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**CLINICAL AND LABORATORY DIAGNOSTIC
AND TREATMENT OF HORSES' BABESIOSIS BY DRUG
AZIDIN-VET™ PRODUCED "BROVAFARMA"**

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Conducted clinical, morphological and biochemical studies of blood of horses for spontaneous babesiosis, as well as urine examination of diseased animals. The peculiarity of the characteristic features of the development of horses babesiosis is the dimness of the fur coat, the pallor of the visible mucous membranes, loss of appetite, hyperthermia, thirst, later on apathy, anorexia, pain in the liver; hepatomegaly, icteric conjunctiva, tachycardia, brownish-red urine.

Regarding the biochemical parameters of blood serum, an increase in the activity of one of the information-diagnostic enzymes – AsAT, which indicates the development of inflammatory processes and is a consequence of hepatitis, which is characterized by cytolytic syndrome. Etiotropic therapy included the use of the drug Azidin-vet in a dose of 1 cm³ per 20 kg body weight intramuscularly, in the form of 3.5% aqueous solution, twice, 24 hours in water.

Key words: horses, diagnosis, liver, enzymes, hepatitis, babesiosis, treatment.

Соловійова Л.М., Єрохіна О.М., Пересунько О.Д., Човгун А.М. Клініко-лабораторна діагностика та лікування бабезіозу у коней препаратом Азидин-вет™ виробництва ТОВ «Бровафарма»

Проведені клінічні, морфологічні та біохімічні дослідження крові коней за спонтанного бабезіозу, а також дослідження сечі хворих тварин. Особливістю, характерними ознаками розвитку бабезіозу у коней є тьмяність шерстного покриву, блідість видимих слизових оболонок, зниження апетиту, гіпертермія, спрага, в подальшому розвивається апатія, анорексія, болючість у ділянці печінки, гепатомегалія, іктеричність кон'юнктиви, тахікардія, коричнево-червоний колір сечі.

Щодо біохімічних показників сироватки крові виявлено підвищення активності одного з інформаційно-діагностичних ензимів – АсАТ, що свідчить про розвиток запальних процесів і є наслідком гепатиту, який характеризується цитолітичним синдромом.

Етіотропна терапія включала в себе використання препарату Азидин-вет™ в дозі 1 см³ на 20 кг маси тіла внутрішньом'язово у вигляді 3,5%-го водного розчину дворазово, упродовж 24 год.

Ключові слова: коні, діагностика, печінка, ферменти, гепатит, бабезіоз, лікування.

Соловьёва Л.Н., Ерохина Е.Н., Пересунько Е.Д., Човгун А.Н. Клинико-лабораторная диагностика и лечение бабезиоза у лошадей препаратом Азидин-вет™ производства ООО «Бровафарма»

Проведены клинические, морфологические и биохимические исследования крови лошадей при спонтанном бабезиозе, а также исследование мочи больных животных. Характерными признаками при развитии бабезиоза у лошадей есть шерстный покров без блеска, бледность видимых слизистых оболочек, снижение аппетита, гипертермия, жажда, в дальнейшем развивается апатия, анорексия, болезненность в области печени, иктеричность конъюнктивы, тахикардия, коричнево-красный цвет мочи.

Относительно биохимических показателей сыворотки крови выявлено повышение активности одного из информационно-диагностических энзимов – АсАТ, что свидетельствует о развитии воспалительных процессов и является следствием гепатита, который характеризуется цитолитическим синдромом.

Этиотропная терапия содержала использование препарата Азидин-вет™ в дозе 1 см³ на 20 кг массы тела внутримышечно в виде 3,5%-го водного раствора дважды, на протяжении 24 часов.

Ключевые слова: лошади, диагностика, печень, ферменты, гепатит, бабезиоз.

Formulation of the problem. Babesiosis of horses is a very common disease in all continents. Damage due to this disease depends not only on the direct death of the animal, but also on forced slaughter, long-term decline in productivity, delayed growth in young, significant costs for clinical, laboratory, and preventive and veterinary and sanitary measures [1, p. 102].

Analysis of recent research and publications. In the territory of Ukraine, the babesiosis of horses is found in the zones of distribution of mites-pencil-sharps pyroplasm: first of all, *Dermacentor pictus* (Polissya) and *Dermacentor marginatus* (Forest-steppe) [2, p. 308]. Due to the fact that mites are most active in the spring period, most babesias are registered in May-June. In the future, the activity of ticks is reduced, and the second peak falls at the end of October – the beginning of November. When the temperature is lower than 5° C, the mites burrow into the leaves, they enter into numbness and in this state overwinter. But there are cases of horses disease on babesiosis in winter as well when they are placed in warm stables [3, p. 17]. Ticks are capable of migration, and as a result they gradually populate new territories, forming new foci of diseases [4, p. 11]. An important feature is the ability to pass the pyroplasmogenic agent to the next generation of mite transvariously, as a result of which the plots of land inhabited by invasive ticks, remain dangerous for decades and are the hearth of disease [5, p. 2]. In the organism of odorless, they persist for 1–2 years [6, p. 39; 7, p. 310].

Diagnosis on babesiosis of horses is put in a complex, based on epizootological data, clinical and pathoanatomical picture, as well as laboratory studies, but microscopy of the smear remains the preferred method of diagnosis of babesiosis [8, p. 422].

In the smears of whole blood, stained by Romanovsky-Gimza or by Mai-Grunwald, the characteristic forms of the pathogen are detected [9, p. 24; 10, p. 5; 11, p. 20]. It forms erythrocytes in the erythrocytes, amoeboid, single and paired pear-like inclusions, and usually they are more than the radius of red blood cells. The pears are joined together by a thin jumper at an acute angle. The number of erythrocytes damaged by babies in the blood of sick horses is 6–10% [9, p. 25; 10, p. 7; 11, p. 22]. When animals die, smears-imprints of parenchymal organs.

In the last decades, serological methods have been developed for the detection of antigens in the serum of babies: RPC (complement fixation reaction), RPZK (extended complement fixation), RIF (immunofluorescence reaction) and RNLL (indirect hemagglutination reaction) and RID (immunodiffusion reaction) for accurate diagnosis [12, p. 560].

In the world practice for the diagnosis of pyroplasmosis, an immunoassay (ELISA), an enzyme-dependent immunosorbent assay (ELISA) [13, p. 42; 14, p. 2; 15, p. 250] is used.

To date, no effective vaccines have been developed to prevent the spread of this invasion, so the basic methods of fighting babesiosis is their early diagnosis. Development of methods of DNA-diagnosis of piroplasmizoses of horses is one of the urgent tasks of modern clinical veterinary medicine. Methods of DNA-diagnosis of animal diseases are already widely implemented in the practice of veterinary medicine [16, p. 44], the high sensitivity of this reaction is detected in the detection of parasitemia at very low levels of invasiveness. Beginning in 1998, the practice of studying baccidiosis of horses is a

method of using PCR-diagnosis (polymerase chain reaction), which is highly effective in mixed infections [13, p. 42].

That is why the study of the occurrence of internal pathology for babesiosis in horses, the substantiation of the informativeness of diagnostic methods, early diagnostic criteria for evaluation of hemocytopoiesis, functional state of the liver, and treatment during the rehabilitation of postbabesiosis state are relevant.

The purpose of the study is to: – establish informative clinical and laboratory tests for the diagnosis of baccidiosis in horses and the effectiveness of treatment with the use of Azidin-vet product manufactured by BrovaPharm.

Material and methods of research. For research, experimental and control groups of animals were formed, with five goals in each. All animals had symptoms of babesiosis. They were held in the private sector of the Polonsky district of Khmelnytsky region, aged 2 to 7 years. The vast majority (4 out of 5 – 80.0%) of horses were male by gender. According to the results of anamnesis, it was found that all diseased animals were attacked by ixodic ticks in the pastures and in the premises where they were kept.

Investigation of the clinical condition of animals was carried out by examination and palpation. For laboratory diagnosis of babesiosis, they took the first drop of blood from the tip of the horse's ear on the substrate, making a smear. The stroke was fixed with Nikiforov liquid (ethanol ether) and dyed azure-eosin for the Romanovsky-Gimza. Detection in red blood cells of blue-colored parasites was the basis for the diagnosis. After the clinical examination of the animals, laboratory blood tests were performed on the hematological parameters. The number of erythrocytes in the blood was determined by the test tube method, in the chamber with Goryaev's net, the hemoglobin content is the hemoglobin-cyanide method.

In serum, the content of albumin, AsAT activity, alkaline phosphatase was determined. For this purpose, a biochemistry universal analyzer RAYTO 1904C of clinical and diagnostic laboratory of the Faculty of Veterinary Medicine of ZNAEU and diagnostic kits were used. The obtained results were processed by statistical methods. For medical purposes, the drug Azidin-vet was used.

Main results of the study. After analyzing the seasonal dynamics of babesiosis, it was noted that most cases of horses' disease were registered in May (29.3%) during the period of mass distribution of ixodic ticks, less frequently in the first decade of June (8.5%). The next wave of diseases of horses for babesiosis was registered in October (3.1%) and November (1.2%). The most optimal for the infection is the ambient temperature in May from 12–13 to 21° C, in which ticks are the most active.

We found that at young age (up to 2 years old) horses had a mild babesiosis, severe clinical course was observed in animals over the age of 2–3 years. During the inspection, it was found that in all 100% of animals the skin was dry, pale, and the hair cover was dull.

Diseased horses had a fever of constant type for 3–4 days, tachycardia, tachypnoe, loss of appetite, thirst, later on apathy and anorexia developed. With the progression of disease of horses, frequent, painful urination was observed. Urine was yellow to red, and on the 3rd and 4th day of illness urine acquired a brownish-red color. The pallor of the mucous membranes (conjunctiva, oral cavity) was detected in 100% of horses, indicating a disturbance in the blood circulation and the development of anemia, only on the 4th day of the disease in 100% of animals, mucous membranes acquired yellow color. The studies we conducted showed that adult horses were ill with babesiosis in severe form, which, in case of untimely therapy, could lead to the death of animals.

In the blood of spontaneously invasive animals, a sharp decrease in the number of erythrocytes was observed to $4.1 \pm 0.7 \text{ T / L}$ ($p < 0.01$). At the time of the appearance of blood urine there was a sharp decrease in the number of red blood cells. At the beginning of the disease in red blood cells little parasites (one in erythrocyte). The highest number of babies in the peripheral blood was observed on the 2nd or 3rd day after detection. There were 1–3 triggers in the field of view of the microscope, sometimes up to 15 forms. Often noted the presence of 2 pathogens in the 1 erythrocyte. Affected erythrocytes were often larger in size and acquired an irregular shape. During an acute course, anisocytosis, pojecylocytosis was observed, indicating a functional insufficiency of the hematopoietic organs and the development of hemolytic anemia, and is a consequence of the disease.

Hemoglobin is hematologic marker for the development of babesiosis in horses. From the examined blood samples taken from sick horses, in 100% of horses it was critical ($60,6 \pm 8,5 \text{ g / l}$), which indicates the development of anemia. In addition to the changes observed in the hemocytopoiesis system, we investigated the functional state of the liver, in particular the study of protein, carbohydrate metabolism and enzyme diagnosis. In 100% of diseased animals, typical symptoms of liver damage were diagnosed: icterus of sclera and visible mucous membranes, pain in the liver and hepatomegaly.

Symptom of a protein metabolism disorder was the decrease in the number of albumins at the onset of the disease to $32.1 \pm 1.31\%$, with the subsequent development of the disease to $29.9 \pm 1.47\%$ (physiological fluctuations 35–45%) [3, p. 5].

In the analysis of the biochemical parameters of blood serum of horses infected by bacillus pathogens, we found that the activity of aspartate aminotransferase (ASAT) was quite high compared with similar indicators of animals in the control group and amounted to $291.3 \pm 10.5 \text{ OD / l}$ ($p < 0, 05$). The level of activity of AsAT in 10 (100%) sick animals was elevated from the first days of manifestation of the disease, indicating the damage to hepatocytes. Hyperfermentemia occurs more quickly than the change in other biochemical parameters, therefore, in acute inflammatory processes in the liver, the activity of enzymes is rapidly increasing, and in the transition to the chronic stage, it decreases somewhat, but does not return to the physiological limits. Therefore, the most significant for liver disease is the growth of the activity of AsAT.

Due to the development of cholestasis, blockage and damage to the bile duct in blood serum of animals increases the activity of alkaline phosphatase (LF). This indicated a pathology outside the hepatic extrahepatic bile duct. Consequently, if jaundice develops, the enzymes mentioned are more informative about the pathological process than conjugated bilirubin, since it pointed to the localization of the defeat.

Therefore, during these periods, elimination in the blood of LF increased ($216.0 \pm 5.2 \text{ OD / l}$), which was why its index was significantly ($p < 0.05$) higher compared to control animals, which meant the development of intrahepatic holestasis in horses.

The patients with babesiosis were isolated, released from all types of work and prescribed dietary nutrition for the usual need for energy and nutrients.

In our practice for performing the experiment, we used Azidin-vet produced by LLC Brovafarma at a dose of 1 cm^3 for 20 kg of body weight intramuscularly, in the form of 3.5% aqueous solution, twice, for 24 hours, for the purpose of complete sterilization of the organism from pathogens. In the horses complications during the introduction of Azidine-Vet was observed. With the development of anemic syndrome for sick animals, with the combined course of several diseases, treatment was carried out in a complex manner, taking into account the etiology and pathogenesis of the disease. Thus, the use of caffeine-benzoate led to the excitation of the vascular center and the strengthening of

the heart. Under the influence of caffeine, the gas exchange in cells was improved, the motor function of the digestive tract was partially regulated, the metabolic processes were restored, and smooth muscle spasms were reduced.

For the treatment of horses with signs of babesiosis also used complex therapy, namely drugs – analgin, dimedrol, antioxidant – ascorbic acid used as part of a desensitizing 5% glucose solution and contrikal, panangin dissolved in 0.9% NaCl – intravenously, jet; furosemid, immunostimulant – catozal, preparations that stimulate hemocytopoiesis – vitamin B₁₂, ferrovit; hepatoprotector tyotriazolin – intramuscularly.

Analyzing the complex treatment of patients with horses with signs of anemia and hepatotoxic syndrome, we found that the therapeutic measures carried out over a period of 10 days lead to a partial restoration of the hemocytopoiesis and the functional state of the liver. The number of erythrocytes and hemoglobin content, although recovering to medium physiological limits, did not reach the values that were in the group of clinically healthy horses. Obviously, the course of rehabilitation should be longer, and the sick animals should be subject to quarterly dispensaries as an important element in controlling health and body functions.

Conclusions:

1. Diagnosis of babesiosis of horses was established in a complex manner, taking into account epizootological data, clinical signs of the disease and the results of microscopy of blood smears with the identification of characteristic forms of babesia.

2. According to the pathology of the hemocytopoiesis system, the most informative are the results of laboratory blood tests, progressive erythrocytopenia, oligochromia, hemolytic anemia. Acute course was observed anisocytosis, poikilocytosis, indicating a functional insufficiency of the hematopoietic organs. Babesiosis unpaired herpes differentiate from infectious anemia.

3. Typical clinical signs for babesiosis of horses are: dimness of the wool, pallor of visible mucous membranes, loss of appetite, hyperthermia, thirst, later on – apathy, anorexia, pain in the area of the liver field, hepatomegaly, conjunctiva icterus, tachycardia, brownish appearance – red color of urine.

4. The obtained results made it possible to analyze the dynamics of biochemical parameters of blood serum. The increase in activity of one of the information-diagnostic enzymes – AsAT, which leads to the development of hepatitis in patients with horses, characterized by cytolytic syndrome.

5. For the treatment of baccidiosis in horses from specific means of basic etiotropic therapy, Azidine-vet was used in a dose of 1 cm³ for 20 kg of body weight intramuscularly, in the form of 3.5% aqueous solution, twice, for 24 hours. From the means of symptomatic Therapy was used drugs – analgin, dimedrol, antioxidant – ascorbic acid in the desensitizing 5% solution of glucose and contrikal, panangin dissolved in 0.9% NaCl – intravenously, jet; furosemid, immunostimulant – catozal, preparations stimulating hemocytopoiesis – vitamin B₁₂, fermented; hepatoprotector tyotriazolin – intramuscularly.

Prospects for further research are the implemented measures for the prevention of babesiosis in horses aimed primarily at the break of the chain: an invasive mite-carrier – a susceptible animal, which includes a complex of organizational-economic and agroeconomic measures aimed at the destruction of biotops of mite-carriers, the organization of cultural pastures, free from arthropods, and grazing on them horses.

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