

## Conference Paper

# Technology for the Use of Reduced Doses of Diminazene Aceturate for the Treatment of Pet Babesiosis

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Babesiosis (piroplasmiasis) is a transmissible parasitic disease, accompanied by fever, anemia, yellowness of the mucous membranes, hemoglobinuria, lost productivity and performance, and with untimely treatment – death of livestock. Therapy to treat livestock babesiosis involves, first of all, the use of anti-babesial drugs. This study aimed to examine the effectiveness of using a reduced diminazene aceturate (DA) dosage against babesiosis in livestock. However, the use of these drugs can cause side effects. The study recommends the use of DA-based drugs against equine babesiosis at a dose of 2.6 mg/kg bodyweight for an active ingredient (AI). With volumetric dosing it is 0.038 cm<sup>3</sup> of a 7 % solution per kg bodyweight, rather than 0.05 cm<sup>3</sup> per kg bodyweight, as indicated in the instructions. The use of a reduced dose of Batrizin (2.5 and 3.0 mg/kg AI) and Fa.Try.Banil. (2.5 mg/kg AI) to treat experimental babesiosis in dogs had a satisfactory anti-babesial effect in the absence of intoxication.

**Keywords:** diminazene aceturate, babesiosis, parasitic disease

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## 1. Introduction

Babesiosis (piroplasmiasis) is a transmissible parasitic disease, accompanied by fever, anemia, yellowness of the mucous membranes, hemoglobinuria, lost productivity and performance, and with untimely treatment – death of livestock.

Being in red blood cells, babesia feed on their cytoplasm and kill them. Babesia wastes and cellular debris have a strong toxic effect on horses. Deep biochemical changes occur, affecting cardiovascular system [1, 2].

A reduced number of red blood cells and loss of hemoglobin entails oxygen deficiency. The body tries to compensate for it with an increasing burden on the heart and lungs. This leads to tachycardia, arrhythmia, shortness of breath, and possible pulmonary edema. Vascular porosity increases, resulting in swollen parts of the body.

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Free hemoglobin resulting from a breakdown of red blood cells is partially filtered by the kidneys, which causes the urine to discolor to red – hemoglobinuria develops (a sign of the disease). Part of the hemoglobin is converted into bile pigments (bilirubin), which causes icteric discoloration of the muscles and mucous membranes. A gastrointestinal syndrome concurrently develops in the body. At the onset of the disease, peristalsis intensifies under the influence of toxins causing short-term diarrhea. Gradually, peristalsis weakens, and flatulence occurs.

If untreated, the disease progresses rapidly and can end up fatally. With timely treatment, all impaired functions are gradually restored. The recovery process is long, and can be complicated by myocarditis, nephritis and hepatitis [3, 4].

Equine babesiosis is a seasonal (spring, May), acute protozoal disease, with symptoms including depression, fever, yellowness, and hemoglobinuria. The disease is recorded in many regions of the Russian Federation [5].

The drugs used against babesiosis often cause side effects. According to many authors, the drugs within a single injection often cause toxic effects. There are two ways to solve this problem: using symptomatic therapy or reducing the dosage of the etiotropic drug.

The study was aimed at the possibility of using and the effectiveness of reduced diminazene aceturate (DA) dosage against babesiosis in livestock

## 2. Methods and Equipment

The study was geared for 7 randomly infected horses of various breeds, aged 2 to 4. The diagnosis was proved by microscopic examination of peripheral blood smears through Romanowsky–Giemsa staining. Microscopic examination enabled to detect round, single and paired pear-shaped babesias. The level of parasitemia was determined based on the average number of parasites per power field with 100 views. Equine babesiosis entries are put in the GIS database. GIS is currently a universal tool for epizootological analysis [2, 6].

There was an acute course of the disease in the new natural foci. The disease began with a sharp rise in temperature to 40–41 °C, accompanied by depression and loss of appetite in a stock. The conjunctiva was initially hyperemic (1–2 days), then it became anemic (from 2–3 days of the disease). On 3–4 days, part of the stock had yellowness (in two cases), as well as spotty and banded hemorrhages on the mucous membrane of the oral cavity. All affected animals had polypnea (20–35 per min), shortness of breath,

tachycardia (80–90 beats/min), increased cardiac impulse and arrhythmia. There was no hemoglobinuria in the sickly horses. Parasitemia in sickly animals amounted to 1.2–2.0%

There was a subacute course of the disease in the fading babesiosis foci. The horses had a temperature rise up to 39.5 °C, rapid fatigue, shortness of breath, tachycardia up to 80 beats/min. Parasitemia amounted to 0.8–1.0 %. Moreover, the owners often continued to use the horses for farming.

Two doses of DA were taken for testing: 3.5 mg/kg bodyweight for an active ingredient (0.05 cm<sup>3</sup>/kg for volumetric dosing) and 2.6 mg/kg bodyweight for an active ingredient (0.038 cm<sup>3</sup>/kg for volumetric dosing). The drug was double administered intramuscularly, with an interval of 24 hours. The piroplasmocidal effectiveness of the drug was tracked against parasitemia. The blood smears were examined before administration of the drug, 24 hours after the 1st injection and 24 hours after the 2nd injection.

There were also two experiments to involve non-pedigree pups, aged 2–4 months, live weight 2–5 kg. The pups got infected through subcutaneously administered blood of babesiosis dogs at a dose of 2 ml bodyweight. The blood was taken from spontaneously infected dogs during a flash of clinical signs. The diagnosis was proved by microscopic examination of smears through Romanowsky–Giemsa staining. The blood for infection was collected in sterile syringes, stabilized with heparin and stored in a refrigerator for at least a day. To assess changes in parasitemia scale, blood smears were daily taken from infected puppies, from the day of infection to the end of the experiment. The temperature, pulse rate and respiration were also measured in the experimental group.

During the experiment, the disease was acute. The incubation period in all animals was within 2–3 days. The average temperature reached its height on the 2nd day from the onset of the disease (39.8–40.20 °C) and was persistent for 2–3 days. The heart rate and respiration increased by

2–2.5 times also on the 2nd day. Parasites in red blood cells began to be detected within 36 hours after infection. By the time of the onset of clinical signs, parasitemia reached an average of 1.5–2.5 %. With slight fluctuations further on, it increased up to 2.9 % by the 5th day. In this period, the treatment began.

### 3. Results

### 3.1. Various DA doses in equine babesiosis

The findings are shown in Table 1. Parasitemia decreased almost 4 times a day after the first injection of the drug in the experimental and control groups. Just a few piroplasmas were found to be present in the smears a day after the second injection. There were no side effects found in the experimental group.

TABLE 1: Comparative results of various DA doses in equine babesiosis

Animal No.	Number of heads	AI dose, mg/kg bodyweight	Frequency of administration	Parasitemia, $X \pm Sx$		
				Before administration	24 hours after the 1 <sup>st</sup> injection	24 hours after the 2 <sup>nd</sup> injection
Experimental	3	2.6	Twice with an	1.80±0.20	0.45±0.01	ea
Control	4	3.5	interval of 24 hours	1.76±0.10	0.40±0.02	ea

Three horses of the control group had muscle twitching, light jactatio capitis, desudation, sharply marked veins on the body. One horse from the control group had muscle tremor and hypersalivation, and one of them had hypotaxia. Sulfocamphocaine at a dose of 10 cm<sup>3</sup> was injected intramuscularly to aid these horses.

These phenomena lasted for an hour, and then weakened. The data suggest that DA at a dose of 2.6 mg/kg bodyweight for AI exhibits a sufficient pyroplasmocidal effect, avoiding toxic impact.

### 3.2. Anti-babesial effect of various Batrizin and Fa.Try.Banil. doses in dog babesiosis

In experiment No. 1, as a means of specific therapy, the dogs were injected intramuscularly with Batrizin in the form of a 7 % solution twice with an interval of 24 hours. In total, the experiment involved 9 puppies that were divided into three equal groups. In group I, the drug was administered at a dose of 3.5 mg/kg for AI, in II – 3.0 mg/kg for AI, and in III – 2.5 mg/kg for AI (Table 2).

Within 24 hours after the first injection of Batrizin, parasitemia decreased on average up to 0.8–1.5 %. Within 24 hours after the second injection of Batrizin it reached 0.2–0.5 %, and after another 24 hours just single specimens of the parasite were visible. On the 5<sup>th</sup> day of treatment, *Babesia* was not found to present in the blood smears.

In experiment No. 2, as a means of specific therapy, the dogs were injected intramuscularly with Fa.Try.Banil. twice with an interval of 24 hours. In total, the experiment

TABLE 2: Average parasitemia in the groups with different Batrizin doses

Group	% parasitemia by day, X+Sx									
	Before administration				1 inj	2 inj	After administration			
	1	2	3	4	5	6	7	8	9	10
I	–	0.5+ 0.07	1.8+ 0.10	2.3+ 0.13	2.9+ 0.10	1.5+ 0.15	0.4+ 0.02	ea	ea	–
II	–	0.6+ 0.04	1.5+ 0.15	2.6+ 0.15	2.7+ 0.08	0.8+ 0.06	0.2+ 0.03	0.2+ 0.03	ea	–
III	–	0.8+ 0.06	1.5+ 0.14	2.5+ 0.14	2.8+ 0.20	1.8+ 0.10	0.5+ 0.05	0.5+ 0.05	ea	–

involved 6 puppies that were divided into two equal groups. In group I, the drug was administered at a dose of 3.3 mg/kg for AI (1 ml per 15 kg bodyweight), in group II –2.5 mg/kg for AI (1 ml per 20 kg bodyweight).

TABLE 3: Average parasitemia in the groups with different Batrizin doses

Group	% parasitemia by day, X+Sx									
	Before administration				1 inj	2 inj	After administration			
	1	2	3	4	5	6	7	8	9	10
I	–	0.8+0.06	2.1+0.18	2.8+0.20	2.9+0.10	1.8+0.11	0.6+0.04	ea	ea	–
II	–	0.6+0.04	1.8+0.11	2.5+0.14	2.7+0.08	1.1+0.09	0.5+0.05	0.2+0.03	ea	–

Within 24 hours following the first injection of Fa.Try.Banil., parasitemia decreased on average up to 1.1–1.8 %. Within 24 hours after the second injection of Fa.Try.Banil., it reached 0.5–0.6 %, and after another 24 hours just single specimens of the parasite were visible. On the 5<sup>th</sup> day of treatment, *Babesia* was not found to be present in the blood smears (Table 3).

The temperature in puppies decreased almost to normal within 12 hours after the first injection of both drugs, and after 24 hours it was already within normal limits and did not rise until the end of the treatment. Heart rate and respiratory rate decreased to normal within 48 hours of treatment.

#### 4. Discussion

There was clinical evidence of equine babesiosis in various areas of the Orenburg region that is permanently unfavorable for this disease [5, 7]. Ixodid ticks *Dermacentor marginatus* and *D. reticulatus* (=pictus) transmit *Babesia caballi* in the Orenburg Region. Adults of both sexes were found to be present with different saturation status on the skin of sickly horses. Ticks parasitize animals annually in the form of two waves: spring (from late March to June) and autumn (from late August to late October). A causative agent is *Babesia* (=Piroplasma) *caballi* Nuttallet Strickland, 1910. In blood smears from sickly horses, piroplasma are oval, ring-shaped, amoebiform and pear-shaped. They are

located in the center of the cell. The size of the parasites is greater than the radius of the red blood cell. Paired pear-shaped forms are areaways interconnected and reach a length of 2.5–4 microns. 1–2 parasites usually reside one red blood cell. Parasitemia averages 0.5–10 %. The peak is related to a sharp rise in body temperature.

Canine babesiosis is a protozoal, natural focal transmissible disease of dogs, foxes and arctic foxes, characterized by fever, impaired cardiovascular and digestive systems, yellowness of the mucous membranes, hemolytic anemia, and hemoglobinuria. A causative agent is *Babesia* (= *Piroplasma*) *canis* Piana et Galli-Valerio, 1895 [7]. The disease is recorded in many regions of the Russian Federation [8].

The therapy involves, primarily, the use of anti-babesial drugs. In recent years, a large number of papers on the treatment of dog babesiosis have been published. The authors noted a high therapeutic activity of DA-based drugs that, as prescribed, are double-administered intramuscularly in the form of a 7 % solution at a dose of 3.5 mg/kg b.w. with an interval of 24 hours.

However, these drugs can cause side effects. According to many authors, the said dosage of the drugs often causes toxic effects, and once exceeding it can cause nervous phenomena (the so-called “azidine psychosis”). The problem can be tackled in two ways: by using symptomatic therapy or by reducing the dose of an etiotropic drug [8, 9].

## 5. Conclusion

All of the above allows the use of DA-based drugs against equine babesiosis at a dose of 2.6 mg/kg b.w. for AI, which, at a volume dosing, accounts for 0.038 cm<sup>3</sup> of a 7% solution per kg bodyweight, rather than 0.05 cm<sup>3</sup>/kg b.w., as prescribed in the instructions.

Thus, there was a short incubation period in the test babesiosis of dogs. Parasitemia increased rapidly and was accompanied by a sharp rise in temperature, pulse rate and respiration. The reduced doses of Batrizin (2.5 and 3.0 mg/kg for AI) and Fa.Try.Banil. (2.5 mg/kg for AI) had a satisfactory anti- babesial effect in the absence of intoxication phenomena.

However, this research is not a supplement to the official instructions for use.

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## Conflict of Interest

The authors have no conflict of interest to declare.

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