The drug Diprokarb for treatment of idiopathic babesial disease in dogs under the conditions of Russia

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Abstract. Study of efficiency of the drug diprokarb with major active ingredient of imidocarb propionate was conducted. One milliliter of diprokarb contains 120 mg of imidocarb propionate. It was studied under the conditions of Moscow and Moscow Region using an example of spontaneous infestation of dogs with babesial disease. All studies were conducted at veterinary clinics using laboratory studies of the blood of infected animals, as well as the study of ixodic ticks that are the major carriers. Monitoring of physical status of animals was being performed from time of the first appearance of babesial disease clinical signs to the complete disappearance of hematozoons from the blood, as well as products of their vital activity. Studies of the animals, which are not demonstrated evident clinical signs of infection, were also conducted during ixodic ticks’ season of activity. Diprokarb was used as the main drug and the symptomatic therapy was conducted when disease is identified. The drug demonstrated 100 % efficiency when applied at the dose of 6 mg per kg of animal body weight.

1. Introduction

Within recent years percentage increase of animals’ incidence rate with babesial disease is noted particularly in spring and in autumn although out-of-season infections of animals are also recorded. It is associated with the increasing anthropogenic load on environment, globalization and observed climatological changes, and thus increasingly greater attention is to this disease is required [1, 2, 3]

Significant role in this is performed: uncontrolled rise of dogs amount, especially stray; absence of effective and free-for-all means of prevention: insanitation of places for walking the dogs; generalization of ixodic ticks and their active breeding. It is also possible to notice unpretentiousness and adaptational capacities of existent ticks, for example, for breeding brown dog-louse Rhipicephalus sanguineus is required only warmth, it can breed in dog-house and even on the dog [4,5].

Genus Ixodes has the largest value. Ticks of this genus can find their host by markedly different ways, for example, existing on plants, tall grass or bushes from where they creep on animals. Rhipicephalus sanguineus finds his host actively, Haller’s organ set on the first pair of legs conduce this. These ticks are very significant for veterinary as they are carrier of pathogenic protozoaires, viruses, bacteria, rickettsiae causing tick-borne fever. Sick animals are the natural reservoir for babesial disease
agents in nature. Small rodents are the transitional reservoir. Ticks of this type attach mainly on the forepart of the body. Ticks attach using hypostome equipped with teeth of mouthparts, after attaching ticks do not move over animal's body, as a rule.

Saliva of tick has anticoagulative features, and also conduce holding power to skin of the host due to tanning agents included in its composition. Preimaginal stages of ticks are settled in ear pavilions, and adult specimens are settled on the head, shield bones and back. Place of lavrae and nymph attaching is responds to structure feature of the host's hair-coat and skin covering (minute structure of skin). In the case of primary attack on carnivores ticks choose places with thinner skin and close arterioloovenular bridge for readily available feed. Mechanical availability of teeth and legs of the carrier was also taken into account as the dog can reach and tear the tick off itself in the case of attaching to the hind quarter of the body.

Female attaches to the host's skin and begins to feed. Male migrates on the animal's body and when contacts with female attaches on female, and fertilization can occur on the animal.

Local reactions and bacteritic inflammations might develop at the place of ticks attaching. So babesial disease of dogs is zoonotic protozoan vector-borne blood parasitic endoglobular disease causing by protozoaires parasite Babesia canis. Stated activators belong to the class Piroplasms included families Babesiidae and Theileriidae. Family Babesiidae includes genus Babesia, Piroplasma and Franciaiella [6,7].

Depending on noted information it was set the task to study the efficiency of administration of drug diprokarb for treatment spontaneous babesial disease of dogs under the conditions of Moscow and Moscow region.

2. Materials and methods
At the first stage occurrence of spontaneous babesial invasion among dogs of different age groups was studied depending on seasons under the conditions of veterinary clinics of moscow and Moscow region. Frequency of occurrence of babesial disease in dogs of different breeds was established. Study of efficiency of administration diprokarb for treatment spontaneous babesial disease of dogs in recommended by manufacturer dose was conducted at the second stage, and then optimal treatment drug dose provided full recovery of sick dog from this disease was tested.

Further we remind briefly questions on biology of babesial disease, epizootology, pathogenesis, clinical picture and treatment of this disease.

Activator is transferred by vector-borne way through intermediate host which role ixodic ticks are played. After penetration into dog's organism protozoaires, as a rule, are introduced into the blood capillaries and further into the red blood cells (but they can also be found in leukocytes and in blood plasma), where their life cycle passes. The shape of parasites' body can be oval, round, amoebiform, anaplasmoid, pear-shaped, pair pear-shaped and other.

In recent years, babesial disease occurs without significant clinical signs, therefore, it is possible to confirm this disease usingmicroscope investigation of blood films stained according to Romanovsky-Giemsa using Diff-Quick paint, as well as seroscopy by antibody-mediated method - PCR. The most confidential test is a blood study by PCR method for the presence of babesia DNA, and in addition it allows determining various subtypes of babezia. In the case of diagnostics in films from peripheral blood, babesia is best detected when stained according to Romanovsky-Giemsa. In this case cytoplasm can be blue-violet coloration, and nucleus is ruby.

Babesia breeding occurs by the mean of schizogenesis in the organism of the tick-carrier. They enter the epithelium cells of the intestinal tract or pass into the body cavity of the tick. Gametogonium is occurred in tick's lumen of the intestine, and 2, 4 and 6 nuclear stages are formed from mononuclear stages. In the future they are migrate to hemolymph, gonads, hypodermis and other organs continuing multiple and binary fission. There they continue fission and penetrate into the cells of various organs, including the salivary glands, and here, as a result of fission, small mononuclear pear-shaped cells form — merozoites, which are an infective stage.
Transovarial transmission is indicative for this disease. When reach into the reproductive glands of female ticks, they enter the ovocyte, while not disrupting their normal development. Ticks larvae hatched out of the egg, molt into the nymphs, and then turn into imago however carrying babesia in themselves before feeding with blood of an infected host. Thus, vertical transphasic (transstaged) transmision of babesia is occured.

It should be noted that babesia are able to circulate in the organism of invertebrates for a long period of time, transovarially up to 40 generations. Therefore when average-expectancy of tick life is 3 years infecting might occur during 120 years.

In practice, from 3 to 80 % of red blood cells can be injured. Babesia breeding in red blood cells is occurred by binary fission and budding.

Babesia breeding is occurred in red blood cells, lysis of which is based not only on the effects of parasites, but also on the appearance of antired-cell antibodies. Clinical progressions appear when the amount of infected red blood cells reaches 3-5 %. At erythrocytoschisis waste products of parasites and heterogeneous proteins came in the blood, which causes a powerful pyrogenic reaction and other toxic signs. Progressive anemia is accompanied by expressed tissue hypoxia and microcirculatory disorders. Cell membranes ("ghosts") of red blood cells and free hemoglobin settle down in the renal capillaries that leads to the development of hematuria and acute renal failure. In the case of massive red blood cells lysis disorders of pigmental metabolism are developed with accumulation of mainly indirect bilirubin in blood. Upon completing of the development cycle, the new generated species are released into the blood channels, where they penetrate into new red blood cells. As the result the amount of free hemoglobin and accumulation of fragments of cellular membrane increases, as well as the amount of metabolite products of the parasites themselves increases in blood.

Acute hemoglobinuria, cymemolytic icterus and anaemia, hepatic, renal and other organs impairment are the results of these processes. Besides, hyperexcitability and nervous system damage are present that are caused by babesia penetration into the brain vascular system.

The severity of the invasion process depends on activator's pathogenicity, resistance of host's organism, amount of parasitizing ticks and other factors. Besides, the tick causes physical damage of animals’ skin areas by its mouthparts, which can infect in future and anaemia can develop in the case of severe invasion in animal.

At the present time among the new drugs against the activators of blood protozoan diseases of animals it should be noted diprocarb, developed and manufactured by Invesa company (Spain).

The main active ingredient of the studied drug diprokarb is imidocarb propionate. One milliliter of diprokarb contains 120 mg of imidocarb propionate. The drug itself is made as clear solutions for injection, practically free of particles, is available in glass darken vials.

Imidocarb the active ingredient of diprokarb is broad spectrum, active against Babesia bovis, Anaplasma marginale, Anaplasma ovis, Nuttallia equi, Babesia equi, Babesia canis, Babesia gipsonii and Ehrlichia canis.

The mechanism of antiprotozoal action of imidocarb lies in process changes of inositol enter into the red blood cells (which is necessary for the parasite), as well as in change of formation and use of polyamines by babesia that leads to their subsequent death.

In the process of performing the work, the blood parasitic drug diprokarb was tested in spring, summer and autumn on spontaneously infected by babesia dogs of different ages and breeds in the recommended by manufacturer dose (on AS) of 3 mg/kg of the animal’s body weight subdermally single dose with the control administration of the drug in 14 days. Taking into account the highest activity of ixodic ticks, the carriers of babesian invasion in the noted seasons and the greatest infection intensity in red blood cells, we came to the conclusion that it is necessary to increase the diprokarb dose of 6 mg/kg of body weight that in the dosage form corresponds to 0.5 ml of the drug per 10 kg of animal body weight subdermally as a single dose. Our further studies conducted with prescription of the stated dose of the drug diprokarb demonstrated propriety of choice, infected dogs had recovered.
3. Results

Total 673 animals were studied on babesial disease among them 542 animals were infected, that is equivalent to 80.5%. Infected puppies at the age of 0-3 months was 67 (12.2%), of 3-6 months was 70 (12.8%), of 6-12 months was 92 (16.8%), of 1-3 years was 159 (29.15%), of 3-6 years was 158 dogs (29.05%). There were 149 animals of hunting breed, 239 draft dogs, and 158 fancy dogs.

The drug was administered to the dogs for therapeutic purpose in the case of confirmed babesial disease. Symptom-directed treatment was also conducted. Diprokarb was administered for dogs treatment at the dose of 0.5 ml per 10 kg of body weight that is equivalent to 6 mg/kg subdermally. Control microscope investigation of blood films in 24 hours demonstrated complete killing of parasites in blood, and repeated administration of diprokarb in 14 days did not required.

Diprokarb was studied on 168 animals - 28 puppies of hunting breed, 5 puppies of Yorkshire terrier breed, 7 puppies of Pommeranter Spitz breed, 63 draft dogs, and 61 crossbreeds of different ages (figure 1).

Symptom-directed treatment was also used during treatment of these animals. Therapeutic benefit was observed within 15-20 days after administration of diprokarb.

It belongs to hazard category 3 substance according to its degree of health effect, it does not have local intolerance, fetotoxic and mutagenic effect in recommended doses. No side effects and allergic reactions were observed when this drug was used, except imperceptible injection site tingling.

After administration the drug imidocarb is quickly absorbed from injection site and penetrate into the majority of organs and body tissues reaching peak concentration within 18-24 hours. Imidocarb does not undergo metabolism in animal's organism and predominantly eliminated in urine.

All animals were bled for study at the initial stage of the disease and during treatment, hematologic indicators were mainly evaluated - general clinical, biochemical, and gas composition of blood was also studied. Coagulogram was taken if it was necessary.

Hospital treatment with careful control of diuresis and changes of hematologic indicators was conducted in severe cases.
Balanced electrolytic solutions were applied for correction pH and electrolytic structure of circulating blood volume. Blood transfusion was conducted in case of severe anaemia (reduction of haematocrit more than 15 %) and hemorrhagic tendency. Types and methods of blood transfusion were selected on a case by case basis.

For preventive purpose diprokarb was administered in apparently healthy hunting dogs at a dose of 0.25 ml per 10 kg (3 mg per kg) of animal body weight as other protective measures as collar and drops were not effective during hunting. After hunting dogs’ owners took off ticks attached and migrated on dog’s body. All ticks were sent for study, a part of them was hematozoons carriers but disease was not registered in dogs that were proved by microscopic study of peripheral blood at an interval of 5 days. Diprokarb was administered by hunters at an interval of 20 days. Any side effects was not reported, blood test was taken and biochemical and clinical blood parameters was evaluated for control of dogs health, short-term increase of blood values such as aspartate aminotransferase, alanine aminotransferase, and cholinesterase were registered but in 2-3 days parameters returned to normal. No side effects and complications were observed.

4. Discussion
Studies were conducted at veterinary clinics using laboratory studies of the blood of infected animals, as well as the study of ixodic ticks that are the major carriers. Monitoring of physical status of animals was being performed from time of the first appearance of babesial disease clinical signs to the complete disappearance of hematozoons from the blood, as well as products of their vital activity. Studies of the animals, which are not demonstrated evident clinical signs of infection, were also conducted during ixodic ticks' season of activity. Diprokarb was used as the main drug and the symptomatic therapy was conducted when disease is identified.

5. Conclusion
Therapeutic efficiency of diprokarb in the case of dogs’ babesial disease was 100 % - complete killing of hematozoons was registered. However dogs aged elder than 6 years died from after-effects of babesial disease more often than puppies as disease progression was complicated by concomitant age-related or acquired during life diseases. Young dogs, as a rule, recovered quicker despite frequent acute progression of babesial disease.

Diprokarb is an effective drug for treatment dogs’ babesial disease when applied at the dose of 6 mg per kg subdermally.

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