ANNOTATION

Dissertation work of Zhabykpayeva Aigul Gabyzkhanovna on the topic: “Study of ixodofauna in the Kostanay region and prevention of babesiosis in dogs” submitted for the degree of Doctor of Philosophy (PhD) in the educational program 8D09101-Veterinary Medicine

Relevance of the research topic. Ixodic ticks (Ixodoidea ticks) are blood-sucking arthropods, belong to the most widespread ectoparasites in the world and are reservoirs and carriers of many pathogens of dangerous viral, bacterial and protozoan diseases of humans and animals. The presence of a single mechanism of transmission of pathogens, common hosts and vectors determines the existence of combined natural foci of human and animal infections: encephalitis, borelliosis, granulocytic anaplosmosis, ehrlichiosis, nuttaliosis, babesiosis and teileriosis, causing significant damage to human and animal health and with considerable mortality.

It is difficult to overestimate the medical and veterinary significance of ixodes ticks, and, nevertheless, it is known that intensive study of the fauna of blood-sucking ticks in Kazakhstan was in Soviet times in the 50-80 years of the 20th century. In the future, studies of the fauna and taxonomy of ixodic mites were only episodic. Various sources provide some information about the faunal complexes of ixodes mites in the zones of the southern, western and eastern regions of Kazakhstan. To date, there is no modern data on the fauna of ixodes ticks in the northern region of the country, and the latest information on this topic can be found only in the literature of the 20th century (50-60 years).

In recent decades, the disturbance of the ecological balance, climatic changes, and intensive anthropogenic impact on faunal complexes have affected the number of ixodes ticks, their epidemiological activity and on the borders of the geographical areas of parasites.

The geographical location of the Kostanay region, bordering on well-known natural foci of blood parasitic infections located in the Chelyabinsk, Orenburg and Kurgan regions of the Russian Federation, as well as the variety of landscape and climatic conditions and wildlife create favorable conditions for the circulation of ixodes ticks infected with various pathogens.

Until now, many aspects of the epizootic manifestation of vector-borne diseases of carnivorous animals, in particular in dogs, have not yet been sufficiently studied and explained in various conditions. The most common and clinically significant disease in dogs is babesiosis, which causes irreparable harm to the animal's health. Every year, the problem of babesiosis becomes more acute, this is facilitated by the formation of biotopes of ixodic ticks in urban areas, as well as an increase in the number of dogs owned by private owners.

Despite the urgency of the problem and the presence of climatic, biological and epizootological prerequisites for the existence of infected ixodic ticks, insufficient attention is paid to the study of canine babesiosis. And, as a result, we have scant information regarding the prevalence, epidemiology, diagnosis and

treatment of vector-borne diseases of dogs, as well as diseases of zoonotic concern. An important aspect is the lack of information about the circulating strain of canine babesiosis in the territory of Kostanay region. Without proven facts of the endemic condition, there is no opportunity to improve the complex of therapeutic and preventive measures against babesiosis in dogs.

In this regard, modern clarification of the ixodofauna, specific vectors of canine babesiosis and epizootology of the disease in order to organize effective preventive and curative measures is relevant.

The purpose of the dissertation research: To study the species diversity of ixodes ticks in the territory of Kostanay region with the identification of vectors of babesiosis of dogs. To study the spread, features of the epizootic process of canine babesiosis and the improvement of protocols for the treatment and prevention of the disease.**The object of research:** ixodic ticks, dogs spontaneously infected with babesiosis.

Research objectives:

1. Monitoring and identification of the species diversity of ixodes ticks in the territory of Kostanay region (geographical distribution).
2. To identify infection of ixodes ticks with the causative agent Babesia spp with molecular genetic identification of babesia in ticks and in blood samples of sick dogs.
3. To analyze the epizootic situation of babesiosis of dogs in the territory of Kostanay region with the study of the features of epizootology: the dynamics of morbidity, seasonality, manifestations of the disease, the influence of pedigree, sexual and age factors on the occurrence of the disease.
4. To study the morphometric parameters of the babesias of dogs found in the Kostanay region and to conduct clinical, hematological and biochemical studies of dogs with babesiosis.
5. To improve preventive measures and the protocol for the treatment of canine babesiosis and introduce it into the practice of veterinary clinics in Kostanay.

Research methods: parasitological (microscopy, collection and accounting of the number of ticks, determination of the extent of invasion, the level of parasitemia); epizootological (monitoring of the spread, analysis of sexual, age and seasonal dynamics); physical (examination, palpation, percussion, auscultation and thermometry); hematological (clinical and biochemical blood analysis); morphometry; PCR-research, molecular genetic sequencing and statistical analysis.

Provisions for protection

The species diversity of ixodes mites in the territory of Kostanay region, depending on the landscape and climatic zones.

The species of ixodes tick D.reticulatus is the main vector of babesiosis of dogs in the territory of Kostanay region.

Babesia canis is the main etiological agent of canine babesiosis in Kostanay region.

The distribution and features of the epizootology of canine babesiosis in the Kostanay region.

Improved preventive measures and protocol for the treatment of canine babesiosis in veterinary clinics of Kostanay and Kostanay region.

Scientific novelty

New and up-to-date data on the species diversity, distribution and seasonality of parasitization of ixodic ticks in the territory of Kostanay region - the northern region of Kazakhstan are presented.

For the first time, ticks living in the territory of Kostanay region were studied for the presence of babesias and the infection of ixodes ticks Babesia canis was determined using molecular genetic methods.

The epizootic situation and the dynamics of the incidence of canine babesiosis in the seasonal and age aspect in the conditions of the northern region of Kazakhstan have been studied.

A scientifically based effective therapeutic protocol with the use of Prednisolone and Dufalac drugs for babesiosis in dogs has been developed and tested and implemented in the practice of veterinary clinics in the city of Kostanay.

Scientifically based improved effective preventive measures are proposed to reduce the spread of the incidence of Babesia canis.

A new method for preparing a permanent preparation of ticks on a slide is proposed. Utility model Patent No. 4171.

The practical significance of the results obtained.

The results obtained expand and clarify information about the ixodofauna of the Kostanay region of Kazakhstan. The role of ixodic ticks in the spread of the pathogen Babesia canis. They make a significant contribution to solving the urgent problem of combating natural focal diseases. Determining the dynamics of the incidence of canine babesiosis in the seasonal and age aspect were additional data revealing the features of the epizootology of invasion in the conditions of the northern region of Kazakhstan.

Effective treatment protocols using Prednisolone and Dufalac drugs have been tested and introduced into their practice in the conditions of veterinary clinics in the city of Kostanay for babesiosis of dogs. Preventive measures have been improved and recommended to prevent babesiosis of dogs. The acts of implementation in the veterinary clinics of Kostanay "VET ZABOTA", "Dogma", "Aibolit", and the clinic of Rudny "Vetlab".

The research results are used in the educational process of the parasitology course at the Department of Parasitology and Tropical Veterinary Medicine of the National University of Bioresources and Environmental Management of Ukraine, Kiev No. 3 dated 03/14/2018.

The results of the work have been tested and presented in the form of:

- practical recommendations for the treatment and prevention of babesiosis in dogs – "Babesiosis of dogs. Distribution in the Kostanay region, diagnosis, treatment and prevention" (approved by the Scientific and Technical Council of the Kostanay Regional University named after A.Baitursynuly", Protocol No. 1 dated 09/13/2024).

- the textbook "Babesiosis of animals (epizootology, biology, diagnostics of a species determinant)" for students in the specialties of veterinary medicine and

biology, to help teachers of higher and secondary specialized educational institutions, veterinarians and specialists of diagnostic centers (approved by the Council of the Faculty of Veterinary Medicine and Animal Husbandry Technology, Kostanay State University named after A.Baitursynov, Protocol No. 3 dated 03/29/2018).

- patent for utility model No. 4171 "Method for preparing a permanent preparation from ticks on a slide".

They are listed in the GenBank database of the National Center for Biotechnology Information of the USA (NCBI) under the number MK070118.1. "Nucleotide sequences of Babesia canis isolated from mites of biotopes of Kostanay region (Babesia canis isolate Kaz-Dr93 small subunit ribosomal RNA gene, partial sequence)".

The results of the research can be used in lecture courses and laboratory classes on ecology, zoology, parasitology, in the performance of theses, master's, doctoral dissertations and scientific research.

Approbation of the results of the dissertation.

- at the International Scientific and Practical Conference of the Institute of Veterinary Medicine "Problems of veterinary medicine, veterinary and sanitary expertise, biotechnology and animal science at the present stage of development of the agro-industrial complex of Russia". Chelyabinsk: South Ural State Agrarian University, - 2018 – pp.75-82

- at the III International Scientific and Practical Conference dedicated to the memory of Doctor of Agricultural Sciences, Professor Bakytzhan Muslimov (December 10, 2020). - Kostanay: A.Baitursynov KRU, 2020. – pp. 68-74

- at the International scientific and practical conference "Baitursynov readings-2024", Kostanay: KRU named afterAkhmet Baitursynuly 2024 – pp. 177-180.

The results of dissertation research were reviewed at the meeting of the Academic Council (2018), the Scientific and Technical Council (2019), and the inter-cathedral meeting of the Akhmet Baytursynuly KRU (2024).

Publications

The results of the dissertation research are reflected in 9 printed works: an article in the foreign rating publication "Open veterinary Journal", percentile-52, 2023 Sep; 13(9):1184-1194, in publications recommended by the KOKSNVO of the Ministry of Internal Affairs of the Republic of Kazakhstan - 4 articles, in the materials of international conferences – 3 articles (Appendix G), as well as the patent for the utility model of the Republic of Kazakhstan No.4171.

The degree of reliability of the results

The reliability of the results is confirmed by a sufficiently large volume of research conducted, adequate to the purpose and objectives of the research, experimental verification of the main provisions of the research hypothesis, data processing by statistical methods, the use of modern high-tech equipment and approbation of the results.

Description of the main research results

1. Monitoring of the species diversity of the ixodofauna (2017-2021) showed that 3 genera of Ixod mites are found in the territory of Kostanay region: the genus Dermacentor, the genus Hyaloma and the genus Rhipicephalus. A significant part of ixodes mites - 98.7% - belongs to the genus Dermacentor, which is represented by 3 species of mites D.reticulatus, D.marginatus, and the less common species D. niveus. From the genus Hyaloma, one species of H. scupence is found in the area. Ticks of the genus Rhipicephalus are represented by one species Rh. schulzei.

2. The prevalence of ixodes ticks in the territory of Kostanay region varies, depending on the landscape and climatic zones. Thus, in all regions of the region, the dominant species of ticks are D. reticulatus (53.2%) and D. marginatus (44.7%). Ticks of the D.reticulatus species are widespread in large numbers in the zone of birch and pine forests with the seizure of steppe areas in the districts of Karabalyksky (6.4%), Fedorovsky (5.8%), and Mendykarinsky (4.5%), as well as in the vicinity and within the cities of Kostanay (18.9%), and Rudny (5.2%). Ticks D. marginatus parasitize in the steppe zone with a dry climate - the Zhitikarinsky district (10.0%), and in the southern districts of Dzhangeldinsky (3.2%), and the city of Arkalyk 4.6%. Rare species of ticks are found in the south of the region: in the Dzhangeldy district, the species H.scupence (0.9%) and Rh. schulzei (0.4%), in the vicinity of Arkalyk, the species D. niveus (0.7%).

3. Molecular genetic methods have established that the main vector of babesiosis of dogs in the territory of Kostanay region are ixodes ticks of the species D.reticulatus. DNA sequencing and phylogenetic analysis confirmed the presence of the parasite of the genus Babesia canis in ticks of the species D.reticulatus and in blood samples of sick dogs.

4. The analysis of the epizootic situation from 2013 to 2023 showed that there are synanthropic foci of babesiosis of dogs in the territory of Kostanay region. The disease is registered annually, unevenly with epizootic fluctuations of rise and decline. Thus, the indicator of the extent of invasion in the region was the highest in 2016 and 2018 and amounted to 41.69% and 42.5%, respectively. A relatively low percentage of morbidity was observed in 2014 - 28.24% and in 2022 – 27.21%. In the remaining years, the average incidence rates of dogs ranged from 31.43% to 39.93%. The spread of babesiosis in dogs is closely related to the spread of ticks that carry the invasion.

5. In total, 2125 dogs were examined in the period 2017-2021, 986 of them were diagnosed with Babesia canis, which is 46.4% of the infection rate. InKostanay and its environs are massively infected with dogs in spring in April and early May, the extent of the invasion reaches up to 74.83%, the second peak of the disease occurs in autumn in September and is 53.8%. The intensity of invasion in spring is 5-20%, in autumn 10-30%. Long-haired and medium-haired breeds are more likely to get sick (German Shepherds 18.3%, Pekingese 11.5%, Central Asian Shepherds 8.7%). Dogs under 4 years of age are more susceptible to babesiosis by 9.1-37.3%. As dogs grow older, there is a tendency to decrease the incidence.

6. Morphometric parameters of babesias found in the blood of dogs have a variety of shapes and sizes typical of the disease. The size of the parasites varies from 0.87 to 5.93 microns, the shapes are irregular, unconnected, rounded, oval,

single and paired pear-shaped. The number of babesias in one erythrocyte ranges from 1 to 12.

7. Clinical manifestations of canine babesiosis range from the acute form of the disease to multiple organ failure, depending on the age of the animal with a risk of mortality. With an increase in the intensity of the invasion of Babesia canis, the degree of pathological changes in the body of dogs increases, which is reflected in a change in the hematological and biochemical profile depending on the course of the disease.

8. It has been established that the most effective protocol for the treatment of dogs with babesiosis is an improved protocol including a combination of drugs Dufalac (orally 0.3 ml / kg 2 times a day for 5 days) and Prednisone (2 mg /kg intramuscularly 1 time a day for 5 days). The treatment protocol provides optimal results: reduces the risk of complications and severity of the disease, a favorable therapeutic response occurs on 5.25 ± 0.3 days, which is 2 times faster than the treatment protocol adopted in the clinics of Kostanay.

9. The study of the effectiveness of the use of insect-acaricides in the conditions of the Kostanay region for the prevention of canine babesiosis has shown that the best protective effect against tick attacks are "Foresto" collars with repellent properties up to 6 months and multicomponent preparations including four strong insect-acaricides - drops "Bars" and "Inspector QuadroC" with a protection period up to 30 days. Leopard and Inspector drops must be used to protect dogs throughout the tick activity season: 6 times every 30 days. If dogs are in the forest area for a long time, in suburban areas, additionally spray the lower part of the animal's body (abdomen, paws) with Leopard spray every 2 days.

Practical recommendations

The results of epizootological monitoring of canine babesiosis in the territory of Kostanay region, as well as on the basis of our own research, developed practical recommendations "Babesiosis of dogs. Distribution in the Kostanay region, diagnosis, treatment and prevention", allow us to give the following recommendations:

1. Practicing veterinarians:

- treatment of canine babesiosis should be comprehensive, in addition to specific antiprotozoal chemotherapy, it should include symptomatic, pathogenetic and immunostimulating therapy. During the rehabilitation period, the animal should be prescribed diet therapy with limited physical activity;

- in case of babesiosis of dogs, in the generally accepted treatment protocol in combination with the specific antiprotozoal drug Pyro-stop and the supportive drugs Hemobalance, Stereofundin and hepatoprotector, we recommend to include Prednisolone at a dose of 2 mg / kg intramuscularly, 3-5 days, depending on the condition of the animal, to prevent immuno-mediated hemolytic anemia and Dufalac at a dose of 0.3 ml in order to prevent complications/ kg 2-3 times a day for 7 days to eliminate toxic substances from the body;

- for early detection of possible complications of the disease, it is necessary to carry out mandatory monitoring of hematological and biochemical parameters of

blood and urine of sick dogs, both during treatment and during the rehabilitation period;

- informing dog owners about ixodic ticks and the diseases they carry. Informing about the beginning of the tick activation period, which begins with the establishment of warm temperatures of +5oC-+12oC in Kostanay region from the end of March to the beginning of April and until November;

- Pretreatment of pets with acaricidal preparations is recommended before and during the seasons of tick activity.

2. Dog owners

It is necessary to carry out general prevention, which consists in the individual protection of dogs from ixodic ticks:

- during the period of maximum activity of ixodic ticks, limit dog walking in forest and park areas with good grass and near reservoirs;

- after each walk, regular inspection of animals for the presence of ticks;

- extraction of ticks within 24 hours from the dog's body (from the moment it starts feeding) and the need to eliminate it;

- the correct extraction of ticks from the body of animals, using special tools;

- treatment of dogs with insect-acaricidal drugs during the tick activity season: Leopard drops or QuadroC Inspector drops must be used to protect dogs during the entire tick activity season 6 times every 30 days. If dogs are in the forest area for a long time, in suburban areas, additionally spray the lower part of the animal's body (abdomen, paws) with Leopard spray every 2 days. Or the use of Foresto collars with repellent properties for up to 6 months.

3. Specialists of the Veterinary Department

For the prevention and control of tick-borne diseases:

- it is necessary to regulate the population of stray dogs in the territory of the city of Kostanay and in the adjacent territories, which will prevent the possibility of new biotopes of invasion and reduce the risk of infection of dogs;

- in order to prevent the introduction of blood parasitic infections into the territory of the region, it is necessary to quarantine newly imported dogs and examine peripheral blood for the presence of babesias;

- to treat dogs with anti-tick drugs traveling to other regions of the country;

- it is necessary to inform the population about endemic areas and control dog walking in these territories;

- in Kostanay, on the territory of park areas and surroundings with high herbage, anti-tick treatments should be carried out annually;

- to carry out deacarization on the territory of dog kennels and aviaries.

The volume and structure of the dissertation. The research materials are presented on 130 pages of computer text. The components of the dissertation work included: introduction, main part and conclusion. The list of references contains 187 titles. The text of the work contains 39 figures, 24 tables and 12 appendices.