Effect of Enrofloxacin for treatment of bovine anaplasmosis and canine babesiosis

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Abstract. The target of the survey is to study the efficacy of Enrofloxacin 5% and its lower doses used for the therapy of bovine anaplasmosis and efficacy of Enrofloxacin 2.5 and 5% used for treatment of canine babesiosis. Enrofloxacin 5% has a pronounced therapeutic effect in treatment of anaplasmosis in cattle used at a dose of 1cm3 per 20 and 30 kg of body weight while using the preparation at a dose of 1cm3 per 40 and 50 kg of body weight the therapeutic effect was practically not observed. At the same time Enrofloxacin has no therapeutic efficacy against canine babesiosis.

1. Introduction
Blood parasite diseases are caused by organisms that live in the blood of their animal hosts. These parasites can range from single-celled protozoa to more complex bacteria and rickettsiae.

Bovine anaplasmosis is an infectious disease of cattle caused by the obligate intercellular bacterium, Anaplasma marginale, which is of the order Rickettsiales. Bovine anaplasmosis, caused by the intraerythrocytic rickettsia Anaplasma marginale (Rickettsiales: Anaplasmataceae), is an economically important disease of cattle which is endemic in tropical and subtropical regions of the world. Anaplasma marginale is host-specific, with only a few reported cases found in sheep and goats. It has only been found to infect erythrocytic cells in cattle under natural conditions. The disease is diagnosed by seeing the parasite on a blood smear. This obligate intracellular pathogen can be transmitted biologically by ticks, mechanically by transfer of infective blood on fomites or the mouthparts of biting insects, and, less commonly, by transplacental transmission from dams to their calves [1, 2, 4, 10].

The tick is considered the primary vector for this disease, and it acquires A. marginale by feeding on infected erythrocytes in cattle. It then acts as a reservoir by replicating in several tissues, but primarily in the midgut and salivary glands, with the latter of greater importance for transmission back to cattle [4]. A. marginale is capable of vertical transmission in tick species, so it is quite an effective reservoir of the disease. Ticks go through four life stages: egg, larvae, nymph (juvenile), and adult. All stages of the tick’s life cycle require feeding on blood, although the target of feeding usually changes at each stage. Ticks usually feed on cattle during the nymph and adult stages, which is when transmission of the disease between species occurs [1, 2, 4, 7, 8, 10].
The incubation period of the infection for cattle varies depending on the dose of the infective agent and ranges from 7 to 60 days, with an average of 28 days. Signs and symptoms include fever, weight loss, abortion, and potentially death (for cattle older than 2 years), although juvenile cattle less than 9 months old are usually asymptomatic [2].

Epidemiologically, they share similar transmission paths and appear to affect cattle at a greater symptomatic intensity as they age. Ticks, biting flies, and stable flies are the most common vectors for anaplasmosis, but mechanical transmission can also occur through fomites or surgical equipment if they are not properly sterilized [2]. Mechanical transmission is likely the only agent to spread bovine anaplasmosis in regions where the aforementioned vectors are absent.

Canine babesiosis is a common tick transmitted disease of dogs worldwide. Canine babesiosis in the Russian Federation is caused by protozoan parasites Babesia canis, which infect the red blood cells. The disease is diagnosed by seeing the parasite on a blood smear.

Babesiosis is primarily spread through the bite of an infected hard tick (gen. Dermacentor and gen. Rhipicephalus). There is also evidence that some direct animal-to-animal transmission may occur, such as when an infected dog with oral lesions or abrasions bites another dog. Recent studies show that Babesia may be transmitted transplacentally (to unborn puppies in the uterus of their mothers). Dogs may also be inadvertently infected through tainted blood transfusion [3, 6].

Dogs infected with babesiosis may present with a wide variety of clinical signs ranging in severity from a sudden collapse with systemic shock, to a hemolytic crisis (the body attacks and destroys the red blood cells called hemolysis), to a subtle and slowly progressing infection with no apparent clinical signs.

Dogs typically present with the acute, severe form of babesiosis, which is characterized by findings such as abnormally dark urine, fever, weakness, pale mucous membranes, depression, swollen lymph nodes, and an enlarged spleen. Blood and urine tests may reveal anemia, thrombocytopenia (low platelets), hypoalbuminemia (low albumin, a blood protein), and bilirubinuria (a pigment from breaking down red blood cells is found in the urine) [3, 6, 9, 10].

2. Purpose
The purpose of this survey is to study the efficacy of Enrofloxacin 5% and its lower doses used for the therapy of bovine anaplasmosis and efficacy of Enrofloxacin 2.5 and 5% used for treatment of canine babesiosis.

3. Materials & Methods

3.1 Animals
Natural infected bull calves (n=40) and experimentally infected dogs (n=12).

3.2. Causative agents
Anaplasma marginale Theiler, 1910 [10] and Babesia canis Piana et Galli-Valerio, 1895 [8].

3.3 Diagnostic tests
The diagnosis was confirmed by microscopic investigation of blood smears stained according to Gimsa method and with the use of serological tests [4, 5, 6].

The treatment effectiveness was being determined daily based on changes of the level of parasitemia in animals. Temperature, pulse and respiratory rate of examined animals were recorded simultaneously.

3.4. Drug
Enrofloxacin 2.5 and 5% were used. Enrofloxacin is a synthetic antibacterial agent from the class of the fluoroquinolone carboxylic acid derivatives. It has antibacterial activity against a broad spectrum of Gram-negative and Gram-positive bacteria. Enrofloxacin works by inhibiting the process of DNA synthesis within the bacterial cells, which results in cell death. This drug is commonly used to treat a
range of bacterial infections, including those of the skin, urinary tract and respiratory system, as well as infections that result from wounds [5].

3.5. Experimental design
Experiment 1. Investigations were carried out on 40 bull calves spontaneously infected with anaplasmosis (the age ranged between 1 and 1.5 years) divided into 4 equal groups. The preparation was injected subcutaneously at a dose of 1 cm$^3$ per 20 (according to the instruction), 30, 40 and 50 kg of body weight within 5 days, respectively. Thus, not more than 10 cm$^3$ of preparation was injected at one point.

Experiment 2. Investigations were carried out on 12 dogs at the age from 8 to 12 months. All animals were divided into 3 groups: 2 experimental and 1 control. The infestation was carried out using a subcutaneous injection of blood from infected with Babesia canis dog at a dose 2 cm$^3$ per 1 animal.

Enrofloxacin 5% at a dose of 1 cm$^3$/10 kg of body weight (therapeutic dose Enrofloxacin 5 mg/kg) was injected to animals of the 1st group within 5 days. Enrofloxacin 2.5% at a dose of 0.2 cm$^3$/1 kg of body weight (therapeutic dose - Enrofloxacin 5 mg/kg) was injected to animals of the 2nd group within 5 days. Animals of the control group stayed untreated.

4. Results
4.1. Experiment 1
Clinical signs of anaplasmosis were present in all bull calves: increased body temperature, anemia, tachycardia and increased respiration, lymph nodes hyperplasia, edemas, thirst and anorexia, atony of the gastrointestinal tract. Blood tests detected the presence of leukocytosis, leukopenia, a sharp drop in the number of erythrocytes, hemoglobin decrease, anisocytosis, poikilocytosis, basophilic aggregates in the erythrocytes, high damage to erythrocytes caused by anaplasmas.

The average values of parasitemia in experimental groups during testing of different doses of Enrofloxacin 5% used for treatment of anaplasmosis in cattle are represented in table 1. During the treatment a decrease of parasitemia in groups I and II with the subsequent disappearance of anaplasmas from blood smears was observed. In groups III and IV a pronounced therapeutic effect was not determined, the preservation of parasites in blood accompanied by a negligible decrease of parasitemia was observed.

| Group | parasitaemia %, X±Sx | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|-------|----------------------|-----|-----|-----|-----|-----|-----|-----|
| I     | 14,8±2,1 12,4±1,8    | 10,1±1,5 | 5,2±1,0 | 1,2±0,2 | -   | -   |     |
| II    | 16,4±2,0 12,1±1,5    | 10,5±0,9 | 6,8±1,0 | 1,2±0,3 | pcs. | -   |     |
| III   | 16,8±1,9 14,2±2,0    | 12,9±1,9 | 13,2±2,1 | 12,8±1,9 | 12,5±1,8 | 12,2±2,0 |     |
| IV    | 15,2±1,9 14,3±2,0    | 14,0±1,8 | 14,2±1,8 | 13,8±1,9 | 14,0±1,6 | 13,6±1,8 |     |

4.2. Experiment 2
Blood from animals of all groups was tested before infestation using microscopic investigation of blood smears stained according to Gimsa method. None of the animals was infected with Babesia canis.

It was determined that after infestation the parasites appear in erythrocytes on the second day along with the subsequently increased parasitemia. Depression, food refusal, temperature increase up to 39.5...40.3°C, tachycardia, labored breathing was detected in all animals, anemia, icteritiousness of the mucous membranes and hemoglobinuria were determined in some animals.
The treatment began on the third day of the experiment. 5 injections in total were made to animals from experimental groups. During the treatment general health deterioration and increase of parasitemia were observed. In groups II and III fell one animal in each group. The average values of parasitemia in experimental groups are represented in table 2.

### Table 2. Average levels of parasitaemia in dogs in experimental groups.

| Group | parasitaemia %, X±Sx |
|-------|----------------------|
|       | 1                    | 2 | 3 | 4 | 5 | 6 | 7 |
| I     | –                    | 0.5±0.07 | 1.2±0.10 | 1.3±0.13 | 1.4±0.10 | 1.5±0.15 | 1.5±0.15 |
| II    | –                    | 0.6±0.04 | 1.5±0.15 | 1.6±0.15 | 1.6±0.08 | 1.6±0.06 | 1.6±0.13 |
| III   | –                    | 0.8±0.06 | 1.5±0.14 | 1.5±0.14 | 1.6±0.20 | 1.7±0.10 | 1.7±0.15 |

### 5. Discussion

During last century a lot of drugs were tested for treatment of anaplasmosis and babesiosis. Nowadays the tetracycline antibiotics are widely used in veterinary medicine for therapy of anaplasmosis in ruminants [1, 7] and diminazene aceturate and imidocarb are used for treatment of canine babesiosis [5, 16].

However, tetracycline antibiotics are not effective enough against the given disease and do not ensure that the animal organism would be totally free of parasites. Moreover, they only have bacteriostatic effects. Anaplasmosis on farms always develops in combination with the whole complex of concomitant bacterial infections which should be treated using a modern strategy of anaplasmosis therapy.

Thereby it is very impotent to increase the number of drugs for treatment of animal blood parasite diseases. According to our results Enrofloxacin 5% has therapeutic effect for treatment of bovine anaplasmosis when used at a dose of 1 cm³ per 20 and 30 kg of body weight (according to the instruction) while at a dose of 1 cm³ per 40 and 50 kg of body weight the therapeutic effect of this preparation was practically not observed. At the same time Enrofloxacin has no therapeutic effect against the infectious agent Babesia canis caused canine babesiosis.

### 6. Conclusion

Based on the performed investigations it was determined that Enrofloxacin 5% has a pronounced therapeutic effect when used for treatment of bovine anaplasmosis at a dose of 1 cm³ per 20 and 30 kg of body weight. At the same time Enrofloxacin has no therapeutic effect against the infectious agent Babesia canis caused canine babesiosis.

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