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The influence of ticks on the spread of vector-borne and arbovirus diseases in the West Kazakhstan region

R.N. Toleuova*[1], B.K. Essimov[2], A.U. Tuyakbayeva[3], G.K. Mussayeva[4], K.I. Akmetov[5], M.A. Berdikulov[6], K.T. Maikhin[7], B. Kazybay[8], G. Assylbekova[9]

[1,2]Abai Kazakh National Pedagogical University, Almaty, Kazakhstan
[3]L.N. Gumilyov Eurasian National University, Astana, Kazakhstan
[4,6,7]Almaty branch «National veterinary reference center», Almaty, Kazakhstan
[5]Toraigyrov University, Pavlodar, Kazakhstan
[8]Kyrgyz National Agrarian University, Bishkek, Kyrgyzstan
[9]Margulan University, Pavlodar, Kazakhstan

*E-mail: *[1]raushan_gul_85@mail.ru, [2]esimov.bolat@mail.ru, [3]akmaral.t@inbox.ru, [4]musaeva_1984@mail.ru, [5]kairat_akhmetov@mail.ru, [6]berdikulov.ma@mail.ru, [7]maikhin67@mail.ru, [8]botakoz28091983@gmail.com, [9]asylbekovage@ppu.edu.kz*

Abstract. The article discusses the findings of a study investigating the impact of ticks on the transmission of vector-borne and arboviral diseases among livestock in the West Kazakhstan region. The epizootic situation with blood-sucking ticks in farm animals has also been studied. The results presented in this article show a potential solution to many problems associated with the spread of tick-borne infections. The analysis of epizootic indicators used for monitoring and zoning the territory of Western Kazakhstan according to the degree of intensity of the epizootic situation and a number of infections among farm animals has been carried out. To monitor and zone the territory of Western Kazakhstan according to the degree of intensity of the epizootic situation for the following viral infections among farm animals (bluetongue, Schmallenberg's disease, nodular dermatitis of cattle, Crimean-Congo haemorrhagic fever, etc.). The methods developed and adapted for the detection and strain differentiation of the virus were used, based on PCR and nucleotide sequencing, followed by analysis of the amplified nucleotide sequences of the genome of isolates of identified animal viruses in Western Kazakhstan. The results are of scientific and practical interest to researchers from neighboring countries: Central Asia, Russia, China, etc.

Keywords: ticks, species composition, vector-borne diseases, arbovirus infections, epizootic situation

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**Corresponding authors*

Introduction

Due to the expansion of logistics ties, animal migration, and climate change, the economy and biological security of many countries are threatened. A significant contribution to the destabilization of the epidemiological situation is made by such exotic viral infections as bluetongue, Schmallenberg's disease, nodular dermatitis of cattle, and Crimean-Congo haemorrhagic fever among people and farm animals.

Several European nations face significant risks related to the introduction and dissemination of viral diseases such as nodular dermatitis in cattle, bluetongue, and Schmallenberg disease. This concern is exacerbated by the unfavorable conditions in animal husbandry and the anticipated importation of livestock from these countries into the Republic of Kazakhstan. Evidence of the existing danger is the fact that cases of importation of seropositive animals for Schmallenberg and bluetongue disease are known in our country. Diagnostic studies on these infections are carried out within the framework of the rules for quarantining animals imported from third countries [1-5].

Despite all the measures taken by various international organizations, the epizootic situation of infectious diseases of animals and birds in the world remains tense. Of particular concern are diseases of Group A, which, according to the classification of the Office International des Epizooties (OIE, World Organisation for Animal Health), are dangerous. It is known that outbreaks of diseases of this group cause enormous socio-economic damage to the state. The Republic of Kazakhstan is clean about these diseases of animals, but every year in some part of our country, as a result of the introduction of infections from unfavorable countries, outbreaks of particularly dangerous diseases are recorded. Many authors consider ticks as the main factor in the source of threats to the livestock of our country, particularly dangerous animal diseases [6-9].

The peculiarity of some transmissive diseases is that their pathogens can remain in the bodies of certain carrier vectors for a long time. The most obvious natural focus of vector diseases is where the spread of infection occurs with blood-sucking arthropods (ticks, fleas, mosquitoes, etc.). The causative agent of the disease constantly circulates along this chain: the carrier animal is a person or an animal. Disease-sensitive animals, pathogens, vectors, or intermediate hosts are the links of the biocenosis associated with a particular biotope. Therefore, natural focal diseases are characterized by strict seasonality, territorial distribution, and clear designation of a specific type of landscape (for example, for the deserts of Central Asia - leishmaniasis of the skin and spirochetosis of ticks, etc.), in contrast to anthroponoses. A natural hearth can contain pathogens of several diseases, as well as various types of animals susceptible to these diseases [10-15].

Active natural foci of Crimean-Congolese hemorrhagic fever are located in Zhambyl, South Kazakhstan, and Kyzylorda regions. The incidence of CCHF is recorded annually in these regions. The main carriers of CCHF in this territory are *Hyalomma anatolicum anatolicum* and *Hyalomma detrium,* Ixod mites that parasitize mainly farm animals. *Boophilus calcaratus* plays an important role in infecting the population. An important role in epidemiology is played by ticks of the genus *Dermacentor niveus,* which are found mainly in the northern part of the hearth - in the lower reaches of the Shu River, in the northern part of the Sarysu and Moyinkum districts of the region [16-18].

The West Kazakhstan region (WKR) borders on the territory of Russia, where there are natural foci of Crimean-Congo hemorrhagic fever (CCHF) and diseases are periodically recorded. In 2007, specific antibodies to the CCHF virus in cattle were detected for the first time in the

western part of the West Kazakhstan region using the complement fixation reaction. 1,871 cattle were studied in this area for three years. Antibodies specific to the virus are found in 25 cows (1.3%). The annual stable circulation of the CCHF virus has been established.

In 2011, 2180 ticks were collected from cattle in the territory of three administrative districts in the west of the West Kazakhstan region and examined in the CCHF. In this area, high budding of *Hyalomma marginatum* cattle was found. This type of tick is the main carrier of CCHF in neighboring regions of Russia. As a result of comprehensive research, a new natural focus of Crimean-Congo hemorrhagic fever was identified in Kazakhstan in the west of the West Kazakhstan region [19].

Research on the causative agent of bluetongue's disease among animals in the Republic of Kazakhstan has so far been unambiguous, and as for the Schmallenberg disease virus, it has not yet been isolated and is not available for research work. In our country, the role of blood-sucking mites in relation to the spread of bluetongue and Schmallenberg diseases and the epizootic situation in these diseases in general have not been monitored, in connection with which there is almost no scientifically based national strategy and measures to combat these diseases [20].

Taking into account the epizootic situation with the participation of blood-sucking mites as a source of infection among farm animals and regional features, the research work planned to be carried out on the scale of West Kazakhstan was not fully carried out.

This study aims to perform monitoring investigations to identify infectious vector-borne diseases affecting both livestock and humans in western Kazakhstan. To achieve this goal, monitoring studies were conducted to assess the species composition and primary host feeders of ticks that pose a risk for transmitting viral vector diseases to farm animals and humans, including bluetongue, Schmallenberg's disease, cattle nodular dermatitis, and Crimean-Congo hemorrhagic fever, among others, in the region.

Materials and research methods

When conducting the study, standard methods recommended by the OIE for the diagnosis of infectious diseases were used. The material for the study was samples of biological material collected by generally accepted parasitological methods from farm animals – ticks. The collection of ticks was carried out by generally accepted methods in places of public gathering (parks, etc.), private farmsteads, herds, and flocks [29,30]. Bacteriological, virological, molecular biological, and serological studies of the collected ticks were carried out.

Ticks collected from animals were placed in empty, dry, hermetically resealable plastic containers. For research, live and dead ticks were delivered to the Almaty branch of the "National Veterinary Reference Center". Several ticks collected from the same animal were analyzed together in a single sample. The processing of collected ticks in the chamber, along with their identification by genus and species, was conducted using specialized keys designed for identifying ticks belonging to the *Ixodidae* family [31].

The identification of the dominant host of ixod ticks was carried out by examining 10% of the total livestock of the selected herd in a particular locality - animals, taking into account the number of ticks and determining the average number of ticks per animal using the following formula:

$$B = (K \times 100): C, \quad (1)$$

where B is the type of animal, K is the number of ticks found in animals of this species, and C is the sum of ticks of this species found in all animal species.

Epizootological monitoring of livestock in West Kazakhstan was conducted through the analysis of statistical data derived from our research reports, along with the findings from virological monitoring studies utilizing PCR techniques.

The mite suspension was frozen and thawed three times, then processed by centrifugation at 600 g for 10 minutes. To isolate the virus, a pool was prepared from several individuals of the same species of ticks. The mites use 2.0 ml tubes containing zirconium-Silicon beads (6 mm SSB60) and DMEM cultivation medium (CAPRICORN Lot No: CP23-6146) (DNA/RNA free Eppendorf). crushed with a homogenizer (LabSafer TS-48/64).

Special PureLink Microbiome DNA Purification Kit "Invitrogen", Catalog Number a29790, Pub to extract (isolate) DNA and RNA from clinical material to extract nucleic acids from tick homogenates. No. MAN0014332 Rev. A.0. a set of reagents was used.

Amplification of tick viruses. The presence of CCHFV, SBV, BTV and LSDV RNA/DNA in ixod ticks was molecularly tested using a single-stage RT-PCR or built-in RT-PCR using a set of special primers for each virus according to the instructions described in real-time commercial RT-PCR kits ("Amplisens® Moscow, Russia). Real-time PCR was performed using a thermocycler Rotor-Gene Q (Qiagen, Hilden, Germany).

According to the level of equipment of the material and technical base, the National Veterinary Reference Center laboratory has a Biosafety Level 3 (BSL-3), which is an advanced institution in the Republic of Kazakhstan that ensures full sanitary, epidemiological, and environmental safety and meets all requirements when working with pathogens of particularly dangerous infections of Agriculture, wild animals, and birds.

Research was conducted using RT-PCR-RV to detect pathogens in ticks by analyzing the DNA/RNA of the bluetongue virus, Schmallenberg virus, bovine nodular dermatitis virus, and Crimean-Congo hemorrhagic fever virus.

Nucleic acids isolated from tick homogenates were used to perform real-time reverse transcription and polymerase chain reaction (RV-RT-PCR) analysis in the course of research conducted at the national veterinary reference center by RV-RT-PCR for the presence of DNA/RNA of ticks Bluetongue, Schmallenberg, bovine nodular dermatitis, Crimean-Congo haemorrhagic fever virus. The reactions were carried out on the Rotor-Gene Q MDX 6plex amplifier (QIAGEN, Germany), which provides high sensitivity and real-time fluorescent signal recording.

To detect viruses and bacteria, a set of PCR reagents with real-time (RV-RT-PCR) hybridization-fluorescent detection was used. The assessment of the genetic material from viral pathogens affecting animals, including Crimean-Congo hemorrhagic fever, Schmallenberg virus, bluetongue, bovine nodular dermatitis, and brucellosis virus RNA, was conducted utilizing the following assays: "Amplisens® CCHFV-fl," "PCR-Schmallenberg-factor," "PCR-Bluetongue-factor," "PCR-nodular dermatitis - cattle-factor," and "PCR - *Brucella spp.* - Dermatitis-cattle-factor." This analysis was performed in accordance with the protocols established by the manufacturers, specifically the federal state budgetary institution "CSP" and LLC "Vet Factor" (Russia) (Table 1).

Table 1

Commercial kits used for the diagnosis of infectious diseases by the RV-RT-PCR method

Tested pathogen	Reagent kit	Manufacturer
Crimean-Congo hemorrhagic fever virus RNA (CCHFV)	AmpliSense® CCHFV-FL	FSBI "CSP", Russia
Schmallenberg virus RNA (SBV)	PCR-SCHMALLENBERG FACTOR	VET FACTOR LLC, Russia

Bluetongue virus RNA (BTV)	THE BLUETONGUE PCR FACTOR	VET FACTOR LLC, Russia
Bovine nodular dermatitis virus (LSDV) DNA	PCR-NODULAR-DERMATITIS-CATTLE-FACTOR	VET FACTOR LLC, Russia
Brucella spp DNA	PCR-Brucella spp.-DERMATITIS-CATTLE FACTOR	VET FACTOR LLC, Russia

Samples were distinguished, obtained and tested in accordance with the methodological instructions of the manufacturers. PCR analysis was carried out in real time using modern thermocyclers.

Research was carried out on 258 adult male and female Ixodes ticks obtained from farm animals to determine the RNA/DNA of bacteria and viruses CCHF, Schmallenberg, bluetongue, nodular dermatitis, as well as *Brucella* spp. The results were processed mathematically in Microsoft Excel.

Results

As a result of identification, ticks were classified as 2 relatives and 6 species of ticks belonging to the Ixodidae family: *Dermacentor pictus* Herm., *Dermacentor marginatus* Sulzer, 1776, *Dermacentor niveus* Neum., 1897, *Hyalloma marginatum* Koch, 1844, *Hyalomma scupense* Schulze, 1918, *Hyalomma anatolicum* Koch, 1844 (Figure 1).

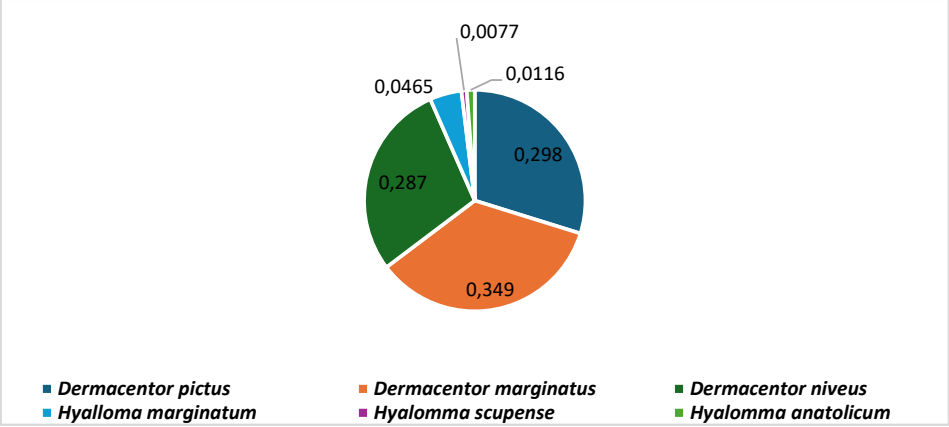

Figure 1. Species composition of ticks detected in the West Kazakhstan region

Ticks of the genus *Dermacentor* are the most common and numerous: of the 258 ticks studied, 241 (93.4%) belong to this genus. The species *Dermacentor marginatus* (34.9%) and *Dermacentor pictus* (29.8%) are found in all territories of the 13 districts of the West Kazakhstan region, where the study was conducted. The smallest were the species of the genus *Hyalloma* (Figure 1).

The smallest type of ixod tick for the fauna of the studied area was the *Hyalomma scupense* species in our research: 2 pieces (0.77%) in a collection.

The distribution of Ixodidae mites in landscape provinces is characterized by a mosaic pattern.

During the entire period of observation, a predominance of females over males was observed (62.8% and 37.2%, respectively), but in July, this ratio was stronger than in late August and early September, which was especially evident in the species *Dermacentor pictus, Dermacentor*

niveus, and very high *Dermacentor marginatus.* The differences are statistically reliable. The decrease in the proportion of females at the end of summer is explained by the later appearance of nymphs at an early age (Table 2).

Table 2

Sexual composition of ticks in western Kazakhstan

Species	Males and females (sample size)	
	♂	♀
Dermacentor pictus	26	51
Dermacentor marginatus	36	54
Dermacentor niveus	29	45
Hyalloma marginatum	3	9
Hyalomma scupense	2	-
Hyalomma anatolicum	-	3
Total	96	162

In areas near settlements, the main feeders for adult ticks among domestic animals are cattle and small cattle. Also, dogs and horses have a high number of ixodids (Figure 2).

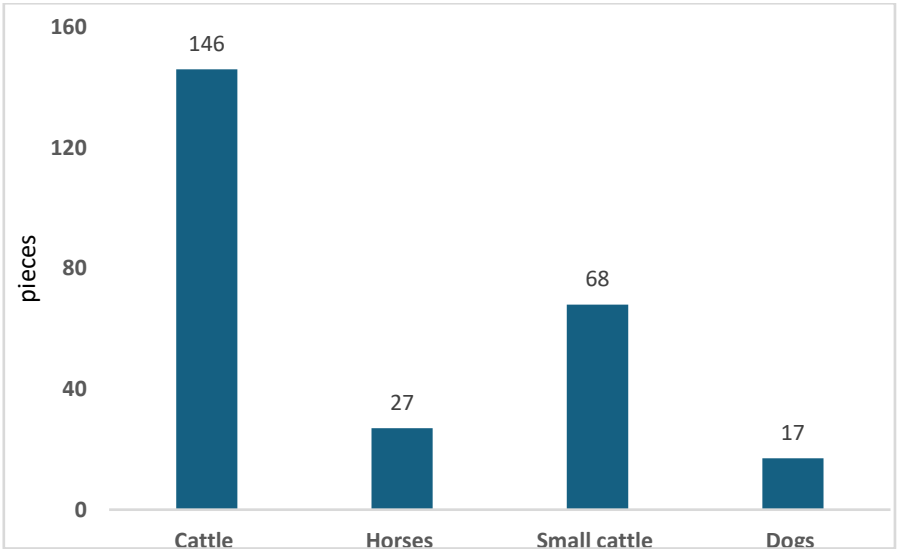

Figure 2. Ticks collected from domestic animals in western Kazakhstan, pieces

Ixodid ticks are known to parasitize a variety of forest-dwelling animals and livestock, as well as numerous species of ground-feeding birds. The primary hosts include large wild mammals such as ungulates and predators, exemplified by deer and foxes, as well as both large and small rodents like hares, squirrels, chipmunks, mice, and ground squirrels. Moreover, adult ticks are also known to actively seek out humans. Due to their inability to move over long distances when hungry, these ticks remain patient, positioned on the tips of grasses or within small bushes, waiting for a potential host to come within reach. Then these parasites act very deftly: tightly cling to wool or clothing, choose a place to suck blood and actively move around the body. In

animals, ticks often attach themselves to the head, especially behind the ears, neck, chest, and groin. In humans, attached parasites are usually found in the armpits, groin, and scalp.

All samples showed a negative result for the presence of infectious agents under study (Table 3).

Table 3

PCR detection of pathogens in blood-sucking insects for infectious diseases of farm animals

Vector	Quantity	CCHF (+)	Schmallenberg (+)	Bluetongue (+)	Nodular dermatitis (+)	Brucella spp. (+)
Ticks (*Hyalomma* and *Dermacentor* spp.)	258	0	0	0	0	0

Note: data on positive samples, % - share of the total

As a result of the conducted studies, it was found that all the studied areas of the West Kazakhstan region are free from the studied infections, which confirms the absence of circulation of pathogens of vector-borne diseases and arbovirus infections.

However, the results confirm the need to constantly monitor the number and infection of ticks, as well as carry out preventive work among the population, and recommend the following recommendations to prevent the spread of diseases:

-continuation of epizootiological surveillance of ticks and other arthropods in regions of western Kazakhstan that are potentially endemic.

- development and implementation of measures for medical and veterinary control and prevention of vector-borne infections.

- use modern methods of molecular diagnostics for early detection of foci of infection and assessment of epizootic risk.

Discussion

The epidemiological uniqueness of *Ixodidae* ticks is that one carrier can contain several pathogens of different species (of a viral or bacterial nature). Currently, tick infections are an important issue that is increasing in importance as new, previously unknown natural-focal diseases are identified in which ixod mites serve as carriers [25]. The practical importance of ticks as pathogens of many natural-focal infections of humans and animals has long attracted the attention of scientists. Numerous studies conducted by domestic and foreign authors have proven the ability of ixodids to store in their own bodies and transfer pathogenic protozoa, bacteria, viruses and other pathogens to feeders [3].

In the modern world, an important task of medical and veterinary science is the study of ticks of the *Ixodidae* family, which are carriers and keepers of vector diseases. According to research by foreign scientists, it was found that the contamination of *Ixodidae* ticks with certain pathogens of natural focal infections is influenced by the species factor [21,22,23,24,25].

Many authors argue that ticks of the *Ixodidae* family are the main carriers of viral pathogens due to their virophoricity and at the same time a reservoir of infections [26,27,28].

In recent decades, there have been significant changes in the conditions of agricultural production in our country, which have led to an increase in biotopes of ixodid ticks suitable for

their presence and an increase in the number of ixodid ones, which has affected the deterioration of the epidemic and epizootic situation for tick-borne diseases.

Studies conducted to study blood-sucking insects, their species composition found in western Kazakhstan and the viral diseases they carry have shown that polymerase-chain reaction is of great importance for species differentiation of insects, as well as the isolation of DNA/RNA of virus pathogens from biological material for sequencing and analysis of genomes is very important.

Monitoring of infectious diseases among sensitive animals allows you to monitor and develop an anti-epizootic action plan. In addition, it is necessary to take into account the natural reservoirs of pathogens, which, in turn, affect the spread of infection. To identify and control such diseases in animals, it is necessary to change the way of determining the source of the pathogen. The identification of new sources of diseases will allow the introduction of effective methods and measures to combat vector diseases of farm animals.

Conclusion

In western Kazakhstan, the main risk of infection with studied infections among the population and farm animals is associated with ticks of the genus *Hyalomma,* which previously revealed positive reactions to viruses and bacteria that pose a threat to livestock and public health. The negative results obtained in the course of this study indicate the absence of active circulation of pathogens of transmission infections during the examination, but do not exclude the possibility of their subsequent occurrence when the epizootic situation changes. The epizootic situation in the West Kazakhstan region remains stable and calm, and cases of vector-borne diseases and arbovirus infections were not recorded in our study. In agriculture, especially in regions with the activity of ticks of the genus *Hyalomma* and *Dermacentor,* it is necessary to regularly monitor and prevent vector-borne diseases and arbovirus infections.

Author Contributions

B.E., M.B., K.M.– concept and supervision of the work; **R.T., G.M., B.K.**– conducting the experiments; **A.T., K.A.** – discussion of the research results; **R.T.** and **G.M.** – writing the text; **G.E.** and **K.A.**– editing the text of the article.

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Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Кенелердің Батыс Қазақстан облысында трансмиссивті және арбовирустық аурулардың таралуына әсері

Р.Н. Төлеуова*[1], Б.К. Есімов[2], А.У. Тұяқбаева[3], Г.К. Мусаева[4], Қ.И. Ахметов[5], М.А. Бердіқұлов[6], Қ.Т. Майхин[7], Б.Ә. Қазыбай[8], Г.Е. Асылбекова[9]

[1,2]Абай атындағы Қазақ ұлттық педагогикалық университеті, Алматы, Қазақстан
[3]Л.Н. Гумилев атындағы Еуразиялық ұлттық университеті, Астана, Қазақстан
[4,6,7]«Ветеринария бойынша ұлттық референттік орталықтың» Алматы филиалы, Алматы, Қазақстан
[5]Торайғыров университеті, Павлодар, Қазақстан
[8]К. И. Скрябин атындағы Қырғыз ұлттық аграрлық университеті.
[9]Ә. Марғұлан университеті, Павлодар, Қазақстан

Аңдатпа. Мақалада кенелердің Батыс Қазақстан облысында ауыл шаруашылығы жануарларының векторлық және арбовирустық ауруларының таралуына әсерін зерттеу нәтижелері келтірілген. Ауыл шаруашылығы жануарларындағы қан соратын кенелердің эпизоотиялық жағдайы да зерттелді. Зерттеу нәтижелері осы мақалада келтірілген кене инфекцияларының таралуына байланысты көптеген мәселелердің ықтимал шешімі болып табылады. Батыс Қазақстан аумағын эпизоотиялық жағдайдың шиеленіс дәрежесі бойынша ауыл шаруашылығы жануарлары арасындағы бірқатар инфекциялар бойынша мониторинг жүргізу және аймақтарға бөлу үшін пайдаланылатын эпизоотологиялық көрсеткіштерге талдау жүргізілді. Ауыл шаруашылығы жануарлары арасында төменде зерттелген (блютанг,

Шмалленберг ауруы, ІҚМ нодулярлық дерматиті, Конго-Қырым геморогиялық қызбасы және т.б.) Батыс Қазақстан аумағына мониторинг жүргізу және аймақтарға бөлу үшін вирустық инфекциялар бойынша эпизоотиялық жағдайдың шиеленісу дәрежесі талданды. Мақалада келтірілген зерттеулерді жүргізуде ПТР және нуклеотидтер секвенциясы негізінде вирусты анықтау және штаммдық саралау үшін әзірленген және бейімделген, кейіннен Батыс Қазақстан аумағында анықталған жануарлар вирустары изоляттары геномының күшейтілген нуклеотидтер тізбегін талдау әдістері пайдаланылды. Нәтижелер көршілес Орта Азия, Ресей, Қытай және т.б. елдердің зерттеушілері үшін ғылыми және практикалық қызығушылық тудырады.

Түйін сөздер: кенелер, түр құрамы, трансмиссивті аурулар, арбовирустық инфекциялар, эпидемиологиялық жағдай

Влияние клещей на распространение трансмиссивных и арбовирусных заболеваний в Западно-Казахстанской области

Р.Н. Толеуова*[1], Б.К. Есимов[2], А.У. Туякбаева[3], Г.К. Мусаева[4], К.И. Ахметов[5], М.А. Бердикулов[6], К.Т. Майхин[7], Б.А. Казыбай[8], Г.Е. Асылбекова[9]

[1,2]Казахский национальный педагогический университет имени Абая, Алматы, Казахстан
[3]Евразийский национальный университет имени Л. Н. Гумилева, Астана, Казахстан
[4,6,7]Алматинский филиал «Национальный референтный центр по ветеринарии», Алматы, Казахстан
[5]Торайгыров университет, Павлодар, Казахстан
[8]Кыргызский национальный аграрный университет имени К.И. Скрябина, Бишкек, Кыргызстан
[9]Павлодарский педагогический университет имени Ә. Марғұлан, Павлодар, Казахстан

Аннотация. В статье приведены результаты исследования влияния клещей на распространение трансмиссивных и арбовирусных заболеваний сельскохозяйственных животных в Западно-Казахстанской области. Также изучена эпизоотическая ситуация с кровососущими клещами у сельскохозяйственных животных. Результаты исследований, приведенные в данной статье, представляют собой потенциальное решение для множества проблем, связанных с распространением клещевых инфекций. Проведен анализ эпизоотологических показателей, используемых для проведения мониторинга и зонирования территории Западного Казахстана по степени напряженности эпизоотической ситуации, по порядку инфекций среди сельскохозяйственных животных. Для проведения мониторинга и зонирования территории Западного Казахстана по степени напряженности эпизоотической ситуации по нижеперечисленным (блютанг, болезнь Шмалленберга, нодулярный дерматит КРС, конго-крымская геморрагическая лихорадка человека и др.) вирусным инфекциям среди сельскохозяйственных животных. Использованы методы, которые разработаны и адаптированы для выявления и штаммовой дифференциации вируса, на основе ПЦР и нуклеотидного секвенирования, с последующим анализом амплифицированных нуклеотидных последовательностей генома изолятов вирусов, выявленных животных на территории Западного Казахстана. Результаты представляют научный и практический интерес для исследователей сопредельных стран: Средней Азии, России, Китая и др.

Ключевые слова: клещи, видовой состав, трансмиссивные заболевания, арбовирусные инфекции, эпизоотическая ситуация

Сведения об авторах:

Толеуова Раушангул Нурлановна – автор для корреспонденции, докторант Казахского национального педагогического университета имени Абая, Достык, 13, 050010, Алматы, Казахстан.

Есимов Болат Кабдушевич – кандидат биологических наук, доцент Казахского национального педагогического университета имени Абая, Достык, 13, 050010, Алматы, Казахстан.

Туякбаева Акмарал Усерхановна – кандидат биологических наук, и.о. доцента Евразийского национального университета имени Л.Н. Гумилева, Сатпаева, 2, 010008, Астана, Казахстан.

Мусаева Гульжан Каленовна – PhD, Алматинский филиал «Национальный референтный центр по ветеринарии», проспект Райымбека, 221 В, 050000, Алматы, Казахстан.

Ахметов Кайрат Имангалиевич – PhD, ассоциированный профессор Торайгыров университета, Ломова, 64, 140008, Павлодар, Казахстан.

Бердикулов Максат Аманбекович – кандидат ветеринарных наук, профессор, Алматинский филиал «Национальный референтный центр по ветеринарии», проспект Райымбека, 221 В, 050000, Алматы, Казахстан.

Майхин Кыдырбай Тажибаевич – кандидат ветеринарных наук, Алматинский филиал «Национальный референтный центр по ветеринарии», проспект Райымбека, 221 В, 050000, Алматы, Казахстан.

Казыбай Ботагоз Акимхановна – аспирант Кыргызского национального аграрного университета им. К.И. Скрябина, ул. Медерова, 68, 720001, Бишкек, Кыргызстан.

Асылбекова Гульмира Ермукановна – кандидат биологических наук, ассоциированный профессор Высшей школы Естествознания Павлодарского педагогического университета, Олжабай батыр, 60, 140000, Павлодар, Казахстан.

Авторлар туралы мәлімет:

Төлеуова Раушангүл Нұрланқызы – хат-хабар авторы, докторант, Абай атындағы Қазақ ұлттық педагогикалық университеті, Достық даңғылы, 13, 050010, Алматы, Қазақстан.

Есімов Болат Кабдушевич – биология ғылымдарының кандидаты, доцент, Абай атындағы Қазақ ұлттық педагогикалық университеті, Достық даңғылы, 13, 050010, Алматы, Қазақстан.

Тұяқбаева Ақмарал Усерханқызы – биология ғылымдарының кандидаты, доцент м.а., Л.Н. Гумилев атындағы Еуразия ұлттық университеті, Қ. Сәтбаев көшесі, 2, 010008, Астана, Қазақстан.

Мұсаева Гүлжан Каленқызы – PhD, «Ветеринария бойынша ұлттық референттік орталық» Алматы филиалы, Райымбек даңғылы, 221 В, 050000, Алматы, Қазақстан.

Ахметов Қайрат Иманғалиұлы – PhD, қауымдастырылған профессор, Торайғыров университеті, Ломов көшесі, 64, 140008, Павлодар, Қазақстан.

Бердіқұлов Мақсат Аманбекұлы – ветеринария ғылымдарының кандидаты, профессор, «Ветеринария бойынша ұлттық референттік орталық» Алматы филиалы, Райымбек даңғылы, 221 В, 050000, Алматы, Қазақстан.

Майхин Қыдырбай Тәжібайұлы – ветеринария ғылымдарының кандидаты, «Ветеринария бойынша ұлттық референттік орталық» Алматы филиалы, Райымбек даңғылы, 221 В, 050000, Алматы, Қазақстан.

Қазыбай Ботагөз Әкімханқызы – аспирант, Қ. Скрябин атындағы Қырғыз ұлттық аграрлық университеті, Медеров көшесі, 68, 720001, Бішкек, Қырғызстан.

Асылбекова Гүлмира Ермұқанқызы – биология ғылымдарының кандидаты, қауымдастырылған профессор, Павлодар педагогикалық университетінің Жаратылыстану ғылымдары жоғары мектебі, Олжабай батыр көшесі, 60, 140000, Павлодар, Қазақстан.

Authors' information:

Toleuova Raushangul – Corresponding author, doctoral student, Abai Kazakh National Pedagogical University, Dostyk, 13, 050010, Almaty, Kazakhstan.

Essimov Bolat – Candidate of Biological Sciences, Associate Professor, Abai Kazakh National Pedagogical University, Dostyk, 13, 050010, Almaty, Kazakhstan.

Tuyakbayeva Akmaral – Candidate of Biological Sciences, Associate Professor, L.N. Gumilyov Eurasian National University, 2 Satpayev st., 010008, Astana, Kazakhstan.

Mussayeva Gulzhan – PhD, Almaty branch «National veterinary reference center», 221b Rayymbek Avenue, 050000, Almaty, Kazakhstan.

Akhmetov Kairat – PhD, Associate Professor, Toraigyrov University, 64 Lomov st., 140008, Pavlodar, Kazakhstan.

Berdikulov Maksat – Candidate of veterinary sciences, Professor, Almaty branch «National veterinary reference center», 221b Rayymbek Avenue, 050000, Almaty, Kazakhstan.

Maikhin Kydyrbai – Candidate of veterinary sciences, Almaty branch «National veterinary reference center», 221b Rayymbek Avenue, 050000, Almaty, Kazakhstan.

Kazybay Botagoz – Postgraduate student of the Kyrgyz National Agrarian University named after K. Scriabin, 68 Mederova St., 720001, Bishkek, Kyrgyzstan.

Assylbekova Gulmira – Candidate of Biological Sciences, Associate Professor, A. Margulan Pavlodar pedagogical University, Olzhabay batyr street, 60, 140000 Pavlodar, Kazakhstan.