ANNOTATION
of the PhD dissertation by Meruyert Akberovna Saduakassova
entitled "Development of genomic test systems for identification and indication of FMD virus topotypes in countries of Central Asia and posing threat to the Republic of Kazakhstan"
submitted for the degree of Doctor of Philosophy (PhD)
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Relevance of the research topic. Livestock farming is a key sector of Kazakhstan's agro-industrial complex, playing a strategic role in ensuring food security, developing export potential, and resilience in rural areas. In 2024, meat production by slaughter weight reached 1.2 million tons, a 4.3% increase compared to the previous year. Processed cattle and small ruminant meat volumes increased by 17%, reaching 270,000 tons. In the first quarter of 2025, beef and lamb exports from Kazakhstan doubled compared to the same period in 2024. The main export destinations were Uzbekistan, the United Arab Emirates, and Iran, demonstrating the growing competitiveness of domestic meat products in foreign markets.

However, further sustainable development of the industry is impossible without ensuring epizootic safety, particularly with regard to particularly dangerous transboundary infections, including foot-and-mouth disease. Even a single outbreak of this disease can lead to significant economic losses, restricted exports, and undermine trust in veterinary control among international partners. This problem is particularly pressing for Kazakhstan, located at the crossroads of intensive trade, logistics, and migration routes connecting Central Asian countries, where various genetic lineages of the foot-and-mouth disease virus (FMDV) continue to circulate.

The genetic variability of circulating FMDV strains significantly complicates epizootological monitoring, molecular diagnostics, and the selection of effective vaccine strains. Under these circumstances, the availability of highly specific and sensitive methods for rapid detection and accurate identification of virus genetic variants, especially those that could be imported into Kazakhstan from neighboring countries, is crucial.

Ensuring epizootic safety and maintaining the territory's foot-and-mouth disease-free status requires the implementation of modern approaches to early infection detection and rapid diagnostics. The use of molecular genetic methods, particularly the development and use of genomic real-time RT-PCR (RT-PCR) test systems, is becoming a key area in implementing the FMD prevention, control, and eradication strategy in the region.

Foot-and-mouth disease virus serotypes O, A, and Asia1 circulate in Central Asian countries, including Kazakhstan. Subtypes of these serotypes exhibit significant topotype/genetic diversity, requiring the use of molecular differentiation methods. Sequencing of the VP1 region and subsequent phylogenetic analysis remains the most reliable method. However, most veterinary laboratories in the region lack the necessary resources, specialized equipment, bioinformatics infrastructure, and qualified personnel to systematically conduct such studies.

In response to this challengeA series of highly specific genomic RT-PCR test systems was developed to detect and identify FMDV topotypes/lineages circulating in Asia. Eight tests were created and validated, targeting the O/ME-SA/PanAsia–PanAsia-2, O/ME-SA/Ind-2001, O/SEA/Mya-98, O/CATHAY, A/ASIA/Iran-05, A/ASIA/G-VII, Asia1/Sindh-08/06, and A/ASIA/Sea-97 FMDV lineages.

The test systems were validated on a large collection of positive samples of various types (epithelium, oropharyngeal fluid, culture supernatants). The results demonstrated high analytical sensitivity (over 96.5% accuracy) and specificity (100%), with a complete absence of cross-amplification between different lineages.

Thus, the developed genomic test systems significantly expand the tools available for epizootic monitoring and enable prompt management decisions when identifying and spreading foot-and-mouth disease outbreaks. Furthermore, their use enables the informed selection of vaccination strains based on the current molecular epizootic picture, which is crucial for improving the effectiveness of preventive measures in Central Asia.

Given the aforementioned relevance, the aim of this dissertation was to develop genomic test systems for the detection and identification of foot-and-mouth disease virus topotypes circulating in Central Asian countries and posing a threat to the epizootic safety of the Republic of Kazakhstan. In accordance with this objective, the following tasks were defined within the dissertation:

- To study the molecular genetic characteristics of foot-and-mouth disease viruses circulating in Asian countries based on the analysis of VP1 gene sequences;
- To select prototype isolates and representative nucleotide sequences characteristic of individual topotypes and genetic lines of foot-and-mouth disease virus;
- To develop topotype-specific oligonucleotides (primers and fluorescent probes) for amplification in the RT-PCR-RV format aimed at differentiating FMDV by topotypes and genetic lines;
- To optimize and validate real-time RT-PCR diagnostic test systems for the indication and identification of foot-and-mouth disease virus by key topotypes;
- To evaluate the sensitivity and specificity of the developed genomic test systems, including cross-reactivity between genetic lines;
- To compare the diagnostic efficiency of the developed genomic test systems with the universal 3D rRT-PCR test system used for the indication of foot-and-mouth disease virus.

Research methods. The study utilized molecular biology (RT-PCR, primer and probe design), virology (isolation and analysis of epithelial samples, oropharyngeal fluid, and culture supernatants), bioinformatics (multiple alignment, phylogenetic analysis), and comparative statistical analysis of test system sensitivity and specificity.

Justification of the novelty and importance of the obtained results

The scientific novelty of the dissertation lies in the development of a complex of diagnostic test systems based on reverse transcription with polymerase

chain reaction in real time (RT-PCR-RV, real-time RT-PCR), designed for the simultaneous indication and identification of foot-and-mouth disease virus topotypes circulating in the countries of Central Asia.

In contrast to the widely used pan-serotype 3D test, which is used in international practice as the “gold standard” for indicating the RNA of the foot-and-mouth disease virus regardless of the serotype, the genomic test systems developed in this work provide not only confirmation of the presence of the virus, but also type-specific identification of the most epizootically significant topotypes and genetic lines (O/ME-SA/PanAsia–PanAsia-2, O/ME-SA/Ind-2001, O/SEA/Mya-98, O/CATHAY, A/ASIA/Iran-05, A/ASIA/G-VII, A/ASIA/Sea-97, Asia-1/Sindh-08).

The test systems were designed using a highly variable region of the VP1 gene as a target, determining the virus's serotype specificity. This approach ensured high sensitivity and specificity, eliminating cross-amplification between strains. The use of TaqMan assay technology increased detection accuracy and eliminated false-positive results, making the developed tests suitable for routine diagnostics and epizootic monitoring.

The developed test systems were compared with a universal 3D test, which confirmed their equivalent sensitivity with a higher resolution in terms of type-specific differentiation of the virus.

The obtained results have significant practical significance, as they ensure accelerated identification of infection sources, control of circulating topotypes and genetic lines of the virus, selection of vaccine strains and, as a result, increased effectiveness of preventive measures against the threat of foot-and-mouth disease introduction into the territory of the Republic of Kazakhstan.

The practical significance is confirmed by the following provisions:

A molecular genetic and phylogenetic analysis of foot-and-mouth disease virus strains circulating in Central and Southeast Asian countries was conducted, which made it possible to substantiate the need to create regionally adapted diagnostic tools.

A set of genomic test systems based on reverse transcription with real-time polymerase chain reaction (RT-PCR-RV) has been developed, ensuring rapid and accurate identification and indication of the most relevant topotypes and genetic lines of the foot-and-mouth disease virus (O/ME-SA/PanAsia, Ind-2001, SEA/Mya-98, CATHAY, A/ASIA/G-VII, A/ASIA/Iran-05, Asia-1, etc.).

The designed primers and probes target the highly variable VP1 region, which determines antigenic and phylogenetic differences between virus lineages, ensuring high diagnostic specificity.

A comparative study was conducted. Validation of the developed test systems with a universal 3D rRT-PCR test system used as the "gold standard" for the indication of foot-and-mouth disease virus, which confirmed the high diagnostic efficiency and reliability of the results obtained.

The possibility of lyophilization of reagents has been demonstrated, which ensures the preservation of activity and stability of test systems during storage and transportation without cold chain, as well as their application in field conditions and laboratories with limited resources.

The obtained results can be used to update the composition of vaccine strains taking into account the changing epizootic situation in the region and to make an informed choice of preventive measures in the event of a threat of pathogen introduction.

The theoretical significance of the work is as follows:

Based on the analysis of nucleotide sequences, principles for selecting unique genetic targets in the VP1 gene region for differentiating foot-and-mouth disease virus topotypes were substantiated, which complements modern understanding of the molecular structure and phylogenetic diversity of FMDV.

The obtained data expand scientific knowledge in the field of molecular epizootology and the evolution of foot-and-mouth disease virus, contributing to the development of approaches to its molecular typing and control.

Based on the obtained scientific results, the Republic of Kazakhstan received patent for utility model No. 2025/0884.2 “Method for diagnosing foot-and-mouth disease virus”, confirming the originality and practical value of the proposed solutions.

Compliance with scientific development directions or state programs
The dissertation was completed within the framework of the state scientific program of the Ministry of Agriculture of the Republic of Kazakhstan (state registration number 0115RK01952), and with the support and funding of international partners of the Department for Environment, Food and Rural Affairs of the United Kingdom (DEFRA, projects SE2943 and SE1129) and the Biotechnology and Biological Sciences Council of the United Kingdom (BBSRC, project BB/E/I/00007017).

The topic of the dissertation research is fully consistent with the national priorities of the Republic of Kazakhstan in the field of veterinary safety, as well as international strategic directions in the field of combating transboundary animal diseases (TADs), including:

The aims and objectives of the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO);

Global strategy for foot-and-mouth disease control - PCP-FMD (Progressive Control Pathway for Foot-and-Mouth Disease);

Concepts for strengthening epizootic monitoring and rapid response to outbreaks of particularly dangerous animal infections.

Thus, the research is not only of scientific and applied nature, but also of strategic importance, contributing to the implementation of international initiatives in the field of biosecurity and the prevention of cross-border infections.

Testing the work. The main provisions and results of the dissertation research were presented at international and national scientific events:

at the international scientific and practical conference of the European Commission on the Control of Foot-and-Mouth Disease (EuFMD) - “Improving global security in the supply of effective foot-and-mouth disease vaccines” (EuFMD open meeting, Italy, 2018);

at the V International Conference of Young Scientists: Biotechnologists, Molecular Biologists and Virologists (ANO Koltsovo Innovation Center, Novosibirsk, 2018);

at the International Conference-Symposium "Astana Biotech 2018" Astana, 2018;

report on the results of dissertation work at the Pirbright Institute (The Pirbright Institute, UK).

Based on the results of the completed dissertation research, 18 scientific papers were published, including:

- in scientific periodicals recommended by the Committee for Control of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan:
- “Izdenister, natizheler. Research, results" (Almaty, 2017);
- «Ғылым және білім», scientific and practical journal of the West Kazakhstan Agrarian and Technical University named after Zhangir Khan (Uralsk, 2019);
- “Kazakhstan zhogary mektebi”, scientific and pedagogical journal (Almaty, 2018);
- in international peer-reviewed scientific journals:
- Journal of Virological Methods (Netherlands, 2018; article: Development and evaluation of a novel real-time RT-PCR to detect foot-and-mouth disease viruses from the emerging A/ASIA/G-VII lineage) - indexed in Web of Science;
- Frontiers in Veterinary Science (Switzerland; article: Establishing a molecular toolbox of lineage-specific real-time RT-PCR assays for the detection and characterization of foot-and-mouth disease viruses circulating in Asia) is an international, peer-reviewed, open-access journal indexed in the Web of Science.

Personal contribution of the applicant and gratitude. This dissertation was completed independently by the author, using their own scientific results and experimental data. The author expresses sincere gratitude to the team of the World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) for their scientific and methodological assistance, as well as for the opportunity to conduct research at this laboratory. Special thanks are extended to Akhmetzhan Akievich Sultanov, Head of the Targeted Financing Program of the Ministry of Agriculture of the Republic of Kazakhstan, for his support and assistance in implementing the research. I sincerely thank my scientific advisor, Doctor of Veterinary Sciences, Professor Kutumbetov Lespek Bekbolatovich for valuable scientific advice and support. Special thanks are extended to my foreign scientific advisor, Dr. Donald King, Ph.D., and to Katarzyna Bachanek-Bankowska for their professional guidance, assistance with laboratory research, and fruitful collaboration.

Volume and structure of the dissertation. The dissertation work is presented on 131 pages of computer text and contains 27 tables, 21 figures and consists of the following sections: introduction, choice of research direction, materials and research methods, results of own research, conclusion, list of used sources, including 157 titles.