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DEVELOPMENT OF A REAGENT KIT FOR THE DETECTION AND TYPING OF THE "WEST" TOPOTYPE OF BLUETONGUE VIRUS SEROTYPE 9 (BTV-9W) IN BIOLOGICAL SAMPLES USING RT-QPCR

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ABSTRACT

Bluetongue (BT), also known as sheep catarrhal fever, is a highly pathogenic viral infection caused by the bluetongue virus (BTV). This disease holds significant economic importance as it is characterized by high mortality rates, reduced productivity, deteriorated animal health, and economic losses.

Kazakhstan is considered free from BT, but the primary vectors of BTV, midges of the genus *Culicoides*, are widely distributed throughout the country. Southern Kazakhstan offers favorable conditions for the reproduction of midges, and the high density of susceptible livestock combined with the import of animals from BTV-endemic regions creates a significant risk of virus spread. This underscores the need for the development of a domestic test system for the detection of BTV via quantitative PCR, capable of differentiating vaccine strains from field strains of the virus.

This study presents the results of development and validation of a PCR test system for the diagnosis of BT. During development, real-time reverse transcription PCR (RT-qPCR) was utilized for the precise detection of BTV, enabling identification of small amounts of viral RNA in biological samples. The kit is based on primers and a fluorescently labeled probe targeting a conserved region of BTV genome (segment 10), ensuring high specificity and ability to detect various virus serotypes. This test system will be in demand in market and will enhance food security in country.

Keywords: *Orbivirus, Bluetongue virus*, diagnosis, sheep catarrhal fever, PCR test system, RT-qPCR

INTRODUCTION

Bluetongue disease (BTD), also known as sheep catarrhal fever, is a viral infection that affects both wild and domestic ruminants. The disease causes significant economic losses in sheep farming and other livestock sectors [1]. According to the classification of the World Organisation for Animal Health (WOAH), BTD belongs to category A as one of the most dangerous infections. Consequently, the detection of BTD requires mandatory reporting to veterinary services and the implementation of quarantine measures.

The Bluetongue virus (BTV) belongs to the genus *Orbivirus* within the *Reoviridae* family. The virus is transmitted through insect bites, particularly by *Culicoides spp.* midges, enabling its spread highly dependent on ecological factors such as climate and geographical barriers affecting vector's flight patterns [2,3]. The viral genome consists of ten linear double-stranded RNA segments with varying degrees of conservation. The second segment, which encodes the major surface protein VP2, is the most variable and determines the viral serotype. The second surface protein, VP5, also plays a role in serotype formation but to a lesser extent [4,5].

Sheep are the most susceptible to BTV, while cattle, goats, and wild ruminants can act as asymptomatic carriers. The characteristic symptoms of the disease include fever, inflammation of mucous membranes, swelling of the head and limbs, degenerative changes in skeletal muscles, and, in severe cases, tissue necrosis and death due to severe exhaustion. The disease does not always present with clear symptoms, complicating early diagnosis [6].

BTV exhibits significant genetic variability, leading to the existence of multiple serotypes. According to the European Commission, 24 classical serotypes of BTV have been identified. However, data from the Pirbright Institute in the UK indicate at least 29 serotypes, including «atypical» serotypes 25-29, which can spread via contact transmission and usually remain clinically silent. Differences in the structure of the VP2 protein, which determines the serotype, result in weak cross-protective immunity, limiting the effectiveness of antibodies generated against one serotype in neutralizing others [7]. Thus, the development of a PCR test system capable of differentiating serotypes is essential for accurate diagnosis and effective epidemiological control.

Considering the serious implications for animal health and economic stability, timely BTD diagnosis is crucial in preventing disease spread. Existing diagnostic methods, including serological and virological techniques, have limitations such as long processing times, low sensitivity in early stages, and cross-reactions with other viruses. Reverse transcription PCR (RT-PCR) is a modern molecular diagnostic tool that allows for rapid and precise detection of viral RNA in biological samples. PCR offers high sensitivity, specificity, and the ability to detect the virus in early stages before clinical symptoms appear. Furthermore, PCR enables serotype differentiation, which is particularly important for epidemiological monitoring and the development of control measures.

During the genetic characterization of BTV variants circulating in Kazakhstan, only the mesogenic topotype "West" of BTV-9 was identified [8]. Strains of this topotype are used for vaccine development against BTV-9. Currently, Kazakhstan lacks domestic test-systems for BTV detection. There-

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Table 1. Data on collected potential insect vectors of BTV

Region	District	Vector	Species	Quantity	Pools
Almaty	Panfilov	*Culicoides* biting midges.	*Trithecoides* spp.	21	1
			Hofmania spp.	40	1
			Oaecata spp.	27	1
			Avaritia spp.	15	1
	Karasai	Keds	*Hippobosca equina*	3	1
Zhambyl	Shuisky	*Culicoides* biting midges.	*Culicoides* spp.	22	1
	Merkensky		*Culicoides* spp.	15	1
	Bayzaksky		*Culicoides* spp.	6	1
	Zhualynsky		*Culicoides* spp.	40	2
Turkestan	Tyulkubassky	*Culicoides* biting midges.	*Remia spp.*	7	1
	Tolebi		*Culicoides* spp.	24	1
	Kazygurt	Keds	*H. equina*	3	2
	Tolebi		*H. equina*	37	15
	Baidibek		*H. equina*	1	1
	Sairam		*H. equina*	5	4
TOTAL:				266	34

fore, the development of a PCR test system capable of detecting RNA from all known BTV serotypes and topotypes while simultaneously differentiating the BTV-9W topotype is necessary. To achieve this goal, at least two loci were selected for detection. The first had to be conserved across all BTV serotypes and topotypes, while the second had to be unique to the BTV-9W topotype, demonstrating significant differences from other sero- and topotypes.

MATERIAL AND METHODS

Biological Samples Collection. Biological samples were collected to validate the PCR test-system. Positive controls included archived PCR-positive samples identified during monitoring studies from from 2018 to 2020. RNA extracted from vaccines containing BTV-4 and BTV-14 serotypes was also used for validation. Archived PCR-positive samples containing genetic material from related and unrelated viruses were pre-screened using primers targeting BTV segments 2 and 10 to assess test system specificity.

Given that the highest risk of BTD introduction and spread was recorded in the Zhambyl, Turkestan, and Almaty regions [8], blood samples from animals and insect vectors were collected in these regions. A total of 547 animal samples from the *Bovidae* family and 266 potential insect vectors were included in the study. Sheep and goats comprised 95% (520) and 5% (27) of the animal samples, respectively. Data on collected potential BTV vectors are presented in Table 1.

Nucleic Acid Extraction and Reverse Transcription. Insect pools were homogenized using a Homogenizer Mixer Mill MM 400 (Retsch) at 25 Hz for 5 minutes with three grinding glass beads and 600 µL phosphate-buffered saline (PBS). After homogenization, samples were centrifuged at 10,000 x g for 1 minute at 4°C. RNA was extracted from blood and insect homogenates using TRIzol (Sigma) following the manufacturer's protocol. Spectrophotometric analysis was performed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Sci.).

Complementary DNA (cDNA) was synthesized using M-MLV reverse transcriptase (SibEnzyme). Each reaction mixture contained 10 µL RNA sample, 1 µL random hexamer primers (Thermo Fisher Sci.), and 9 µL reverse transcription buffer, following the manufacturer's protocol. The reaction mixture was incubated at 25°C for 10 minutes, followed by 37°C for 60 minutes, and then heated at 70°C for 10 minutes.

PCR Analysis. To validate the developed PCR test system, collection of biological samples positive and negative for BTV RNA were analyzed using classical PCR. The first PCR reaction was performed using external primers "BTV-S10-F" and "BTV-S10-R" targeting the tenth segment of the dsRNA, which encodes the NS3/3A surface proteins of the virus [9]. The second reaction used two pairs of internal primers "BTV_Seg10_F" and "BTV_NS3_R" [9,10]. Additionally, PCR detection of BTV-9 "West" serotype was performed using primers "BTV-9W-S2-F" and "BTV-9W-S2-R," target-

Table 2. Primers for detection and typing BTV using classic PCR

Designation	Sequence
BTV_Seg10_F	(5')TGCTATCCGGGCTGATCCAAA
BTV-S10-R	(5')ACCTYGGGGCGCCACTC
BTV_NS3_R	(5')GCGTACGATGCGAATGCAGC
BTV-9W-S2-F	(5')GTTAAAAAATCGCATATGTCAG
BTV-9W-S2-R	(5')GTAAGTGTAAAATCCCCCCC

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ing the second dsRNA segment encoding the VP2 surface protein [11]. The primers using classic PCR are listed in Table 2.

Amplification was conducted using Hot-Start Taq DNA polymerase (NEB) according to the manufacturer's protocol. Amplification conditions were as follows: 95°C for 5 minutes, followed by 40 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. PCR products were analyzed using 1.5% agarose gel electrophoresis and visualized under UV light.

Design, Synthesis and Purification of qPCR Primers and Probes. To develop primers and fluorescently labeled probes for the RT-qPCR assay, an *in-silico* analysis was conducted using multiple bioinformatics tools. MEGA-X was employed to identify genomic regions conserved within the target topotype, while RNAstructure 6.0.1 (National Institutes of Health) was used to predict secondary structures of the primers and potential intermolecular interactions between primers and probes. Additionally, Tm Calculator (Thermo Fisher Scientific) was utilized to determine and optimize the annealing temperature of the primers.

The primers synthesis was performed using an automated oligonucleotide synthesizer, and purified by high-performance liquid chromatography (HPLC) at the Organic Synthesis Laboratory of LLP «National Center for Biotechnology» (Astana, Kazakhstan).

Generation of Genetic Constructs. Molecular cloning of amplicons was performed using the commercial TOPO™ TA Cloning Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. For this purpose, amplicons were eluted from the gel and directly cloned into the linearized pCR2.1-TOPO-TA vector system. Transformation of *Escherichia coli* strain *DH5 Alpha* competent cells was carried out using a heat shock method (90 seconds at 42°C). Vector NTI 8 (Thermo Fisher Scientific) was used to generate DNA construct maps.

Sanger DNA Sequencing. To verify the nucleotide sequences of the cloned inserts, the obtained plasmids were sequenced using the ABI 3500XL Genetic Analyzer (Applied Biosystems) with the BigDye® Terminator v3.1 kit (Applied Biosystems), following the manufacturer's protocol.

***In Vitro* Transcription.** To obtain RNA controls, plasmids based on the pCR2.1-TOPO-TA vector were linearized at the *EcoRI* restriction site and then used as templates for in vitro transcription with T7 phage RNA polymerase (Thermo Fisher Scientific). The synthesized RNA was precipitated using 3M LiCl and utilized as positive controls, including for evaluating the linearity of the test system. The concentration of RNA used for primer sensitivity testing was determined using the Qubit Fluorometer (Invitrogen, USA) and the Qubit™ RNA High Sensitivity (HS) Kit (Invitrogen) according to the manufacturer's instructions.

Optimization of the Test-system. For RT-qPCR, an in-house M-MLV reverse transcriptase and HotStart-Taq polymerase (SibEnzyme) were used with a standard HS-Taq buffer (SibEnzyme), supplemented with 0.15 mg BSA (Thermo Fisher Scientific), 0.5 mM dNTPs (SibEnzyme), and 2 mM $MgCl_2$ (SibEnzyme). Primers and TaqMan probes were added at final concentrations of 400 nM and 100 nM, respectively. The template consisted of total and control RNA in amounts ranging from 1 fg to 1 ng, isolated from biological samples.

Statistical Analysis. Statistical analysis was performed using EpiInfo (CDC). Clopper-Pearson confidence intervals (95% CI) based on the beta distribution were used to calculate 95% confidence intervals. Differences between sample groups were analyzed using Student's unpaired t-test, and differences were considered statistically significant at $p < 0.05$.

RESULTS

Development of qPCR Targets for Detection and Typing of Primers and Probes. The qPCR test system includes two detection loci: a conserved region, which enables the detection of RNA from all known serotypes, and a highly divergent region, which allows differentiation of the BTV-9W topotype.

Segment 10 (Seg-10) was selected as the primary target for detecting BTV RNA from all variants, as it contains both variable and highly conserved regions and is involved in the formation of the BTV-9W topotype. Segment 2 (Seg-2), the most variable among the BTV genome segments, was chosen as the target for the differential identification of BTV-9W genetic variants. The detection locus for BTV RNA within Seg-10 was 96 bp, while the locus for differential identification of the BTV-9W topotype within Seg-2 was 154 bp. The primers used in this study are listed in Table 3.

Cloning of Seg-2 and Seg-10 of BTV-9W and RNA Control Synthesis. To generate positive controls for the test system, fragments of the viral genome containing the target loci for BTV detection and typing were cloned. For this purpose, amplicons of 825 bp, synthesized using the primers BTV-S10_Nhe-Xba-F (5'-CAGTCGACGTCAGCGTTAAAAAGTGTCGCTGCCAT) and BTV-S10_Sac-Sal_R (5'-AATCTAGAGCTCCTCCCCCGTTAKACAGCAG) (Seg-10), as well as a 542 bp amplicon obtained with BTV-9W-2F and BTV-9W-S2-qPCR-R primers (Seg-2), were eluted from an agarose gel and directly cloned into the linearized pCR2.1-TOPO-TA vector.

Table 3. Primers and probes used in the reagent kit for the detection and typing of BTV-9W by RT-qPCR

Designation	Sequence
BTV-9W-S10-qPCR-F	5`-TGGAYAAAGCRATGTCAAA
BTV-9W-S10-qPCR-R	5`-CATCATCACGAAACGCTTC
BTV-9W-S2-qPCR-F	5`-ACCGTTCGGGAAATTCATG
BTV-9W-S2-qPCR-R	5`-GAATGTGTCRAGTCTATCAGC
BTV-9W-S10-qPCR-probe	5`-FAM-GCTGCATTCGCATCGTACGC-BHQ1
BTV-9W-S2-qPCR-probe	5`-JOE-ACCGTTCGCCCAGTTGAAGAGGCA-BHQ1

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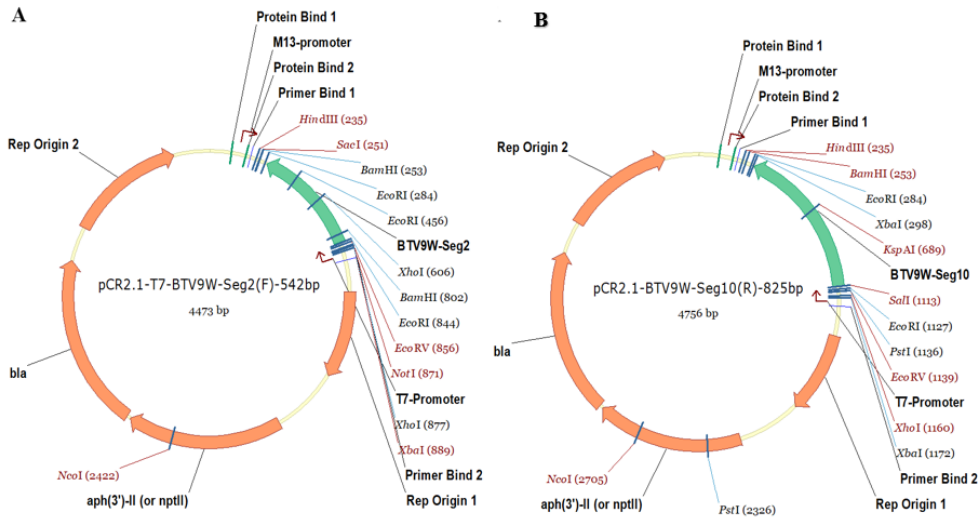


Designations: A – plasmid map of pCR2.1-T7-BTV9W-Seg2(F)-542bp;
B – plasmid map of pCR2.1-T7-BTV9W-Seg10(F)-825bp.
Figure 1 – plasmid maps of DNA constructs generated using Vector NTI 8

Following ligation, the resulting DNA clones were analyzed using restriction analysis and PCR to confirm the integration of the corresponding DNA fragments into the vector.

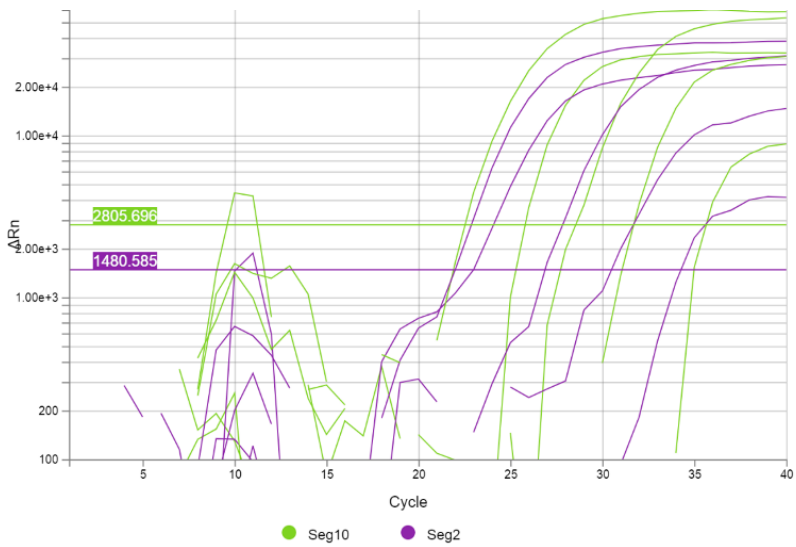
The nucleotide sequences of the cloned regions in the DNA constructs pCR2.1-BTV9W-Seg10-825bp and pCR2.1-BTV9W-Seg2-542bp were verified by Sanger sequencing. Forward sequencing was performed using a T7 promoter primer (5'-TAATACGACTCACTATAGGG), while reverse sequencing was conducted using an M13-rev primer (5'-AGCGGATAACAATTTCACACAGGA). Figure 1 presents the DNA construct maps of pCR2.1-T7-BTV9W-Seg10(F)-825bp and pCR2.1-T7-BTV9W-Seg2(F)-542bp.

To obtain RNA controls, the plasmids pCR2.1-BTV9W-Seg10-825bp and pCR2.1-BTV9W-Seg2-542bp were linearized at the EcoRI restriction site and subsequently used as templates for *in vitro* transcription with T7 phage RNA polymerase. The synthesized RNA was precipitated using 3M LiCl and employed as positive controls, including for assessing the linearity of the test system.

Furthermore, viral genome regions were subcloned into the pET23c bacterial expression vector. The resulting plasmids, pET23c-BTV9W-Seg10-825bp and pET23c-BTV9W-Seg2-542bp, were used to transform *E. coli BL21*(DE3) expression strain cells, enabling *in vivo* synthesis of BTV9W-Seg10 (S10) and BTV9W-Seg2 (S2) RNA for scaling up the production of control RNA for the qPCR test-system.

Evaluation of Sensitivity and Efficiency for Target Duplexing. To assess the analytical sensitivity of the assay, serial 10-fold dilutions of synthesized S2 and S10 RNA (starting from a concentrated stock of 10^9 copies/µL) were prepared, with final concentrations ranging from 10^8 to 10^3 copies/µL. Figure 2 presents the threshold cycle (Ct) values obtained from real-time RT-PCR for various concentrations of the control RNA.

The efficiency plot for real-time RT-qPCR, based on four reaction replicates, is shown in Figure 3. Since the most suit-



Designations: Seg10 - RNA S10; Seg2 - RNA S2. The analysis was performed using the QuantStudio 5 instrument.
Figure 2 – Results of RT-qPCR of a dilution series of synthesized control RNAs S10 and S2 with both sets of primers and probes.

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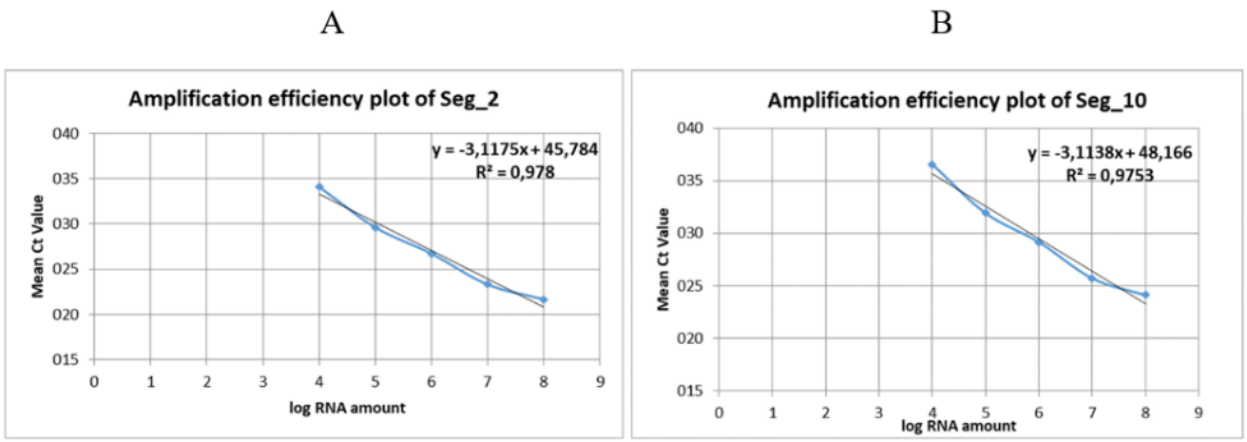

Designations: The RT-qPCR efficiency is presented as an approximating function (logarithmic trendline). (A) – for RNA S2; (B) – for RNA S10. The number of replicates for each dilution is n = 4. Data were obtained using the QuantStudio 5 instrument.

Figure 3 – RT-qPCR results for a dilution series of synthesized control RNAs S2 and S10.

able approximation function for the sensitivity curve was found to be a logarithmic function (with high coefficients of determination $R^2 = 0.978$ and 0.9753), it was concluded that the real-time RT-qPCR assay demonstrates high efficiency under the given conditions.

Evaluation of Thermal Stability in RT-qPCR. The thermal stability of primers and probes was assessed using RNA controls in triplicate experiments. As shown in Table 4, the optimal temperature range for the test system was determined to be 54–62°C, as the Ct values obtained in this range did not differ significantly from each other ($p \leq 0.05$). Figure 4 presents representative real-time RT-qPCR results for serial dilutions of synthesized S10 and S2 RNA controls, with the primer and probe annealing temperature set at 60°C.

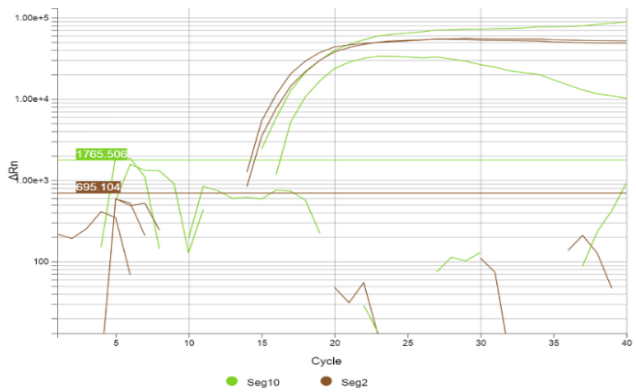

Figure 4 – Results of RT-qPCR thermal stability testing for dilutions of synthesized control RNAs S2 and S10 using the QuantStudio 5 amplifier.

Evaluation of Test Kit Specificity. The analytical specificity of the test system for potential cross-reactions was assessed using genetic material from viruses affecting ungulate mammals, which the system is intended to diagnose. Testing was conducted using archival samples containing the genetic material of the following viruses: Bovine viral diarrhea virus (BVDV) types 1 and 2, Sheep pox virus (SPPV), Orf virus (ORFV), Lumpy skin disease virus (LSDV), Bovine papular stomatitis virus (BPSV), Infectious bovine rhinotracheitis virus (IBR), Parainfluenza virus type 3 (PI3), Bovine respiratory syncytial virus (BRSV). Analysis of these samples did not reveal any false-positive results for either of the target loci (Seg-2 BTV and Seg-10 BTV), as confirmed by representative results shown in Figure 5.

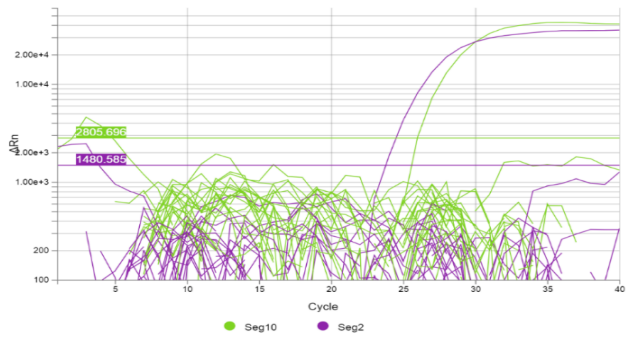

Figure 5 – RT-qPCR results for various samples containing DNA and RNA from related and unrelated livestock viruses, as well as synthesized control RNAs S2 and S10, using the QuantStudio 6 amplifier.

To further evaluate specificity, related viruses BTV-4, BTV-14, and BTV-9W were tested. Table 5 presents the specificity analysis results for the developed real-time RT-qPCR duplex assay, using samples containing RNA from related bluetongue virus (BTV) strains (BTV-4, BTV-14, and BTV-9W (both vaccine and field strains)), as well as genetic material from unrelated bovine and small ruminant viruses. As expected a positive signal in the JOE (S2) and FAM (S10) channels was detected only for the BTV-9W topotype. BTV-4 and BTV-14 strains produced positive results only in the FAM (S10) channel, no false-positive results were observed in samples containing nucleic acids from unrelated viruses. Thus, the specificity of the developed duplex RT-qPCR assay was determined to be 100%.

The conducted analysis confirmed the high sensitivity of the duplex primer and fluorescent probe set for detecting BTV genetic material, with an estimated limit of detection of approximately 2.093 genome copies, corresponding to an analytical sensitivity of 1.0×10^4 copies per reaction. Additionally, the assay demonstrated high accuracy in differentiating the BTV-9W topotype, with a detection limit of approximately 2.0948 genome copies at the same analytical sensitivity level. Furthermore, the assay demonstrated 100% specificity.

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Table 5. Specifity analysis of the developed primer and fluorescent probe set (n=4).

Virus type	Ct value, FAM channel (Seg-10)	Ct value, JOE channel (Seg-2)	Analysis result
Related viruses (BTV)			
BTV-4	24.54±1.01	Neg.	+
BTV-14	23.15±1.25	Neg.	+
BTV-9W (vaccine strain	21.28±0.95	21.06±0.98	+
BTV-9W (field strain)	22.45±0.90	22.73±0.89	+
Unrelated viruses of large and small ruminants			
BVDV-1	Neg.	Neg.	—
BVDV-2	Neg.	Neg.	—
PI3	Neg.	Neg.	—
IBR	Neg.	Neg.	—
SPPV	Neg.	Neg.	—
ORFU	Neg.	Neg.	—
LSDV	Neg.	Neg.	—
BPSU	Neg.	Neg.	—

DISCUSSION

The reagent kit developed in this study for the detection and typing of BTV-9W based on RT-qPCR demonstrates high sensitivity, specificity, and efficiency. The strategic selection of two genomic targets Segment 10 (Seg-10) for universal detection and Segment 2 (Seg-2) for specific identification of BTV-9W enabled the creation of a duplex PCR test system with robust analytical performance.

Seg-10 was selected as a universal target due to the presence of both conserved and variable regions, which allows for the detection of multiple BTV serotypes. Seg-2, being the most variable segment in the BTV genome, enabled reliable differentiation of the BTV-9W topotype, as confirmed by duplex fluorescence signals in both the FAM and JOE channels. The precise design of primers and fluorescently labeled probes targeting amplicons of 96 bp (Seg-10) and 154 bp (Seg-2) ensured optimal amplification efficiency and minimal non-specific activity.

Sensitivity assessment revealed a detection limit of approximately 2.093 genome copies for both Seg-2 and Seg-10, with a logarithmic correlation across a dynamic range of 10^3 to 10^8 copies/µL and high reproducibility. Thermal stability testing confirmed a broad operating temperature range (54–62°C), with optimal Ct values observed at 60°C, indicating high robustness of the test system under standard laboratory conditions.

Specificity analysis demonstrated no cross-reactivity with either related orbiviruses or unrelated viral pathogens of livestock, confirming 100% specificity. Among the BTV strains tested, only BTV-9W yielded positive signals for both targets, while BTV-4 and BTV-14 were detected exclusively through Seg-10, as anticipated.

The use of synthetic RNA controls and bacterial expression systems for RNA synthesis contributed to the standardization and reproducibility of the method. This, in turn, facilitates scalability of production and quality control of the reagent kit.

In conclusion, the developed RT-qPCR reagent kit is a reliable diagnostic tool for broad epizootiological surveillance and specific typing of the BTV-9W topotype in both clinical and field samples. This assay can be effectively applied in veterinary epidemiology, disease monitoring, and outbreak control in endemic regions.

CONCLUSION

Bluetongue (BT) is an economically significant disease affecting ruminant livestock, with the greatest impact on sheep farming. Although no officially registered outbreaks of bluetongue have been reported in Kazakhstan, several scientific studies indicate the presence of seropositive animals in the southern regions of the country.

This study aimed to develop an RT-qPCR-based test system for the differential diagnosis of genetic variants of bluetongue virus (BTV). The proposed RT-qPCR assay enables the effective differentiation of the non-pathogenic vaccine-like BTV strain (BTV-9W) from other virus strains that could potentially enter the country through imported livestock or wild ungulates from regions where bluetongue outbreaks are regularly reported.

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БЛЮТАНГ ВИРУСЫНЫҢ ҮЛГІЛЕРІН ҚҰСҚАЛАУ ЖӘНЕ ТИПТЕРГЕ ТАЛДАУ ҮШІН 9 СЕРОТИПІН “WEST” ТОПОТИПТІН (BTV-9W) КТ-КПТР-РТ ӘДІСІНЕ АРЛАЛҒАН РЕАГЕНТТЕР ЖИНАҒЫН ӘЗІРЛЕУ

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ТҮЙІН

Қойлардың қабыну безгегі (ҚҚБ, блютанг), немесе «көгерген тіл», ретінде белгілі (BTV) қоздыратын жоғары белсенді вирустық індет екені белгілі. Бұл індет экономикалық тұрғыдан өте маңызды, себебі ол жоғары өлім-жітіммен, өнімділіктің төмендеуімен, мал басының жағдайының нашарлаумен және экономикалық шығындармен смпатталады.

Қазақстан ҚҚБ бойынша қауіпсіз елдер қатарына жатқанына карамастан, BTV-дің негізгі тасымалдаушылары – Culicoides туысына жататын есеккүрттар ел аумағында кеңінен таралған. Қазақстанның оңтүстігінде есеккүрттар көбеюіне қолайды жағдайлар қалыптасқан, ал вирусқа сезімтал малдың баршылығы және эпидемиялық BTV аймақтарынан жануарларды импорттау блютанг вирусының таралу қаупін арттырады. Осыған байланысты кең таралған вакциналық штаммдарды оларды табиғи штаммдардан ажыратуға арналған ПТР әдісі арқылы BTV отандық тест-жүйесін әзірлеу қажеттілігі өте жоғары.

Бұл жұмысқа ҚҚБ баламасына арналған ПТР-тест жүйесін әзірлеу және валидациялау нәтіжелері ұсынылған. BTV-ны дәл анықтаудың молекулярлық құралы ретінде кері транскрипция әдісіне негізделген нақты уақыт режиміндегі ПТР усынылады, ол алынған үлгілерде вирус РНҚ-сының аз мөлшерінен анықтауға мүмкіндік береді. Жиынтықтың негізгін BTV геномының консервативті бөлігін (Seg10) нысанаға алатын праймерлер мен флуоресцентпен таңбаланған зонд құрайды, бұл жоғары қаблетті және вирус серотиптерінің әр түрлілігін ажыратуды қамтамасыз етеді. Ұсынылып отырған тест-жүйесі нарықта сұранысқа ие болып, елдің азық-түлік қауіпсіздігін арттыруға септігін тигізеді.

Кілт сөздер: *Orbivirus, Bluetongue virus*, диагностика, қойлардың қабыну безгегі, КТ-кПТР-РТ, ПТР тест-жүйесі

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РАЗРАБОТКА НАБОРА РЕАГЕНТОВ ДЛЯ ОБНАРУЖЕНИЯ И ТИПИРОВАНИЯ ТОПОТИПА “WEST” СЕРОТИПА 9 (BTV-9W) ВИРУСА БЛЮТАНГА МЕТОДОМ ОТ-КПЦР-РВ В БИОЛОГИЧЕСКИХ ОБРАЗЦАХ

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АННОТАЦИЯ

Катаральная лихорадка овец (КЛО, блютанг), известная как болезнь синего языка, является высокопатогенной вирусной инфекцией, вызываемой вирусом катаральной лихорадки овец (Bluetongue virus, BTV). Данная болезнь имеет важное экономическое значение, так как характеризуется высокой смертностью, снижением продуктивности, ухудшением здоровья животных и экономическими потерями.

Казахстан считается благополучным по КЛО, но основные переносчики BTV — мокрецы рода *Culicoides* — широко распространены на территории страны. На юге Казахстана сложились благоприятные условия для размножения мокрецов, а высокая плотность восприимчивого скота и импорт животных из эндемичных по BTV районов создают высокий риск распространения вируса. В связи с вышесказанным возникает необходимость разработки отечественной тест-системы для выявления BTV методом количественной ПЦР, способной отличать вакцинные штаммы вируса от полевых.

В данной работе представлены результаты по разработке и валидации ПЦР тест-системы для диагностики КЛО. В процессе разработки для точного выявления BTV использовался метод ПЦР с обратной транскрипцией в режиме реального времени, который позволяет обнаруживать небольшие количества вирусной РНК в биологических образцах. Основой набора являются праймеры и флуоресцентно меченый зонд, нацеленные на консервативный участок генома

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BTV (Seg 10), что обеспечивает высокую специфичность и возможность обнаружения различных серотипов вируса. Разрабатываемая тест-система будет востребована на рынке и повысит продовольственную безопасность в стране.

Ключевые слова: *Orbivirus, Bluetongue virus,* диагностика, катаральная лихорадка овец, ОТ-кПЦР-РВ, ПЦР тест-система