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Optimisation of PCR conditions for the detection of infectious bovine rhinotracheitis

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Abstract. The relevance of this study is determined by the need to improve the efficiency of laboratory diagnostics of persistent viral infections in cattle, which are characterised by low pathogen concentrations and difficulty in detection using traditional methods. The aim of the study was to develop and optimise a polymerase chain reaction (PCR) for accurate identification of bovine infectious rhinotracheitis virus based on analysis of the gB gene. Modern methods of molecular biology, genetic engineering, and bioinformatics analysis were used, including the collection, processing, and alignment of nucleotide sequences. During the study, nucleotide sequences of the gB gene of bovine infectious rhinotracheitis virus were collected and analysed, which allowed the identification of its conserved, variable, and polymorphic regions. Based on the results of the analysis, a highly specific pair of primers was designed and synthesised to ensure selective amplification of the target viral genome fragment. DNA of the bovine infectious rhinotracheitis virus was isolated to optimise amplification conditions. Optimal temperature parameters of the polymerase chain reaction and the quantitative composition of reagents were also determined, ensuring high sensitivity and specificity of the method. The results confirmed that the efficiency of diagnostics can

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be improved through rational selection of primers and reaction parameters. The practical value of this work is that the obtained data and developed methodological approaches can be used by veterinary diagnostic laboratories and research institutions to improve PCR diagnostics of bovine infectious rhinotracheitis virus

Keywords: primers; BoHV-1; PCR diagnostics; gB gene; reaction optimisation

Introduction

Infectious bovine rhinotracheitis (IBR), caused by Bovine herpesvirus-1 (BoHV-1), is one of the key viral infections of cattle, causing significant economic damage: reduced productivity, reproductive losses, and deterioration of herd health. One of the main problems in controlling BoHV-1 is its ability to establish latent infection: after a clinical or subclinical period, the virus often becomes latent and can be reactivated by stress, immunosuppression or other triggering factors (Ostler & Jones, 2023). In this regard, reliable epidemiological monitoring, timely diagnosis and control measures require the use of modern, sensitive and specific methods for detecting the virus.

In recent years, new studies have emerged that have significantly advanced the methodology for diagnosing BoHV-1 IBR in cattle – not only at the level of serological screening, but also molecular identification of active infection. First, in 2022, a highly sensitive method was developed and verified – digital droplet PCR (ddPCR) for the detection of BoHV-1 in seminal fluid, with a detection threshold of approximately 4.5 DNA copies per reaction, which significantly exceeds the sensitivity of classical qPCR (Yu *et al.*, 2022). This method is particularly relevant in the context of international semen trade control, as well as when it is necessary to detect the virus in asymptomatic, latent carriers. Secondly, a comparative study of various nucleic acid methods conducted by H. Aslim & O. Bulut (2024) showed that both real-time PCR (qPCR) and real-time loop-mediated isothermal amplification (LAMP) have high specificity and sensitivity when analysing nasal swabs; these methods can be used as a more rapid (compared to cultivation) diagnosis of active infection. In addition, according to A. Karabasova *et al.* (2025), a comprehensive approach to diagnosis – a combination of serological and molecular methods – is recommended in a recent study from Kazakhstan, which evaluated the sensitivity and specificity of enzyme-linked immunosorbent assay (ELISA) and qPCR. The authors noted that ELISA remains a reliable tool for mass herd screening (sensitivity ~90%, specificity ~95%), while qPCR detected active infection in only a small proportion of animals, indicating the hidden (latent) nature of the infection in many.

It is also important to consider data from epidemiological studies conducted in recent years, which show that BoHV-1 is still widespread among cattle in various countries. For example, in Colombia, a seroepidemiological study by C. González *et al.* (2025) showed a significant seroprevalence of BoHV-1 among cattle, even in the presence of other bovine respiratory disease complex (BRDC) viruses. In Ireland, as part of the national testing programme, the BoHV-1 status of nearly 190,000 animals was tested: the seropositive rate was 11.4% at the animal level and 48.8% at the farm level; with large herds and active animal rotation considered important risk factors (Brock *et al.*, 2025). Data from D. Barrett *et al.* (2024) confirm significant seroprevalence and co-infection with BVD (bovine viral diarrhoea). In addition, a review of control programmes in Europe shows variability in approaches to the eradication of IBR (infectious bovine rhinotracheitis) (Iscaro *et al.*, 2021). An example of a successful national control programme is presented in Slovakia, where a combination of testing and vaccination has reduced the spread of the virus in herds (Mandelik *et al.*, 2021).

Thus, recent studies show that molecular diagnostic methods (ddPCR, qPCR, LAMP) are becoming increasingly sensitive and accessible for routine use, and their combination with serological tests increases the reliability of detecting both latent and active forms of infection. At the same time, epidemiological data confirm the widespread circulation of BoHV-1 even in the absence of pronounced clinical symptoms, which emphasises the need for constant monitoring, while the standardisation and validation of diagnostic samples remain key elements of effective national control and prevention programmes. The aim of this study was to develop and optimise a PCR method suitable for accurate laboratory identification of IBR virus in cattle and further use in veterinary diagnostics.

Materials and Methods

The work was carried out at the shared-use laboratory at the Research Institute for Biological Safety Problems (Gvardeysky, Kazakhstan) between January and September 2024. The object of study was the DNA of the infectious rhinotracheitis virus from the Aktyubek strain, obtained from the institute's microorganism collection. and engineered plasmid DNA containing a fragment of the gB gene of the BoHV-1 was also used as a positive control (PC). The gB gene was chosen because of its high conservatism among IBR cattle isolates and structural stability. In addition, gB is present in all known subtypes of IBR in cattle, which allows the developed method to effectively detect both classical and circulating field variants of the pathogen (Abukhadra *et al.*, 2022).

Primers were selected for the detection of BoHV-1 with multiple alignment of the nucleotide sequences of the virus genes. The analysis used the sequences of IBR virus isolates obtained from various geographical regions and presented in the GenBank database (n.d.). Sequence alignment was performed using MEGA version 11 software with the ClustalW algorithm. The specificity of the selected oligonucleotide primers was verified using the BLAST service (n.d.). Virus DNA was extracted from virus-containing material in a biosafety level 2 (BSL-2) laboratory using the innuPREP DNA Mini Kit 2.0 (InnuScreen, Germany) in accordance with the manufacturer's instructions. The quality and concentration of the isolated DNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). For specificity testing experiments, DNA and cDNA samples of the following viruses were also obtained from the institute's collection: cowpox virus strain Cowpox-Cam, bovine nodular dermatitis virus strain Atyrau/KZ/2016, bovine viral diarrhoea virus strain Georgian, bovine parainfluenza-3 virus strain Belarus.

The primers were designed to amplify conservative flanking regions surrounding polymorphic domains, ensuring reliable detection of the target region regardless of sequence variations. The gene site was selected based on analysis of available sequences in GenBank (n.d.) databases and multiple alignment using the ClustalW algorithm, which guarantees specificity and universality of amplification. To ensure high sensitivity and specificity of PCR, optimal primers were selected – short oligonucleotide sequences 20 nucleotides long, complementary to the target sites of the genetic material under study. The sequences of the selected primers are presented in Table 1.

Table 1. Primer sequences for the identification of IBR in cattle

Primer name	5' – 3' sequence	Tm	GC%	Product size, bp
BoHV-1_gB_F	AGCGGCGTCTACCTGACCTA	60	58	133
BoHV-1_gB_R	ACCGCGTAGAGCAGCGAGAA	60	58	

Note: Tm – melting temperature of the DNA fragment; GC% – percentage of GC pairs (proportion of guanine and cytosine in the sequence); bp – base pairs (unit of DNA length)
Source: developed by the authors

To optimise the PCR conditions, a series of experiments were conducted with selected primers. To select the optimal annealing temperature for the primers, gradient PCR was performed at temperatures of 50, 52.5, 55, 57.5, 60, and 62.5°C using the standard composition of the reaction mixture from a commercial kit and a final primer concentration of 400 nM. The Taq polymerase concentration was optimised in the range from 0.5 to 4 units. The standard primer concentrations for PCR are 200-600 nM in the reaction mixture (Lumiprobe, n.d.). During optimisation, concentrations ranging from 50 nM to 1 μM were tested. The recommended concentration of $MgCl_2$ in the reaction mixture is 1-4 mM, but the optimal value is determined experimentally. In the experiments conducted, the range of final $MgCl_2$ concentrations from 0.5 to 6 mM was investigated.

During the studies, the specificity of the selected PCR parameters for detecting BoHV-1 was verified using DNA and cDNA of infectious bovine rhinotracheitis virus, bovine pox virus, bovine nodular dermatitis virus, bovine viral diarrhoea virus, and bovine parainfluenza-3 virus. Constructed plasmid DNA containing a fragment of the gB gene of the BoHV-1 was used as a positive control in the PCR. Deionised water was used as a negative control (NC). To determine the sensitivity of the selected PCR conditions, tenfold dilutions of plasmid DNA carrying a fragment of the gB gene of the infectious bovine rhinotracheitis virus were prepared and used. The range of final concentrations of plasmid DNA with a specific insert in the reaction mixture was from 1 ng to 1 μg. The number of copies of plasmid DNA was calculated based on its length and mass using formula (1):

$$\text{Copies} = \frac{m}{L \times 660} \times 6.022 \times 10^{23}, \tag{1}$$

where m is the amount of DNA (g); L is the length of the plasmid (base pairs); 660 g/mol is the average molecular weight of 1 base pair of double-stranded DNA; 6.022×10^{23} is Avogadro's number.

Results and Discussion

To select the optimal conditions for PCR with the BoHV-1_gB_F and BoHV-1_gB_R primers, experiments were conducted on gradient PCR, enzyme concentration selection, primers, and $MgCl_2$. The results of gradient PCR showed that the selected primers effectively amplified the target region at annealing temperatures of 55-62.5°C. The optimal temperature for further experiments was selected as 57°C, at which no non-specific products were observed. Analysis of the effect of Taq polymerase concentration showed that the most effective amplification of the target product was observed at an enzyme concentration of 1 unit and above. A Taq polymerase concentration of 1 unit was selected for subsequent experiments. Assessment of the effect of primer concentration showed that specific amplification was observed at all concentrations studied, but the most intense formation of amplicons occurred at concentrations of 400 nM and above. In further studies, a final primer concentration of 600 nM was used. Selection of the $MgCl_2$ concentration showed that amplification of the specific product was observed at concentrations of 1.5-6 mM. For further reactions, a concentration of 2.5 mM $MgCl_2$ was selected as providing the best balance of specificity and amplification efficiency. Thus, to optimise the PCR conditions with the selected primers, experiments were conducted to select the key reaction parameters. The optimal annealing temperature for the BoHV-1_gB_F and BoHV-1_gB_R primers was determined by gradient PCR and was 57°C, at which there were no non-specific products. The concentration of Taq polymerase was selected as 1 unit, which ensured effective amplification. The optimal concentration of primers was 600 nM, and $MgCl_2$ was 2.5 mM. As a result of the studies, the conditions for PCR for the diagnosis of IBR virus in cattle by PCR were optimised. Further studies were conducted to determine the specificity and sensitivity parameters of the primers (Figs. 1, 2). The results of determining the specificity of the developed PCR method for the identification of IBR virus in cattle showed that a specific PCR product with a size of 133 bp is produced only in the BoHV-1 DNA sample and in the positive control, no amplicons were observed in other samples, which confirms the specificity of the developed PCR diagnosis of IBR virus in cattle.

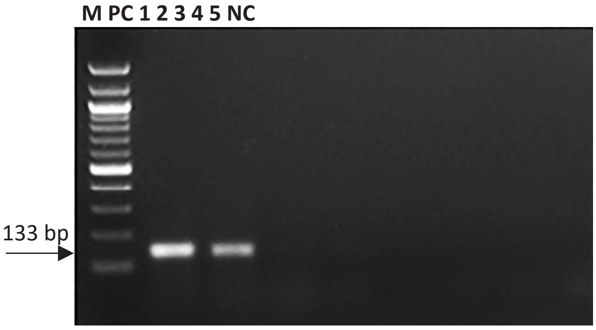

Figure 1. Determination of primer specificity for IBR diagnosis in cattle

Note: M – DNA molecular weight marker; PC – positive control; 1 – DNA of the infectious rhinotracheitis virus strain Aktyubek; 2 – DNA of the nodular dermatitis virus strain Atyrau/KZ/2016; 3 – Cowpox-Cam cowpox virus DNA; 4 – Georgian bovine viral diarrhoea cDNA; 5 – bovine parainfluenza-3 virus strain Belarus cDNA; NC – negative control

Source: authors' development

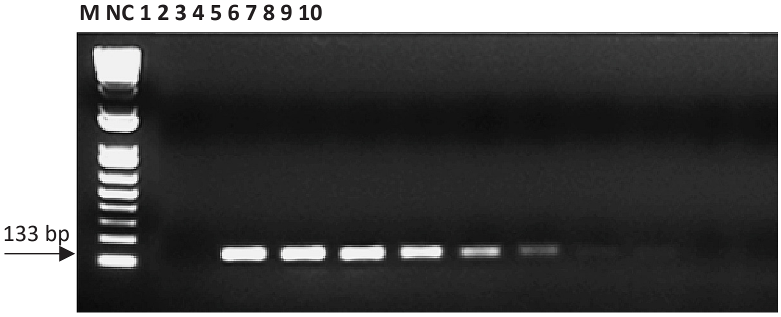

Figure 2. Determination of the sensitivity of selected PCR conditions for the diagnosis of IBR in cattle

Note: M – DNA molecular weight marker; NC – negative control; 1 – 1 ng (nanogram); 2 – 100 pg (picogram); 3 – 10 pg (picogram); 4 – 1 pg (picogram); 5 – 100 fg (femtograms); 6 – 10 fg (femtograms); 7 – 1 fg (femtograms); 8 – 100 ag (attograms); 9 – 10 ag (attograms); 10 – 1 ag (attogram)

Source: authors' development

The selected PCR conditions allow reliable detection of plasmid DNA containing a region of the gB gene of the infectious rhinotracheitis virus of cattle at a concentration of 10 fg, which corresponds to $\sim 1.7 \times 10^3$ copies of the DNA molecule, indicating the high sensitivity of the selected PCR method. A conservative region of the gB gene of the infectious rhinotracheitis virus, characterised by high specificity due to the absence of similarity to the gene sequences of other viruses infecting cattle, was used as a target for PCR. Using the designed primers, optimisation of the reaction mixture composition and temperature-time amplification parameters, the developed method for diagnosing IBR in cattle showed high sensitivity and specificity.

The development of PCR-based diagnostic systems for detecting bovine infectious rhinotracheitis virus remains one of the priority tasks of modern veterinary diagnostics due to the high contagiousness of the infection and the difficulty of detecting the virus in its early stages. In this study, the classical PCR method aimed at detecting the gB gene amplicon was optimised, demonstrating high sensitivity and specificity when the reaction is performed manually using standard equipment. These results are consistent with the general trend of increasing the diagnostic efficiency of molecular techniques and are comparable with data in the literature. Current research is mainly focused on qPCR for BoHV-1, which provides even higher analytical sensitivity and quantitative assessment of viral load. In particular, L. Xu *et al.* (2024) described a SYBR Green II qPCR method with a detection limit of approximately 7.8×10^1 copies/μL, which demonstrated good sensitivity and repeatability without cross-amplification with other cattle pathogens. When using this method, the frequency of positive detections in clinical samples was higher than with conventional PCR, confirming the superiority of qPCR in terms of analytical parameters. Similarly, according to Z. Yu *et al.* (2022), ddPCR for the detection of BoHV-1 in semen demonstrates excellent sensitivity (up to 4.5 copies), especially for latent infections. According to S. Pawar *et al*. (2014), the LAMP assay for semen also provides rapid detection without complex equipment.

In addition to purely technical differences between classical PCR and qPCR, the choice of targets for detection remains important. Evaluation analyses and meta-reviews emphasise that BoHV-1 glycoproteins (gB, gC, gD, gE, etc.) are reliable targets for primer development due to the conservatism of certain gene regions and their widespread use in diagnostics. This choice is also confirmed by a number of international studies, including J. Zhang *et al*. (2025), where g family genes served as the main target for molecular diagnosis of the virus. It is important to note that a number of studies use alternative detection methods that can provide other levels of sensitivity comparable to qPCR. For example, RPA-LFD (recombinant polymerase amplification with lateral flow) for herpesvirus, which provides visual detection of the virus within 30 minutes with a sensitivity of about 1 $TCID_{50}$, which is comparable to sensitive molecular methods and is suitable for rapid diagnosis in the field (Wu *et al*., 2025). Another example is combined RPA methods for the simultaneous detection of BVDV and BoHV-1, which demonstrate a sensitivity of BoHV-1 of about 10^4 copies/μL, which, according to L. Jiang *et al*. (2024), is slightly inferior to qPCR, but remains high enough for practical diagnosis. As pointed out by P. Horwood & T. Mahony (2011), multiplex approaches, including the simultaneous detection of multiple respiratory viruses, increase the efficiency of screening. Classical PCR for gB remains reliable for regional laboratories, as shown in the article by B. Muylkens *et al*. (2007), where the conservatism of gB is confirmed for the detection of various subtypes.

A comparison of the results indicates that, despite the speed and simplicity of conventional PCR, qPCR and RPA methods have advantages in terms of sensitivity and potential automation, which is important for large diagnostic centres. However, the development of optimised classical PCR methods, as in this study, remains relevant for regional and resource-limited laboratories where access to qPCR equipment is limited. Epidemiological data from different regions confirm the widespread circulation of BoHV-1 and the need for optimised methods (Raaperi *et al*., 2010; Dias *et al*., 2013). Thus, the results of this study are consistent with current scientific trends in the molecular diagnosis of infectious bovine rhinotracheitis: increased requirements for sensitivity and specificity, the use of modern analytical platforms, and the analysis of multiplex methods.

Conclusions

In this study, a PCR method for detecting bovine infectious rhinotracheitis virus was optimised, demonstrating high specificity and sensitivity. The optimal reaction parameters include an annealing temperature for the BoHV-1_gB_F and BoHV-1_gB_R primers of 57°C, a Taq polymerase concentration of 1 unit, primers – 600 nM, $MgCl_2$ – 2.5 mM, which ensures effective amplification of the target region without the formation of non-specific products. The specificity of the method was confirmed by testing on a panel of viruses affecting cattle, including nodular dermatitis virus, cowpox virus, viral diarrhoea and parainfluenza-3. A PCR product with a size of 133 bp was formed exclusively in samples of BoHV-1 DNA and the positive control, and was absent in the other samples, which indicates the high specificity of the primers. The sensitivity of the method was 10 fg of plasmid DNA, corresponding to ~1.7×10^3 copies of the molecule, which allows the detection of infection at early stages and ensures reliable diagnosis even at low concentrations of viral material.

The developed PCR method is an effective tool for rapid diagnosis of IBR in cattle, combining accuracy, sensitivity and reproducibility. Prospects for further research include the introduction of real-time quantitative PCR to assess viral load using fluorescent probes, which will not only detect the presence of the virus but also determine its concentration in biological material. Expansion of the tested panel of strains from different geographical regions is necessary to verify the universality of the method and its applicability to a variety of field isolates. Integration of the developed PCR diagnostics with other molecular and serological approaches will create a basis for comprehensive monitoring of infections in cattle. An important direction is to validate the method on an expanded set of clinical samples obtained from animals with different clinical statuses and from different farms, which will allow assessing the practical effectiveness of the developed methodology in the context of surveillance and prevention of IBR in cattle.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Abukhadra, B.A., Mosad, S.M., & El-Rahman, S.A. (2022). Molecular identification of bovine alpha herpesvirus subtype 1.1 in field isolates in Egypt by phylogenetic analysis of glycoprotein B gene. *Advances in Animal and Veterinary Sciences*, 10(8), 1739-1746. doi: 10.17582/journal.aavs/2022/10.8.1739.1746.

[2] Aslim, H.P., & Bulut, O. (2024). Detection of BoHV-1 – comparative evaluation of real time PCR, real time LAMP and subtyping. *Acta Scientiae Veterinariae*, 52(1), article number 1954. doi: 10.22456/1679-9216.139938.

[3] Barrett, D., Lane, E., Lozano, J.M., O'Keeffe, K., & Byrne, A.W. (2024). Bovine herpes virus type 1 (BoHV-1) seroprevalence, risk factor and bovine viral diarrhoea (BVD) co-infection analysis from Ireland. *Scientific Reports*, 14(1), article number 867. doi: 10.1038/s41598-023-50433-5.

[4] BLAST. (n.d.). Retrieved from https://blast.ncbi.nlm.nih.gov/Blast.cgi.

[5] Brock, J., Guelbenzu-Gonzalo, M., Lozano, J.M., Lane, E.A., Gunn, M., Brady, S., Thulke, H.H., & Graham, D.A. (2025). Prevalence and risk factors for bovine herpesvirus type 1 (BoHV-1) infection in Irish beef herds: Results from the National Beef Welfare Scheme 2023. *Irish Veterinary Journal*, 78, article number 22. doi: 10.1186/s13620-025-00308-0.

[6] Dias, J.A., Alfieri, A.A., Ferreira-Neto, J.S., Gonçalves, V.S., & Muller, E.E. (2013). Seroprevalence and risk factors of bovine herpesvirus 1 infection in cattle herds in the state of Paraná, Brazil. *Transboundary and Emerging Diseases*, 60(1), 39-47. doi: 10.1111/j.1865-1682.2012.01316.x.

[7] GenBank. (n.d.). Retrieved from https://www.ncbi.nlm.nih.gov/genbank/.

[8] González, C.B., Briñez, K., Tobón, J.C., Sanjuanelo Corredor, D.W., Bauermann, F.V., & Guzmán Barragán, B.L. (2025). Cross-sectional seroprevalence study of bovine herpesvirus 1, bovine respiratory syncytial virus, and parainfluenza virus 3 in cattle from Villavicencio, Colombia. *BMC Veterinary Research*, 21, article number 24. doi: 10.1186/s12917-024-04461-6.

[9] Horwood, P.F., & Mahony, T.J. (2011). Multiplex real-time RT-PCR detection of three viruses associated with the bovine respiratory disease complex. *Journal of Virological Methods*, 171(2), 360-363. doi: 10.1016/j.jviromet.2010.11.020.

[10] Iscaro, C., Cambiotti, V., Petrini, S., & Feliziani, F. (2021). Control programs for infectious bovine rhinotracheitis (IBR) in European countries: An overview. *Animal Health Research Reviews*, 22(2), 136-146. doi: 10.1017/S1466252321000116.

[11] Jiang, L., Zhang, G., Wang, P., Niu, X., Liu, Q., Zhang, S., Gao, W., & Li, Y. (2024). Simultaneous detection of bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) using recombinase polymerase amplification. *Scientific Reports*, 14, article number 10169. doi: 10.1038/s41598-024-56869-7.

[12] Karabasova, A., Turkeev, M.K., Tulepov, B.S., Lesov, B.E., Akshalova, P.B., Tujmebaeva, K.Zh., Akylbaj, A.K., Kanatbaev, S.G., & Kajmoldina, S.E. (2025). Analysis of serological and molecular genetic methods for the diagnosis of infectious rhinotracheitis in cattle in Kazakhstan. *Science and Education*, 1(78), 172-186. doi: 10.52578/2305-9397-2025-1-1-172-186.

[13] Lumiprobe. (n.d.). *DNA amplification with ProbeMaster® UNI PCR/qPCR reaction mixture, 5x*. Retrieved from https://ru.lumiprobe.com/protocols/pcr-qpcr-master-mix-5x?utm_source.

[14] Mandelik, R., Bires, J., Ozsvari, L., Hodnik, J.J., & Vilcek, S. (2021). Infectious bovine rhinotracheitis control program in Slovakia. *Frontiers in Veterinary Science*, 8, article number 675521. doi: 10.3389/fvets.2021.675521.

[15] Muylkens, B., Thiry, J., KIBRen, P., Schynts, F., & Thiry, E. (2007). Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. *Veterinary Research*, 38(2), 181-209. doi: 10.1051/vetres:2006059.

[16] Ostler, J.B., & Jones, C. (2023). The bovine herpesvirus 1 latency-reactivation cycle, a chronic problem in the cattle industry. *Viruses*, 15(2), article number 552. doi: 10.3390/v15020552.

[17] Pawar, S.S., Meshram, C.D., Singh, N.K., Sonwane, A.A., Saini, M., Rautmare, S.S., Muglikar, D.M., Mishra, B.P., & Gupta, P.K. (2014). Rapid detection of bovine herpesvirus 1 in bovine semen by loop-mediated isothermal amplification (LAMP) assay. *Archives of Virology*, 159(4), 641-648. doi: 10.1007/s00705-013-1869-2.

[18] Raaperi, K., Nurmoja, I., Orro, T., & Viltrop, A. (2010). Seroepidemiology of bovine herpesvirus 1 (BHV1) infection among Estonian dairy herds and risk factors for the spread within herds. *Preventive Veterinary Medicine*, 96(1-2), 74-81. doi: 10.1016/j.prevetmed.2010.06.001.

[19] Wu, Y., Zhang, W., Yi, C., He, K., Hu, C., Ye, G., & Zhang, A. (2025). Rapid, sensitive, and visible RPA-LFD assay for BoHV-1 and BoHV-5. *Microbiology Spectrum*, 4, 13(3), article number e0089524. doi: 10.1128/spectrum.00895-24.

[20] Xu, L., *et al.* (2024). Establishment of a real-time fluorescent quantitative PCR detection method and phylogenetic analysis of BoAHV-1. *BMC Veterinary Research*, 20, article number 180. doi: 10.1186/s12917-024-04025-8.

[21] Yu, Z., Zhao, Z., Chen, L., Yan, H., Cui, Q., Ju, X., Yong, Y., Liu, X., Ma, X., & Zhang, G. (2022). Development of a droplet digital PCR assay to detect bovine alphaherpesvirus 1 in bovine semen. *BMC Veterinary Research*, 18, article number 125. doi: 10.1186/s12917-022-03235-2.

[22] Zhang, J., Cao, J., Cao, L., Zhang, C., Gong, L., Kang, X., Li, D., & Zhang, Y. (2025). Research progress on the diagnostic techniques and vaccine development of bovine herpesvirus types 1. *Frontiers in Veterinary Science*, 12, article number 1703336. doi: 10.3389/fvets.2025.1703336.

Ири мүйүздүү малдын инфекциялык ринотрахеитин аныктоо үчүн ПЧР коюунун параметрлерин оптималдаштыруу

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Аннотация. Бул иликтөөнүн актуалдуулугу малдын узакка созулган вирустук инфекцияларын лабораториялык диагностиканы жогорулатуу зарылдыгында турат, анткени бул инфекциялардын учурунда оору жараткан агенттин концентрациясы аз болуп, аны салттуу ыкмалар менен аныктоо кыйынчылык жаратат. Изилдөөнүн максаты – ири мүйүздүү малдын инфекциялык ринотрахеити вирусун так идентификациялоо үчүн gB генинин анализине негизделген полимеразалык чынжыр реакциясын (ПЧР) оптималдаштыруу. Ишти аткарууда молекулярдык биологиянын заманбап ыкмалары, гендик инженерия жана биоинформатика колдонулду, анын ичинде нуклеотиддик секвенциялардын жыйноосу, иштетилиши жана салыштырылышы. Изилдөө учурунда ири мүйүздүү малдын инфекциялык ринотрахеити вирусунун gB генинин нуклеотиддик секвенциялары жыйналып, анализденди, натыйжада анын консервативдик, вариабелдүү жана полиморфтук аймактары аныкталды. Анализдин негизинде жогорку спецификалык таасири бар праймерлерди синтездөө жана иштеп чыгуу жүргүзүлүп, вирус геномунун максаттуу фрагментин амплификациялоого мүмкүндүк берилди. Иш учурунда ири мүйүздүү малдын инфекциялык ринотрахеити вирусунун ДНКсын бөлүп алуу жүргүзүлүп, амплификация шарттарын оптималдаштыруу үчүн негиз түзүлдү. Ошондой эле полимеразалык чынжыр реакциясынын температуралык параметрлери жана реагенттердин сандык курамы оптималдаштырылып, методдун жогорку сезгичтиги жана спецификтиги камсыздалды. Изилдөө натыйжалары праймерлерди жана реакция параметрлерин рационалдуу тандоо аркылуу диагностиканын эффективдүүлүгүн жогорулатууга мүмкүнчүлүк берерин көрсөттү. Практикалык мааниси – алынган маалыматтар жана иштелип чыккан методикалык ыкмалар ветеринардык диагностикалык лабораториялар жана илимий-изилдөө мекемелер тарабынан ири мүйүздүү малдын инфекциялык ринотрахеити вирусунун ПЦР-диагностикасын өркүндөтүү үчүн колдонулушу мүмкүн

Негизги сөздөр: праймерлер; BoHV-1; ПЧР-диагностика; gB гени; реакцияны оптималдаштыруу

Оптимизация параметров постановки ПЦР для выявления инфекционного ринотрахеита крупного рогатого скота

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Аннотация. Актуальность данной работы определяется необходимостью повышения эффективности лабораторной диагностики персистирующих вирусных инфекций крупного рогатого скота, для которых характерны низкие концентрации возбудителя и затрудненность его выявления традиционными методами. Цель исследования заключалась в разработке и оптимизации полимеразной цепной реакции (ПЦР) для точной идентификации вируса инфекционного ринотрахеита крупного рогатого скота на основе анализа гена gB. Для выполнения работы использованы современные методы молекулярной биологии, генной инженерии и биоинформатического анализа, включая сбор, обработку и выравнивание нуклеотидных последовательностей. В ходе исследования были собраны и проанализированы нуклеотидные последовательности гена gB вируса инфекционного ринотрахеита крупного рогатого скота, что позволило выявить его консервативные, вариабельные и полиморфные участки. На основании результатов анализа была разработана и синтезирована высокоспецифичная пара праймеров, обеспечивающая селективное амплифицирование целевого фрагмента генома вируса. В ходе работы было проведено выделение ДНК вируса инфекционного ринотрахеита крупного рогатого скота, необходимое для оптимизации условий амплификации. Также установлены оптимальные температурные параметры полимеразной цепной реакции и подобран количественный состав реагентов, обеспечивающие высокую чувствительность и специфичность метода. Результаты исследования подтвердили возможность повышения эффективности диагностики путем рационального подбора праймеров и параметров реакции. Практическая ценность работы заключается в том, что полученные данные и разработанные методические подходы могут быть использованы ветеринарными диагностическими лабораториями и научно-исследовательскими учреждениями для совершенствования ПЦР-диагностики вируса инфекционного ринотрахеита крупного рогатого скота

Ключевые слова: праймеры; BoHV-1; ПЦР-диагностика; gB ген; оптимизация реакции