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UDC 619:616.39-07(574)(045) **Research article**

Epizootic situation and comprehensive diagnosis of equine salmonellosis-associated abortion in the Republic of Kazakhstan

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Abstract

Background and Aim. Abortion associated with equine salmonellosis is an infectious disease that significantly affects the productivity of herd-based horse breeding in the Republic of Kazakhstan. The disease is characterized by late-term abortions and is associated with considerable economic losses in the agricultural sector. The aim of this study was to detect *Salmonella* DNA using molecular genetic methods.

Materials and Methods. The study was based on pathological material collected from horses in nine regions of Kazakhstan during the period from 2023 to 2025.

Results. PCR analysis revealed that the proportion of positive samples ranged from 25% to 33.3%, depending on the year. Enzyme-linked immunosorbent assay (ELISA) of 309 equine serum samples detected antibodies to *Salmonella abortus equi* in 29 cases (10.6%). Microbiological investigations using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) confirmed the presence of *Salmonella spp.*, as well as opportunistic microorganisms such as *Enterobacter hormaechei, Pantoea agglomerans*, and *Escherichia hermannii.*

Conclusion. The obtained results confirm the circulation of the pathogen among horses and emphasize the need for ongoing epizootiological surveillance. Furthermore, they highlight the importance of continued molecular and genetic studies, including whole genome sequencing, to enhance understanding of the disease's epidemiology and to optimize preventive measures.

Keywords: abortion; diagnostics; epizootiology; horses; MALDI-TOF; salmonellosis.

Introduction

Herd-based horse breeding holds a significant position in the agricultural sector of the Republic of Kazakhstan. As of January 1, 2025, the equine population exceeded 4.2 million, placing the country among the leading nations within the Commonwealth of Independent States in terms of horse numbers. Horses serve not only as draft and transport animals in remote areas, but also as important sources of

meat and milk. The production and processing of mare's milk into koumiss holds cultural and economic importance. However, the characteristic practices of herd management –particularly year-round grazing – create favorable conditions for the spread of infectious diseases, among which salmonellosis-associated abortion poses a particular threat.

Equine abortion remains one of the most significant problems in horse breeding, leading to substantial economic losses and reduced genetic potential in herd populations. It is estimated that infectious causes account for approximately 30–50% of all equine abortions globally, with bacterial pathogens being responsible in over 60% of confirmed infectious cases .Among these, *Salmonella enterica* subsp. *enterica* serovar abortus equi (*S. abortus equi*) holds particular importance as an abortifacient pathogen causing systemic infection and placentitis in pregnant mares [1].

Severe outbreaks of *S. abortus equi* have been reported in foals in Italy, with high morbidity and mortality rates, particularly in mixed infections [2]. Similar cases have been reported in Argentina [3] and central Italy [4], where *S. abortus equi* was identified as a primary abortifacient agent. In Xinjiang, China, a seroprevalence of 20.9% was detected among 971 horses tested using ELISA [5]. Australian studies on stud farms also confirm the circulation of multiple *Salmonella* serotypes [6], while pooled environmental testing in equine hospitals in the U.S. supports the pathogen's persistence in clinical settings [7].

Further research from Italy has shown public health implications associated with *S. abortus equi* in horses slaughtered for meat [8]. Russian researchers have described thrombohemorrhagic syndromes in young livestock associated with salmonellosis [9]. In Argentina, *S. abortus equi* was recognized as an emergent cause of abortion in equines [3].

In Kazakhstan, foundational research was provided by *Sultanov* et al. [1], while recent molecular characterization of local isolates was carried out by *Mussayeva* et al. [10]. According to *Issabekov* et al. [11], bacterial abortion remains a persistent problem in the northern regions of Kazakhstan. These findings are supported by official data from the Committee for Veterinary Control and Supervision of the Ministry of Agriculture, which reported 217 cases of equine abortion between 2021 and 2024, with bacterial causes identified in 132 cases (60.8%) and *S. abortus* equi confirmed in 14.3% of these [12].

New serological approaches using recombinant outer membrane proteins have been proposed for diagnostic improvements [13], while recent experimental vaccine development in Kazakhstan demonstrates active efforts toward prevention [14].

The aim of this study is to assess the current epizootic status of salmonellosis-associated abortion in horses in Kazakhstan. This is achieved by detecting *S. abortus equi* DNA using PCR, evaluating antibody prevalence via ELISA, performing microbiological identification using MALDI-TOF MS, and analyzing the regional and temporal dynamics of positive cases.

Materials and Methods

Ethical approvals

This study was approved by the Local Ethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry Technology at the S. Seifullin Kazakh Agrotechnical Research University, meeting held on November 8, 2023 (Protocol No. 3).

This study was conducted within the framework of the project IRN AR22783162 "Epidemiology and Molecular Genetic Analysis of the Causative Agent of Equine Salmonellosis-Associated Abortion" and covers the period from 2023 to 2025. Research materials including serum samples and pathological specimens were collected from horse farms located in the Karaganda, North Kazakhstan, Abai, Mangystau, Pavlodar, Atyrau, Ulytau, West Kazakhstan, and Akmola regions. A combination of molecular biological, serological, and microbiological diagnostic methods was employed to detect *Salmonella* infection.

PCR was used to detect *Salmonella* DNA in pathological material collected as part of epizootiological surveillance. According to the summary analysis of the epizootic situation, a total of 34 samples were examined. These samples were collected from 15 aborted equine fetuses originating from farms located in the Karaganda, North Kazakhstan, and Akmola regions. From each fetus, pathological material was sampled from internal organs – primarily liver, lungs, spleen, and intestines – depending on the condition and integrity of the carcass. Sampling was performed by veterinary personnel during the spring and autumn periods of 2023-2025, which coincide with seasonal abortion peaks in herd-based

horse breeding. The samples were placed into sterile containers, cooled to 4 °C immediately upon collection, and transported to the laboratory within 24 hours for further molecular analysis.

Diagnosis was performed using a certified real-time PCR test kit from VetFactor. DNA extraction was carried out using the QIAamp DNA Mini Kit according to the manufacturer's instructions (Qiagen, Germany). Amplification was performed using a CFX96 thermal cycler (Bio-Rad, USA). Reliability of the results was ensured through the inclusion of positive and negative control samples.

An ELISA was conducted in 2025 on 309 serum samples collected from horses across nine regions. The ELISA plates were sensitized with a thermostable extract of *S. abortus equi* (10 μg/mL). An anti-horse conjugate (Cusabio, China) diluted 1:2000 was used, along with TMB substrate (Immunobiotech, Russia) and a stop solution of 0.02 M H2SO4 Serum samples were diluted from 1:100 to 1:800, and in some series up to 1:6400. Optical density was measured at 450 nm. A result was considered positive if the optical density was twice (or more) of the negative control.

Microbiological analysis was conducted in 2025 on samples from aborted horse fetuses obtained from farms in the Karaganda and Kostanay regions. The analysis included tissue samples from the liver, lungs, kidneys, spleen, and intestines.

One gram of biological material was collected from each organ and transferred under sterile conditions for further processing. The samples were thoroughly homogenized in 9 mL sterile physiological saline, maintaining a 1:10 ratio to ensure uniform bacterial distribution. The homogenized material was allowed to stand briefly to allow larger tissue fragments to settle, after which the suspension was centrifuged to remove residual tissue debris. The resulting supernatants were incubated in a thermostat under optimal temperature conditions, creating an environment conducive to the activation and initial proliferation of any Salmonella present.

The prepared suspensions were streaked onto Endo agar (for *Enterobacteriaceae detection*) and Salmonella-Shigella (SS) agar using a sterile Drigalski spatula. The plates were incubated aerobically at 37 °C for 18-24 hours. Colonies with characteristic morphology were subcultured for purification.

Real-time PCR was conducted using a certified VetFactor diagnostic kit (VetExpert, Kazakhstan), optimized for *Salmonella spp*. detection. DNA extraction from pathological samples was performed with the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. Each PCR reaction (25 μL) included 5 μL of DNA template, primers/probes from the kit, and Taq polymerase mix. Amplification was carried out on a CFX96 thermal cycler (Bio-Rad, USA) under the following conditions: initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 60 seconds. Fluorescence was detected in the FAM channel. Each run included positive and negative controls to validate results.

Indirect ELISA was developed using a thermostable extract of *Salmonella abortus equi* (10 μg/mL) coated on 96-well microplates in carbonate buffer (pH 9.6), incubated overnight at 4 °C. After blocking and washing, horse serum samples (diluted 1:100 to 1:6400) were added and incubated at 37 °C for 1 hour. Detection was carried out using horseradish peroxidase–conjugated anti-horse IgG (Cusabio, China; 1:2000), followed by TMB substrate and a 0.02 M sulfuric acid stop solution. Absorbance was measured at 450 nm. A result was considered positive if the OD value was at least twice that of the negative control.

Identification of the isolated microorganisms was conducted using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The analysis was performed at the National Center for Biotechnology of the Republic of Kazakhstan (Astana, Kazakhstan). Colonies grown on Endo and Columbia agar were transferred to a polished steel target plate using a sterile loop. After air drying, 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution was applied to each spot. Spectra acquisition was carried out using a Bruker Microflex LT mass spectrometer (Bruker Daltonics, Germany). Identification was performed using the Bruker Biotyper software and database. Score values ≥2.0 were considered reliable for species-level identification.

Results and Discussion

PCR

According to epizootiological surveillance data collected between 2023 and 2025, a total of 34 pathological samples from horses across nine regions of Kazakhstan were tested. Positive results were observed in 5 out of 16 samples (31%) in 2023; 2 out of 6 samples (33.3%) in 2024; and 3 out of 12

samples (25%) in 2025. This confirms the continued circulation of *Salmonella spp*. among herd-based horse populations, particularly in the Karaganda, North Kazakhstan, and Akmola regions.

ELISA

In total, 309 serum samples from mares across nine regions were analyzed. Antibodies to *S. abortus equi* were detected in 29 cases, accounting for 10.6% of all samples tested (Table 1).

Table 1 – Number of samples tested for *Salmonella abortus equi*

№	Region	Number of Samples Tested	Number of Positive Samples	Prevalence, %
1	Karaganda	39	7	17.94
2	Pavlodar	40	3	7.5
3	Akmola	31	5	16.12
4	North Kazakhstan	34	7	20.58
5	Mangystau	30	0	0
6	Abai	38	4	10.52
7	Atyrau	33	2	6.06
8	Ulytau	37	1	2.7
9	West Kazakhstan	27	0	0
	Total	309	29	

To visualize the seroprevalence of Salmonella abortus equi in different regions, a bar chart was constructed (Figure 1). The highest prevalence rates were observed in North Kazakhstan (20.6%) and Karaganda (17.9%) regions, while no antibodies were detected in samples from Mangystau and West Kazakhstan regions.

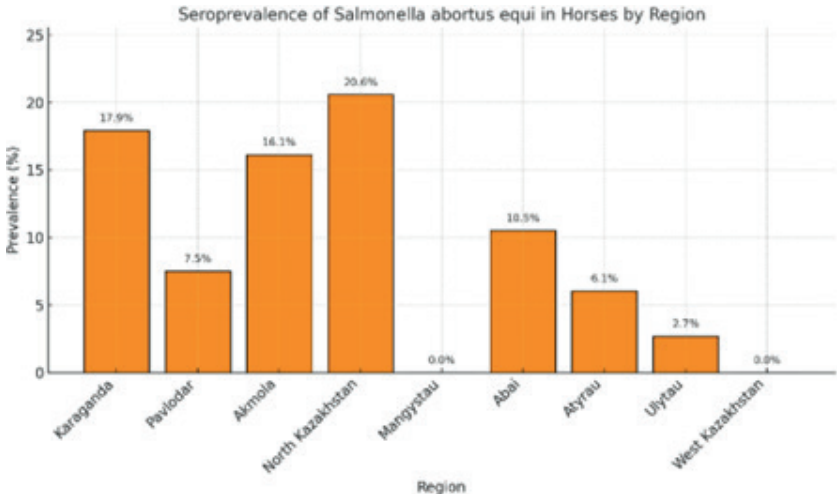

Figure 1 – Regional distribution of seroprevalence rates (%) of *Salmonella abortus equi* in horse serum samples from nine regions of Kazakhstan

In certain cases, high antibody titers of up to 1:1600 were observed, which may indicate persistent carriage or prior infection.

Microbiological Investigations

A bacteriological analysis was conducted on aborted equine fetal tissues received from farms located in the Karaganda and Kostanay regions. MALDI-TOF mass spectrometry performed on isolated colonies revealed the presence of *Enterobacter hormaechei* in lung-derived samples. This microorganism belongs to the *Enterobacteriaceae* family and is considered an opportunistic pathogen, capable of causing various infections in both animals and humans, particularly in immunocompromised individuals.

Further MALDI-TOF analysis identified colonies isolated from a liver sample as *Lelliottia amnigena*. Also, a member of the *Enterobacteriaceae* family, this is an environmental and commensal organism occasionally associated with opportunistic infections (Figure 2).

Rank (Quality)	Matched Pattern	Score Value
1 (-)	Salmonella sp (enterica st Stanley) 15 LAL	1.662
2 (-)	Salmonella sp (enterica st Dublin) Sa05_188 VAB	1.596
3 (-)	Salmonella sp (enteritidis) 25089078 (PX) MLD	1.569
4 (-)	Salmonella sp (enterica st Hadar) Sa05_506 VAB	1.563
5 (-)	Salmonella sp (choleraesuis) 08 LAL	1.506

Figure 2 – Results of mixed-sample analysis: proteins characteristic of *Salmonella spp.* identified

Colonies isolated from liver and lung samples were cultured on Endo agar and Columbia agar for further investigation. Colonies obtained from the spleen were distributed as follows: three Petri dishes with Endo agar, and one with Columbia agar. Subsequent analysis will help clarify the species identity of the isolates and confirm the presence of *Salmonella spp.* (Figure 3).

Result Overview

Organism names which are in blue and underlined are linked to the matching hint table below.

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
A7 (+++) (A)	A7	Salmonella sp	2.437	Salmonella sp	2.4
B7 (++) (A)	B7	Salmonella sp	2.220	Salmonella sp	2.01
C7 (+++) (A)	C7	Salmonella sp	2.334	Salmonella sp	2.23
D7 (+++) (A)	D7	Salmonella sp	2.437	Salmonella sp	2.311
E7 (+++) (A)	E7	Salmonella sp	2.345	Salmonella sp	2.297
F7 (+++) (A)	F7	Enterobacter hormaechei	2.482	Enterobacter hormaechei	2.462
G7 (+++) (A)	G7	Enterobacter hormaechei	2.4	Enterobacter hormaechei	2.379
H7 (+++) (A)	H7	Enterobacter hormaechei	2.488	Enterobacter hormaechei	2.468
A8 (++) (A)	A8	Escherichia hermannii	2.237	Escherichia hermannii	2.191
B8 (+++) (A)	B8	Escherichia hermannii	2.394	Escherichia hermannii	2.346
C8 (+++) (B)	C8	Lelliottia amnigena	2.403	Lelliottia amnigena	2.381
D8 (+) (B)	D8	Lelliottia amnigena	1.95	Lelliottia amnigena	1.917

Figure 3 – MALDI-TOF mass spectrometry result of a colony isolated from the spleen

The following pathogens were identified in tissue samples from the liver, lungs, intestines, and spleen of aborted equine fetuses from farms in the Karaganda region: *Salmonella spp., E. hormaechei, Escherichia hermannii, and L. amnigena.* In contrast, samples from farms in the Kostanay region (Vladimirovka village) revealed the presence of *Escherichia fergusonii* (kidneys), *Pantoea agglomeran*s (lungs, liver, spleen, and heart), and *Mixta calida* (intestines). The use of MALDI-TOF mass spectrometry confirmed the polyetiological nature of microbial associations. In several cases, the isolation of *Salmonella spp.* coincided with the presence of opportunistic bacteria, which may enhance the pathogenicity of the infectious process.

The data obtained confirm the ongoing epizootic concerns regarding salmonellosis-associated abortion in horses within several regions of the Republic of Kazakhstan. Although no official outbreak foci have been reported, the detection of relevant species via PCR and ELISA indicates ongoing pathogen circulation and the potential threat posed by latent or chronic carriers. The proportion of PCR-positive results from samples collected between 2023 and 2025 ranged from 25% to 33%. This may be

influenced by regional disease prevalence, as well as the quality of pathological material collected. The highest proportions of positive cases were recorded in the Karaganda, Akmola, and North Kazakhstan regions, aligning with serological and microbiological findings from 2025.

ELISA testing confirmed the presence of antibodies to *S. abortus equi* in 10.6% of serum samples. High antibody titers (up to 1:1600) observed in some animals suggest prior infection or an ongoing chronic condition. The positive results observed in samples obtained from multiple regions – from Abai to Atyrau – point to a broad geographic distribution of the pathogen, with no clear regional confinement.

Noteworthy are the microbiological findings from aborted fetuses, where MALDI-TOF mass spectrometry not only identified *Salmonella spp.*, but also co-isolated several opportunistic bacteria, including *E. hormaechei, E. hermannii, P. agglomerans, E. fergusonii, L. amnigena*, and *Mixtacalida.* These findings highlight the polyetiological nature of the disease, wherein multiple microorganisms may concurrently contribute to pathogenesis. Mixed infections are likely to exacerbate clinical severity and complicate diagnostic efforts.

The results underscore the importance of incorporating comprehensive diagnostic methods including PCR, ELISA, and MALDI-TOF in infectious disease surveillance systems for herd-based horse breeding. This is particularly relevant for remote areas of Kazakhstan where veterinary infrastructure is limited and horses remain integral to traditional lifestyles and local economies.

This study not only confirmed the presence of *Salmonella* infection in horses but revealed important aspects of its polyetiology and regional epizootic patterns, providing a foundation for the development of targeted preventive and veterinary measures.

Conclusion

These findings confirm the widespread prevalence of salmonellosis-associated abortion in horses in Kazakhstan, and demonstrate the continued circulation of *S. abortus equi* among the equine population. The use of diagnostic methods such as PCR, ELISA, and microbiological testing facilitates accurate pathogen identification and improves diagnostic outcomes. Moreover, this study highlights the polyetiological nature of the disease, evidencing the necessity for further investigation into the interactions of multiple microorganisms in equine salmonellosis pathogenesis. The continued use of molecular and serological techniques is essential for effective surveillance and prevention strategies.

Authors' Contributions

TB, ZhB and GE: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript., DSH, AK, AS, IA and SA: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript".

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Conflicts of Interest

The authors declare that they have no competing interests.

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