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EPIZOOTIC SITUATION OF BLUETONGUE IN SHEEP IN THE REPUBLIC OF KAZAKHSTAN BASED ON RESEARCH RESULTS IN 2021-2025

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The article presents the results of a study of biological samples collected from sheep from farms located in various regions of the Republic of Kazakhstan. The study aimed to assess the epizootic situation of bluetongue disease. A total of 25 samples were collected: 15 samples were tested using real-time reverse transcription polymerase chain reaction (RT-qPCR) with the MagMAX™ Viral RNA Isolation Kit (Applied Biosystems), and 10 samples were tested for antibodies against BTV VP7 using the ID Screen® Bluetongue Competition ELISA Kit (ID-Vet, France). Total RNA was extracted from 15 EDTA-anticoagulated blood samples using the MagMAX™ Viral RNA Isolation Kit (Applied Biosystems) as per the kit instructions for use. For the competitive ELISA (c-ELISA), absorbance (optical density) was measured at a wavelength of 450 nm. Reverse transcription and amplification were performed using the Applied Biosystems 7500 Fast Real-Time PCR System using the following thermocycling conditions: 45°C for 10 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 45 s.

All 25 biological samples tested using modern, highly sensitive diagnostic methods yielded negative results, indicating the absence of bluetongue virus circulation in sheep farms in the Republic of Kazakhstan during the period 2021–2025.

Keywords: *bluetongue, ELISA, sheep, PCR, virus.*

2021-2025 ЖЫЛДАРДАҒЫ ЗЕРТТЕУ НӘТИЖЕЛЕРІ НЕГІЗІНДЕ ҚАЗАҚСТАН РЕСПУБЛИКАСЫНДАҒЫ ҚОЙ БЛЮТАНГІНІҢ ЭПИЗООТИЯЛЫҚ ЖАҒДАЙЫ

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Мақалада блютангтың эпизоотиялық жағдайын зерттеу мақсатында жүргізілген, Қазақстан Республикасының әртүрлі өңірлеріндегі шаруашылықтарға тиесілі қойлардан іріктеп алынған биологиялық материалды зерттеу нәтижелері келтірілген. 2021-2025 жылдар ішінде барлығы 25 сынама іріктеліп алынды, оның ішінде 15 үлгі MagMAX ™ Viral RNA Isolation Kit (Applied Biosystems) жиынтығын қолдана отырып, нақты уақыттағы кері транскрипциялы полимеразды тізбекті реакциямен (RT-PCR) (qPCR) сынақтан өткізілді және 10 үлгі ИФТ-да ID Screen® bluetongue competition Kit (ID-Vet, Франция) жиынтығын пайдалана отырып, BTV-VP7 қарсы антиденелердің болуына зерттелді. Жалпы РНК тест нұсқаулықтарына сәйкес MagMAX ™ Viral RNA Isolation Kit (Applied Biosystems) жиынтығының көмегімен ЭДТА-дан 15 қан үлгісінен шығарылды.

c-ELISA жүргізу кезінде тест нәтижелерінің жұтылуы (оптикалық тығыздығы) толқынның ұзындығы 450 нм болғанда есептелген. Кері транскрипция және амплификация Applied Biosystems 7500 Fast Real-Time PCR System жүйесінде термоциклер бағдарламасын пайдалана отырып жүргізілді: 10 минут ішінде 45 °C, 10 минут ішінде 95 °C, содан кейін 15 с ішінде 95 °C бойынша 40 цикл және 45 с ішінде 60 °C. Флуоресценция 60 °C-45 с кезеңінде өлшенді.

Қазіргі заманғы жоғары сезімтал тестілерді қолдана отырып, биологиялық материалдың барлық 25 сынамасын зерттеу кезінде теріс нәтижелер алынды, олар 2021-2025 жылдар ішінде Қазақстан Республикасының қой шаруашылығы шаруашылықтарында блютанг вирусы айналымының жоқтығын көрсетеді.

Түйінді сөздер: *блютанг, вирус, ИФТ, қойлар, ПТР.*

ЭПИЗООТИЧЕСКАЯ СИТУАЦИЯ БЛЮТАНГА ОВЕЦ В РЕСПУБЛИКЕ КАЗАХСТАН ПО РЕЗУЛЬТАТАМ ИССЛЕДОВАНИЙ В 2021-2025 ГОДАХ

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В статье приведены результаты исследований биологического материала, отобранного у овец, принадлежащих хозяйствам различных регионов Республики Казахстан, проведенных с целью изучения эпизоотической ситуации блютанга. Всего было отобрано 25 проб, из которых 15 образцов было протестировано полимеразной цепной реакцией с обратной транскрипцией в реальном времени (RT-PCR) (qPCR), с применением набора MagMAX™ Viral RNA Isolation Kit (Applied Biosystems), и 10 образцов было исследовано на наличие антител против BTV-VP7 с использованием ИФА набора ID Screen® bluetongue competition Kit (ID-Vet, Франция). Общая РНК была экстрагирована из 15 образцов крови с ЭДТА с помощью набора MagMAX™ Viral RNA Isolation Kit (Applied Biosystems) в соответствии с инструкциями теста.

При проведении c-ELISA поглощение (оптическая плотность) результатов теста считывалось при длине волны 450 нм. Обратная транскрипция и амплификация проводились на системе Applied Biosystems 7500 Fast Real-Time PCR System с использованием программы термоциклера: 45

°C в течение 10 мин, 95 °C в течение 10 мин, затем 40 циклов по 95 °C в течение 15 с и 60 °C в течение 45 с. Флуоресценция измерялась на этапе 60 °C – 45 с.

При исследованиях всех 25 проб биологического материала с применением современных высокочувствительных тестов получены отрицательные результаты, которые указывают на отсутствие циркуляции вируса блютанга в овцеводческих хозяйствах Республики Казахстана в течение 2021-2025 годов.

Ключевые слова: *блютанг, ИФА, овцы, ПЦР, вирус.*

Introduction. Bluetongue is an arthropod-transmitted viral disease of ruminants and certain other animals that was recognized and described more than 100 years ago in southern Africa. Bluetongue virus (BTV) infection of ruminants and vector Culicoides insects is enzootic throughout tropical and temperate regions of the world; however, there have been drastic recent regional alterations in the global distribution of BTV infection, particularly in Europe since 1998. [1, p. 107].

Bluetongue (BT) disease or catarrhal fever is a non-contagious, insect-borne, viral disease of ruminants, mainly sheep and less frequently cattle. BT disease results in both direct and indirect economic losses. High morbidity and mortality, abortions, stillbirths, abnormalities in the fetus, low birth weight in young ones, reduced milk yield, lowered fertility rate, early culling, meat and fleece losses, etc. contribute to direct losses whereas trade restrictions imposed on animal movement, germplasm, and animal products, expenditure for vaccination, diagnosis, vector control and treatment of clinically pretentious animals are some of the indirect losses encountered due to BT [2, p. 147, 3, p. 65]. Due to its economic impact on animal livestock, BT is listed as a multispecies disease by World Organization for Animal Health (OIE) [4, p. 401].

After a few sporadic incursions, BTV started to circulate in Europe between 1998 and 2005 with the detection of at least 5 serotypes (BTV 1, 2, 4, 9 and 16). Initially, the virus spread remained limited to the Mediterranean Basin. A first BTV-8 epidemic swept across northwestern Europe between 2006 and 2010, first reported in the Netherlands before spreading to Belgium, Germany, France and Luxembourg [5, p. 521]. Between 2010 and 2023, different BTV outbreaks were notified in northern Europe associated with BTV-8 re-emergence as well as incursions of other BTV serotypes (e.g., BTV-4). Currently, a new BTV epidemic is spreading across Europe, this time linked to BTV-3. Although BTV-3 was first identified in Europe in Italy in 2017 [6, p. 273], a new incursion was detected in the Netherlands in 2023 [7, p. 109, 8, 1552].

To date, BTV has been detected on every continent except Antarctica. The disease derives its name from one of its hallmark signs, cyanosis of the tongue (“blue tongue”), resulting from vascular thrombosis, edema, and hemorrhage caused by viral damage to endothelial cells [9, pp. 3070, 10, pp. 153]. Clinical manifestations include high fever, inflammation of the tongue and intestinal mucosa, lameness due to sore hooves, and necrotic foci in the nasal and buccal mucosa [11, pp.19448]. Accurate diagnosis of BT is vital for disease control and for maintaining safe international trade in ani mals and animal products [12, p. 318]. Tentative diagnosis may rely on clinical signs and gross lesions, but confirmation requires laboratory testing. Standard methods include detection of viral RNA by reverse transcription poly merase chain reaction (RT-PCR) and virus isolation in mammalian or insect cell cultures or embryonated chicken eggs [13, p. 481]. Molecular typing and characterization provide precise identification, while group-specific antigen cap ture enzyme-linked immunosorbent assay (ELISA) is commonly used to confirm viral isolates [14, p. 1741]. Globally, BT imposes considerable economic losses estimated at approximately USD 3 billion annually [15, p. 65]. Direct losses include high morbidity and mortality, abortions, stillbirths, low birth weight, reduced fertility, decreased milk yield, and premature culling [16, p. 147]. Indirect losses stem from restrictions on trade in live rumi nants, animal products, and germplasm, as well as costs associated with vaccination, diagnostics, vector control, and supportive treatment of affected animals [16, p. 148].

Although there are no official data on cases of bluetongue in the country [17, p. 71], given the complex global epizootic situation of this disease and the risk of its introduction, this study aimed to investigate the epizootic situation of bluetongue in sheep in the Republic of Kazakhstan.

Objectives:

1. To examine blood serum and whole blood samples collected from small ruminants in various regions of Kazakhstan for the presence of antibodies against BTV VP7 using the competitive ELISA (c-ELISA);

2. To perform molecular analysis of oral mucosal swabs, internal organs, pathological material, and whole blood samples collected from small ruminants in various regions of Kazakhstan using real-time RT-PCR.

Materials and methods

Study Setting and Design

These studies were conducted at S. Seifullin Kazakh Agro Technical Research University.

The study material included oral mucosal swabs, internal organs, pathological material, blood serum, and whole blood samples collected from small ruminants in the following locations: the village of Karasay, Zaisan District, East Kazakhstan Region (n = 6); the village of Akadyr, Shet District, Karaganda Region (n = 6); the village of Oktyabrskoye, Karasu District, Kostanay Region (n = 2); the Zhappas farm, Kaztalovsky District, West Kazakhstan Region (n = 1); the village of Sarykol, Sarykol District, Kostanay Region (n = 2); the Mibulak rural district, Ulytau District, Ulytau Region (n = 2); the M. Mamyr rural district, Karkaraly District, Karaganda Region (n = 3); and the village of Zhartas, Abai District, Karaganda Region (n = 3). Sample

collection and testing for bluetongue in small ruminants from the above-mentioned regions were carried out between 2021 and 2025.

Ethics statement

The samples were collected by qualified veterinarians following standard sample collection techniques without injury or stress to the animals. The animal breeders were always informed of the objectives and the nature of the analysis. All animal procedures are in agreement with the S. Seifullin Kazakh Agro Technical Research University and the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan (CVCS of MoA of RK), which are in accordance with international ethical standards (European Union Directive 2010/63/EU) legislation and ARRIVE (Animal Research Reporting of In Vivo Experiments) guidelines.

Molecular assays

Viral RNA extraction. All samples (n = 15) was randomly selected for molecular analysis by real-time RT-PCR. Total RNA was extracted from the 15 EDTA blood samples using the MagMAX™ Viral RNA Isolation Kit (Applied Biosystems) as per the test's instructions. Real-time reverse transcription-polymerase chain reaction (RT-qPCR). BTV RNA was detected using the LSI VetMAX™ BTV NS3 All Genotypes kit (Applied Biosystems) according to the manufacturer's instructions. This kit targets the segment 10 of the viral genome that encodes NS3 protein, and it is based on specific duplex detection of the BTV by a FAM™-NFQ-labeled TaqMan® probe and the internal positive control by VIC™–TAMRA™. The reverse transcription and amplification were performed on an Applied Biosystems 7500 Fast Real-Time PCR System using the thermal cycler program: 45 °C for 10 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 45 s. Fluorescence was measured at the 60 °C-45 s step. The interpretation of the test results was done according to the manufacturer's instructions. A sample is positive for BTV if there is a sigmoidal amplification curve in the FAM-NFQ channel and the Ct value is not higher than 40.

The International Epizootic Bureau's Manual on Diagnostic Tests and Vaccines for Terrestrial Animals was used to conduct the study [18, pp. 75017].

Viral RNA extraction. All samples (n = 15) were randomly selected for molecular analysis by real-time RT-PCR. Total RNA was extracted from 15 EDTA blood samples using the MagMAX™ Viral RNA Isolation Kit (Applied Biosystems) according to the test instructions. Real-time reverse transcription polymerase chain reaction (RT-PCR) (qPCR). BTV RNA was amplified using the LSI VetMAX™ BTV NS3 All Genotypes Kit (Applied Biosystems) according to the manufacturer's instructions. This kit targets segment 10 of the viral genome, which encodes the NS3 protein, and is based on specific duplex detection of BTV using a FAM™-NFQ-labeled TaqMan® probe and an internal positive control using VIC™–TAMRA™. Reverse transcription and amplification were performed on an Applied Biosystems 7500 Fast Real-Time PCR System using the following thermocycler program: 45 °C for 10 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 45 s. Fluorescence was measured at the 60 °C-45 s stage. Test results were interpreted according to the manufacturer's instructions. A sample is considered positive for BTV if a sigmoidal amplification curve is observed in the FAM-NFQ channel and the Ct value does not exceed 40. The International Epizootic Bureau's Manual on Diagnostic Tests and Vaccines for Terrestrial Animals [18, pp. 75017] was used to conduct the study.

Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA)

All the collected 10 sera were tested for antibodies against BTV-VP7 using ID Screen® bluetongue competition Kit (ID-Vet, France). The c-ELISA test was performed according to the manufacturer's instructions. The absorbance (optical density) of the ELISA test results was read at a wavelength of 450 nm. The percentage of inhibition was calculated using the following formula: % inhibition = [(OD sample/OD negative controls) × 100]. Serum samples with a percentage of inhibition lower than or equal to 35% were considered positive to BTV-VP7 antibodies, greater than 35% and less than or equal to 45% are considered doubtful, and higher than 45% are considered negative. The International Epizootic Bureau's Manual on Diagnostic Tests and Vaccines for Terrestrial Animals was used to conduct the study [18, pp. 75017].

Statistical Analysis

Statistical analysis of the data was performed using standard mathematical methods on a personal computer with Microsoft Office 2016 software.

Results

The results of the study of samples taken from sheep belonging to farms in various regions of the Republic of Kazakhstan, conducted during 2021-2025, are shown in Table 1.

As shown in Table 1, of the six samples collected in 2021 from a farm in the East Kazakhstan Region, one sample was tested for antibodies against BTV VP7 using the ID Screen® Bluetongue Competition ELISA Kit, and five samples were tested using real-time reverse transcription polymerase chain reaction (RT-qPCR). All results were negative.

Of the six samples collected in 2022 from a farm in the Karaganda Region, three samples were tested using ELISA and three samples were tested using real-time reverse transcription polymerase chain reaction (RT-qPCR); all test results were negative.

All three samples collected in 2023 from farms in the Kostanay and West Kazakhstan Regions were tested using real-time reverse transcription polymerase chain reaction (RT-qPCR), and all yielded negative results.

Table 1 – Results of testing biological material samples using ELISA and PCR

No.	Name of the farm	Name of study and material	Years					Results
			2021	2022	2023	2024	2025	
			Number of samples tested by ELISA and PCR					
1	S. Karasay, Zaisan District, East Kazakhstan Region	ELISA, blood serum	1					negative
		PCR, oral mucosal wash and whole blood	3					negative
		PCR, internal organs, and a piece of the brain	1					negative
		ELISA, blood serum	1					negative
2	P. Akadyr, Shet District, Karaganda Region	ELISA, blood serum		3				negative
		PCR, swab from the oral mucosa		3				negative
3	S. Oktyabrskoye, Karasusky District, Kostanay Region	PCR, pathological material			2			negative
4	Zhappas Agricultural Cooperative, Kaztalovsky District, West Kazakhstan Region	PCR, internal organs			1			negative
5	S. Sarykol, Sarykol District, Kostanay Region	PCR, internal organs				1		negative
		ELISA, blood serum				1		negative
6	Mibulaksky Rural District, Ulytau District, Ulytau Region	ELISA, blood serum				2		negative
7	Rural district of M. Mamyr Karkaralinsky district Karaganda region	ELISA, blood serum					2	negative
		PCR, pathological material					1	negative
8	S. Zhartas, Abai District, Karaganda Region	PCR, internal organs					3	negative
	Total:		25					25

In 2024, of the two samples collected from a farm in the Kostanay Region, one sample was tested using ELISA and one sample was tested using PCR, and one sample collected from the Karaganda Region was tested using PCR. All tests yielded negative results.

When testing six samples collected in 2025 from farms in the Karaganda Region, two samples were examined using ELISA and four samples were examined using real-time reverse transcription polymerase chain reaction (RT-qPCR). All test results were negative.

Thus, of the 25 samples collected from sheep on farms in various regions of the Republic of Kazakhstan, 15 samples were tested using real-time reverse transcription polymerase chain reaction (RT-qPCR) following RNA extraction with the MagMAX™ Viral RNA Isolation Kit (Applied Biosystems), and 10 samples were tested for antibodies against BTV VP7 using the ID Screen® Bluetongue Competition ELISA Kit (IDvet, France).

Overall, the consistently negative results obtained using modern, highly sensitive diagnostic methods indicate the absence of bluetongue virus circulation in sheep farms in the Republic of Kazakhstan during the period 2021–2025.

Discussion. Bluetongue is a non-contagious arthropod-borne viral disease of livestock, causing severe economic losses. A highly concerted effort is needed to control this disease in endemic areas. This is further hampered by the existence of 27 BTV serotypes, which largely fail to cross-protect (most of which are circulating in India). This has made the goal of protective immunization against the disease particularly difficult

to achieve. Live attenuated or inactivated vaccines, based on all circulating local Indian strains of the virus, are not available. However, recent developments of inactivated or sub-unit vaccines may help to control the disease in the subcontinent. This may be the only alternative for the coming days. Continuous monitoring of the different strains of bluetongue virus circulating in a particular area is necessary for developing relevant diagnostics and vaccines. Surveillance and epidemiology in endemic areas are of great significance to control the entry of viruses into newer regions, including our country.

The bluetongue virus is actively evolving due to mutations and genetic reassortment among its serotypes. On the one hand, this represents a risk factor for the emergence of novel pathogenic serotypes; on the other hand, it necessitates preventive measures aimed at their timely detection. Global climate change contributes to the expansion of the habitats of insect vectors of bluetongue virus, thereby increasing the risk of disease emergence in new territories and the formation of endemic zones. The globalization of trade and economic relations also remains a significant factor in the spread of bluetongue through the movement of infected breeding livestock. The epizootic situation in neighboring Russia confirms that this route of bluetongue virus introduction is of particular importance. Therefore, control of bluetongue spread should include quarantine measures for imported livestock, vaccination–especially in endemic areas–and systematic surveillance studies.

To assess the epizootic situation of bluetongue in various regions of the Republic of Kazakhstan during 2021–2025, 25 samples were collected from sheep from farms in different regions of the country. Of these, 15 samples were tested using real-time reverse transcription polymerase chain reaction (RT-qPCR) following RNA extraction with the MagMAX™ Viral RNA Isolation Kit (Applied Biosystems), and 10 samples were tested for antibodies against BTV VP7 using the ID Screen® Bluetongue Competition ELISA Kit (IDvet, France).

All tests yielded negative results, indicating the absence of bluetongue virus circulation in sheep farms in the Republic of Kazakhstan during the period 2021–2025.

The results obtained are consistent with data reported by domestic researchers [12, p. 319; 17, p. 72; 19, p. 491].

Conclusion

A study was conducted on 25 samples collected from sheep from farms located in various regions of the Republic of Kazakhstan. The samples were tested using real-time reverse transcription polymerase chain reaction (RT-qPCR) and a competitive enzyme-linked immunosorbent assay (c-ELISA) to detect antibodies against the BTV VP7 protein.

Examination of the biological samples using modern, highly sensitive diagnostic methods yielded negative results, indicating the absence of bluetongue virus circulation in sheep farms in the Republic of Kazakhstan during the period 2021–2025.

Control of bluetongue spread should include compliance with quarantine measures for imported livestock, vaccination–especially in endemic areas–and systematic surveillance studies.

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СОВЕРШЕНСТВОВАНИЕ ВОСПРОИЗВОДСТВА МОЛОЧНОГО СТАДА С ИСПОЛЬЗОВАНИЕМ УЛЬТРАЗВУКОВОЙ ДИАГНОСТИКИ И СЕКСИРОВАННОГО СЕМЕНИ

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В данной статье рассмотрены современные подходы к совершенствованию воспроизводства молочного стада на основе применения ультразвуковой диагностики и дифференцированных схем гормональной коррекции. Целью исследования являлась оценка эффективности использования ультразвукового метода для ранней диагностики стельности и выявления гинекологических патологий у коров, а также анализ результатов их последующего лечения. Исследования проведены в условиях крестьянского хозяйства на поголовье 133 коров. Животные находились в одинаковых условиях содержания и кормления, соответствующих зоотехническим нормам. Установлено, что УЗИ по сравнению с ректальной пальпацией позволяет выявлять большее количество особей с репродуктивными нарушениями (24 случая против 19), а также обеспечивает более точное определение физиологического состояния животных. В структуре выявленных патологий преобладали гипофункция яичников, эндометрит и фолликулярные кисты. Применение дифференцированных схем лечения позволило восстановить репродуктивную функцию у 79,2% животных, при этом наибольшая эффективность отмечена при гипофункции яичников (85,7%), наименьшая – при эндометрите (66,7%). Полученные результаты свидетельствуют о высокой практической значимости инновационных методов диагностики в системе управления воспроизводством молочного стада на базе хозяйств региона.

Ключевые слова: *молочное скотоводство, воспроизводство стада, ультразвуковая диагностика, гинекологические патологии, гипофункция яичников, гормональная терапия, сексированное семя.*

СҮТТІ ІРІ ҚАРА МАЛ ТАБЫНЫҢ ҰДАЙЫ КӨБЕЮІН УЛЬТРАДЫБЫСТЫҚ ДИАГНОСТИКА ЖӘНЕ ЖЫНЫСЫ СҰРЫПТАЛҒАН ҰРЫҚТЫ ҚОЛДАНУ АРҚЫЛЫ ЖЕТІЛДІРУ

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