination that is used to treat naturally occurring canine babesiosis is a doxycycline-enrofloxacin-metronidazole combination with and without diminazene aceturate (Lin *et al.*,2010, Vial *et al.*,2006). The overall efficacy of combination of doxycycline-enrofloxacin-metronidazole in conjunction with and without administration of diminazene aceturate for *B. gibsoni* is 85.7% and 83.3%, respectively, with a mean recovery time of 24.2 and 23.5 days, respectively (Lin *et al.*,2010). This means concomitant use of intramuscular diminazene aceturate may not improve the efficacy of a doxycycline-enrofloxacin-metronidazole combination in management of canine babesiosis caused by *B. gibsoni*.

CONCLUSIONS

Although the incidence of canine babesiosis has been increasing, effective and crucial therapeutic modality is unavailable. Single drug treatment or combined treatment with diminazene aceturate, atovaquone, clindamycin, metronidazole, doxycycline, and pentamidine do not eliminate the parasites from the peripheral blood. Thus, a broad understanding of treatment protocols is required when implementing an appropriate treatment plan against canine babesiosis. Furthermore, it is also important to consider factors such as drug availability, dosage regimes and their adverse effects, convenience to use, and the cost. Importance of follow up treatments needs to be explained to the clients as a part of the management strategies of canine babesiosis. The combination therapy might be more effective rather than a single therapy. However, the combination treatments are generally used as the second-line of treatments when conventional therapies fail. Many studies are required for analyzing the correlations of these drugs when used in combination with each other, and to determine the best efficient composition for the inhibition of the growth of parasite. Most importantly, possible relapses and the development of drug-resistant isolates are matters of concern. Thus, the development of novel, more effective antibabesial drugs against canine babesiosis is urgently required.

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