

Project Title: IRN No. AP09259983 "Development of an express test for the diagnosis of salmonella abortion of horses based on monoclonal antibodies".

Relevance: Equine husbandry in the Republic of Kazakhstan is a vital sector of animal husbandry, with a current horse population exceeding 3,180,000. Salmonella abortion in mares is an infectious disease characterized by abortions and the birth of non-viable foals. The economic impact includes the loss of reproductive capacity in broodmares, reduced foal production, decreased mare productivity, and expenses for veterinary drugs and disinfection. The development of this project aims to enhance animal health and improve the efficiency of disease diagnostics. The primary diagnostic method is bacteriological, but it is insufficiently sensitive, time-consuming, and highly dependent on the quality of the material. According to recommendations, PCR can be used, but its application is challenging due to high equipment and test system costs. ELISA may be used but does not allow for the differentiation of the causative agent.

This project involves the development of a domestic ELISA test for the detection and simultaneous differentiation of the causative agent of salmonella abortion in horses. The test will enable analysis outside laboratories, providing results within 15 minutes. It can be used for monitoring studies instead of labor-intensive bacteriological analysis. The development is in demand not only in Kazakhstan but also in CIS countries where this infection is widespread. Currently, there are no analogs of this technology in the Republic of Kazakhstan.

Objective: To develop a domestic express test based on monoclonal antibodies for the diagnosis of Salmonella abortion in horses, allowing for the rapid and accurate detection of infected animals.

Research Team Members:

Project Leader: Borovikov S.N., candidate of biological sciences, Professor. H-Index: 3, Profile (<http://orcid.org/0000-0002-9721-9732>).

Research Team Members:

Abdrahmanov S.K., D.Sc., Senior Researcher. H-index: 6, Profile (<http://orcid.org/0000-0003-3707-3767>).

Akibekov O.S. – Ph.D., Senior Researcher. H-index: 2, Profile (<http://orcid.org/0000-0002-8647-0083>).

Zhumalin A.Kh., Master, Researcher. H-index: 2, Profile (<http://orcid.org/0000-0003-2057-4186>).

Kuibagarov M.A., Ph.D., Researcher. H-index: 1, Profile (<https://orcid.org/0000-0001-7428-7620>).

Shevtsova E.S., Researcher. H-index: 5, Profile (<https://orcid.org/0000-0002-7221-5866>).

Syzdykova A.S., Master, Researcher. H-index: 3 Profile (<http://orcid.org/0000-0002-8647-0083>).

Expected and Achieved Results:

During the project, 247 samples of biological and pathological material were collected from various farms. PCR and ELISA analysis revealed the presence of *Salmonella spp* DNA and specific antibodies.

The complete genome characterization of three strains of *Salmonella serovar abortus equi* isolated from material samples in the Republic of Kazakhstan was conducted for the first time. *E. coli* strains producing recombinant OmpX *S. abortus equi* protein were created. Recombinant OmpX *Salmonella enterica* proteins were developed and purified. The specificity of recombinant proteins was determined, demonstrating high efficiency in studying sera from horses in affected farms.

Hybrid cells producing antibodies to OmpX *S. abortus equi* were obtained by hybridizing immune splenocytes with a myeloma cell line. Their immunochemical characteristics were studied, indicating their potential use in developing an express test for detecting the causative agent of salmonella abortion in horses.

Components were manufactured, and assembly of the express test for detecting the causative agent of salmonella abortion in horses in material samples was carried out. Laboratory tests of the developed test confirmed its effectiveness, providing results within 15 minutes outside the laboratory.

Normative-technical documentation for the production and application of the ELISA test for the express diagnosis of salmonella abortion in horses has been developed and approved. The test can be recommended for implementation in veterinary practice.

A positive decision on the patent application for the method of obtaining an antigen of outer membrane proteins from bacteria of the genus *Salmonella spp* was obtained in the Republic of Kazakhstan.

The following articles have been published as a result of the research:

Borovikov S.N., Syzdykova A.S. Development of an enzyme-linked immunosorbent assay for serological diagnosis of Salmonella abortion in horses // Bulletin of Science of the Kazakh Agro-Technical University named after S. Seifullin (interdisciplinary). - 2022. - No3 (114). – Part 2. - P. 111-119.

Borovikov S.N., Syzdykova A.S., Zhumalin A.Kh. The use of Salmonella abortus equi protein antigen for serological diagnosis of mares abortion // European Academic Science and Research. – 2022. – P.15-16.

Borovikov S., Syzdykova A., Akibekov O. and Tursunov K. The Use of Various Tests for the Serological Diagnosis of Salmonella Abortion in Horses in Kazakhstan // Int J Vet Sci. - 2023. - P.1-4 CiteScore Q2.

Sergey Borovikov, Anara Ryskeldina, Kanat Tursunov, Alfiya Syzdykova, Orken Akibekov. Expression of recombinant Salmonella enterica OmpX protein and study of its possible use for serological diagnosis of Salmonella abortion in mares // Veterinary World.-2023, 16(9):1790-1795. CiteScore Q1.

Sergey Borovikov, Marat Kuibagarov, Orken Akibekov, Anna Muranets. Clinical case of Salmonella detected in an aborted mare fetus and its characteristics // Int J Vet Sci. - CiteScore Q2 (accepted for publication).

Target Consumers of Scientific Products: "Republican Veterinary Laboratory" Ministry of Agriculture of the Republic of Kazakhstan, regional and district

laboratories; "National Reference Center for Veterinary Medicine"; entities engaged in horse breeding.

Information for Potential Users:

An express test for the diagnosis of salmonella abortion in horses has been developed, allowing analysis of biological and pathological material outside laboratory conditions and providing reliable results within 15 minutes. The use of the test in veterinary practice will enable on-site monitoring of infection, replacing the lengthy and labor-intensive bacteriological analysis. Additionally, the test can be used for a rapid and reliable assessment of the quality of horse meat for microbial contamination by *Salmonella spp.*