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Research article

PCR-based identification of animal species in commercial sausage products using cytochrome b gene markers

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Abstract

Background and Aim. Authentication of meat species in processed products is important for food quality control and consumer protection. This study evaluated the applicability of a PCR assay targeting the mitochondrial cytochrome b (*Cyt b*) gene for species identification in commercial sausage products.

Materials and Methods. Reference samples of horse, cattle, sheep, pig, and chicken meat were analyzed together with 15 commercial sausage products. Total DNA was extracted and amplified using species-specific *Cyt b* primers. PCR products were analyzed by agarose gel electrophoresis and selected amplicons were confirmed by sequencing.

Results. Species-specific amplification products corresponding to the expected fragment sizes were obtained for all investigated animal species. Analysis of commercial sausage products revealed both agreement and discrepancies between declared and detected meat species composition. Sequencing confirmed the identity of selected PCR products.

Conclusion. The *Cyt b*-based PCR assay proved suitable for qualitative identification of animal species in thermally processed meat products and may be useful for routine authenticity testing of meat products.

Keywords: meat adulteration; *Cyt b;* PCR; food control; meat species identification.

Introduction

Meat adulteration through the substitution of high-value meat species with less expensive alternatives remains a significant concern for food safety, consumer protection, and regulatory authorities [1, 2]. Species substitution may lead to economic fraud, mislabeling, and violations of religious or ethical dietary requirements [3]. Processed meat products such as sausages are particularly vulnerable to adulteration because morphological characteristics of the original raw materials are lost during processing, making visual identification impossible [4].

Reliable authentication of meat species therefore requires analytical methods capable of detecting species-specific markers in complex and thermally processed food matrices [5]. Various approaches have been proposed for meat authentication, including physicochemical, spectroscopic, immunological, and

molecular genetic techniques [6, 7, 8]. Among these, polymerase chain reaction (PCR)-based methods are considered highly reliable because DNA remains detectable even after substantial technological processing [9, 10].

Mitochondrial DNA markers are particularly suitable for species identification owing to their high copy number and relative resistance to degradation. The cytochrome b (*Cyt b*) gene has been widely used for the authentication of meat products and species differentiation due to the presence of both conserved and species-specific regions. Although several multiplex PCR systems have been described for simultaneous detection of multiple animal species, singleplex PCR assays remain attractive because of their simplicity, reduced risk of primer competition, and ease of result interpretation [11, 12].

The present study was based on the hypothesis that species-specific *Cyt b* primers can provide reliable identification of animal species in thermally processed meat products. Therefore, the objective of this study was to evaluate the applicability and analytical specificity of a *Cyt b*-based PCR assay for detecting declared and undeclared animal species in commercial sausage products.

Materials and Methods

Samples and reagents. In this study, muscle tissue from horse meat (n = 5), beef (n = 5), lamb (n = 5), pork (n = 5), and chicken (n = 5) were used as reference samples. In addition, commercial meat products were analyzed, including boiled sausages (n = 4), smoked sausages (n = 7), and frankfurters (n = 4). All samples were stored at −20 °C until DNA extraction.

DNA Extraction and PCR Amplification.

Total DNA was extracted from meat products using the DNA-EXTRAN-2 kit (Syntol, Russia) according to the manufacturer's instructions. PCR reactions were performed in a total volume of 25 μL containing 2 μL of genomic DNA, 1 μL of each primer, 0.5 μL dNTP mixture, 2.5 μL of 10× reaction buffer, 0.2 μL Taq DNA polymerase, and nuclease-free water. Amplification was performed in a T100 thermal cycler (Bio-Rad, USA) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 53–65 °C for 30 s, and extension at 68 °C for 40 s, with a final extension at 68 °C for 5 min. PCR amplification products were purified using the QIAquick PCR Purification Kit (Qiagen, Singapore) according to the manufacturer's protocol. Selected PCR amplicons were subjected to Sanger sequencing using an ABI 3730xl DNA Analyzer (Applied Biosystems, USA). Sequencing data were collected using 3730 DNA Analyzer Data Collection Software v3.0 and analyzed using Sequencing Analysis Software v5.3.1 (Applied Biosystems, USA). Consensus sequences were aligned and compared with reference *Cyt b* sequences retrieved from the GenBank database to confirm species identity.

DNA extracted from reference samples of each animal species served as positive controls. A no-template control containing nuclease-free water instead of DNA was included in each PCR run to monitor possible contamination.

Species-specific primers targeting the *Cyt b* gene, used for PCR amplification, are presented in Table 1.

Table 1. Primers used for amplification of the *Cyt b* gene

Animal species	Primer sequence (5'→3')	Amplicon size, bp	Annealing temperature (°C)
Horse	F: AGCCACCCTTACCCGATTTTT R: GTCCGCCGATTCATGTTAGTGTC	500	59
Cattle	F: ACGAGGCTTATATTATGGGTCTTA R: ATTGGCCGGGGTGTAGTTATC	500	53
Sheep	F: TCCCAGCCCCCTCAAACAT R: AAAAGCCCCCTCAGATTCATTC	550	60
Pig	F: ACGCAAACGGGGCATCA 3' R: GGGGGAGTGTTAAGTGGGTTTG	450	65
Chicken	F: CCTTTGTGGGCTATGTTCTC R: GGGGGTTACTAGTGGGTTTG	400	55

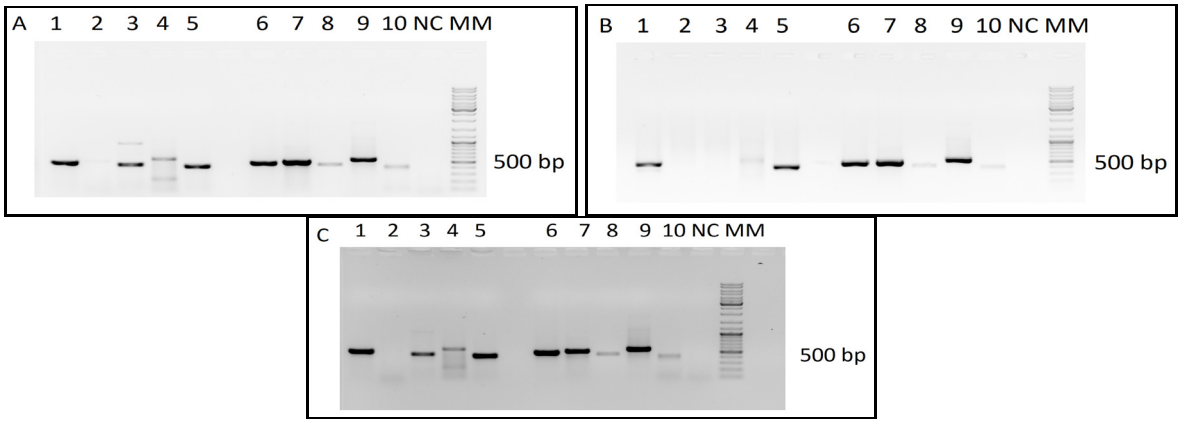
PCR products were analyzed by electrophoresis in a 1% agarose gel. To prepare the gel, 0.4 g of agarose was added to 40 µL of 1× TAE buffer and melted in a microwave oven. The solution was then cooled to 50–60 °C, after which 2.5 µL of ethidium bromide solution was added. The mixture was poured into a casting tray to form a thin gel layer. Using special combs, wells were formed in the gel for sample loading. For electrophoretic analysis, 10 µL of each PCR product was mixed with 2 µL of 6× loading dye and loaded into the agarose gel wells. The electrophoresis was performed under the following conditions: electric field strength of approximately 6 V/cm for 50 minutes. The results of electrophoresis were visualized by UV irradiation of the gel using a transilluminator.

Statistical Analysis

The present study was designed as a qualitative PCR-based species identification assay. Therefore, quantitative statistical comparison of amplification signals was not performed. Results were interpreted based on the presence or absence of species-specific amplicons of the expected size. The proportion of samples showing agreement between declared and detected species composition was calculated descriptively. Because of the limited sample size, estimates of analytical sensitivity, specificity, and limit of detection were not determined and should be evaluated in future studies.

Results

PCR analysis of three types of sausage products using species-specific primers targeting the *Cyt b* gene of cattle, horse, pig, sheep, and chicken revealed DNA fragments of 500, 450, and 400 base pairs, as shown in Figure 1. Amplification of the *Cyt b* gene segment in cattle corresponded to a fragment size of 500 base pairs. The amplicon sizes of the mitochondrial DNA fragments of pig and chicken were 450 bp and 400 bp, respectively, which correspond to the expected fragment sizes.



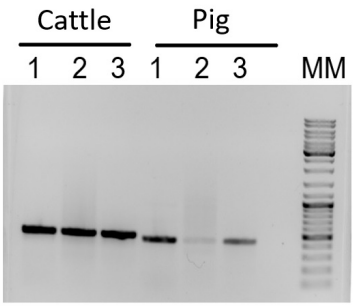
Note: smoked sausage (A), boiled sausage (B), frankfurters (C)

Lane 1 – primers targeting *Cyt b* of cattle; Lane 2 – primers targeting *Cyt b* of horse; Lane 3 – primers targeting *Cyt b* of pig; Lane 4 – primers targeting *Cyt b* of sheep; Lane 5 – primers targeting *Cyt b* of chicken; Lane 6 – DNA of cattle; Lane 7 – DNA of horse; Lane 8 – DNA of pig; Lane 9 – DNA of sheep; Lane 10 – DNA of chicken; NC – negative control.

Figure 1. Electrophoregram of PCR analysis of sausage products

Since the composition of the analyzed sausage products did not indicate the presence of pork components, a control PCR analysis was performed using species-specific primers targeting the *Cyt b* gene of cattle and pig.

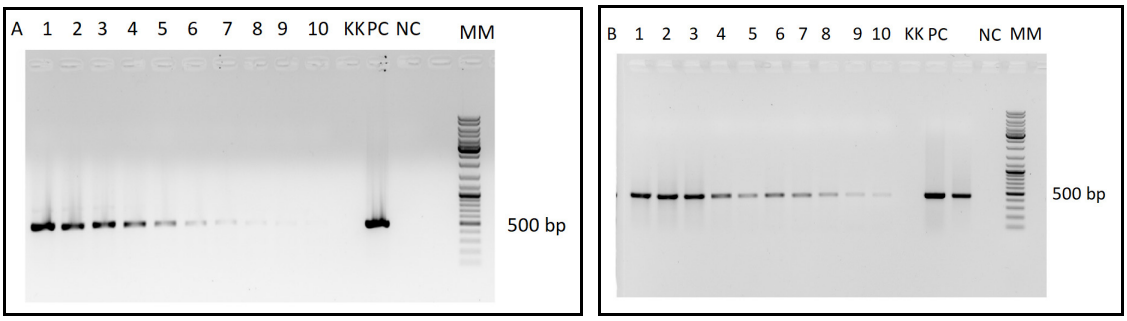
To increase the specificity of the reaction, the annealing temperature was increased to 70 °C, while the optimal annealing temperatures were 53 °C for primers targeting the *Cyt b* gene of cattle and 57 °C for primers targeting the *Cyt b* gene of pig (Figure 2).



Lane 1 – smoked sausage; Lane 2 – boiled sausage; Lane 3 – frankfurters.
Figure 2. Electrophoresis of PCR analysis of sausage products using species-specific primers targeting the *Cyt b* gene of cattle and pig

As shown in Figure 2, the increase in annealing temperature confirms the presence of pig DNA in samples of smoked sausage and frankfurters. In samples of boiled sausage, trace amounts of pig DNA were detected, which may be associated with contamination of production equipment.

The analytical sensitivity of the quantitative PCR assay was assessed using serial dilutions of DNA isolated from animal biological specimens. The assay demonstrated a detection limit ranging from 62.5 pg to 1.9 pg of DNA per 25 µL reaction volume (Figure 3).



Lane 1 – 1000 pg; Lane 2 – 500 pg; Lane 3 – 250 pg; Lane 4 – 125 pg; Lane 5 – 62.5 pg; Lane 6 – 31.2 pg; Lane 7 – 15.6 pg; Lane 8 – 7.5 pg; Lane 9 – 3.9 pg; Lane 10 – 1.9 pg; Lane 11 – 0.9 pg; PC – DNA extracted from beef (positive control); NC – reaction mixture without DNA (negative control); KK – DNA from a non-target animal species; MM – DNA molecular weight marker.
Figure 3. Analytical sensitivity of primers targeting the cytochrome b (*Cyt b*) gene of cattle (A), pig (B)

The simultaneous detection of species-specific amplicons of cattle and pig DNA in the same samples indicates a mixed species composition of the meat raw materials. Differences in band intensity were observed; however, because the assay was qualitative, no conclusions regarding the relative proportion of species-specific DNA were made. The electrophoretic profile indicates the presence of biological material from both cattle and pigs in the sausage products, which is important for quality control, detection of adulteration, and verification of product labeling compliance.

For validation of the PCR analysis based on primers targeting the *Cyt b* gene, four types of boiled sausages, seven types of smoked sausages, and four types of frankfurters were examined. Table 2 presents the results of species identification of meat raw materials in sausage products of various types using PCR based on analysis of the *Cyt b* gene. The declared composition of the products was compared with the actual animal DNA detected in the sausage samples.

Table 2. Results of composition determination in sausage product samples

No.	Product type	Composition	Animal DNA detected by PCR based on the Cyt b gene
1	Boiled sausage No. 1	Beef Chicken meat Spices	Cattle DNA Chicken DNA
2	Boiled sausage No. 2	Beef Pork meat Spices	Cattle DNA Pig DNA Chicken DNA
3	Boiled sausage No. 3	Beef Chicken meat Spices	Cattle DNA Pig DNA Chicken DNA
4	Boiled sausage No. 4	Pork Chicken meat Spices	Pig DNA Chicken DNA
5	Smoked sausage No. 1	Pork Chicken meat Spices	Pig DNA Chicken DNA
6	Smoked sausage No. 2	Beef Chicken meat Spices	Cattle DNA Chicken DNA
7	Smoked sausage No. 3	Beef Chicken meat Spices	Cattle DNA Pig DNA Chicken DNA
8	Smoked sausage No. 4	Beef Chicken meat Spices	Cattle DNA Chicken DNA
9	Smoked (Krakow-style) sausage No. 1	Chicken meat Spices	Chicken DNA
10	Smoked (Krakow-style) sausage No. 2	Pork Beef Spices	Cattle DNA Pig DNA
11	Smoked (Krakow-style) sausage No. 3	Beef Spices	Cattle DNA Pig DNA
12	Frankfurters No. 1	Beef Chicken meat Spices	Cattle DNA Chicken DNA
13	Frankfurters No. 2	Beef Spices	Cattle DNA
14	Frankfurters No. 3	Beef Chicken meat Pork Spices	Cattle DNA Chicken DNA Pig DNA
15	Frankfurters No. 4	Beef Chicken meat Spices	Cattle DNA Chicken DNA Pig DNA

DNA of animal species was successfully detected in all analyzed samples, confirming the suitability of the PCR method based on the *Cyt b* gene for the analysis of thermally processed meat products. The obtained data demonstrate both consistencies and discrepancies between the declared composition and the actual species composition of the sausages. In several samples (boiled sausages No. 1 and No. 4; smoked sausages No. 1, No. 2, and No. 4; frankfurters No. 1–3), the detected DNA corresponded to the meat species indicated on the label. This indicates correct labeling and the absence of substitution of meat raw materials in these products.

At the same time, in samples of boiled sausage No. 2 and No. 3, as well as in smoked sausage No. 3 and smoked (Krakow-style) sausages No. 1–3, the presence of animal DNA not declared in the product composition was detected (pig or chicken DNA in the absence of corresponding labeling). In samples of smoked (Krakow-style) sausage No. 2 and frankfurters No. 4, DNA from several animal species was detected simultaneously, indicating either the use of mixed meat raw materials or possible technological cross-contamination. The detection of chicken DNA in products where it is not declared may indicate a partial substitution of more expensive meat raw materials with cheaper alternatives, or the use of shared production lines without adequate cleaning procedures.

The data presented in the table confirm the high informative value of PCR analysis targeting the *Cyt b* gene for monitoring the authenticity of meat products. The detected discrepancies between the declared and actual species composition indicate possible cases of adulteration or violations of production technology, which highlights the necessity of molecular genetic control within the system of veterinary sanitary inspection and quality monitoring of meat products.

To confirm the presence of pig DNA in sausage products where it was not declared, additional analyses were conducted to isolate and determine the nucleotide sequence of the amplified fragment. Sequencing of selected amplicons showed high similarity to reference *Cyt b* sequences available in GenBank, supporting the species identification results obtained by PCR. (Figure 3).

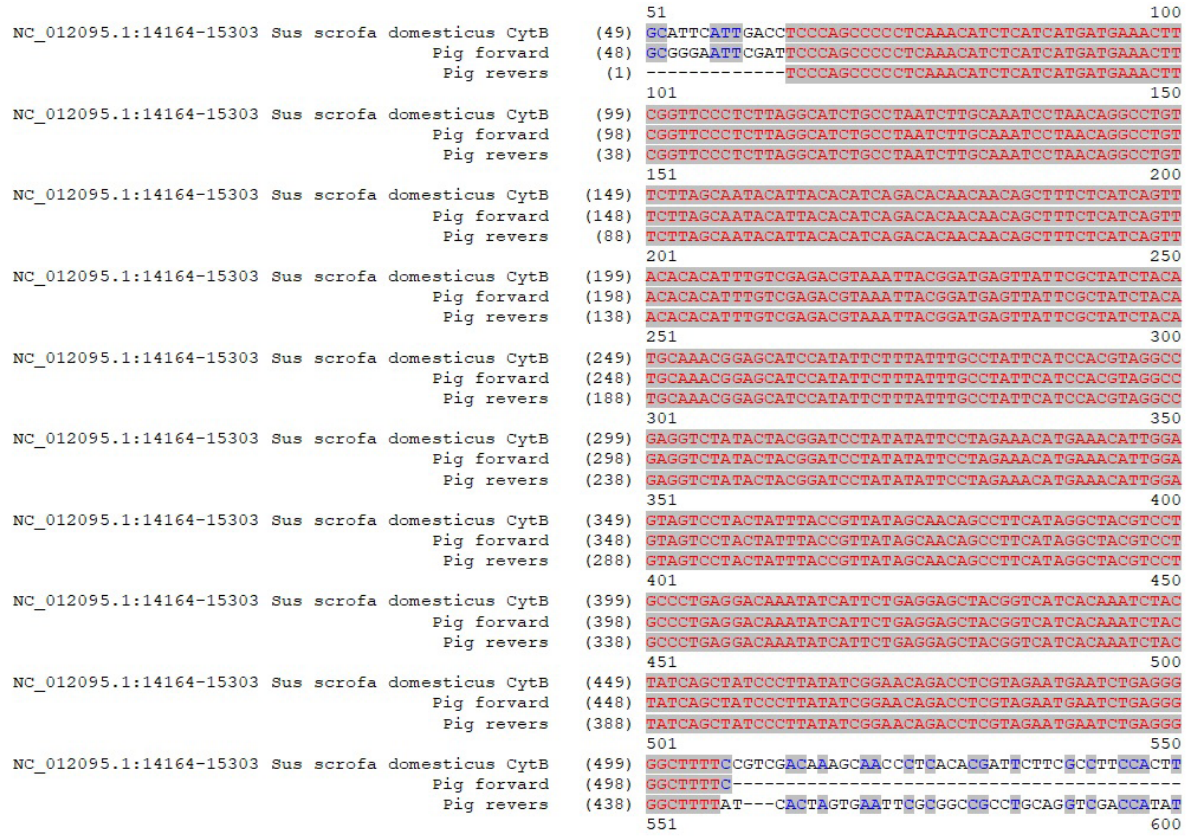


Figure 3. Alignment of the reference nucleotide sequence with the Cyt b DNA fragment isolated from a sausage product

Figure 3 shows that the nucleotide sequences of the *Cyt b* gene fragment isolated from sausage products almost completely coincide with the reference sequence of Sus scrofa domesticus (NC_012095). Throughout the analyzed region, a high degree of nucleotide conservation is observed, which is characteristic of the mitochondrial *Cyt* b gene and confirms its suitability as a marker gene for species identification of meat raw materials. Distinct regions of complementary binding of the forward and reverse primers (Pig forward and Pig reverse) can be clearly identified, indicating the correct primer design and the specificity of PCR amplification. The absence of significant mismatches in the primer-binding regions suggests a high efficiency of amplification of the target pig DNA fragment, even under conditions of technological processing of meat products. Minor nucleotide variations observed at several positions are not systematic and may be attributed to intraspecific variability or sequencing characteristics. Overall, the obtained data demonstrate the high efficiency and specificity of the molecular genetic identification method based on analysis of the *Cyt b* gene.

Discussion

The present study demonstrated the applicability of a PCR assay targeting the mitochondrial *Cyt b* gene for qualitative identification of animal species in commercial sausage products. Species-specific amplification products of the expected size were obtained for cattle, horse, sheep, pig, and chicken DNA, indicating successful amplification of the selected target regions. Furthermore, amplification products were detected in thermally processed meat products, confirming the suitability of mitochondrial *Cyt b* markers for analysis of processed food matrices. Analysis of commercial sausage products revealed both agreement and discrepancies between the declared and detected species composition. In several samples, the detected DNA corresponded to the species listed on the product label, whereas in other cases undeclared animal species were identified. The presence of undeclared DNA may indicate substitution of meat raw materials, inaccurate labeling, or technological cross-contamination during processing. However, because the assay was designed as a qualitative method, the relative proportion of each species present in mixed products could not be determined.

To further verify the PCR results, selected amplicons were subjected to nucleotide sequence analysis. Comparison of the obtained sequences with reference *Cyt b* sequences demonstrated high sequence similarity, supporting the species identification results obtained by PCR. These findings are consistent with previous studies reporting the usefulness of mitochondrial *Cyt b* markers for authentication of meat products and detection of species substitution. The practical applicability of the assay was additionally demonstrated through the analysis of commercially available sausage products. The ability to detect animal DNA in thermally processed samples suggests that the method may be useful for routine monitoring of meat authenticity in veterinary and food control laboratories [12].

Several limitations of the present study should be acknowledged. First, the assay was evaluated as a qualitative method and does not provide quantitative information regarding the proportion of meat species in mixed products. Second, cross-reactivity was evaluated only for the investigated animal species and was not assessed using DNA from additional species. Third, technical PCR replicates were not performed, and therefore assay reproducibility was not formally evaluated.

In addition, the relatively small number of reference samples does not allow estimation of diagnostic sensitivity and specificity with statistical confidence. Blinded evaluation of samples was not performed, as the declared composition of commercial products was known to the investigators during analysis. Although species identification was based on objective PCR amplification and sequence analysis results, the possibility of observational bias cannot be completely excluded. Future studies should therefore focus on determination of the limit of detection, assessment of assay reproducibility, blinded sample analysis, evaluation of analytical performance characteristics, and validation using larger sample sets and experimentally prepared meat mixtures.

Conclusion

The present study demonstrated the applicability of a *Cyt b*-based PCR assay for qualitative identification of cattle, horse, sheep, pig, and chicken DNA in commercial sausage products. The method successfully detected both declared and undeclared animal species in thermally processed products. The obtained results indicate that *Cyt b*-based PCR may serve as a useful tool for routine meat authenticity testing. However, further studies are required to determine analytical sensitivity, limit of detection, and diagnostic performance characteristics of the assay.

Authors' Contributions

SB, AB: Planned the study and drafted and revised the manuscript. KT, AS: Performed the extraction of DNA and PCR analysis. KM, AB: Performed the analysis date. All authors have read, reviewed, and approved the final manuscript.

Acknowledgments

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) for language editing and improvement of grammar. The authors reviewed and edited the output and take full responsibility for the content of the publication.

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Conflicts of Interest

The authors declare that they have no conflicts of interest. Aitbay Bulashev serves as the Scientific Editor of CAJVS; however, he did not participate in the peer review process or in the decision-making regarding this manuscript.

References

1 Kowalczyk, S. (2021). Quality and methods of adulteration of meat and fish products on the Polish market in 2010-2020. *Rocz Panstw Zakl Hig,* 72(4), 361–372. DOI: 10.32394/rpzh.2021.0183.

2 Han, F., Huang, X., J.H.A., Zhang, D., Feng, F. (2020). Detection of Beef Adulterated with Pork Using a Low-Cost Electronic Nose Based on Colorimetric Sensors. *Foods,* 9(2), 1–15.

3 Vaithiyanathan, S., Vishnuraj, M.R., Narender Reddy, G., Srinivas, C. (2021). Authentication of camel meat using species-specific PCR and PCR-RFLP. *J Food Sci Technol*., 58(10), 3882–3889. DOI: 10.1007/s13197-020-04849-w.

4 Rohman, A. (2018). The employment of Fourier transform infrared spectroscopy coupled with chemometrics techniques for traceability and authentication of meat and meat products. *J Adv Vet Anim Res*., 6(1), 9–17. DOI: 10.5455/javar.2019.f306.

5 Doosti, A., Ghasemi Dehkordi, P., Rahimi, E. (2014). Molecular assay to fraud identification of meat products. *J Food Sci Technol.*, 51(1), 148–52.

6 Mualim, M., Latif, H., Pisestyani, H., Rahayu, P. (2024). Analysis of species adulteration in beef sausage using real-time polymerase chain reaction in Makassar, Indonesia. *Vet World.*, 17(10), 2355–2364.

7 Cahyadi, M., Wibowo, T., Pramono, A., Abdurrahman, Z.H. (2020). A Novel Multiplex-PCR Assay to Detect Three Non-Halal Meats Contained in Meatball using Mitochondrial 12S rRNA Gene. *Food Sci Anim Resour.,* 40(4), 628–635. DOI: 10.5851/kosfa.2020.e40.

8 Wadood, S.A., Boli, G., Xiaowen, Z., Hussain, I., Yimin, W. (2020). Recent development in the application of analytical techniques for the traceability and authenticity of food of plant origin. *Microchemical Journal,* 152, 104295.

9 Fanelli, V., Mascio, I.., Miazzi, MM., Savoia, M.A., De Giovanni, C., Montemurro, C. (2021). Molecular Approaches to Agri-Food Traceability and Authentication: An Updated Review. *Foods,* 10(7), 1644. DOI: 10.3390/foods10071644.

10 Karppinen, K., Avetisyan, A., Hykkerud, A.L., Jaakola, L. (2022). A dPCR Method for Quantitative Authentication of Wild Lingonberry (Vaccinium vitis-idaea) versus Cultivated American Cranberry (V. macrocarpon). *Foods,* 11(10), 1476. DOI: 10.3390/foods11101476.

11 Ulberth, F., Koeber, R. (20025). Reference materials for food authentication. *Anal Bioanal Chem.*, 417(12), 2427–2438. DOI: 10.1007/s00216-025-05743-0.

12 Afifa Khatun, M., Hossain, A., Hossain, M.S., Kamruzzaman Munshi, M., Huque, R. (2021). Detection of species adulteration in meat products and Mozzarella-type cheeses using duplex PCR of mitochondrial cyt b gene: A food safety concern in Bangladesh. *Food Chem (Oxf)*, 2, 100017. DOI: 10.1016/j.fochms.2021.100017.

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Research article

Clinical trials of a test kit for the visual diagnosis of uterine diseases in cows

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Abstract

Background and Aim. The prevalence of uterine diseases in cows is reported to range from 14.3% to 50% of obstetric and gynecologic disorders, compromising reproductive function and increasing economic losses. Clinical diagnosis is most informative in the early postpartum period, whereas laboratory methods, despite high specificity, are often impractical for routine on-farm use. This study aimed to develop a test kit for visual diagnosis of uterine diseases in cows and to determine its diagnostic performance.

Materials and Methods. The study was performed on a commercial dairy farm («En-Dala» LLP) in Holstein-Friesian cows (n=100) in their first or second lactation. The design features of the test kit for visual diagnosis of uterine diseases in cows (catheter length, catheter diameter) were determined using a comparative approach. The performance of clinical methods (rectal and vaginal examinations), laboratory methods (Whiteside test and cytology), and two prototype reagents included in the visual test kit was evaluated by calculating sensitivity, specificity, diagnostic accuracy, PPV, and NPV. The diagnostic parameters of the test (foam formation, discoloration time) were determined using descriptive statistics and a comparative method.

Results. An optimal catheter length of 40 cm and diameter of 0.6 cm were established, and a 50-mL syringe with appropriate design features was selected for aspiration of cervicovaginal mucus (CVM). Prototype 1 (10% H_2O_2 + 0.01% methylene blue) showed the most favorable diagnostic profile (Sens. 90.3%, Spec. 100.0%, Acc. 94.2%, PPV 100.0%, NPV 87.5%) versus the other methods, maintaining maximal specificity regardless of days in milk. Diagnostic criteria for test use were defined as foam formation >1 cm and discoloration within ≤4 min, with stratification by clinical course.

Conclusion. The developed test kit for visual diagnosis of uterine diseases in cows is a rapid and highly specific diagnostic method, enabling its use for screening the herd for uterine disease regardless of the days in milk.

Keywords: clinical diagnosis; clinical effectiveness; dairy cattle; uterine disease.

Introduction

Uterine diseases in cows remain among the most significant causes of impaired reproductive performance and economic losses in dairy cattle production (add a reference). The prevalence of

these conditions may range from 14.3% to 50% of all obstetric and gynecologic disorders recorded in dairy cows, which contributes to cow infertility and represents a limiting factor for agriculture sector development [1, 2]. According to data reported by international researchers within the European Union, costs associated with endometritis are approximately €30 per cow [3]. A key feature of the clinical course of uterine diseases in cows is their confusing dynamic: variation in the inflammatory process, the severity of clinical signs, and the differential effectiveness of diagnostic methods depending on days in milk necessitate a stratified diagnostic approach to these conditions [4].

Under on-farm conditions, the most accessible approaches for diagnosing uterine disease remain clinical methods (rectal and vaginal examination) add reference. However, the effectiveness of these methods depends on the veterinarian's expertise and the days in milk, whereas in subclinical cases they are largely uninformative [5, 6]. In recent years, ultrasonography has also been widely implemented on farms for the diagnosis of uterine disease, based on detection of intrauterine exudate, measurement of endometrial wall thickness, and identification of abnormal contents within the uterine lumen; however, these findings are not universal diagnostic indicators in subclinical disease [7, 8]. Therefore, a combined «clinical examination + ultrasonography + cytology» approach is currently recommended when monitoring reproductive tract status in cows at different days in milk points to improve diagnostic accuracy [9].

Research efforts are ongoing to identify rapid diagnostic solutions for uterine diseases in cows that enable accurate diagnosis based on characteristics of uterine contents and the micro and macroscopic structure of the organ itself: changes in the structure of the organ, the cellular composition of the uterine walls, changes in the chemical composition of uterine secretions. For example, histologic examinations have revealed endometrial changes in cows with subclinical endometritis, characterized by a vascular response, strands of collagen fibers, and focal inflammatory lesions [10]. Morphometric measurements of the reproductive tract in cows make it possible to develop a standardized sampling protocol for histologic assessment during biopsy, which in turn facilitates procedural standardization and, consequently, improves diagnostic accuracy [11]. The use of endoscopic systems of various designs may also represent an effective approach for diagnosing uterine disease in cows, as supported by the use of visual insemination system – the AlphaVision device (IMV Technologies, France) which not only increased the proportion of successful inseminations but also identified reproductive system abnormalities in 14% of the study animals [12].

An additional approach involves using the physicochemical properties of uterine contents as a source of diagnostic markers for uterine disease in cows. One example is the Metrisor device (Firat University, Turkey), developed based on gas sensors that detect intrauterine gases produced by pathogenic microflora in the presence of inflammation and enable on-farm diagnosis of metritis in cows with up to 80% accuracy [13]. Evaluation of the enzymatic activity of cervicovaginal mucus has shown that, in combination with dyes, this marker may also serve as a valuable diagnostic tool; accordingly, a new laboratory method for diagnosing uterine diseases in cows was developed [14]. A logical continuation of this work was to adapt the laboratory method for field use by developing an appropriate device and test reagent formulation, followed by validation under farm conditions.

The aim of this study was to develop a test kit for visual diagnosis of uterine diseases in cows and to determine the diagnostic performance parameters when the test is applied. It was expected that, thanks to the optimal design of the test and the composition of the reagents, a method would be developed for diagnosing uterine diseases in cows, allowing for diagnoctics to be carried out in a short time.

Materials and Methods

Ethical approval

The Local Ethics Committee of S. Seifullin Kazakh Agrotechnical Research University (KATRU) approved the study (Protocol No. 3, November 3, 2022). Study planning and implementation were conducted in accordance with the Law of the Republic of Kazakhstan «On Responsible Treatment of Animals» (No. 97-VII LRK, December 30, 2021) [15].

The study was conducted from May to August 2025 at the commercial dairy farm «En-Dala LLP» (Akmola Region) and at the Veterinary Medicine Research Laboratory of the Institute of Animal Science and Veterinary Medicine, KATRU.

The study population comprised Holstein-Friesian cows in their first (n=50) or second (n=50) lactation at 10-91 and more days in milk (d.m.), maintained under a free-stall housing system with ad libitum access to feed and water and milked twice daily.

To determine the sampling device parameters for cervico-vaginal mucus (CVM) collection, catheters with a length of 40 cm and diameters of 0.4 cm, 0.6 cm, and 0.8 cm were compared by assessing insertion depth and the volume of mucus collected. The mucus volume within the catheters was calculated using the cylinder volume formula:

$$V = \pi \times r2 \times h \quad (1)$$

where:

V – the volume of the contents;

π – a constant approximately equal to 3.14;

r – the radius of the catheter lumen;

h – the height of the column of collected mucus.

For these measurements, cows (n=100) were randomly selected into 5 groups depending on the days in milk: Group I, cows (n = 20) at 10–20 days in milk; Group II, cows (n = 20) at 21–30 days in milk; Group III, cows (n = 20) at 31–60 days in milk; Group IV, cows (n = 20) at 61–90 days in milk; and Group V, cows (n = 20) at ≥91 days in milk.

To evaluate the effectiveness of the prototype diagnostic reagents, a comparative analysis of diagnostic methods for uterine diseases was performed. These included clinical methods (rectal and vaginal examination) and laboratory methods (Whiteside test, cytologic examination, and the prototype diagnostic reagents for the visual diagnostic test kit).

Rectal examination was used to assess uterine position, size, consistency, and rigidity, as well as the condition of the uterine horns and body; particular attention was paid to uterine wall thickening, fluctuation, asymmetry of the horns, and pain on palpation. Indicators of uterine disease were considered to include uterine enlargement, uneven wall consistency, and the presence of fluctuation [16].

Vaginal examination included inspection of the mucosa and cervix and collection of CVM. The collected discharge samples were placed in a sterile container with a screw-cap lid, and CVM was evaluated according to a test card for diagnosing the physiologic status of the reproductive tract in cows [17].

The Whiteside test of CVM was performed by placing 1 mL of CVM into a glass test tube, followed by addition of 1 mL of 5% sodium hydroxide solution. The resulting mixture was heated over an alcohol burner to boiling and then cooled under running tap water. An animal was considered diseased if the tube contents turned lemon yellow; in clinically healthy animals, the tube contents did not change [18].

For cytologic evaluation of CVM, mucus samples were collected using Minitube cytology brushes (Minitube GmbH, Germany), and smears were stained using the Romanowsky-Giemsa method. In each smear, 200–300 cells were counted at 1000 × magnification using an Olympus CX41 microscope (Olympus Corporation, Japan). Smears were evaluated by quantifying epithelial cells and leukocytes, specifically polymorphonuclear neutrophils (PMNs) [19].

The first prototype diagnostic reagent for the visual diagnostic test kit for uterine diseases in cows was based on a method for the visual diagnosis of acute and chronic uterine disorders in cows [20]. Using this prototype, a CVM sample was collected, and the free end of the catheter was sealed with a cap. Next, 1 mL of a 10% hydrogen peroxide solution and 1 mL of a 0.01% methylene blue solution were sequentially introduced into the mucus through the catheter lumen. An animal was classified as diseased if foam formation and discoloration of the device contents occurred; in clinically healthy animals, no foam formation was observed and the contents retained their original color [21].

As the second prototype diagnostic reagent for the visual diagnostic test kit for uterine diseases in cows, a 10% hydrogen peroxide solution and a 0.03% 4-chloro-1-naphthol solution were used. The procedure was performed analogously to that of the first prototype. An animal was classified as diseased if foam formation occurred and the device contents turned dark gray; in clinically healthy animals, foam formation did not occur or was minimal, and the color of the contents remained unchanged.

To determine the clinical effectiveness of diagnostic methods for uterine diseases in cows, sensitivity (Sens.), specificity (Spec.), diagnostic accuracy (Acc.), positive predictive value (PPV), and negative predictive value (NPV) were calculated using the following formulas [22]:

$$Sens. = \frac{TP}{TP + FN} \quad (2)$$

where:
Sens. – sensitivity;
TP – number of true positive samples;
FN – number of false negative samples.

$$Spec. = \frac{TN}{FP + TN} \quad (3)$$

where:
Spec. – specificity;
TN – number of true negative samples;
FP – number of false positive samples.

$$\boldsymbol{Acc. = Sens. \times Prev. \times Spec. \times (1 - Prev.)} \quad (4)$$

where:
Acc. – diagnostic accuracy;
Sens. – sensitivity;
Prev. – prevalence;
Spec. – specificity.

$$PPV = \frac{Sens. \times Prev.}{Sens. \times Prev. + (1 - Spec.) \times (1 - Prev.)} \quad (5)$$

where:
PPV – positive predictive value;
Sens. – sensitivity;
Prev. – prevalence;
Spec. – specificity.

$$NPV = \frac{Spec. \times (1 - Prev.)}{(1 - Sens.) \times Prev. + Spec. \times (1 - Prev.)} \quad (6)$$

where:
NPV – negative predictive value;
Spec. – specificity;
Prev. – prevalence;
Sens. – sensitivity.

This study used a combined reference in the form of vaginal examination and cytology. To avoid incorporation bias, the performance of the vaginal examination was evaluated against a composite reference excluding vaginal examination, and the performance of the cytology was evaluated against a composite reference excluding cytology.

Diagnostic parameters obtained when using the visual diagnostic test were determined using standard metric measurements and descriptive statistical methods. The evaluated parameters included foam height and the time to color change of the catheter contents.

Statistical analyses were performed using Jamovi (The jamovi project, version 2.7.6) and MedCalc (MedCalc Software Ltd, version 23.4.8) [23, 24]. The statistical analysis was predominantly descriptive. Continuous (quantitative) variables were summarized as the mean and standard deviation (M ± SD); the standard deviation was calculated using the standard sample formula and used as the primary measure of variability. Diagnostic performance was assessed using 2×2 contingency tables (true positives, false

positives, false negatives, and true negatives) against the reference diagnosis (clinical examination combined with cytology), with calculation of sensitivity, specificity, diagnostic accuracy, and the positive (PPV) and negative (NPV) predictive values. All hypothesis tests were two-sided, and results were considered statistically significant at $P < 0.05$.

Results

To determine the catheter specifications for CVM collection for the visual diagnostic test kit for uterine diseases in cows, 40 cm catheters with diameters of 0.4 cm, 0.6 cm, and 0.8 cm were used. Catheter insertion depth was measured using a ruler placed inside the catheter, and the volume of collected mucus was calculated using the cylinder volume formula. The measurement results are presented in Table 1.

Table 1. Determination of catheter length and diameter for a visual diagnostic test kit for uterine diseases in cows at different days in milk

Group no.	Clinical status	D=0.4 см		D=0.6 см		D=0.8 см	
		l, cm	V, mL	l, cm	V, mL	l, cm	V, mL
I (10-20 d.m.)	diseased	30.1 ± 0.53	0.97 ± 0.04	31.8 ± 0.42	1.12 ± 0.06	29.5 ± 0.61	0.95 ± 0.02
	clinically healthy	24.73 ± 0.32	0.51 ± 0.05	25.68 ± 0.39	0.71 ± 0.04	23.4 ± 0.44	0.63 ± 0.04
II (21-30 d.m.)	diseased	27.5 ± 0.55	0.95 ± 0.04	28.3 ± 0.63	1.16 ± 0.06	27.3 ± 0.65	0.97 ± 0.05
	clinically healthy	21.8 ± 0.4	0.52 ± 0.03	22.4±0.5	0.69 ± 0.05	21.5 ± 0.64	0.62 ± 0.04
III (31-60 d.m.)	diseased	23.5 ± 0.56	0.88 ± 0.05	24.4 ± 0.35	1.01 ±0.05	22.5 ± 0.34	0.91 ± 0.05
	clinically healthy	20.1 ± 0.61	0.49 ± 0.03	20.2 ± 0.76	0.65 ± 0.04	19.3 ± 0.82	0.57 ± 0.05
IV (61-90 d.m.)	diseased	23.4 ± 0.65	0.76 ± 0.05	24.47 ± 0.75	1.01 ± 0.03	22.5 ± 0.43	0.85 ± 0.03
	clinically healthy	18.21 ± 0.21	0.45 ± 0.06	18.53 ± 0.26	0.61 ± 0.06	17.81 ± 1.29	0.51 ± 0.05
V (≥91 d.m.)	diseased	21.75 ± 0.82	0.71 ± 0.03	22.85 ± 1.31	0.98 ± 0.02	21.47 ± 0.92	0.76 ± 0.03
	clinically healthy	17.75 ± 0.43	0.45 ± 0.05	18.06 ± 0.33	0.5 9 ± 0.04	17.4 ± 0.56	0.47 ± 0.04

The data in Table 1 show that, across all groups and for all catheter diameters, the mean insertion depth was statistically significantly greater in diseased animals ($P < 0.05$) than in clinically healthy animals. In Group I, when using a catheter with a 0.6-cm diameter, the insertion depth into the vagina up to the cervix was 31.8 ± 0.42 cm in diseased cows, whereas it was 25.68 ± 0.39 cm in clinically healthy cows. A similar trend was observed for the volume of collected CVM samples, with values in diseased animals being 1.5–2.0 times higher than those in clinically healthy animals.

When analyzing data from cows with uterine disease, a catheter with a diameter of 0.6 cm – particularly at 10–60 days in milk – enabled collection of the largest volume of CVM compared with catheters of 0.4 cm and 0.8 cm. This may be attributable to mucus viscosity and catheter lumen patency: a 0.4 cm catheter may become occluded, whereas a 0.8 cm catheter may generate insufficient negative pressure for aspiration.

These results indicate that the initially selected catheter length of 40 cm for the CVM sampling device is sufficient for aspiration of the contents, and that the optimal catheter diameter is 0.6 cm, which allows collection of an adequate amount of CVM for analysis.

The next stage of the study was to compare the clinical effectiveness of diagnostic methods for uterine diseases in cows at different days in milk. For this purpose, clinical and laboratory diagnostic methods were used, including the prototype diagnostic reagents for the visual diagnostic test for uterine diseases in cows. The results are presented in Table 2.

Table 2. Clinical effectiveness of diagnostic methods for uterine diseases in cows at different days in milk (n=65)

Days in milk	Diagnostic method	Diagnostic performance metrics (%)				
		Sens.	Spec.	Acc.	PPV	NPV
10–20	Rectal examination	94.7	33.3	86.4	90.0	50.0
	Vaginal examination	94.7	75.0	91.3	94.7	75.0
	Whiteside test	42.9	50.0	44.4	75.0	20.0
	Cytology	44.4	100.0	54.6	100.0	28.6
	Prototype 1	94.1	100.0	95.0	100.0	75.0
	Prototype 2	84.2	100.0	86.4	100.0	50.0
21–30	Rectal examination	66.7	66.7	66.7	66.7	66.7
	Vaginal examination	83.3	83.3	83.3	83.3	83.3
	Whiteside test	33.3	25.0	28.6	25.0	33.3
	Cytology	33.3	100.0	66.7	100.0	60.0
	Prototype 1	83.3	100.0	91.7	100.0	85.7
	Prototype 2	60.0	100.0	75.0	100.0	60.0
31–60	Rectal examination	16.7	57.1	38.5	25.0	44.4
	Vaginal examination	66.7	85.7	76.9	80.0	75.0
	Whiteside test	75.0	25.0	50.0	50.0	50.0
	Cytology	33.3	100.0	62.5	100.0	53.6
	Prototype 1	83.3	100.0	91.7	100.0	85.7
	Prototype 2	80.0	100.0	90.9	100.0	85.7
61–90	Rectal examination	50.0	85.7	77.8	50.0	85.7
	Vaginal examination	66.7	71.4	70.0	50.0	83.3
	Whiteside test	50.0	50.0	50.0	50.0	50.0
	Cytology	50.0	100.0	88.9	100.0	87.5
	Prototype 1	85.7	100.0	91.7	100.0	83.3
	Prototype 2	50.0	75.0	66.7	50.0	75.0
≥91	Rectal examination	25.0	87.5	66.7	50.0	70.0
	Vaginal examination	33.3	87.5	72.7	50.0	77.8
	Whiteside test	50.0	50.0	50.0	50.0	50.0
	Cytology	33.3	100.0	75.0	100.0	71.4
	Prototype 1	80.0	100.0	87.5	100.0	75.0
	Prototype 2	50.0	50.0	50.0	50.0	50.0
Total	Rectal examination	65.7	73.3	69.2	74.2	64.7
	Vaginal examination	79.4	86.7	82.8	87.1	78.8
	Whiteside test	53.9	40.0	47.8	53.9	40.0
	Cytology	40.5	100.0	66.7	100.0	56.7
	Prototype 1	90.3	100.0	94.2	100.0	87.5
		79.3	83.3	80.9	88.5	71.4

According to the data in Table 2, the highest diagnostic performance parameters were observed for Prototype 1 of the visual diagnostic test: sensitivity (Sens.) 90.3%, specificity (Spec.) 100.0%, diagnostic accuracy (Acc.) 94.2%, positive predictive value (PPV) 100.0%, and negative predictive value (NPV) 87.5%. This method exceeded vaginal examination in diagnostic accuracy by 11.4% and exceeded Prototype 2 of the visual diagnostic test kit by 13.3%. Cytology demonstrated absolute specificity (100.0%) and positive predictive value (100.0%); however, it showed low sensitivity (40.5%), which resulted in moderate diagnostic accuracy (66.7%) and a limited negative predictive value (56.7%). Among the clinical diagnostic methods in cows, vaginal examination showed the most balanced performance metrics (sensitivity 79.4%, specificity 86.7%, diagnostic accuracy 82.8%); rectal examination was inferior to vaginal examination across all effectiveness parameters, by 13.7% in sensitivity, 13.4% in specificity, and 13.6% in diagnostic accuracy. The Whiteside test was the least clinically effective diagnostic method, underperforming Prototype 1 of the visual diagnostic test kit by 36.4% in sensitivity, 60.0% in specificity, and 46.4% in diagnostic accuracy.

Based on the stratified analysis, a pronounced dependence of several diagnostic methods on days in milk was identified. At 10–20 days in milk, clinical diagnostic methods demonstrated high sensitivity (Sens. 94.7%); however, for rectal examination, specificity was 41.7% lower than that of vaginal examination, with a diagnostic accuracy of 86.4%. Vaginal examination showed a more balanced sensitivity/specificity profile (94.7%/75.0%) and high diagnostic accuracy (91.3%). During the same period, sensitivity of Prototype 1 and Prototype 2 was 94.1% and 84.2%, respectively, and diagnostic accuracy was 95.0% and 86.4%, respectively, with absolute specificity (100.0%). In contrast, cytological examination, despite also showing absolute specificity (100.0%), had low sensitivity (44.4%) and diagnostic accuracy (54.6%). The Whiteside test exhibited the lowest diagnostic informativeness among the methods evaluated.

During the 21–30 days in milk, vaginal examination showed stable sensitivity, specificity, and diagnostic accuracy values of 83.3%, exceeding rectal examination by 16.6% for these parameters. Sensitivity of Prototype 1 was higher than that of Prototype 2 by 23.3% (83.3% vs 60.0%, respectively), and diagnostic accuracy was higher by 16.7% (91.7% vs 75.0%), while maintaining absolute specificity (100.0%). During this period, cytological examination demonstrated absolute specificity (100.0%); however, as in the previous time interval, it had low sensitivity (33.3%) and diagnostic accuracy (66.7%). The Whiteside test was non-informative during this postpartum interval (Sens. 33.3%, Spec. 25.0%, Acc. 28.6%).

Beginning in the 31-60 days in milk, the informativeness of clinical diagnostic methods declined substantially: sensitivity, specificity, and diagnostic accuracy for rectal examination were 16.7%, 57.1%, and 38.5%, respectively. In contrast, vaginal examination yielded higher sensitivity (+50.0%), specificity (+28.6%), and diagnostic accuracy (+38.4%) than rectal examination. During this interval, Prototype 1 and Prototype 2 showed the highest sensitivity and diagnostic accuracy (Sens. 83.3% and 80.0%; Acc. 91.7% and 90.9%, respectively) while maintaining absolute specificity (100.0%). Cytology results in this period were similar to those observed at 21–30 days in milk: despite absolute specificity (100.0%), sensitivity was 33.3% and diagnostic accuracy was 62.5%. The Whiteside test demonstrated relatively high sensitivity (75.0%) but low specificity and diagnostic accuracy (25.0% and 50.0%, respectively).

During the 61–90 days in milk, clinical diagnostic methods showed moderate performance. Sensitivity of vaginal examination (66.7%) exceeded that of rectal examination by 16.7%, whereas specificity of rectal examination (85.7%) was higher than that of vaginal examination by 14.3%, and diagnostic accuracy (77.8%) was higher by 7.8%. Prototype 1 exhibited the highest diagnostic performance in this period and markedly outperformed Prototype 2: sensitivity of Prototype 1 (85.7%) was higher than that of Prototype 2 by 35.7%, and specificity (100.0%) and diagnostic accuracy (91.7%) were each higher by 25.0%. Cytology during this period also demonstrated absolute specificity (100.0%); although sensitivity remained low (50.0%), diagnostic accuracy was the highest across all time intervals (88.9%). The Whiteside test was of limited informativeness, as in the previous intervals (Sens., Spec., and Acc. each 50.0%).

In the latest period (≥91 days in milk), clinical rectal and vaginal diagnostic methods were characterized by high specificity (87.5%) and moderate diagnostic accuracy (66.7% and 72.7%, respectively), but low sensitivity (25.0% and 33.3%). In this period, Prototype 1 was the most effective

diagnostic method, with sensitivity of 80.0%, specificity of 100.0%, and diagnostic accuracy of 87.5%, which were 30.0%, 50.0%, and 37.5% higher, respectively, than those of Prototype 2. Cytology showed diagnostic performance similar to that in the previous period; however, sensitivity decreased by 16.7% and diagnostic accuracy by 13.9%. The clinical effectiveness of the Whiteside test did not change compared with the previous interval, and the method remained of limited informativeness.

Thus, considering overall diagnostic performance and the stability of results across days in milk intervals, Prototype 1 of the visual diagnostic test kit was the most effective. Among the clinical methods, vaginal examination was the most informative. Cytological examination provided maximal specificity but had low sensitivity and moderate diagnostic accuracy. The Whiteside test exhibited the lowest diagnostic informativeness across postpartum periods.

The next stage of the study was to determine the diagnostic parameters of the developed visual diagnostic test kit for uterine diseases in cows. For this purpose, during CVM testing, foam height (cm) and the time to color change of the catheter contents (min) were measured in both diseased and clinically healthy animals. The measurement results are presented in Table 3.

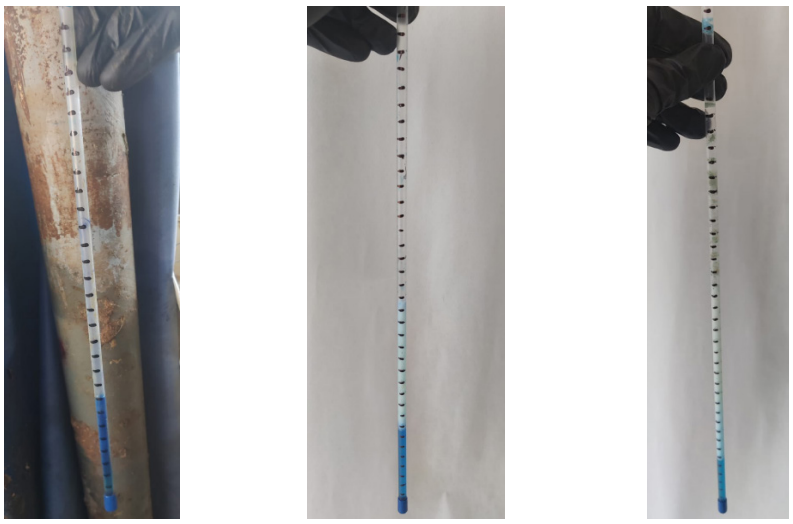
Table 3. Determination of diagnostic parameters of the visual diagnostic test kit for uterine diseases in cows (n = 45)

Clinical course of uterine disease	n	Reaction readout	
		foam height (cm)	time to color change of catheter contents (min)
Clinically healthy animals	15	0.22 ± 0.42	25.5 ± 3.5
Acute	15	17.5 ± 10.1	3.5 ± 0.35
Chronic	15	5.62 ± 3.93	4.5 ± 0.45

According to Table 3, clinically healthy animals exhibited a minimal reaction when the visual diagnostic test for uterine disease was applied, characterized by negligible foam formation (0.22 ± 0.42 cm) and the longest time to color change of the catheter contents (25.5 ± 3.5 min), reflecting low reaction intensity in the absence of pathology. In contrast, animals with uterine disease showed an accelerated color change: 3.5 ± 0.35 min in acute cases and 4.5 ± 0.45 min in chronic cases. These findings indicate a clear separation between diseased and clinically healthy animals based on the time parameter, which was markedly reduced in the presence of pathology.

With respect to foam formation, the highest value was observed in acute cases (17.5 ± 10.1 cm), whereas in chronic cases the value was substantially lower (5.62 ± 3.93 cm). In clinically healthy animals, foam formation was virtually absent, indicating that foam height reflected not only the presence or absence of pathology but also gradation by clinical course: the most pronounced reaction corresponded to acute disease, whereas a moderate reaction was characteristic of chronic disease.

Collectively, these data indicate that the developed visual diagnostic test kit produces a characteristic reaction pattern depending on the animals' clinical status: clinically healthy animals show no foam or only minimal foam formation with a prolonged time to color change (Figure 1a); chronic disease is characterized by moderate foam formation with rapid color change (Figure 1b); and acute disease is characterized by abundant foam formation and the fastest color change of the catheter contents (Figure 1c).



a b c

a – clinically healthy animal; b – chronic course; c – acute course

Figure 1. Results of using the test for visual diagnosis of uterine diseases in cows

Discussion

Uterine diseases in cows, particularly metritis and endometritis of various forms, remain among the leading causes of deteriorated herd reproductive performance and reduced cow productivity, while the severity of clinical signs becomes «attenuated» as time postpartum increases [25, 26]. This factor drives demand for rapid diagnostic methods that meet the following requirements: ease of use under on-farm conditions and the ability to screen the herd for uterine disease, thereby reducing the time from diagnosis to initiation of potential treatment when pathology is detected [27, 28].

When using the visual diagnostic test for uterine diseases in cows, two diagnostic criteria were selected: an animal was considered diseased if foam formation exceeded 1 cm and discoloration of the catheter contents occurred within 4 min. This approach yields high specificity because it requires the simultaneous presence of two visual reaction indicators, thereby reducing the likelihood of false-positive results when pathology is absent and helping to minimize unjustified costs and production losses [29].

The obtained reaction parameter data in diseased and clinically healthy animals confirm the validity of the selected approach: in clinically healthy cows, the reaction was virtually absent (foam 0.22 ± 0.42 cm; discoloration within 25.5 ± 3.5 min), whereas acute disease showed a distinct reaction pattern (foam 17.5 ± 10.1 cm; discoloration within 3.5 ± 0.35 min) and chronic disease was associated with a moderate reaction (foam 5.62 ± 3.93 cm; discoloration within 4.5 ± 0.45 min). The more moderate reaction observed in chronic cases is consistent with the literature, which indicates that chronic or subclinical uterine disease is characterized by less pronounced or absent clinical signs, thereby necessitating more sensitive markers or adjustment of diagnostic parameters [25, 30].

Comparative analysis of the diagnostic performance of methods for detecting uterine disease in cows showed that Prototype 1 (10% H_2O_2 + 0.01% methylene blue) of the visual diagnostic test provided the most stable effectiveness metrics: overall sensitivity reached 90.3%, specificity 100.0%, diagnostic accuracy 94.2%, PPV 100.0%, and NPV 87.5%. Notably, the specificity of Prototype 1 remained at 100.0% regardless of days in milk, which is critically important for on-farm screening because it minimizes unnecessary therapeutic interventions. In contrast, Prototype 2 (10% H_2O_2 + 0.03% 4-chloro-1-naphthol) ultimately showed more variable performance, with sensitivity of 79.3%, specificity of 83.3%, and diagnostic accuracy of 80.9%; a trend toward reduced performance was observed in later postpartum intervals, declining to 50.0% for all parameters at ≥91 days in milk. This may indicate lower reaction robustness or greater dependence on mucus composition as the inflammatory profile changes over the course of disease. Such postpartum-period dependence is conceptually consistent with the notion that the pathogenesis and manifestations of inflammatory uterine disease differ by clinical course (acute, chronic, and subclinical) and, consequently, that a universal marker or diagnostic feature may behave differently across postpartum time points [31, 32].

With respect to clinical diagnostic methods, the following pattern was observed: vaginal examination showed a higher overall performance profile (Sens. 79.4%, Spec. 86.7%, Acc. 82.8%) compared with rectal examination (Sens. 65.7%, Spec. 73.3%, Acc. 69.2%). The effectiveness of clinical methods for diagnosing uterine disease in cows was maximal in the early postpartum period (10–20 days in milk), which is consistent with literature reporting more overt clinical signs during the acute course of uterine disease [25, 33].

In our study, cytological examination demonstrated maximal specificity (100.0%) with relatively low sensitivity (40.5%), which is consistent with available literature describing cytology as a highly specific tool for diagnosing uterine disease [34]. In our study, cytology was used specifically as a high-specificity reference method, whereas the developed test was intended as a screening approach designed to identify a larger number of disease cases while maintaining a low false-positive rate. Population-based studies emphasize the need for a multimodal diagnostic strategy, as some animals may be negative based on clinical findings yet positive on cytology; a combined approach provides a more objective assessment of herd-level disease prevalence [32, 35].

It should be noted that for methods based on assessment of vaginal discharge or cervicovaginal mucus, a standardized sampling procedure is essential. Comparative studies have shown that the diagnostic performance of different discharge assessment approaches (Metricheck and vaginal speculum) differs in sensitivity and specificity despite similar diagnostic workflows [36]. Therefore, the objective of determining the device parameters for the visual diagnostic test helped reduce sample variability, which in turn improved the accuracy of the study.

From a practical perspective, the results suggest that the visual diagnostic test aligns with a cow-side diagnostic approach: it is rapid, reproducible, highly specific, and provides an easily interpretable readout. For example, reagent strip-based tests and leukocyte esterase assays are also positioned as rapid diagnostic methods that enable assessment of inflammatory status under on-farm conditions without laboratory infrastructure [28]. From the standpoint of commercial dairy production, a test with high sensitivity and specificity could potentially reduce the proportion of undiagnosed uterine disease cases, which is important for preserving herd reproductive performance. This is critical because reproductive system disorders are associated with both a lower probability of achieving pregnancy and a higher risk of gestational pathologies in breeding animals [37].

Based on the study results, the proposed visual diagnostic test for uterine diseases in cows can be integrated into on-farm diagnostic protocols for the breeding herd as a rapid, reproducible, and accurate diagnostic method, enabling timely diagnosis and implementation of necessary therapeutic and preventive measures. Subsequently, it is planned to validate the developed test on a larger number of animals on other dairy farms in Kazakhstan.

Conclusion

A test kit was developed for the visual diagnosis of uterine diseases in cows at different days in milk, based on collection of cervicovaginal mucus and detection of an inflammation biomarker via a chemical reaction. The visual diagnostic test kit for uterine diseases in cows includes a graduated catheter (40 cm in length and 0.6 cm in diameter), a 50- μL syringe, a coupling for attaching the syringe to the catheter, a protective cap for the free end of the catheter, and two 5-μL syringes containing the diagnostic reagents. When two reagent formulations were compared, Prototype 1 (10% H_2O_2 + 0.01% methylene blue) was preferred over Prototype 2 (10% H_2O_2 + 0.03% 4-chloro-1-naphthol) and demonstrated high diagnostic performance (sensitivity 90.3%, specificity 100.0%, diagnostic accuracy 94.2%). The established criterion for a positive test result (foam formation >1 cm and discoloration of the catheter contents within 4 min) ensured unambiguous result interpretation and high test specificity. Based on clinical trial results, the visual diagnostic test demonstrated high diagnostic effectiveness relative to a composite reference standard (clinical signs plus cytology findings), supporting its use for screening the breeding herd at different postpartum time points.

Authors' Contributions

IJ: developed the study concept, justified the study design, supervised all stages of the research, edited the initial version of the manuscript; AM: developed the experimental protocol, conducted the

animal study, contributed to the writing of the methodological section; ZhZh: prepared the literature review, introduction, reference list, edited the manuscript text; AA: performed the statistical analysis, interpreted the results, contributed to the preparation of the Results and Discussion sections; YeK: conducted the animal study, was responsible for the technical implementation of the device, preparation of reagents, contributed to the preparation of the Introduction section; AZ: provided project administration, conducted the animal study, was responsible for preparing the device and reagents for research, served as the corresponding author.

Statement on Competing Interests (Conflict of Interest)

The authors declare that they have no conflicts of interest.

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References

1 Tokaev, Z., Muratbaev, D.M., Zajnettinova, D.B., Kadralieva, B.T., Aitpaeva, Z.S. (2024). Faktory besplodiya u vysokoproduktivnyh korov [Infertility factors in highly productive cows]. *Nauka i obrazovanie,* 1:3(76), 202-210. DOI: 10.52578/2305-9397-2024-3-1-202-210. [*In Russian*].

2 Umitzhanov, M., Turebekov, O.T., Omarbekova, G.K., Akimzhan, N.A., SHynybekova, SH. S. (2024). Rasprostranennost' besplodiya i vosstanovlenie funkcii polovyh organov korov [Prevalence of infertility and recovery of cow reproductive functions]. *Issledovaniya, rezul'taty,* 4(104), 25-32. DOI: 10.37884/4-2024/03. [*In Russian*].

3 Wicaksono, A., van den Borne, B.H.P., Aardema, H., van Werven, T., Hogeveen, H., Steeneveld, W. (2025). Estimating the costs of interrelated reproductive disorders in dairy farms. *Journal of Dairy Science*, 108(8), 8508-8528. DOI: 10.3168/jds.2024-26053.

4 Jakupov, I.T., Abultdinova, A.B., Mamytbekova, G.K., Zharkimbaeva, Z.Z., Yeszhanova, G.T., Zabrodin, A.G. (2023). The effectiveness of uterine disease diagnosis methods in cows depending on postpartum days. *Herald of Science of S. Seifullin Kazakh Agro Technical Research University: Veterinary Sciences,* 3(003), 68-74. DOI: 10.51452/kazatuvc.2023.3(003).1520.

5 Kushaliev, K.ZH., Shámshidin, Á.S., Gabdullin, D.E., Harzhau, A., Ginayatov, N.S., Batyrgaliev, E.A., Kulbaev, R.M., Ahmetova, G.M. (2025). Akushersko-ginekologicheskii monitoring i prichiny reproduktivnyh narushenii u korov [Obstetric and gynecological monitoring and causes of reproductive disorders in cattles]. *Nauka i obrazovanie*, 2(3(80), 42-53. DOI: 10.52578/2305-9397-2025-3-2-42-53. [*In Russian*].

6 Gabdullin, D.E., Tagaev, O.O., Dzhulanov, M.N., Koibagarov, K.U. (2023). BQO jaǵdaıynda súrti baǵyttaǵy sıyrlardyń akýsherlik-gınekologıalyq aýrýlarynyń taralýy [Prevalence of obstetrical and gynecological diseases of dairy cows in West Kazakhstan region]. *Ǵylym jáne bilim,* 1:4(73), 205-211. DOI: 10.52578/2305-9397-2023-4-1-205-211. [*In Kazakh*].

7 Alikhanov, K.D., Jakupov, I.T., Abultdinova, A.B., Kuzerbayeva A.T., Mamytbekova, G.K. (2022). Ultrasound diagnostics parameters of uterine pathologies in cows. *Science and education,* 1:4 (69), 79-86. DOI: 10.52578/2305-9397-2022-4-1-79-86.

8 Uzyntleuova, A.D., Dzhulanov, M.N., Koibagarov, K.U., Zhumageldiev, A.A., Abzhalieva, A. B., Hizat, S. (2023). Sıyrlardyń jasyryn endometrıtin últradybystyq zertteý dıagnostıkasy [Ultrasound diagnosis of occurrent endometritis in cows]. *Ǵylym jáne bilim,* 1:2(75), 49-58. DOI: 10.52578/2305-9397-2024-2-1-49-58. [*In Kazakh*].

9 Bimenova, Zh.Zh., Shoramanov, B.R., Mahashev, E., Nesipbaeva, A.K., Turgumbekov, A.A., Ahmetova, M.S., Ziyabek, D.B., Qasymbek, A.S. (2025). Sharýashylyq jaǵdaıynda endometrıtterdi balaý, aldyn alý jáne zamanaýı emdeý ádisterin ońtaılandyrý [Optimization of modern methods of treatment, prevention and diagnosis of endometritis in farm conditions]. *Ǵylym jáne bilim,* 2:3(80), 284-292. DOI: 10.52578/2305-9397-2025-3-2-284-292. [*In Kazakh*].

10 Uzyntleuova, A.D., Dzhulanov, M.N., Koibagarov, K.U., Dzhulanova, N.M., Mahmutov, A. K., Abzhalieva, A.B. (2023). Patogistologicheskaya kartina endometriya korov pri skrytom endometrite [Pathohistological picture of the endometrium of cows with latent endometritis]. *Nauka i obrazovanie*, 1:2(75), 51-69. DOI: 10.52578/2305-9397-2024-2-1-58-69. [*In Russia*n].

11 Tegza, A.A., Khassanova, M.A., Yablochkova, G.S., Sebenov, N.T. (2023). To the issue of the technique of endometrial biopsy in cows. *3i: intellect, idea, innovation - intellekt, ideya, innovaciya*, 4, 46-53. DOI: 10.52269/22266070_2023_4_46.

12 Rakeckii, V.A., Mustafin, B.M., Tlegenova, Zh.Zh., Aubakirov, M.Zh. (2025). Effektivnost' primeneniya pribora Alpha Vision pri opredelenii sostoyaniya genitalii pered osemeneniem i pri iskusstvennom osemenenii korov [Efficiency of using the alpha vision device in determining the state of genitalia before insemination and artificial insemination of cows]. *3i: intellect, idea, innovation - intellekt, ideya, innovaciya,* 1(3), 48-56. DOI: 10.52269/KGTD253148. [*In Russian*].

13 Risvanli, A., Tanyeri, B., Yildirim, G., Tatar, Y., Gedikpinar, M., Kalender, H., Safak, T., Yuksel, B., Karagulle, B., Yilmaz, O., Kilinc, M.A. (2024). Metrisor: A novel diagnostic method for metritis detection in cattle based on machine learning and sensors. *Theriogenology*, 223, 115-121. DOI: 10.1016/j.theriogenology.2024.05.002.

14 Jakupov, I.T., Yeszhanova, G.T., Mamytbekova, G.K., Birimzhanova, D.A., Zabrodin, A.G., Abultdinova, A.B., Zharkimbaeva, Zh.Z. (2025). Laboratornaya diagnostika poslerodovyh patologii u korov [laboratory diagnostics of postpartum pathologies in cows]. *Nauka i obrazovanie,* 1.1(78), 99-111. DOI: 10.52578/2305-9397-2025-1-1-99-111. [*In Russian*].

15 Law on Responsible Treatment of Animals, No. 97-VII LRK of December 30, 2021. Republic of Kazakhstan. https://adilet.zan.kz/eng/docs/Z2100000097.

16 Sheldon, I.M. (2019). *The Metritis Complex in Cattle.* In Noakes, D.E., Parkinson, T.J., England G.C.W. (Eds.), Veterinary Reproduction and Obstetrics. Elsevier. 408-433.

17 Jakupov, I., Kuzerbayeva, A., Karabayeva, Zh. (2016). Entwicklung einer Farbkarte zur Unterscheidung von Lochien bei Kühen mit und ohne Störung der Uterusinvolution. *Tierarztliche Praxis, Ausgabe G: Grosstiere/Nutztiere,* 44(06), 368-370. DOI: 10.15653/TPG-160691.

18 Bedewy, R.B., Rahaway, M.A. (2019). Comparative study for detection of subclinical endometritis in local cows. *Advances in Animal and Veterinary Sciences*, 7(4), 289-294. DOI: 10.17582/journal.aavs/2019/7.4.289.294.

19 Karis, P., Jaakson, H., Ling, K., Opsomer, G., Siimon, L., Reimann, E., Pärn, P., Gross, J.J., Henno, M., Kurykin, J., Valdmann, M., Ots, M., Waldmann, A. (2025). Associations of cytological endometritis with insulin resistance and gene expression in adipose tissue of transition dairy cows. *Journal of Dairy Science*, 108(11), 12567-12576. DOI: 10.3168/jds.2025-26835.

20 Sposob vizual'noi diagnostiki ostryh i hronicheskih zabolevanii matki u korov [Method for visual diagnosis of acute and chronic uterine diseases in cows]. (2024). Patent RK № 9618 na poleznuyu model' [Patent for utility model]. Jakupov I.T.; Yeszhanova G.T.; Zharkimbayeva Zh.Z.; Abultdinova A.B.; Mamytbekova G.K.; Zabrodin A.G.; Kurmangaliyev E.E.; zayavitel' i patentoobladatel' Kazahskii agrotehnicheskii issledovatel'skii universitet im. S.Seifullina. - zayavl. 21.06.2024; opubl. 27.09.2024. Bul. №39. [*In Russian*].

21 Jakupov, I., Wehrend, A., Abulgazimova, G., Zabrodin, A., Kurmangaliyev, Y., Abultdinova, A. (2026). Effectiveness of clinical and laboratory methods and a new method of visual diagnosis of uterine inflammation in cows on different days after calving. *International Journal of Veterinary Science,* 15(2), 553-563. DOI: 10.47278/journal.ijvs/2025.174.

22 Gardner, I.A., Greiner, M. (2006). Receiver-operating characteristic curves and likelihood ratios: improvements over traditional methods for the evaluation and application of veterinary clinical pathology tests. *Veterinary clinical pathology*, 35(1), 8-17. DOI: 10.1111/j.1939-165x.2006.tb00082.x.

23 The jamovi project (2025). *Jamovi.* (Version 2.7.6) [Computer Software]. Retrieved from https://www.jamovi.org.

24 MedCalc Software Ltd. (2026). *Diagnostic test evaluation.* (Version 23.4.8) [Web application]. https://www.medcalc.org/en/calc/diagnostic_test.php.

25 Bogado Pascottini, O., LeBlanc, S.J., Gnemi, G., Leroy, J.L.M.R., Opsomer, G. (2023). Genesis of clinical and subclinical endometritis in dairy cows. *Reproduction,* 166(2), 15-24. DOI: 10.1530/REP-22-0452.

26 Várhidi, Z., Csikó, G., Bajcsy, Á.C., Jurkovich, V. (2024). Uterine Disease in Dairy Cows: A Comprehensive Review Highlighting New Research Areas. *Veterinary Sciences,* 11(2), 66. DOI: 10.3390/vetsci11020066.

27 Kusaka, H., Kimura, T., Nishimoto, N., Sakaguchi, M. (2022). Combined use of non-laboratory methods for the practical diagnosis of endometritis in postpartum dairy cows. *Veterinary Medicine and Science*, 8, 2585-2592. DOI:10.1002/vms3.904.

28 Bartolome, N.D., Jongsuwanwattana, R., Asawakarn, S., Suadsong, S., Sangpradit, K., Swangchan-Uthai, T. (2025). Assessment of the physical and chemical properties of vaginal discharge for the diagnosis of endometritis in dairy cattle. *Veterinary World*, 18(5), 1322-1332. DOI: 10.14202/vetworld.2025.1322-1332.

29 Denis-Robichaud, J., Barbeau-Grégoire, N., Gauthier, M.-L., Dufour, S., Roy, J.-P., Buczinski, S., Dubuc, J. (2025). Validity of purulent vaginal discharge, esterase, luminometry, and three bacteriological tests for diagnosing uterine infection in dairy cows using Bayesian latent class analysis. *Preventive Veterinary Medicine,* 239, 106521. DOI: 10.1016/j.prevetmed.2025.106521.

30 Druker, S.A., Sicsic, R., van Straten, M., Goshen, T., Kedmi, M., Raz, T. (2022). Cytological endometritis diagnosis in primiparous versus multiparous dairy cows. *Journal of Dairy Science,* 105(1), 665-683. DOI: 10.3168/jds.2020-20064.

31 Kelly, E.T., McAloon, C.G., O'Grady, L., Duane, M., Somers, J.R., Beltman, M.E. (2022). Reproductive tract disease in Irish grazing dairy cows: Retrospective observational study examining its association with reproductive performance and accuracy of 2 diagnostic tests. *Journal of Dairy Science,* 105(6), 5471-5492. DOI: 10.3168/jds.2021-21404.

32 Tobolski, D., Krupa, M., Polak, Z., Pascottini, O.B., Opsomer, G., Barański, W. (2025). Exploring the influence of endometritis diagnostic criteria on uterine involution, milk yield and fertility in dairy cows. *BMC Veterinary Research,* 21(1), 276. DOI: 10.1186/s12917-025-04727-7.

33 Wang, H., Yan, Z., Wu, X., Zhang, Y., Wei, Y., Zhao, X. (2021). Using vaginal discharge score (VDS) grading system to evaluate the effect of clinical endometritis on reproductive performance of dairy cows in China. *Animal Reproduction,* 18(1), e20200228. DOI: 10.1590/1984-3143-AR2020-0228.

34 Wagener, K., Gabler, C., Drillich, M. (2017). A review of the ongoing discussion about definition, diagnosis and pathomechanism of subclinical endometritis in dairy cows. *Theriogenology,* 94, 21-30. DOI: 10.1016/j.theriogenology.2017.02.005.

35 McDougall, S., Aberdein, D., Bates, A., Burke, C.R. (2020). Prevalence of endometritis diagnosed by vaginal discharge scoring or uterine cytology in dairy cows and herds. *Journal of Dairy Science,* 103(7), 6511-6521. DOI: 10.3168/jds.2019-18048.

36 Ishiyama, D., Magata, F., Matsuda, F. (2025). Comparison of vaginal examination methods to evaluate urovagina and purulent vaginal discharge in periestrous dairy cows. *Journal of Reproduction and Development,* 71(1), 17-23. DOI: 10.1262/jrd.2924-071.

37 Bruinjé, T.C., Morrison, E.I., Ribeiro, E.S., Renaud, D.L., LeBlanc, S.J. (2024). Associations of inflammatory and reproductive tract disorders postpartum with pregnancy and early pregnancy loss in dairy cows. *Journal of Dairy Science,* 107(3), 1630-1644. DOI: 10.3168/jds.2023-23976.

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Research article

Parasite fauna of wild ungulates in Kazakhstan: current state and analysis

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Abstract

Background and Aim. Wild ungulates are a vital component of Kazakhstan's biodiversity, encompassing 12 species occupying diverse ecological niches. However, the current structure and epizootiological significance of their parasite fauna remain insufficiently synthesized. This review aims to summarize the contemporary data on parasitic diversity in wild ungulates across Kazakhstan, analyze its host associations, and identify key ecological and epidemiological patterns.

Materials and Methods. A systematic literature search was conducted in Scopus, Web of Science, and RSCI databases, as well as Kazakhstani peer-reviewed journals. Keywords in Kazakh, English, and Russian included the following terms: wild ungulates, parasite diversity, host-parasite relationships, epidemiology, Kazakhstan. The timeframe covered publications from 1989 to 2026, with earlier foundational works included for baseline comparisons. From 120 initially identified sources, 50 peer-reviewed publications were selected after screening.

Results. The parasite fauna exhibits high species richness, with a clear predominance of geohelminths over biohelminths and primary localization in the digestive tract. Dozens of helminth species have been recorded in key hosts, including saiga (*Saiga tatarica*), argali (*Ovis ammon*), maral (*Cervus elaphus sibiricus*), roe deer (*Capreolus pygargus*), Siberian ibex (*Capra sibirica*), and goitered gazelle (*Gazella subgutturosa*). The community structure is complex, showing a significant overlap between host species. Critically, a substantial proportion of parasites are shared between wild and domestic animals, indicating active cross-species transmission in both natural and anthropogenically transformed ecosystems.

Conclusion. The parasitic communities of wild ungulates in Kazakhstan are characterized by structural complexity and extensive faunal exchange with livestock, highlighting significant epidemiological risks. These findings underscore the necessity for continuous epizootiological monitoring and the development of integrated control strategies to mitigate the impact of parasitic infections on both wildlife conservation and livestock health.

Keywords: wild ungulates; parasite fauna; biodiversity; systematic review.

Introduction

Wild ungulates represent a crucial component of the natural and cultural heritage of Kazakhstan, and their conservation is of key importance for maintaining biodiversity and ecosystem stability. The country's natural biocenoses are inhabited by 12 species of ungulates representing various taxonomic groups and ecological niches. In this regard, their comprehensive study, including the analysis of parasite

fauna, holds significant scientific importance for understanding the functioning of ecosystems in Asia and adjacent regions.

The territory of Kazakhstan is characterized by pronounced landscape and climatic heterogeneity, ranging from arid deserts and semi-deserts to steppe, forest-steppe, and mountainous ecosystems [1]. Such environmental diversity determines the distribution, migration patterns, and population density of wild ungulates, including key species such as saiga (*Saiga tatarica*), argali (*Ovis ammon*), Siberian ibex (*Capra sibirica*), maral (*Cervus elaphus sibiricus*), roe deer (*Capreolus pygargus*), and goitered gazelle (*Gazella subgutturosa*) [2–4]. These hosts form complex ecological networks and serve as reservoirs for a wide range of parasitic organisms with different life cycles and transmission strategies [5].

Parasites are an integral component of natural ecosystems and play a significant role in regulating host population dynamics, structuring communities, and maintaining ecological balance. At the same time, parasite–host interactions in wild ungulates are of considerable applied importance, as many helminths, protozoa, and arthropod ectoparasites are shared between wild and domestic animals [6]. This overlap facilitates pathogen circulation across wildlife-livestock interfaces, contributing to the emergence and persistence of epizootic and zoonotic diseases. In regions with intensive pastoralism, which is typical for Kazakhstan, such interactions are especially pronounced due to overlapping grazing areas and seasonal movements of animals [7].

Despite the recognized importance of parasite fauna in wild ungulates, available data for Kazakhstan remain fragmented and are often limited to individual host species, specific regions, or particular taxonomic groups of parasites. Earlier studies reported a high diversity of helminths, with a predominance of geohelminths and a primary localization in the gastrointestinal tract. However, recent environmental changes, including climate variability, anthropogenic pressure, and transformation of natural landscapes, may significantly affect parasite diversity, transmission pathways, and host-parasite relationships [8].

In addition, advances in molecular biology have substantially expanded the possibilities for studying parasite fauna, allowing for more accurate species identification, detection of cryptic taxa, and clarification of phylogenetic relationships. The integration of classical parasitological approaches with molecular-genetic methods provides new insights into the structure and dynamics of parasite communities in wild ungulates and enhances the reliability of epidemiological assessments. While individual aspects of wildlife parasitology in the region have been addressed in specific contexts, a unified, large-scale synthesis has historically been lacking.

This manuscript represents the first comprehensive, multi-host systematic review dedicated exclusively to the parasite fauna of wild ungulates across the entire territory of Kazakhstan. Unlike previously published regional reports or species-specific papers that focused restrictedly on isolated host populations, this study uniquely consolidates fragmented, long-term national data with contemporary global findings.

By evaluating these integrated data through the lens of the global One Health concept and climate-driven ecosystem shifts, this review aims to summarize existing knowledge on the diversity, host associations, and ecological features of parasite fauna in wild ungulates of Kazakhstan. Furthermore, it establishes an unprecedented analytical framework to evaluate potential risks associated with parasitic infections at the wildlife–livestock–human interface, highlight critical research gaps, and outline strategic priorities for future veterinary biosecurity and wildlife conservation in Central Asia.

Materials and Methods

The review is devoted to the study of the parasitic fauna of wild ungulates in Kazakhstan. Among wild caprine bovids in Kazakhstan, representatives of the genera Saiga, Capra, and Ovis are found. The only representative of the genus Saiga is the saiga antelope, alongside which the Siberian ibex (*Capra sibirica*), as well as urial and argali (*Ovis* spp.), inhabit. On the territory of Kazakhstan, three populations of saiga are distinguished – the Ural, Ustyurt, and Betpak-Dala populations, which emphasizes its wide geographical distribution. According to aerial survey data, the saiga antelopes population demonstrates a stable positive trend: in 2023 it exceeded 1.9 million individuals, in 2024 it amounted to 2,833,600 individuals, and in 2025 it reached 3.9–4.1 million individuals [9–11].

The Siberian ibex is distributed in the mountain systems of the Tien Shan, the Zhetysu (Dzungarian) Alatau, and the Southern Altai; its population is estimated at 15 000–20 000 individuals. Among wild sheep, rare and protected forms are of particular importance. The Ustyurt urial is listed in the Red Book of Kazakhstan, as are subspecies of argali (*Ovis ammon ammon, O. ammon nigrimontana, O. ammon collium, O. ammon karelini*), whereas *O. ammon* severtzovi is likely extinct. The Ustyurt mouflon (O. vignei arkal) also inhabits Kazakhstan. The total number of argali is about 19 thousand individuals, with significant populations concentrated in the State National Nature Park "Altyn-Emel" and the "Almaty State Nature Reserve" [12, 13].

The only representative of gazelles is the goitered gazelle (*Gazella subgutturosa*), listed in the Red Book of Kazakhstan. Its population is about 15 thousand individuals, including approximately 5.5 thousand in the State National Nature Park "Altyn-Emel" [14, 15].

The family Cervidae is represented by three genera – *Cervus, Capreolus,* and *Alces*. The red deer (*Cervus elaphus*), including the Altai maral (*C. elaphus sibiricus*), is widely distributed in mountainous regions, whereas the Bukhara deer (*C. elaphus bactrianus*) is a rare species (about 1140 individuals). The total number of red deer is about 13 thousand individuals [14, 16]. The Siberian roe deer (*Capreolus pygargus*) reaches about 100 thousand individuals and is distributed throughout the country.

Among perissodactyls, the Turkmenian kulan (*Equus hemionus kulan*) is noted, with a population of about 4.1 thousand individuals, as well as Przewalski's horse, maintained under reintroduction conditions [14].

Thus, the diversity of hosts [17] creates the prerequisites for the formation of complex parasitic systems.

Correspondingly, the parasite fauna of wild ungulates is characterized by high species richness and a complex structure. According to studies by *K.K. Baitursinov* [18, 19], conducted in 1983–2007, various species of wild animals were examined using the method of complete helminthological dissection. A wide distribution of nematodes of the family Trichostrongylidae, including the genera *Trichostrongylus, Ostertagiella, Nematodirus, Marshallagia,* and *Nematodirella,* was established.

Studies conducted in the West Kazakhstan region (Ural population) revealed that saiga antelopes are infested with a range of helminth species. Coproscopic examinations identified four helminth species: *Oesophagostomum venulosum, Trichuris skrjabini, Nematodirus gazellae,* and *Moniezia expansa,* as well as oocysts of the protozoan *Eimeria elegans / E. manafovae.* In addition to these findings, other studies in the same region have reported four additional nematode species belonging to four different genera: *Ostertagia ostertagi* (intensity: 18.2 worms per host), *Cooperia onchophora (*17.3), *N. spathiger* (35.2), and *Trichostrongylus axei* (12.8). These intensity values indicate a moderate to high level of infection in this population compared with other regions of Kazakhstan [20–23].

An analysis of climatic, natural, and anthropogenic factors affecting seasonal patterns of helminth infestation has shown that environmental conditions play a crucial role in the survival and transmission of helminth larvae. The main source of helminth infestation in saiga populations is watering sites, where larvae accumulate and remain viable under favourable conditions. Therefore, the creation of additional, ecologically safe watering points and the greening of surrounding areas are recommended as practical measures to reduce infection rates and improve the health status of wild ungulate populations. As a result of contacts between wild and domestic animals, pathogen exchange and helminth circulation among different animal species can occur in the West Kazakhstan region. Consequently, it can be hypothesized that saiga antelopes of the Ural population may contribute to the maintenance of helminth foci in the region, but direct evidence of parasite transmission from saigas to livestock under natural conditions is not yet available. Further molecular epidemiological studies are needed to confirm the directionality of cross-species transmission [24–26].

Regarding to domestic scientists works in 2014–2017 of the total number of parasite species recorded across all wild ungulate hosts in Kazakhstan (73 species), the distribution by host species was as follows: in saiga antelope – 34 species (46.6%); in argali – 28 species (38.6%); in maral – 17 species (21.9%); in roe deer – 14 species (17.8%); in wild boar, Siberian ibex, and kulan – 10 species each (13.7% each); in Ustyurt urial – 9 species (12.3%); in moose – 6 species (8.2%); and in goitered gazelle – 4 species (5.5%). Among all recorded parasite species, 30 (41.1%) belong to biohelminths and

43 (58.9%) to geohelminths. Most parasite species are localized in the digestive tract. Studies of maral (2015–2017) revealed 11 parasite species, including *M. expansa, O. venulosum, Capillaria bovis, T. skrjabini, Bunostomum phlebotomum, N. spatiger, Haemonchus contortus, Strongylata* sp., as well as *E. robusta, E. gallivalerioi,* and *E. cervi*. The prevalence of infection ranged from 33.17 to 100% [27–34].

According to data by *A. Smagulova* et al. [35], a wide spectrum of gastrointestinal parasites was identified in wild ungulates, including *Dama dama, O. ammon collium, Cervus elaphus, C. pygargus, Alces alces, O. ammon, Saiga tatarica, Bos mutus,* and *O. gmelina*; these included strongylid-type eggs, *Eimeria, Trichuris, Nematodirus, Capillaria,* and *Dicrocoelium lanceolatum*. The highest prevalence of infection was noted in *D. dama* (66.6%), whereas *S. tatarica* recorded the highest mean intensity of infection; meanwhile, *A. alces, O. ammon,* and *C. pygargus* were characterized by a relatively low prevalence (less than 15%) and moderate intensity of infection (approximately 4–5 helminths per infected host).

Among 36 examined wild ungulates, infection was identified in 3 animals: *Taenia multiceps* was found in 2 out of 25 roe deer (*C. pygargus*) with cysts localized in the femoral muscle, and *Taenia hydatigena* was identified in 1 out of 9 moose (*A. alces*) with cysts localized in the liver. Both of the 2 examined red deer were free from cysticercosis [36].

In four out of seven moose examined in the Akmola and Kostanay regions, third-stage larvae of Cephenemyia ulrichii were discovered [37].

In the studies of *F.D. Akramova* et al. [38], the presence of 26 helminth species was established in wild ungulates. Similar infection rates have been identified in Northwestern Uzbekistan [39–42]. In the biogeocenoses of Karakalpakstan, the artiodactyl fauna is represented by five wild ungulate species: *Sus scrofa nigripes* (wild boar), *C. elaphus bactrianus* (Bukhara deer), *G. subgutturosa* (goitered gazelle), *S. tatarica* (saiga antelope), and *O. orientalis* arcal (arkal sheep). A total of 26 helminth species were identified in these hosts, belonging to the classes Cestoda (6 species), Trematoda (2 species), and Nematoda (18 species). The species composition varied by host: wild boar harbored 14 helminth species, Bukhara deer – 11, saiga antelope – 13, and goitered gazelle – 14. Common parasites shared among these ungulate species included representatives of the genera *Taenia, Echinococcus, Fasciola, Schistosoma, Gongylonema,* and *Setaria*. Based on their life cycles, the identified helminths can be divided into two ecological groups: monoxenous (direct life cycle, without intermediate hosts) and heteroxenous (requiring one or more intermediate hosts). Monoxenous species included representatives of the families Trichosephalidae (3 species), Trichostrongylidae (2 species), Dictyocaulidae (2 species), Syphaciidae (1 species), and Ascarididae (1 species). Heteroxenous development, involving obligatory intermediate hosts, was characteristic of 18 species, including 6 cestodes, 2 trematodes, and 10 nematodes. The high proportion of heteroxenous species emphasizes the importance of intermediate host communities in maintaining parasite transmission in this arid region [43].

Studies conducted in the Caucasus revealed a high prevalence of mixed protostrongylid infections in wild and domestic ruminants, with roe deer and tur showing the highest helminth diversity and infection rates due to extensive migration and frequent contact with livestock pastures. The dominant lung nematodes included *Protostrongylus hobmaeri, P. kochi,* and *Muellerius capillaris*, particularly in tur populations. Infection intensity and prevalence were influenced by season, altitude, host ecology, and mollusk abundance. Wild ungulates occupying mountain pastures shared numerous helminth species with sheep and goats, suggesting active parasite exchange between wildlife and domestic animals across the Caucasus region [44, 45].

Discussion

Detailed summaries by species are of particular importance [26]. In the goitered gazelle, 21 species of helminths have been registered, including F*asciola hepatica, Avitellina centripunctata, Multiceps multiceps (larvae), Taenia hydatigena (larvae), Taenia sp., Haemonchus longistipes, Marshallagia marshalli, Nematodirella gazelli, Nematodirus dogieli, N. gazellae, N. mauritanicus, N. oiratianus, N. spathiger, Ostertagiella circumcincta, O.occidentalis, Parabronema skrjabini, Setaria labiato-papillosa, Skrjabinema ovis, Skrjabinodera saiga, Trichuris skrjabini, Trichuris sp.,* and *Trichinella spiralis.*

Taxon-specific distribution, geographic localization, and diagnostic methods of major parasites identified in wild ungulates of Kazakhstan

Host Species	Parasite Taxonomic Group	Representative Parasite Species / Genera	Geographic / Regional Distribution	Primary Diagnostic Methods
Saiga antelope (*Saiga tatarica*)	Nematoda, Cestoda, Protozoa	*Nematodirus gazellae, Oesophagostomum venulosum, Trichuris skrjabini, Moniezia expansa, Ostertagia ostertagi, Eimeria elegans*	West Kazakhstan (Ural population), Betpak-Dala, Ustyurt ecosystems	Coproscopy, complete helminthological dissection, Molecular (PCR)
Argali (*Ovis ammon*)	Nematoda, Cestoda, Trematoda	*Fasciola hepatica, Dicrocoelium lanceatum, Marshallagia marshalli, Protostrongylus hobmaieri, Echinococcus granulosus (larvae)*	Tien Shan, Zhetysu Alatau, National Park "Altyn-Emel"	Complete helminthological dissection, morphological identification
Altai maral (*Cervus elaphus sibiricus*)	Nematoda, Cestoda, Protozoa	*Moniezia expansa, Capillaria bovis, Haemonchus contortus, Eimeria robusta, Eimeria cervi*	East Kazakhstan region, mountainous zones	Coproscopic profiling, post-mortem diagnostics
Siberian roe deer (*Capreolus pygargus*)	Nematoda, Cestoda, Trematoda	*Dicrocoelium lanceatum, Capreocaulus capreoli, Dictyocaulus eckerti, Taenia multiceps (larvae)*	Widespread throughout Northern, Central, and Eastern Kazakhstan	Morphological analysis, Molecular tissue screening (PCR)
Siberian ibex (*Capra sibirica*)	Nematoda, Cestoda, Trematoda	*Cystocaulus vsevolodovi, Neostrongylus zvetkovi, Protostrongylus kochi, Spiculocaulus austriacus*	High-altitude systems of Tien Shan and Altai mountains	Complete helminthological dissection, larvoscopy
Goitered gazelle (*Gazella subgutturosa*)	Nematoda, Cestoda, Trematoda	*Haemonchus longistipes, Nematodirella gazelli, Parabronema skrjabini, Skrjabinodera saiga*	Arid deserts, National Park "Altyn-Emel"	Coproscopy, historical baseline dissections
Moose (*Alces alces*)	Nematoda, Cestoda, Diptera (Arthropoda)	*Taenia hydatigena (larvae), Cephenemyia ulrichii (larvae)*	Northern Kazakhstan (Akmola and Kostanay regions)	Morphological diagnostic keys, Molecular barcoding (COI *gene*)

Overall, in the argali, 47 species of helminths were identified, including *Fasciola hepatica, Dicrocoelium lanceatum, Taenia hydatigena* (larvae), *Multiceps multiceps* (larvae), *Moniezia benedeni, M. alba, Parabronema skrjabini, Skrjabinema ovis, Chabertia ovina, Trichostrongylus colubriformis, T. probolurus, Ostertagia ostertagi, O. occidentalis, O. circumcincta, O. orloffi, O. trifida, O. trifurcata, Marshallagia marshalli, M. mongolica, M. schumakovitschi, Haemonchus contortus, N. abnormalis, N. archari, N. dogieli, N. oiratianus, N. spathiger, Dictyocaulus filaria, Protostrongylus hobmaieri, P. skrjabini, P. davtiani, P. raillieti, Spiculocaulus leuckarti, Cystocaulus ocreatus, Trichuris ovis, T. skrjabini, Capillaria sp., Setaria labiato-papillosa, N. gazellae, Nematodirella gazelli, N. longissimespiculata, Taenia ovis* (larvae), *Echinococcus granulosus* (larvae), *Ostertagiella davtiani*, and *Muellerius capillaris*.

In the Siberian roe deer, 22 species were identified, including *Dicrocoelium lanceatum, Moniezia expansa, Taenia cervi* (larvae), *T. hydatigena* (larvae), *Capreocaulus capreoli, Chabertia ovina, Dictyocaulus eckerti, Haemonchus contortus, Marshallagia marshalli, N. filicollis, N. oiratianus, N.*

spathiger, Ostertagiella circumcincta, O. occidentalis, O. trifurcata, Parabronema skrjabini, Setaria capreola, Spiculocaulus austriacus, Trichuris skrjabini, Trichostrongylus colubriformis, T. probolurus, and *T. vitrinus.*

In the Siberian ibex, 28 species were registered, including *Dicrocoelium lanceatum, Moniezia benedeni, Multiceps multiceps* (larvae), *M. skrjabini* (larvae), *Taenia hydatigena* (larvae), *Cystocaulus vsevolodovi, Marshallagia marshalli, M. mongolica, M. schumakovitschi, Nematodirus abnormalis, N. dogieli, N. filicollis, N. oiratianus, N. spathiger, N. sugatini, Neostrongylus zvetkovi, Ostertagia ostertagi, Ostertagiella circumcincta, O. occidentalis, Protostrongylus davtiani, P. dikmansi, P. kochi, P. raillieti, Skrjabinema ovis, Spiculocaulus austriacus, S. leuckarti, S. orloffi,* and *Trichuris skrjabini.*

In saiga antelopes, 49 helminth species have been recorded, of which 16 are common to all populations, including *Taenia hydatigena* (larvae), *Avitellina centripunctata, Skrjabinema ovis, Ostertagiella occidentalis, Marshallagia marshalli, M. mongolica, Trichuris skrjabini, as well as additionally Echinococcus granulosus* (larvae), *Protostrongylus skrjabini, Trichostrongylus axei, T. probolurus, Haemonchus contortus, N. abnormalis, N. gazellae, N. mauritanicus* and *Nematodirella longissimespiculata.*

In natural biocenoses adjacent to areas of developed livestock breeding, active exchange of parasites between wild and domestic animals is observed.

A commonality of helminth fauna has been noted between saiga antelopes and sheep, argalis and sheep, ibexes and sheep, goitered gazelles and small ruminants, and kulans and horses. Among coccidia, representatives of the genus *Eimeria* are widely distributed, including *E. robusta, E. gallivalerioi,* and *E. cervi,* which are detected in various species of ungulates with high infection intensity [47, 48].

The overlap of parasite fauna between wild and domestic ungulates translates into specific risks. Trichostrongylidae nematodes, prevalent in saiga and argali [18–22], can reduce weight gain and milk production in sympatric livestock. *Fasciola hepatica* and *Dicrocoelium lanceatum,* documented in argali and goitered gazelle [26, 35, 38], cause liver condemnation and economic losses. From a public health perspective, *E. granulosus* (hydatid cysts) in saiga and argali [38, 46] poses a zoonotic risk via infected dogs, while Trichinella spiralis in goitered gazelle [26] threatens consumers of undercooked game meat. These findings suggest that wildlife parasitology should be integrated into national veterinary surveillance, particularly at wildlife–livestock interfaces. Despite the substantial amount of accumulated data, modern studies remain limited. This is due to the labor-intensive nature of helminthological research, difficulties in accessing habitats, and restrictions on sampling protected species.

Conclusion

The conducted analysis of literature data on the parasite fauna of wild ungulates in Kazakhstan demonstrates that it is characterized by high species richness, a complex structure, and a significant degree of overlap with parasites of domestic animals. This review summarizes information on 12 host species inhabiting various landscape and climatic zones, ranging from deserts to mountain ecosystems. The most parasitologically studied species proved to be the saiga antelope (*S. tatarica*), argali (*O. ammon*), maral (*C. elaphus sibiricus*), roe deer (*C. pygargus*), Siberian ibex (*C. sibirica*), and goitered gazelle (*G. subgutturosa*).

It has been established that geohelminths predominate in the parasite fauna (58.9%) over biohelminths (41.1%), with the gastrointestinal tract being the primary site of parasite localization. The widest spectrum of helminth species was recorded in the saiga antelope (49 species) and argali (47 species), reflecting both the ecological characteristics of these hosts and their higher level of study. A high degree of helminth fauna commonality between wild and domestic ruminants has been revealed, indicating the formation of a unified parasitic system driven by shared use of pastures and watering sites, as well as the ongoing reduction of natural wildlife habitats.

At the same time, the available data suggest a possible decline in parasite species diversity in the natural biocenoses of Kazakhstan, although direct long-term comparative studies are lacking. This potential trend may be related to anthropogenic ecosystem transformation, climate change, and host population fragmentation, but the current evidence remains circumstantial. Further targeted monitoring is required to confirm whether species richness is genuinely decreasing or whether apparent differences reflect uneven sampling effort over time [49].

Consequently, the conservation of wild ungulate biodiversity in Kazakhstan is impossible without regular monitoring of their parasite fauna [50]. Priority directions for future research should include: (1) systematic surveys of all key host species across all major populations using standardized protocols; (2) integration of classical and molecular genetic diagnostic methods; (3) assessment of climate change and anthropogenic pressure impacts on parasite community structure; and (4) development of predictive models for epizootic risks at the wildlife–livestock–human interface. Addressing these tasks will not only expand fundamental knowledge on the functioning of natural foci but also provide a scientifically sound basis for conservation strategies of vulnerable species and for the control of parasitic diseases in a changing environment.

Authors' Contributions

EK, AZh: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. EA, AA: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript.

Statement on Competing Interests

The authors declare that they have no conflicts of interest.

References

1 Dimeyeva, L., Salmukhanbetova, Z., Malakhov, D. (2022). Mapping the rangelands of wild ungulates in the Barsakelmes Nature Reserve (Kazakhstan). *Arid Ecosystems,* 12, 473–480. DOI:10.1134/S2079096122040047.

2 Altyn Dala Conservation Initiative. (2024). *Global atlas of ungulate migration: Saiga movement data.*

3 Bekenov, A.B., Eszhanov, B.E., Makhmutov, S. (1995). *Qazaqstan sútqorektileri [Mammals of Kazakhstan]*. Almaty: Ǵylym. [*In Kazakh*].

4 Mukhametzhanov, M.D., Esmukhanbetov, D.N., Karagoishin, Zh.M. (2020). Almaty qoryǵyndaǵy maral men eliktiń bıologıasy jáne olardyń san-mólsheriniń dınamıkasy [Biology of maral and rob deer in the Almaty nature reserve and their quantitative dynamics]. *Qazaqstan zoologıa habarshysy*, 1: 2, 105–114. [*In Kazakh*].

5 Hoberg, E.P., Brooks, D.R. (2008). A macroevolutionary mosaic: Episodic host-switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography,* 35.

6 Baitursinov, K.K. (2008). Ekologicheskie osnovy profilaktiki gelmintozov dikih kopytnyh zhivotnyh v usloviyah Kazahstana [Ecological bases of preventive measures of helminthosis of wild animals in Kazakhstan]. *Rossiiskii parazitologicheskii zhurna*l, 1. [*In Russian*].

7 Poloz, S.V. (2020). Ekologicheskie osobennosti formirovaniya ustoichivosti modelnyh vidov dikih zhivotnyh v sisteme «parazit–hozyain» [Ecological features of the stability formation of model species of wild animals in the "parasite–host" system]. *Ekologiya i zhivotnyi mir,* 1, 15–20. [*In Russian*].

8 Pelgunov, A.N., Maklakova, L.P. (2013). Parasitological aspects of wild ungulate acclimatization and introduction. *Russian Journal of Parasitology*, 3, 67–75.

9 Ortomedia. (2023). Chislennost' sajgakov v Kazahstane [*The number of saigas in Kazakhstan*]. https://ortcom.kz/ru/deyatelnost-pravitelstva/1684838122. [*In Russian*].

10 Vlast.kz. (2024). *Process regulirovaniya chislennosti sajgakov priostanovlen do 2025 goda [The process of regulating the number of saigas has been suspended until 2025].* https://vlast.kz/novosti/58894-process-regulirovania-cislennosti-sajgakov-priostanovlen-do-2025-goda-minekologii.html. [*In Russian*].

11 Arasha.kz. (2025). S*aigaki v Kazahstane: chislennost', rekord 2025 [Saigaki v Kazahstane: chislennost', rekord 2025].* https://ru.arasha.kz/2991-saigaki-v-kazakhstane-chislennost-rekord-2025. [*In Russian*].

12 Kol'sai-Koldery. (2026). *Fauna Kazahstana: sibirskii gornyi kozel [Fauna of Kazakhstan: Siberian Mountain goat].* https://kolsai-koldery.kz/science/fauna/?id=30. [*In Russian*].

13 Tabigat Media. (2023). *Kopytnye Kazahstana [Ungulates of Kazakhstan]*. https://tabigat.media/wildlife/Kopytnye-Kazahstana [*In Russian*].

14 Vecher.kz. (2025). *Uchet dikih zhivotnyh proshel v nacparke Altyn-Emel [Registration of wild animals took place in the Altyn-Emel National Park].* https://vecher.kz/ru/article/uchet-dikih-jivotnyh-proshel-v-nasparke-altyn-emel.html. [*In Russian*].

15 Institut zoologii Respubliki Kazahstan. (2025). S*otrudniki Instituta zoologii prinyali uchastie v ezhegodnom zimnem uchete kopytnyh v nacional'nom parke «Altyn-emel'» [The staff of the Institute of Zoology took part in the annual winter ungulate census in the Altyn-Emel National Park].* (2025). https://zool.kz/eng/news/the-staff-of-the-institute-of-zoology-participated-in-the-annual-winter-survey-of-ungulates-in-altyn-emel-national-park/ [*In Russian*].

16 Wild-life.kz. (n.d.). *Kazahstanskii gornyi baran [Kazakhstan mountain sheep].* https://wild-life.kz/ru/kazahstanskii-gornyi-baran/ [*In Russian*].

17 Adilet. (2025). *Ob utverzhdenii Koncepcii sohraneniya i ustoichivogo ispol'zovaniya bioraznoobraziya v Respublike Kazahstan na 2026-2035 gody [On approval of the Concept of Conservation and Sustainable Use of Biodiversity in the Republic of Kazakhstan for 2026-2035]*. https://adilet.zan.kz/rus/docs/P2500001206. [*In Russian*].

18 Baitursinov, K.K. (2008). Ekologicheskie osnovy profilaktiki gel'mintozov dikih kopytnyh zhivotnyh v uslovijah Kazahstana [Ecological bases of preventive measures of helminthosis of wild animals in Kazakhstan]. *Rossiiskii parazitologicheskii zhurnal,* 1, 1–7. [*In Russian*].

19 Baitursinov, K.K. (2008). Faktory formirovanija obshhnosti gel'mintofauny dikih i domashnih kopytnyh Kazahstana [Factors of formation of similarity of helminthofauna of wild and domestic ungulate in Kazakhstan]. *Rossiiskii parazitologicheskii zhurnal,* 4, 5–12. [*In Russian*].

20 Sidihov, B.M., Orynbai, N., Tenizbai, G. (2019). Invazirovannost' gel'mintami pishhevaritel'nogo trakta sajgakov v Zapadno-Kazahstanskoi oblasti [Helminths Infection of Cattle Depending on Natural Climatic Conditions of West Kazakhstan Region]. *Dostizhenijya vuzovskoi nauki*, 202–204. [*In Russian*].

21 Kushaliyev, K., Ussenov, Z., Alimbekov, S., Mullakaev, O., Kozhayeva, A., Khairushev, A. (2023). Study of the saiga helminth fauna and Ural sheep in western Kazakhstan. *Open Veterinary Journal*, 13, 485. DOI:10.5455/OVJ.2023.v13.i4.11.

22 Kushaliyev, K.Zh., Kozhayeva, A.R., Sadenov, M.M., Khayrushev, A.R. (2022). Gel'mintozy saigakov na territorii ih migracii [Helminthoses of saigas in their migration territory]. *Nauka i obrazovanie*, 1(66), 61–71. DOI:10.52578/2305-9397-2022-1-1-61-71. [*In Russian*].

23 Kidiraliyev, E.Zh., Zhaksylykova, A.A., Kydyrkhanova, E.A., Kushaliyev, K.Zh., Shevtsov, A., et al. (2024). Molecular evaluation of Eimeria spp. infection in the Volga–Ural saiga population of Kazakhstan. *International Journal for Parasitology: Parasites and Wildlife,* 24. DOI: 10.1016/j.ijppaw.2024.100966.

24 Baitursinov, K.K. (2009). Kratkie dannye po biologii i zarazhennosti gel'mintami sajgaka (Saiga tatarica) [Brief data on biology and infection with helminths of saiga (Saiga tatarica).]. *Vestnik KazNU. Seriya biologicheskaya,* 3(42), 83–87. [*In Russian*].

25 Kushaliyev, K., Sarybayeva, D., Kozhayeva, A., Khamzina, A., Sundetkaliyeva, G. (2024). Epizootic map of helminth infections in Ural saiga populations. Global BioScience Journal, 1(4), 79–86.

26 Absatirov, G., Smagulov, D., Bozymov, K., Shalmenov, M., Nassambayev, Y., Yessengaliyev, K., Baitlessova, L., Girisgin, A.O. (2025). Risks to the growth, conservation and management of the Ural saiga population. *Diversity,* 17(9), 595. DOI:10.3390/d17090595.

27 Suleimenov, M.Zh., Abdybekova, A.M., Tlepov, A.A., Tuganbaev, A., Dzhusupbekova, N.M. (2014). Rasprostranenie vozbuditelei parazitarnyh zoonozov v Kazahstane [Prevalence of parasitic zoonose causative agents in Kazakhstan]. *Teoriya i praktika bor'by s parazitarnymi boleznyami,* 15, 296–298.

28 Kerimbaeva, R.A., Abdybekova, A.M., Dzhusupbekova, N.M. (2015). Qazaqstandaǵy maraldardyń ektoparazıtteri [Ectoparasites marals in Kazakhstan]. *Izdenister, nátıjeler*, 3, 53–56. [*In Kazakh*].

29 Abdybekova, A.M., Dzhusupbekova, N.M., Akhmetova, G.D., Abdibaeva, A.A., Zhaksylykova, A.A. (2016). Shyǵys Qazaqstan oblysy maraldarynyń parazıtofaýnasy [Parasitofauna of deer of the East Kazakhstan region]. *Vestnik nauki KATU im. S. Seifullina*, 4–6. [*In Kazakh*].

30 Abdybekova, A.M. (2016). *Rekomendacii po profilaktike i lecheniju gel'mintozov maralov [Recommendations for the prevention and treatment of maral helminthiasis]*. Almaty: 18. [*In Russia*n].

31 Abdybekovaa, A.M., Dzhusupbekova, N.M., Abdibaevaa, A.A., Zhaksylykova, A.A. (2017). Effektivnost' lekarstvennyh form preparatov, razrabotannyh dlja lecheniya gel'mintozov maralov, olenei i drugih dikih kopytnyh [Efficiency of medicinal forms of preparations developed for treatment of helminthoses of marals, deer and other wild copy]. *Izvestiya NAN RK,* 3(39), 34–40. [*In Russian*].

32 Abdybekovaa, A.M., Dzhusupbekova, N.M., Abdibaevaa, A.A., Zhaksylykova, A.A. (2017). Ekonomicheskii ushherb, nanosimyi parazitami v maralovodcheskih hozyaistvah Vostochno-Kazahstanskoioblasti [Economic damage influenced By parasites in maral breeding farms Of the Eastern Kazakhstan region]. *Izvestiya NAN RK*, 4(40), 10–18. [*In Russian*].

33 Shimit, L.D. (2020). *Maralovodstvo [Maral breeding]*. Kyzyl: TuvGU, 83. [*In Russian*].

34 Abdybekova, A.M., Sultanov, A.A., Dzhusupbekova, N.M., Abdibayeva, A.A., Zhaksylykova, A.A., Kerimbaeva, R.A., Akhmetova, G.D., Torgerson, P.R. (2017). Parasites of farmed marals in Kazakhstan. *Small Ruminant Research,* 153, 142–145.

35 Smagulova, A., Uakhit, R., Manapov, N., Lider, L., Kiyan, V. (2025). The main helminths and protozoa of the digestive tract of domestic and wild ungulates in northern and central Kazakhstan. *Herald of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences,* 3(011), 43–51. [*In Russian*].

36 Kiyan, V., Smagulova, A., Uakhit, R., Hermosilla, C., Lider, L., Jazina, K., Manapov, N. (2025). Molecular identification of cryptic cysticercosis: Taenia spp. in wild and domestic intermediate hosts in Kazakhstan. *Diversity*, 17, 655. DOI:10.3390/d17090655.

37 Bauer, C., Uakhit, R., Smagulova, A., Jazina, K., Lyalchenko, A., Kiyan, V. (2025). Where there are moose (Alces alces) in Eurasia, there are moose nose botflies: First morphological and molecular identification of Cephenemyia ulrichii (Brauer, 1862) in Kazakhstan. *International Journal for Parasitology: Parasites and Wildlife*, 27, 101086. DOI: 10.1016/j.ijppaw.2025.101086.

38 Akramova, F.D., Shakarbaev, U.A., Akbarov, A.A., Ubbiniyazova, J.K., Toremuratov, M. Sh., Saparov, K.A., Azimov, D.A. (2023). Gel'mintofauna polorogih (Artiodactyla: Bovidae) severo-zapadnogo Uzbekistana [Helminth fauna of cavicornians (artiodactyla: bovidae) in North-Western Uzbekistan]. *Teoriya i praktika bor'by s parazitarnymi boleznyami*, 24, 40–45. DOI:10.31016/978-5-6048555-6-0.2023.24.40-45. [*In Russian*].

39 Azimov, D.A., Dadaev, S.D., Akramova, F.D., Saparov, K.A. (2015). *Gel'minty zhvachnyh zhivotnyh Uzbekistana [Helminths of ruminants of Uzbekistan]*. Tashkent: Fan, 223. [*In Russian*].

40 Azimov, D.A., Akramova, F.D., Shakarboev, Je.B., i dr. (2019). *Shistosomoz zhivotnyh [Animal schistosomiasis]*. Tashkent: Fan, 320. [*In Russian*].

41 Ivashkin, V.M., Oripov, A.O., Sonin, M.D. (1989). *Opredelitel' gel'mintov melkogo rogatogo skota [Small cattle helminth Detector]*. Moskva: 256. [*In Russian*].

42 Anderson, R.C. (2000). *Nematoda parasites of vertebrates: Their development and transmission.* New York: CAB International, 650.

43 Akramova, F.D., Toremuratov, M., Shakarbaev, U.A., Azimov, D.A., Rakhmonova, L.A., Akbarov, A.A., Erkinova, L.U. (2020). Ekologicheskii analiz gel'mintofauny dikih parnokopytnyh [Ecological analysis of helminth fauna of wild artiodactyles]. *Rossiiskii parazitologicheskii zhurnal,* 14(4), 11–23. [*In Russian*].

44 Gadaev, H.H. (2015). Gel'mintofauna domashnih i dikih zhvachnyh na pastbishah Chechenskoj Respubliki [Helminth fauna of domestic and wild ruminants on the pastures of the Chechen Republic]. *Rossiiskii parazitologicheskii zhurnal*, 2, 8–12. [*In Russian*].

45 Гадаев, Х.Х. (2013). Ekologicheskie osnovy formirovaniya gel'mintofauny u dikih zhivotnyh [Ecological basis formation of helminthes fauna of wild animals]. *Rossiiskii parazitologicheskii zhurnal,* 1, 6–8. *[In Russian*].

46 Bajtursinov, K.K. (2008). Vliyanie gel'mintov na dinamiku populyacii dikih kopytnyh zhivotnyh [The influence of helminths on the population dynamics of wild ungulates]. *Vestnik KazNU im. al'-Farabi. Seriya ekologicheskaya,* 1(22), 3–8. [*In Russian*].

47 Morgan, E., Shaikenov, B., Torgerson, P., Medley, G., Milner-Gulland, E. (2005). Helminths of Saiga antelope in Kazakhstan: implications for conservation and livestock production. *Journal of wildlife diseases,* 41, 149–62. DOI:10.7589/0090-3558-41.1.149.

48 Abdybekova, A.M., Zhaksylykova, A.A., Kushaliyev, K.Zh., Kidiraliyev, E.Zh., Kozhayeva, A.R., Kuzhebayeva, U.Zh., Grachev, A., Shevtsov, A., Budke, C.M. (2023). A survey of the parasites of Ural saiga antelopes and Turkmenian kulans of Kazakhstan. *International Journal for Parasitology: Parasites and Wildlife,* 21, 232–236. DOI: 10.1016/j.ijppaw.2023.06.006.

49 Chelsea, L.W. (2025). Parasites in a Changing World: Troublesome or in Trouble? *Annual Review Animal Biosciences.* 13, 303–323. DOI:10.1146/annurev-animal-111523-102039.

50 Glamazdin, I.G., Medvedev, I.N., Kutuzov, D.D., Fayzullina, I.I., Nazarova, S.V., Sysoeva, N.Y., Komarov, N.E., Bannikova, O.A., Gubanova, M.I., Bakaeva, K.V., et al. (2024). Main Parasitic Infestations of Wild Ungulates Used for Food. *Journal of Biochemical Technology,* 15(2), 59–63. DOI:10.51847/iKmPtRdbFp.

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Review article

Horse breeding and horse meat production in Kazakhstan: current status, safety challenges and future development prospects

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Abstract

Background and Aim. The global agri-food system is facing systemic challenges driven by climate change, depletion of land and water resources and increasing demand for animal protein. Productive horse breeding represents a strategically important, environmentally sustainable pathway for diversifying the meat market. The aim of this study is to provide a comprehensive analysis of the current state of horse breeding and horse meat production in the Republic of Kazakhstan, including an assessment of the veterinary and sanitary, environmental, and biological factors affecting the quality, nutritional value and safety of horse meat products.

Materials and Methods. The study was based on statistical data, analytical reports and published scientific literature addressing various aspects of horse breeding and horse meat production.

Results. The conducted analysis demonstrates that global productive horse breeding is on the threshold of a conceptual shift. The present review advances a fundamentally new position: horse meat should be considered not merely as an ethnocultural niche or a marginal regional specialty, but as a strategically important, environmentally sustainable protein system of global significance.

Conclusion. From the perspective of the One Health systems concept, horse breeding and horse meat production are regarded as an interconnected system linking animal health, human health and environmental health. Contemporary approaches to the sustainable development of the industry require the consideration of environmental, veterinary and sanitary, and biological factors that affect the safety and quality of the products. The study identified key methodological and infrastructural limitations that impede the development of the sector and highlighted the role of horse meat as a strategic source of protein in addressing global food security challenges.

Keywords: animal protein; food security; horse meat; One Health; productive horse breeding; review.

Introduction

The modern structure of global food security system is undergoing fundamental transformations. According to long-term macroeconomic forecasts, aggregate global demand for animal-origin products continues to demonstrate a steady upward trend through 2034 (Food and Agriculture Organization, 2025). In this multifactorial context, the horse meat markets represents a unique segment, evolving from a local traditional product of nomadic cultures to the status of a functional superfood [1].

Historically, horse meat played a central role in the diets of the Eurasian steppe populations. Archaeological and genetic evidence indicates that the early domestication of horses in the settlements of the Botai culture on the territory of modern Kazakhstan (around 3500 BC) was accompanied by the active use of their meat and milk [2]. Today, this product attracts close attention from the global scientific community. Studies published in leading journals such as *Meat Science, Animals,* and *Toxins* have demonstrated that the meat of equids possesses exceptional nutritional properties: a high concentration of heme iron, an optimal ratio of polyunsaturated fatty acids and high dietary value [3, 4, 5, 6].

The significance of this study lies in the need for a comprehensive assessment of the current state of horse breeding, the factors influencing the quality and safety of horse meat products, and the development of evidence-based approaches to improving the competitiveness of the domestic horse meat industry.

Today, the Republic of Kazakhstan is at the forefront of the development of this sector. The domestic market is demonstrating historic records of horse meat consumption [7].

Current literature sources indicate a growing interest in horse meat as a high-protein and dietary product with high nutritional and biological value. Moreover, the ambitious Comprehensive Livestock Development Plan for 2026–2030 approved by the Government of the Republic of Kazakhstan provides an institutional basis for doubling exports of domestic meat [8].The aim of this study is to conduct a comprehensive analysis of the current state of horse breeding and horse meat production in the Republic of Kazakhstan, including an assessment of the veterinary and sanitary, environmental, and biological factors affecting the quality, nutritional value, and safety of horse meat products.The research novelty of the study lies in its comprehensive examination of the sector from veterinary and sanitary, environmental, and biological perspectives, as well as in the integration of contemporary approaches to ensuring food security and promoting the sustainable development of horse breeding within the context of the modernization of the country's agro-industrial sector.

At the same time, the current state of horse meat production requires further improvement of veterinary and sanitary control, food safety monitoring systems, and the implementation of international quality standards and product traceability systems.

Materials and Methods

This review article was prepared based on a bibliographic search of scientific publications retrieved from electronic databases such as Google Scholar, PubMed, Web of Science and Scopus, as well as reports and statistical data from international sources such as FAOSTAT and the analytical platform ReportLinker.

Taking into account various aspects of the study, the results were divided into categories in accordance with the following approaches: the global meat market and the positioning of horse meat, macroeconomic patterns of international trade, assessment of the current state and development prospects of horse breeding, shifts in consumer behavior and nutraceutical characteristics of horse meat, including its dietary profile, which has a beneficial effect on human health.

The analysis also covered regional studies in the field of veterinary and sanitary control, characterizing the influence of environmental and biological factors on the quality, nutritional value and safety of horse meat.

Particular attention was paid to the limitations of molecular genetic research, the potential of genomic selection (GBLUP), as well as the transition from descriptive toxicology to a conceptual assessment of risks associated with exposure to xenobiotics.

Results

1. The global meat market and the positioning of horse meat: A macroeconomic analysis

1.1. Leading producing countries and production dynamics

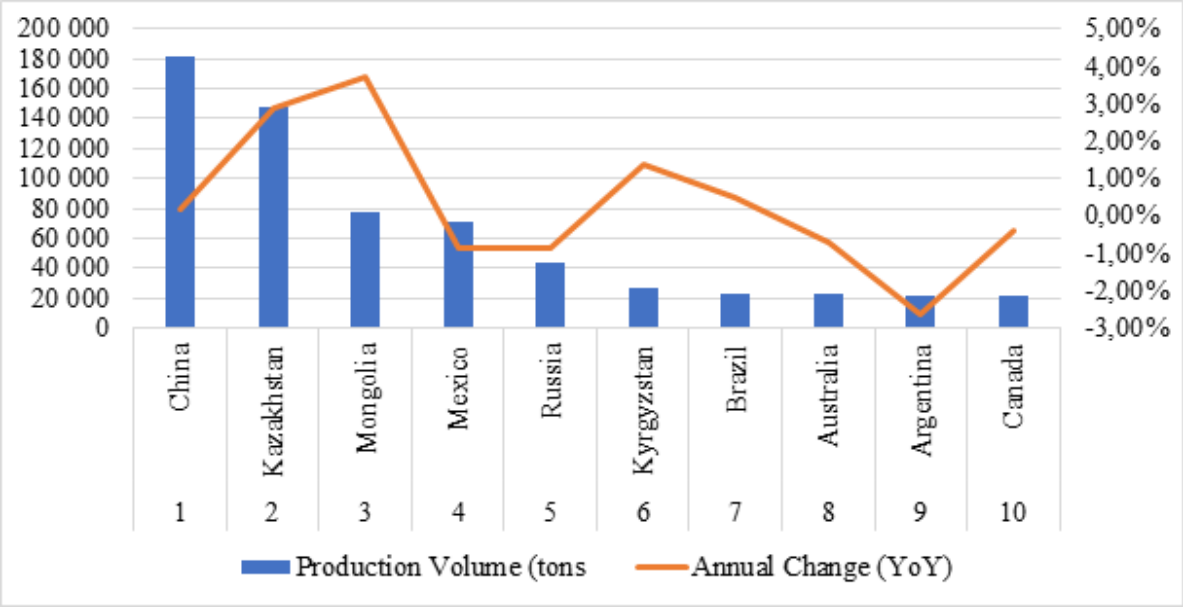
Global horse meat production demonstrates a high degree of concentration in the countries of the Asian region and Latin America. An analysis of aggregated data from FAOSTAT and the analytical platform ReportLinker makes it possible to identify the states that determine the architecture of global supply [11, 12].

Although poultry and pork account for the largest share of consumed animal protein, horse meat occupies a niche but important position. Its production is considered a more environmentally sustainable

alternative to beef due to lower methane emissions per unit of output [1]. The world's modern horse meat market is characterized by structural and trade asymmetry, where the production center is shifted to the countries of Asia and South America, whereas mostadded value is generated in the importing countries of the European Union [9]. Global production is concentrated in several countries, among which China (generating 24.3% of the global volume) and Kazakhstan (19.8%) dominate. In contrast, the largest net importers, shaping pricing policy, are Italy and Belgium, with the latter serving as a key logistical hub for the distribution of imported Latin American raw materials throughout Europe (Figure) [10].

This imbalance means thatproducing countries (such as Mongolia, accounting for 10.4% of production) often remain at the lower end of the value-added curve, acting exclusively as suppliers of low-cost raw materials [9].

However, the available data remain limited due to the opacity of transnational supply chains and insufficient statistical accounting in developing countries. The main limitation of current economic research is the lack of empirical models assessing the impact of non-tariff barriers on pricing. At the macroeconomic level, such asymmetry means that without the creation of strong domestic clusters of deep processing, producing countries will continue to lose economic rent in favor of European transit hubs.



Top 10 horse meat producing countries in the world

FAOSTAT statistics and the analytical platform ReportLinker indicate a decline in horse slaughter volumes in the countries of the Americas and Australia. At the same time, the countries of Central and East Asia demonstrate accelerated growth. Kazakhstan ranks second globally and demonstrates one of the highest CAGR rates (+3.17%), being surpassed in growth rate only by Mongolia (+6.26%) [11, 12].

1.2. The structure of international trade: Export-import flows

In 2024, the total volume of global horse meat trade experienced a correction due to logistical constraints in Europe. Nevertheless, the market maintains a clear division of roles between exporters and high-valueimporters.

Italy holds the leading position in imports (23,237,219 kg; 142,178,974 USD), significantly surpassing Belgium (11,935,024 kg; 60,285,965 USD) and Japan (4,677,055 kg; 35,395,333 USD). Switzerland is characterized by high unit prices despite relatively low volumes, which indicates a premium segment, while the Netherlands demonstrates moderate volumes at comparatively low unit values. The key exporters are Mongolia, Poland, Spain and Argentina [13].

1.3. Sociocultural determinants of consumption

The perception of horse meat varies from being a food taboo to being regarded as a delicacy. The studies conducted among chefs in France have identified several profiles: from "conservatives"

to “pragmatists,” who consider horse meat as a sustainable alternative to beef due to its low carbon footprint and high nutritional value [14, 15].

2. Integration into the global Halal market

For Kazakhstan, oriented toward exports to the countries of the Middle East and Southeast Asia, compliance with Halal standards is critically important. The Halal meat market is in a phase of accelerated expansion [16, 17]. In Islamic law (Fiqh), the majority of scientists recognize horse meat as fully permissible (halal). This is based on authentic hadiths. According to these reports, during the time of Prophet Muhammad, his companions consumed horse meat. This provides religious justification for the production and export of Kazakhstani horse meat to global Muslim markets [18].

3. Kazakhstan as a strategic production model

The Republic of Kazakhstan serves as an illustrative model for analyzing the potential and barriers of commercial horse breeding. The country possesses significant territorial resources and shows rapid growth in domestic consumption. However, its integration into the global export market is constrained by structural inefficiencies. The industry exhibits pronounced fragmentation of the value chain: large agricultural holdings, due to economies of scale, achieve profitability levelsof 18–22%, while numerous small producers demonstrate profitability not exceeding 5–8% [19].

This profitability gaplimits the scalability of production and reduces overall competitiveness. This divergence can be explained by dependence on imported feed additives, a shortage of modern processing infrastructure and the absence of consolidated cooperative linkages among farmers. The transition from an exclusive focus on domestic consumption to export expansion requires the implementation of integrated cluster models (such as the “Kaz Meat Cluster” project), that are capable of mitigating transaction costs. Strategically, this means that the success of the Kazakhstani model will depend not on a simple increase in livestock numbers, but on the technological modernization of the entire logistics chain “from farm to table” [20].

4. Nutritional value and functional properties

The biochemical profile of horse meat is characterized by a high content of easily digestible protein and micronutrients with a minimal level of lipids compared to beef and pork [21, 22]. The crude protein content is approximately21.1%, which is comparable to beef. However, intramuscular fat content ranges from only 2.0% to 6.0%. The baseline cholesterol index in horse meat is also at a nutritionally favorable level of 50–61 μg /100 g. Of particular value is the profile of micronutrients responsible for hematopoiesis: the concentration of heme iron in horse meat can reach 34.21 μg/100 g, which radically exceeds the indicators of beef (on average 2.50 μg/100 g) and pork (17.42 μg/100 g) [23, 24].

However, published data on the precise nutritional value of horse meatoften vary. This inconsistency can be explained by the influence of heterogeneous factors, such as the age of the animal, breed, type of muscle tissue studied and feeding system. The main limitation of modern reviews is the extrapolation of data obtained from isolated regional populations to the entire species. The biochemical density of horse meat and its high iron content indicate its high potential not simply as a source of basic calories, but as a specialized functional product for the correction of iron-deficiency and metabolic conditions in high-risk populations.

5. Lipidomics and health implications

The lipid profile of horse meat is of significant scientific interest due to the specific features of equine digestion (a monogastric digestive system with hindgut fermentation in the cecum), which allow the absorption of polyunsaturated fatty acids (PUFAs) from pasture grasses without complete bacterial biohydrogenation, characteristic of ruminant animals [21]. Horse meat is distinguished by a high protein content and a low level of intramuscular fat. Meat from pasture-raised horses is characterized by an optimal ratio of Omega-6 to Omega-3 fatty acids, while the absolute content of cardioprotective alpha-linolenic acid (Omega-3) reaches 1.4%, which significantly exceeds the indicators of beef (0.1%) and pork (0.6%). At the same time, the concentration of linoleic acid is about 11.1%, forming a favorable PUFA: SFA index at the level of 0.29. From a physiological perspective, such a lipid profile may contribute to a reduced atherogenic risk, making horse meat an anti-inflammatory component of the diet [25, 26, 27, 28, 29].

Despite these biochemical advantages, the clinical evidence base remains fragmented. Most conclusions about the health benefits of horse meat are based on the extrapolation of in vitro properties

of individual identified fatty acids, rather than on long-term dietary interventions. A critical limitation is the lack of controlled randomized clinical trials in humans that could reliably confirm the epidemiological advantages of regular horse meat consumption compared to conventional types of red meat [5].

5.1. Micronutrients and vitamins

Horse meat has been reportedto contain a high level of retinol (30 vs. 12 or 5 μg/100 g) and a lower niacin content(1.6 vs. 5.9 or 5.7 mg/100 g) compared to beef and pork, respectively. The riboflavin content in horse meat was similar to that in beef and pork (0.21 vs. 0.19 or 0.16 μg/100 g), respectively. The thiamine content in horse meat was higher than in beef but lower than in pork (0.20 vs. 0.07 or 0.56 μg/100 g), respectively. The bright color of horse meat is due to the high concentration of myoglobin and heme iron, which has high bioavailability. In addition, by-products (bones) are a rich source of calcium and phosphorus, which creates prospects for the production of dietary supplements [3].

6. Toxicology, ecotoxicology and biological safety

Access to international markets is strictly regulated by biological and ecotoxicological standards regulating the levelsof heavy metals, radionuclides and pesticides.

6.1. Biological safety: The problem of sarcocystosis

A key threat to export (especially to Japan, where basashi, a dish made from raw horse meat, is popular) is infection with the parasite Sarcocystis fayeri. This intracellular parasite produces an actin-depolymerizing factor (ADF), which is a potent enterotoxin causing severe food poisoning [30, 31].

In addition to sarcocystosis, a serious threat to animal health and the quality of the obtained meat is posed by parascaridosis. According to recent studies by scientists of the NJSC “Shakarim University” conducted on indigenous horses of the Zhabe breed, this parasitic invasion has a pronounced negative impact on the physiological condition of animals. It has been established that infection significantly reduces key hematological indicators: the hemoglobin level decreases by 23.5–36% and a decrease in the number of erythrocytes, total protein and glucose is also recorded, which directly worsens the development and well-being of the animal [31].

6.2. Ecotoxicological assessment: heavy metals, pesticides, and radionuclides

In the context of environmental safety, the most important aspect is the ecotoxicological monitoring of horse meat produced in various regions of Kazakhstan. The studies show that the level of accumulation of xenobiotics in the muscle tissue of herd-raised horses directly depends on the industrial and historical specifics of the grazing region.

A detailed regional analysis published in Veterinary World in 2026 showed that levels of anthropogenic contamination vary by region. Exceedance of maximum permissible concentrations (MPC) for cadmium (Cd) was recorded in horse meat samples at the level of 0.105 ± 0.015 μg/kg. The industrial Karaganda region proved to be the most susceptible to contamination with toxic elements, where exceedances of MPC for lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn) were recorded in almost all types of meat. In the Akmola region, exceedances for copper were noted in beef, lamb and horse meat, as well as locally for cadmium in horse meat (Shortandy district). At the same time, in the Abai region, exceedance of MPC in meat was detected exclusively for cadmium [33].

According to the conducted study, beef samples from various zones of the Semipalatinsk nuclear test site in most cases comply with organoleptic standards, however, the presence of small amounts of radioactive substances may lead to their accumulation in the body, thereby causing various pathological changes in organs and tissues. The conducted studies indicate the need to strengthen radiation monitoring and to prioritize radioisotope analysis in assessing the safety of meat products [34].

7. The domestic market of Kazakhstan: macrostatistics and breeding

In 2025, Kazakhstan updated historical records of horse meat consumption. In the fourth quarter, consumption reached 2.35 kg per capita and by the end of the year it was 7.63 kg. The share of horse meat in the meat consumption of the people of Kazakhstan increased to 9%, driven by faster growth in beef prices [7].

As of January 1, 2026, the highest annual growth is observed in poultry farming (+7.6%), as well as in horse breeding (+6.7%), which indicates the most dynamic development of these sectors. Moderate growth rates are recorded for cattle (+3.2%) and for sheep and goats (+2.2%), which indicates stable but less intensive development of these areas. The lowest growth is observed in pig farming (+1.0%), which indicates slower rates of expansion of this sector compared to other types of livestock [37].

7.1. Zootechnical, physicochemical and organoleptic indicators of horse meat of Kazakhstani breeds

According to the data of the Food and Agriculture Organization of the United Nations (FAO DAD-IS), 8 horse breeds are officially registered in Kazakhstan: Kushum, Mugalzhar, Kostanay, Akhal-Teke, Aday, Kazakh, Zhabe (an intra-breed type of the Kazakh breed) and Karabair [35]. Each of these breeds possesses its own unique zootechnical profile. Nevertheless, it is the indigenous breeds and their derivatives that form the basis of the country's meat balance due to year-round pasture-based management [36].

The meat-and-dairy direction of horse breeding in Kazakhstan is represented by the Mugalzhar and Kushum breeds, that characterized by high live weight (up to 656 kg) and a slaughter yield of up to 60%, as well as by the Kazakh breed (Zhabe type), that distinguished by high adaptability and the ability to obtain forage by digging through snow (tebenevka). The Aday and Karabair breeds possess combined productivity and satisfactory working qualities. The Kostanay and Akhal-Teke breeds are primarily oriented toward sporting use, and their meat productivity is of secondary importance [19, 35, 36, 38, 39, 40]. Scientific breeding makes it possible to significantly improve the physicochemical and organoleptic parameters of horse meat from indigenous horses while preserving its exceptional dietary value [41].

The horse meat is characterized by high nutritional value due to its significant protein content, favorable amino acid and fatty acid profile, as well as a reduced level of undesirable lipid fractions. Organoleptic indicators vary depending on the anatomical localization of muscles, but, in general, good characteristics of tenderness and color parameters are noted, which confirms its high quality as a food product (Table) [42].

Physicochemical and organoleptic indicators of horse meat (indigenous and improved breeds of the Republic of Kazakhstan)

№	Category	Indicator	Characteristics / Values	Research base
1	Physicochemical	Mass fraction of protein	20.0% - 24.0% (High protein density against the background of low intramuscular fat content)	General population of local breeds
		Trans fatty acids (TFA)	1.85% - 3.46% (under pasture-based management). This is significantly lower than in lamb (about 8%) and beef (up to 9.6%)	Pasture-raised
		Amino acid profile	20 amino acids identified (9 essential). L-lysine, L-glutamic acid, and L-aspartic acid predominate. High EAA/NEAA index	Kazakh horse (in various muscles)
		Fatty acid profile	19 acids identified (8 saturated, 7 monounsaturated, 4 polyunsaturated). Optimal n-6/n-3 PUFA ratio	Kazakh horse (Semitendinosus muscle)

Table continued

2	Organoleptic	Tenderness	The maximum degree of tenderness (reduction in shear force) is observed in the lumbar muscle Psoas major (PM)	Kazakh horse
			he lightness (L*) value is highest in the Longissimus dorsi; the redness (a*) value is maximal in the diaphragm	Kazakh horse

Discussion

8. Integration of the "One Health" concept

The "One Health" paradigm postulates an inseparable, mechanistic link between environmental conditions, animal health and human well-being. In the context of horse breeding, this concept describes strict cause-and-effect cascades: the state of the environment (soil degradation, industrial pollution) directly determines the toxicological safety of the feed base [33]. The quality of feed and climatic stressors modulate the immune status of the animal, which increases its susceptibility to zoonotic infections and accelerates the bioaccumulation of heavy metals.

The consumption of contaminated meat closes this chain, transmitting the accumulated toxicological and microbiological risks directly to the human population. This creates a negative feedback loop, where anthropogenic climate change depletes pasture resources, which steadily reduces animal resilience, and consequently, the quality of food products. This cascade pathway implies that ensuring food and epidemiological safety is impossible without the primary ecological remediation of agrobiocenoses. However, empirical data confirming these interconnections remain fragmented due to the fact that veterinary supervision, environmental monitoring, and healthcare services function in institutional isolation.

9. Research gaps and future directions

A critical analysis of the existing literature reveals several fundamental gaps that hinder the systemic transformation of the industry. First, there is a lack of international standardization in research methodologies, from muscle sampling protocols to analytical chemistry calibrations, which makes reliable meta-analyses difficult. Second, a critical shortage of genomic and transcriptomic data persists; the absence of dense reference SNP panels for meat-oriented horses completely blocks the application of marker-assisted selection. Third, the macroeconomic analytics of the global market suffers from weak granularity, failing to account for the impact of informal sectors of the economy on pricing. Finally, there is no harmonized international toxicological regulation that takes into account the specifics of the physiology and metabolism of heavy metals in the horse organism. Eliminating these institutional and methodological gaps should become the main priority for international research consortia in the coming decade.

Conclusion

This review demonstratesdemonstrates that global productive horse breeding is on the threshold of a conceptual shift. The present review advances a fundamentally new position: horse meat should be considered not merely as an ethnocultural niche or a marginal regional specialty, but as a strategically important, environmentally sustainable protein system of global significance.

Documented nutritional advantagessuch as record levels of heme iron, an ideal amino acid profile and enrichment with omega-3 polyunsaturated fatty acids make it a valuable dietary product.

The rational use of this resource offers a real mechanism for reducing the carbon intensity of the meat industry while simultaneously providing the population with high-quality micronutrients. Nevertheless,

the realization of this macroeconomic potential is impossible without critically overcoming current barriers. The industry requires the urgent integration of advanced genomic selection tools (GBLUP), the implementation of precision mathematical models for managing toxicological risks (EDI, THQ), and the restructuring of disproportionate trade chains. At all stages of the integration process, the implementation of the HACCP system is substantiated as an integral part of veterinary and sanitary examination, ensuring the identification and control of biological, chemical, and physical risk factors and guaranteeing the safety of products of animal origin.

Kazakhstan possesses significant potential for the further development of horse breeding. The sector is increasingly recognized not only as a traditional branch of animal husbandry but also as a promising source of environmentally sustainable and nutrient-rich meat products. In this context, government support, the modernization of livestock production systems and the growing focus on product quality and export potential create favorable conditions for the industry's long-term sustainable development.

Further development of the sector should be based exclusively on the strict interdisciplinary paradigm of “One Health,” which will ensure the necessary balance between the intensification of the agrarian economy, the preservation of fragile steppe ecosystems, and the long-term protection of human health.

Authors’ Contributions

ZhT: developed the study concept, conducted the literature review, and prepared the initial draft of the manuscript. KI: participated in data analysis and revised the manuscript. AS: supervised the study and assisted with the methodology. SD: carried out editing and final proofreading of the manuscript. All authors have read, reviewed, and approved the final version of the manuscript.

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Statement on Competing Interests

The authors declare that they have no conflicts of interest.

References

1 Hankerson, B.R., Müller, D. (2026). The other red meat: Environmental and nutritional advantages of horsemeat over beef and other meats. *Agricultural Systems*, 234, 104665. DOI: 10.1016/j.agsy.2026.104665.

2 Librado, P., Khan, N., Fages, A., et al. (2021). The origins and spread of domestic horses from the Western Eurasian steppes. *Nature,* 598, 634–640. DOI: 10.1038/s41586-021-04018-9.

3 Lorenzo, J.M., Sarriés, M.V., Tateo, A., Polidori, P., Franco, D., Lanza, M. (2014). Carcass characteristics, meat quality and nutritional value of horsemeat: A review. *Meat Science,* 96(4), 1478–1488. DOI: 10.1016/j.meatsci.2013.11.024.

4 Iskhan, Q., Kärimov, A. (2025). *Zhylqy túligi. Ǵylymi-tanymdyq zhinaq [Horse food. Scientific and educational collection]*. Semeydegi Shäkarim universiteti. «Alash-tanym». [*In Kazakh*].

5 Lee, C.E., Seong, P.N., Oh, W.Y., Ko, M.S., Kim, K.I., Jeong, J.H. (2007). Nutritional characteristics of horsemeat in comparison with those of beef and pork. *Nutrition research and practice,* 1(1), 70–73. DOI: 10.4162/nrp.2007.1.1.70.

6 Ustinova, A.V., Aslanova, M.A. (2011). Konina - cennoe syr'e dlya proizvodstva produktov detskogo pitaniya [Horse meat is a valuable raw material for the production of baby food]. *Myasnaya industriya*, 7, 22–24. [*in Russian*].

7 Xinhua. (2026). *Horse meat consumption in Kazakhstan hits record high.* https://english.news.cn/20260321/4a02fee52a384a619b8c46687ac8bbe8/c.html

8 Prime Minister of Kazakhstan. (2026). *Government approves the Comprehensive Livestock Development Plan for 2026–2030.* https://primeminister.kz/en/news/government-approves-the-comprehensive-livestock-development-plan-for-2026-2030-30976

9 Stanciu, S. (2015). Horse meat consumption - between scandal and reality. *Procedia Economics and finance,* 23, 697–703. DOI: 10.1016/S2212-5671(15)00392-5.

10 Jastrzębska, E., Daszkiewicz, T., Górecka-Bruzda, A., Feliś, D. (2019). Current situation and prospects for the horse meat market in Poland and the world. *Med. Weter*., 75(4), 196–202. DOI: 10.21521/mw.6203.

11 Food and Agriculture Organization of the United Nations (FAO). (2025). FAOSTAT: Crops and livestock products. https://www.fao.org/faostat/

12 ReportLinker. (2024). *Global Horse Meat Production by Country.* https://www.reportlinker.com/dataset/813a6540b502f21a85fd99a8365dcef231881f0b

13 Tridge. (2026). *Horse meat market overview.* https://www.tridge.com/market-overview/horse-meat

14 Lamy, A., Costa, S., Vial, C., Badji, I., Carrère, M., Rollet, P., Amiot, M.J. (2023). Horsemeat consumption in France: Determinants and sustainable market perspectives. *Meat Science,* 198, 109083. DOI: 10.1016/j.meatsci.2022.109083.

15 Vial, C., Lamy, A., Sebbane, M. (2025). Chefs saddle up Perceptions of horse meat as a sustainable gastronomic alternative in France. *Foods,* 14(4), 638. DOI: 10.3390/foods14040638.

16 The Business Research Company. (2026). *Halal food global market report 2026.* https://www.thebusinessresearchcompany.com/report/halal-food-global-market-report

17 Mordor Intelligence. (2024). *Halal Meat Market Size & Share Analysis.* https://www.mordorintelligence.com/industry-reports/halal-meat-market

18 Urazbakhtin, A.A. (2024). Praktiki pitaniya v islame kak sredstvo poznaniya bozhestvennykh otkrovenii Korana. Kriticheskii analiz dozvolennogo i nedozvolennogo [Nutritional practices in Islam as a means of understanding the divine revelations of the Quran. Critical analysis of the permitted and prohibited]. *Obshchestvo: filosofiya, istoriya, kul'tura,* 12, 258–264. DOI: 10.24158/fik.2024.12.31. [*in Russian*].

19 Kabylbekova, D., Assanbayev, T.S., Kassymbekova, S., Kantanen, J. (2024). Genetic studies and breed diversity of Kazakh native horses: A comprehensive review. *Advancements in Life Sciences,* 11: 1, 18–27.

20 Daribayeva, A., Alina, G., Nurgaliyeva, A., Ukubassova, G., Tursumbayeva, M. (2025). Kazakhstan's meat market: Analysis of production, consumption, and export. *Scientific Horizons*, 28(5), 90–101. DOI: 10.48077/scihor5.2025.90.

21 Balji, Y., Knicky, M., Zamaratskaia, G. (2020). Perspectives and safety of horsemeat consumption. *International Journal of Food Science and Technology*, 55(3), 942–952. DOI: 10.1111/ijfs.14390.

22 Ustinova, A.V., Dydykin, A.S., Aslanova, M.A., Soldatova, N.E. (2012). Myasnye produkty dlya detei po standartu khalyal' [Meat products for children according to the halal standard]. *Myasnye tekhnologii,* 8, 42–45. [*in Russian*].

23 Belaunzaran, X., Bessa, R.J., Lavín, P., Mantecón, A.R., Kramer, J.K., Aldai, N. (2015). Horse-meat for human consumption Current research and future opportunities. *Meat Science*, 108, 74–81. DOI: 10.1016/j.meatsci.2015.05.006.

24 Čobanović, N., Grković, N., Suvajdžić, B., Vićić, I., Karabasil, N. (2023). Horse carcass and meat quality—current knowledge and research gaps. *Meat Technology*, 64(2), 160–165. DOI: 10.18485/meattech.2023.64.2.29.

25 Lušnic Polak, M., Mervič, U., Demšar, L. (2017). Nutritional value of horse meat and products on the Slovenian market. *Meso: prvi hrvatski časopis o mesu,* 19(1), 73–81. DOI: 10.31727/m.19.1.1.

26 Seong, P.N., Park, K.M., Kang, G.H., Cho, S.H., Park, B.Y., Chae, H.S., Van Ba, H. (2015). The differences in chemical composition, physical quality traits and nutritional values of horse meat as affected by various retail cut types. *Asian-Australasian Journal of Animal Science*s, 29(1), 89. DOI: 10.5713/ajas.15.0049.

27 Ivanković, A., Pećina, M., Bittante, G., Amalfitano, N., Konjačić, M., Kelava Ugarković, N. (2025). Meat Production Potential of Local Horse Breeds: Sustainable Conservation Through Valorization. *Animals*, 15(13), 1911. DOI: 10.3390/ani15131911.

28 Beldarrain, L.R., Morán, L., Sentandreu, M.Á., Insausti, K., R. Barron, L.J., Aldai, N. (2021). Muscle and subcutaneous fatty acid composition and the evaluation of ageing time on meat quality parameters of Hispano-Bretón horse breed. *Animals*, 11(5),1421. DOI: 10.3390/ani11051421.

29 Irikura, D., Saito, M., Sugita-Konishi, Y., Ohnishi, T., Sugiyama, K.-I., Watanabe, M., Yamazaki, A., Izumiyama, S., Sato, H., Kimura, Y., Doi, R., Kamata, Y. (2017). Characterization of Sarcocystis fayeri's actin-depolymerizing factor as a toxin that causes diarrhea. *Genes to Cells,* 22(9), 825–835. DOI: 10.1111/gtc.12515.

30 Kamata, Y., Saito, M., Irikura, D., Yahata, Y., Ohnishi, T., Bessho, T., Inui, T., Watanabe, M., Sugita-Konishi, Y. (2014). A toxin isolated from Sarcocystis fayeri in raw horsemeat may be responsible for food poisoning. *Journal of Food Protection*, 77(5), 814–819. DOI: 10.4315/0362-028X.JFP-13-351.

31 Akhmetzhanova, A., Dyusembaev, S., Muratbayev, D., Nurzhumanova, Z. (2024). Indicators of the blood composition of medicated horses. *Наука и образование*, 4(77), 249–257. DOI: 10.52578/2305-9397-2024-4-1-249-257.

32 Bainiyazov, A., Adilbekov, Z., Mustafina, R., Zhuzzassarova, G., Suranshiyev, Z., Suleimenov, S., Serikova, A. (2026). Technogenic contamination of livestock- and fish-derived food products with heavy metals and radionuclides in industrial and post-nuclear regions of Kazakhstan. *Veterinary World,* 19(3), 1052-1068.

33 Duyssembayev, S., Serikova, A., Ikimbayeva, N., Balgabaikyzy, A., Zhexenayeva, A. (2023). Thequality of beef in the conditions of the former Semipalatinsk testsite. *Journal of Animal Physiology and Animal Nutrition,* 107, 1328–1335. DOI: 10.1111/jpn.13821.

34 Orazymbetova, Z., Ualiyeva, D., Dossybayev, K., Torekhanov, A., Sydykov, D., Mussayeva, A., Baktybayev, G. (2023). Genetic diversity of Kazakhstani Equus caballus (Linnaeus, 1758) horse breeds inferred from microsatellite markers. *Veterinary Sciences,* 10(10), 598. DOI: 10.3390/vetsci10100598.

35 Iskhan, K., Akimbekov, A., SHamekova , M., Muhatai, G., Orynaliev, K. (2025). Myasnaya i molochnaya produktivnos' loshadei kazahskoi porody [Meat and dairy productivityof kazakh-bred horses]. *Izdenister Natigeler,* 1(105), 5–12. DOI:10.37884/1-2025/01. [*in Russian*].

36 Byuro nacional'noi statistiki. (2026). Byuro nacional'noi statistiki Respubliki Kazahstan [Bureau of National Statistics of the Republic of Kazakhstan]. https://stat.gov.kz/ [*in Russian*].

37 Amin, A., Rashit, U., Kaster, N., Zhassulan, O., Korgan, O., Kairat, I. (2025). Breeding Progress in Mugalzhar Horses: The Irtysh Type as a Model for Meat Production Efficiency. *American Journal of Animal and Veterinary Sciences*, 20(3), 328-339. DOI: 10.3844/ajavsp.2025.328.339.

38 Zhassulan, O., Amin, A., Shokhan, A., Zhumagaliyeva, G., Murat, K., Kairat, I. (2025). Assessment of Meat Productivity of Kushum Horses in the Almaty Region. *American Journal of Animal and Veterinary Sciences*, 20(3), 356–364. DOI: 10.3844/ajavsp.2025.356.364.

39 Kargayeva, M.T., Baimukanov, D.A., Karynbayev, A.K., Alikhanov, O., Zhunusov, A.M. (2020). Productive-biological features of aday breed kazakh horses. *Eurasian Journal of Biosciences,* 14(1), 329–335.

40 Iskhan, K., Akimbekov, A., Mukinov, Kh., Mukinov, E. (2024). Creation of beskaragai factory type and shoynkara and baytor lines of Kazakh horse breed. *Journal of Osh State University. Agriculture: Agronomy, Veterinary and Zootechnics,* 3(2), 272–281. DOI: 10.52754/16948696_2024_2(7)31.

41 Igenbayev, A., Amirkhanov, S., Ospankulova, G., Kardenov, S., Baytukenova, S., Shariati, M.A. (2022). Determination of the fatty acid composition and fatty acids trans-isomers in the horse, stall horse, mutton, beef and pork meat. *Slovak Journal of Food Sciences*, 16. DOI: 10.5219/1799.

42 Frontiers in Animal Science. (2025). *Muscle fiber characteristics and nutritional composition in anatomical regions of the Kazakh horse. Frontiers in Animal Science.*

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Research article

Optimization of culture conditions for vaccinia virus propagation

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Abstract

Background and Aim. Monkeypox virus has recently re-emerged as one of the most significant zoonotic pathogens and a major global public health concern. Due to its genetic and antigenic similarity to other members of the *Orthopoxvirus* genus, the use of model viruses is essential for the development of vaccines and diagnostic approaches. In this context, Vaccinia virus is widely regarded as a key model system for *Orthopoxvirus* research and is extensively used in the development of vaccines and diagnostic tools. The aim of this study was to determine the optimal conditions for the cultivation of vaccinia virus.

Materials and Methods. Vaccinia virus was cultivated in Vero, HeLa, and LK cell lines. Viral replication activity was evaluated based on cytopathic effect observed in cell cultures and the level of viral biological activity. In addition, the effects of fetal bovine serum concentration, multiplicity of infection, and incubation temperature on viral replication were investigated. For each parameter, the efficiency of viral propagation was comparatively analyzed to determine optimal cultivation conditions.

Results. The results demonstrated that the highest level of viral replication was observed in HeLa cells. The optimal infection dose for efficient viral propagation was determined to be 0.00369 $TCID_{50}$/cell. Furthermore, 2% fetal bovine serum was sufficient to ensure stable and efficient viral growth. The most favorable incubation temperature was 37 °C, which supported the maintenance of viral biological activity.

Conclusion. The optimized cultivation conditions identified in this study enable efficient biomass production of vaccinia virus. These findings provide an important scientific basis for further fundamental and applied research on Orthopoxviruses, including the development of vaccines and diagnostic systems.

Keywords: cultivation parameters; biological activity; *Orthopoxvirus*; vaccinia virus; temperature.

Introduction

Monkeypox is a zoonotic infectious disease caused by the monkeypox virus, a member of the genus *Orthopoxvirus.* The virus demonstrates a high degree of genetic homology with other clinically significant Orthopoxviruses, including variola virus, vaccinia virus, and cowpox virus [1]. Members of the *Orthopoxvirus* genus are characterized by their high pathogenic potential for both humans and animals, represent a continuing concern for public health and veterinary medicine.

Historically, variola virus, the causative agent of smallpox, was responsible for widespread epidemics over several centuries, leading to significant morbidity and mortality worldwide. Following the global vaccination campaign, smallpox was officially declared eradicated in 1980 [2]. Nevertheless, the continued existence of related Orthopoxviruses in natural animal reservoirs sustains the risk of zoonotic transmission, and sporadic epizootic and zoonotic cases are still reported in endemic regions [3].

Initially, the zoonotic nature of monkeypox was identified in the Democratic Republic of the Congo, whereas evidence of human-to-human transmission was subsequently reported in Abia State in southeastern Nigeria [4, 5]. By the end of the 20th century, the epidemiological profile of monkeypox had expanded, and the first documented fatal cases were recorded [6]. In the early 21st century, the geographic spread of the virus extended to a transcontinental level, with confirmed cases reported in the United States and the United Kingdom [7, 8]. In 2022, outbreaks of the disease were reported across multiple countries within the European Union [9, 10], prompting the World Health Organization (WHO) to declare monkeypox a Public Health Emergency of International Concern. This decision was prompted by the spread of the infection beyond previously endemic regions and the need for a coordinated global response [11].

Vaccinia virus historically played a crucial role in the eradication of variola virus, which induces a robust immune response and enabled the interruption of smallpox transmission [12]. Based on this virus, first-generation vaccines such as *Dryvax* and *Lister* were developed in the mid-20th century, making a decisive contribution to global vaccination campaigns and the eventual eradication of variola virus [13]. A later improved vaccine, ACAM2000, demonstrates high immunogenicity; however, its use is limited by safety concerns, as it is a replication-competent virus [14]. Next-generation vaccines, including JYNNEOS and MVA-BN, based on the modified vaccinia Ankara strain, are characterized by strong immunogenicity and improved safety profiles, allowing their use even in immunocompromised individuals. Notably, the JYNNEOS vaccine has demonstrated high efficacy in the prevention of monkeypox [15].

In addition, vaccinia virus antigens are widely used in the diagnosis of monkeypox. Several studies have demonstrated that vaccinia virus antigens exhibit high sensitivity in detecting the humoral immune response induced by monkeypox virus infection [16–18]. Moreover, neutralizing antibodies generated against vaccinia virus have been shown to effectively neutralize monkeypox virus in neutralization assays [19].

Cell culture systems and cultivation conditions for vaccinia virus propagation have been previously described in the scientific literature [20–22]. Therefore, the significance of the present study lies in the investigation of the cultural properties of the vaccinia virus strain in order to obtain a highly biologically active viral suspension for further application in the development of diagnostic methods.

Thus, the presented data indicate that vaccinia virus represents an important biological agent in the context of biosafety related to *Orthopoxvirus* infections. Accordingly, the maintenance, cultivation, and investigation of this virus are of significant importance for scientific research.

The aim of this study was to select an optimal system and parameters for the cultivation of vaccinia virus.

Materials and Methods

Refreshing of the museum strain

In the first stage of the study, vaccinia virus was passaged in lamb kidney (LK) cell culture. Cells were grown in 25 cm^2 culture flasks using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, antibiotics (penicillin, 100 IU/mL; streptomycin, 100 µg/mL), and 1% L-glutamine. Prior to infection, the cell monolayer was washed with phosphate-buffered saline (PBS), after which a virus-containing suspension was added, and adsorption was carried out at 37 °C for 1 h. Subsequently, maintenance medium (DMEM containing 2% serum) was added, and the cultures were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

A total of three consecutive passages of the virus were performed as cytopathic effects (CPE) developed, with daily microscopic monitoring of their progression.

Selection of cell cultures and optimization of vaccinia virus cultivation conditions

In order to select the most suitable cell line providing efficient viral replication, both continuous and primary trypsinized cell cultures maintained at the Laboratory of Cell Biotechnology (RIBSP) were

used. The study employed African green monkey kidney (Vero), human cervical carcinoma (HeLa), and lamb kidney (LK) cell lines.

The susceptibility of cell cultures to the virus was assessed by infecting monolayers of Vero, HeLa, and LK cells, followed by incubation at 37 °C in a 5% CO_2 atmosphere for 5 days, with daily microscopic observation of the development of CPE. To optimize infection conditions, cells were infected at doses ranging from 0.000369 to 3.69 $TCID_{50}$/cell, and the effects of temperature (33 ± 0.5 °C, 36.5 ± 0.5 °C, and 39 ± 0.5 °C) and serum concentration (2.5%, 10%) on the development of viral infection.

Determination of the biological activity of the viral strain under study

The infectious activity of the virus was determined by cell culture titration. The infectious titer was calculated using the Reed and Muench method and expressed in lg $TCID_{50}/cm^3$.

Statistical analysis of data

Statistical analysis of the data was performed using GraphPad Prism software. Differences between groups were evaluated and considered statistically significant at $p < 0.05$.

Results

Vaccinia virus was passaged sequentially in LK cell culture to assess the dynamics of CPE development and viral adaptation to the cellular system. During cultivation, daily microscopic observations were performed to monitor the condition of the cell monolayer and the degree of morphological changes.

During the first passage, CPE was weakly expressed and minimal within the initial 1–2 days. By day 4, CPE development reached approximately 30–40%, indicating the onset of active virus –cell interaction. By day 6, the level of cell monolayer destruction increased to 60–70%, reflecting pronounced viral replication in the system. The dynamics of viral growth during the first passage are presented in Figure 1, illustrating the temporal development of the cytopathic process.

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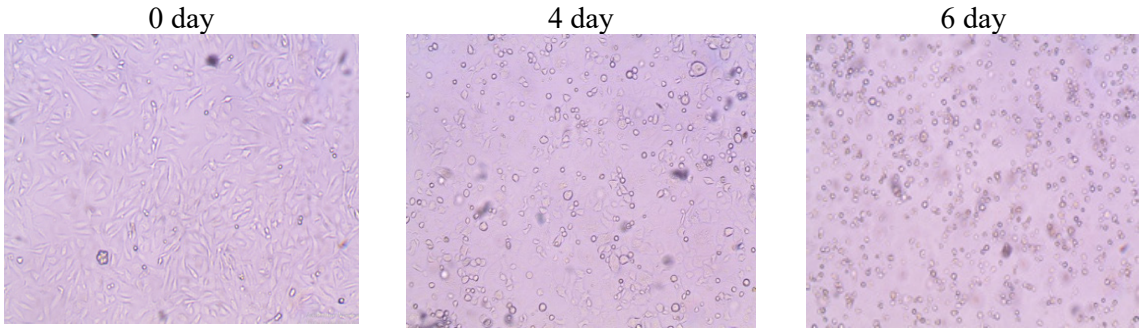

Figure 1. Cytopathic effect of vaccinia virus in LK cell culture during the first passage (×2000)

During the study, the smallpox vaccine virus was cultured in monolayers of Vero, HeLa, LK cell lines, with successive passages and assessment of the dynamics of CPE.

In the first passage, the development of CPE in the studied cell cultures was gradual. In the Vero culture, the first signs of cytopathic changes were observed on day 3, whereas in HeLa cells, cytopathic effects were detected as early as day 2, and in the LK culture – on day 3. By day 6 of cultivation, the degree of monolayer damage reached 70–80% in all cell types, indicating active viral replication.

In the second passage, an acceleration of CPE development was observed in all investigated cell lines; however, the dynamics of its manifestation varied depending on the cell type. In Vero cells, CPE was detected on day 2, with pronounced monolayer destruction (80–90%) observed by day 4 of cultivation. In HeLa cells, initial signs of CPE also appeared on day 2, while the maximum level of cell damage (80–90%) was recorded by day 3. In LK cells, CPE development occurred slightly more slowly: initial changes were observed on day 2, whereas pronounced CPE reaching 80–90% was achieved by day 5 of cultivation.

In the third passage, a similar trend of accelerated CPE development was observed in all investigated cell lines. The cytopathic effect during the third passage is shown in Figure 2.

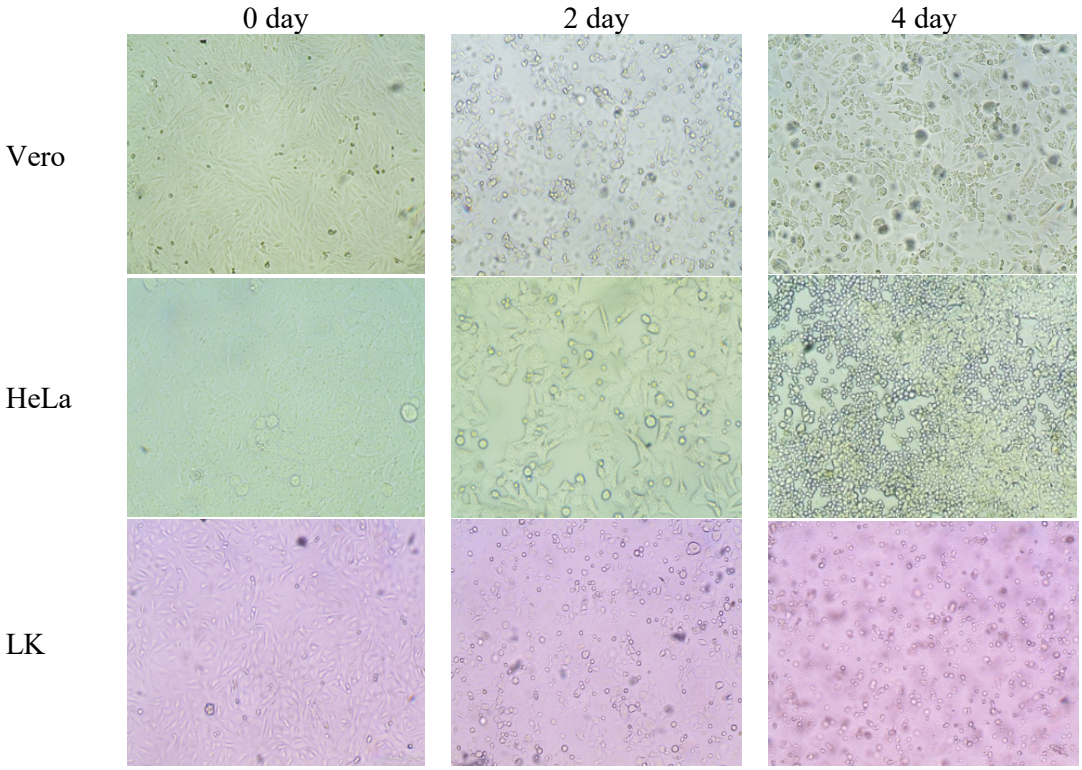

Figure 2. Morphological changes in cell cultures infected with vaccinia virus during the third passage

The obtained results indicate that the most pronounced replication of vaccinia virus was observed in HeLa cell culture, where cytopathic effect developed more rapidly compared to the other investigated cell lines. The infectivity data confirmed these observations: the highest viral titer was recorded in HeLa cells, reaching 8.50 ± 0.083 lg $TCID_{50}/cm^3$, whereas lower values were obtained in Vero and lamb kidney (LK) cells, amounting to 5.75 ± 0.08 and 6.33 ± 0.08 lg $TCID_{50}/cm^3$, respectively. The dynamics of vaccinia virus biological activity are presented in Figure 3.

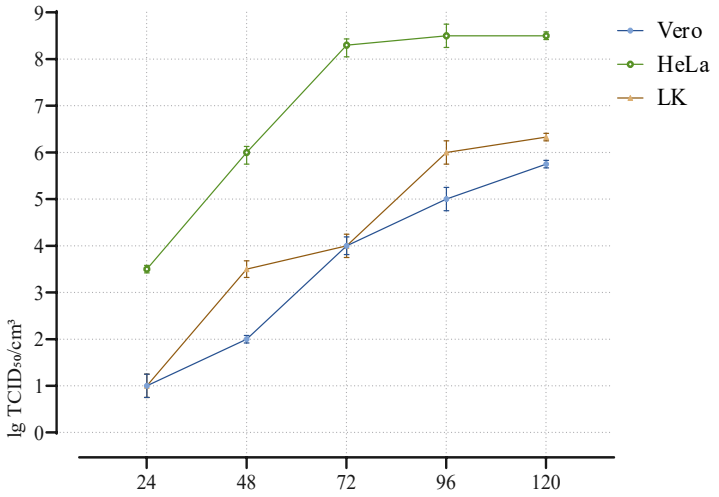

Figure 3. Dynamics of the biological activity of vaccinia virus

The infectious dose of vaccinia virus in HeLa cell culture was determined. It was established that viral biological activity depends on the magnitude of the infectious dose. At high virus concentrations (3.69; 0.369; 0.0369; and 0.00369 $TCID_{50}$/cell), a stable high level of biological activity was observed, with an infectious titer of 8.16 ± 0.083 lg $TCID_{50}/cm^3$. When the dose was reduced to 0.000369 $TCID_{50}$/cell, the infectious activity decreased to 6.6 ± 0.016 lg $TCID_{50}/cm^3$, accompanied by a reduction in the severity of CPE (Figure 4).

Based on the obtained results, an infectious dose of 0.00369 $TCID_{50}$/cell was selected as the optimal condition, under which stable levels of biological activity and pronounced CPE of vaccinia virus were maintained.

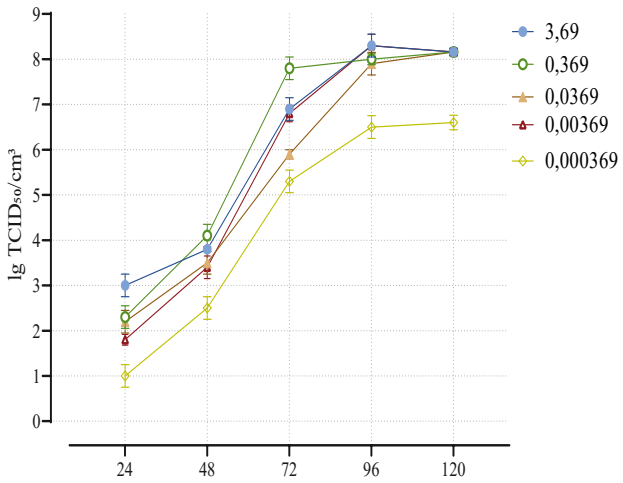

Figure 4. Dynamics of vaccinia virus biological activity at different infectious doses

During the selection of fetal bovine serum concentration in the culture medium, it was found that the biological activity of vaccinia virus showed only minor variation depending on serum content. At 2% and 5% FBS, the biological activity was 7.5 ± 0.083 lg $TCID_{50}/cm^3$, whereas at 10% FBS it reached 8.00 lg $TCID_{50}/cm^3$. No significant differences were observed between the tested conditions (Figure 5).

Thus, a 2% FBS concentration was determined to be optimal, as it ensures a sufficient level of viral biological activity while allowing more economical use of culture medium components.

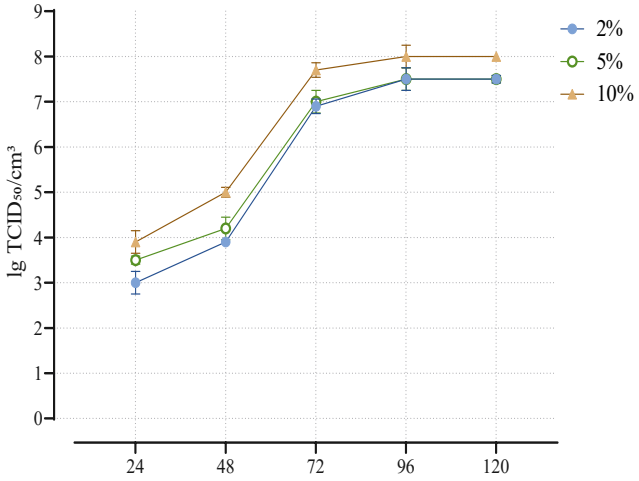

Figure 5. Biological activity of vaccinia virus at different fetal bovine serum concentrations

In the experiment aimed at determining the optimal cultivation temperature for the Orthopoxvirus strain, three temperature regimes (33 °C, 37 °C, and 39 °C) were evaluated. Cell monolayers were

infected with vaccinia virus at an infectious dose of 0.00369 $TCID_{50}$/cell, followed by incubation under the specified temperature conditions.

It was established that viral biological activity depended on the incubation temperature. At 33 °C, the infectious titer was 3.75 ± 0.25 lg $TCID_{50}/cm^3$, whereas at 39 °C it reached 6.5 ± 0.00 lg $TCID_{50}/cm^3$. The highest viral activity was observed at 37 °C, where the titer reached 7.50 lg $TCID_{50}/cm^3$. These results indicate that 37 °C is the optimal temperature for virus cultivation. The results are presented in Figure 6.

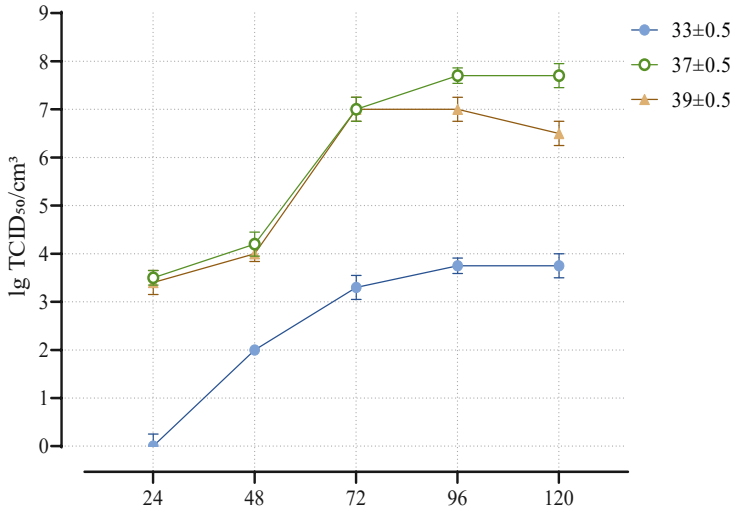

Figure 6. Biological activity of vaccinia virus at different cultivation temperatures

Discussion

Monkeypox, caused by viruses of the genus *Orthopoxvirus*, has recently regained significant scientific attention due to changes in its epidemiological pattern. In this context, vaccinia virus represents an important model for studying the biology of *Orthopoxviruses,* including viral replication mechanisms and theoptimization of cultivation conditions.

The results of the present study demonstrated that vaccinia virus efficiently replicates in various cell cultures; however, the level of replication is strongly dependent on the cell line used. The highest biological activity and the most rapid development of CPE were observed in HeLa cells, indicating their high susceptibility to the virus. These findings are consistent with classical studies showing that continuous cell lines provide more efficient viral replication compared to primary cultures [20].

In Vero and LK cells, virus replication was less pronounced, and CPE developed more slowly. Nevertheless, the ability of vaccinia virus to efficiently replicate in LK cells was previously demonstrated. For instance, *Subramanyam* et al. [21] showed that LK cells can support active viral replication and may be used for virus cultivation, which supports the results obtained in the present study.

In this study, high viral titers were observed at infection doses ranging from 3.69 to 0.00369 $TCID_{50}$/cell. At the lowest dose (0.000369 $TCID_{50}$/cell), a significant reduction in viral titer was detected. Thus, the effective infectious dose was determined to be 0.00369 $TCID_{50}$/cell. Although a high viral titer was also observed at a dose of 3.69 $TCID_{50}$/cell, this condition was considered unsuitable, as high infection doses induce rapid cytotoxic effects in cells, leading to alterations in viral phenotypic properties and the formation of free virions in subsequent passages.

The accumulation of virus in cell cultures depends not only on the cell type, temperature, and infectious dose, but also on the composition of the culture medium, including serum concentration. In serum-free conditions, cells experience a deficiency of essential nutrients, leading to cellular damage and accelerated senescence, which in turn negatively affects viral replication. Conversely, excessively high serum concentrations may promote the presence of non-specific inhibitors that suppress viral replication. Therefore, in the present study, a 2% serum concentration was used, which enabled the achievement of a high viral titer.

Viral replication in cell cultures is also temperature-dependent, and this factor is regulated by the viral genome. For most viruses, the optimal growth temperature is 36–37 °C. In the present study, 33 °C was shown to be unfavorable for the replication of the vaccinia virus strain, whereas at 39 °C an increase in viral titer was observed during the first 72 hours, followed by suppression of viral replication between 96 and 120 hours. Our findings demonstrate that a temperature of 37 °C provides optimal conditions for achieving a high viral titer of the vaccinia virus strain.

Conclusion

The present study demonstrated that vaccinia virus efficiently replicates in various cell cultures, with the highest biological activity observed in HeLa cells. The optimal infectious dose was determined to be 0.00369 $TCID_{50}$/cell, ensuring stable viral replication. It was shown that a 2% fetal bovine serum concentration is sufficient to maintain high viral biological activity, while the optimal cultivation temperature is 37 °C.

The established cultivation parameters provide favorable conditions for vaccinia virus propagation and may be of interest for further fundamental and applied research.

Authors' contributions

OS, NO, MS, BU, MK: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript; ZhK: wrote the original draft; LB, KZh: writing-review & editing.

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Statement on Competing Interests

The authors declare that they have no conflicts of interest.

References

1 Mitjà, O., Ogoina, D., Titanji, B.K., Galvan, C., Muyembe, J.J., Marks, M., Orkin, C.M. (2023). Monkeypox. *The Lancet*, 401(10370), 60–74. DOI:10.1016/S0140-6736(22)02075-X.

2 World Health Organization. (2023). *Smallpox overview*. https://www.who.int/emergencies/situations/smallpox

3 Bunge, E.M., Hoet, B., Chen, L., Lienert, F., Weidenthaler, H., Baer, L.R., et al. (2022). The changing epidemiology of human monkeypox – A potential threat? A systematic review. *PLOS Neglected Tropical Diseases*, 16(2), e0010141. DOI:10.1371/journal.pntd.0010141.

4 Ladnyj, I.D., Ziegler, P., Kima, E. (1972). A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. *Bulletin of the World Health Organization,* 46(5), 593–597.

5 Arita, I., Henderson, D.A. (1976). Monkeypox and whitepox viruses in West and Central Africa. *Bulletin of the World Health Organization,* 53(4), 347–353.

6 Likos, A.M., Sammons, S.A., Olson, V.A., Frace, A.M., Li, Y., Olsen-Rasmussen, M., et al. (2005). A tale of two clades: Monkeypox viruses. *Journal of General Virology*, 86(10), 2661–2672.

7 Centers for Disease Control and Prevention. (2003). U*pdate: Multistate outbreak of monkeypox – Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin. Morbidity and Mortality Weekly Report (MMWR).* https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5226a5.htm

8 Vaughan, A., Aarons, E., Astbury, J., Balasegaram, S., Beadsworth, M., Beck, C.R., et al. (2018). Two cases of monkeypox imported to the United Kingdom. *Eurosurveillance,* 23(38), 1800509. DOI:10.2807/1560-7917.ES.2018.23.38.1800509.

9 European Centre for Disease Prevention and Control. (2023). *Weekly communicable disease threats report.* https://www.ecdc.europa.eu/sites/default/files/documents/communicable-disease-threats-report-week-32-2023_0.pdf

10 World Health Organization. (2022). *Multi-country monkeypox outbreak: Situation update (2022 DON396).* https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON396

11 World Health Organization. (2022). *Second meeting of the International Health Regulations Emergency Committee regarding the multi-country outbreak of monkeypox*. https://www.who.int/news/item/23-07-2022-second-meeting-of-the-international-health-regulations-%282005%29-%28ihr%29-emergency-committee-regarding-the-multi-country-outbreak-of-monkeypox

12 Kaynarcalidan, O., Moreno Mascaraque, S., Drexler, I. (2021). Vaccinia virus: From crude smallpox vaccines to elaborate viral vector vaccine design. *Biomedicines,* 9(12), 1780. DOI:10.3390/biomedicines9121780.

13 Sánchez-Sampedro, L., Perdiguero, B., Mejías-Pérez, E., García-Arriaza, J., Di Pilato, M., Esteban, M. (2015). The evolution of poxvirus vaccines. *Viruses,* 7(4), 1726–1803. DOI:10.3390/v7041726.

14 Wahid, M., Mandal, R.K., Sikander, M., Khan, M.R., Haque, S., Nagda, N., Ahmad, F., Rodriguez-Morales, A.J. (2025). Safety and efficacy of repurposed smallpox vaccines against mpox: A critical review of ACAM2000, JYNNEOS, and LC16. *Journal of Epidemiology and Global Health,* 15(1), 88. DOI:10.1007/s44197-025-00432-8.

15 Dalton, A.F. (2023). Estimated effectiveness of JYNNEOS vaccine in preventing mpox: A multijurisdictional case-control study – United States. *Morbidity and Mortality Weekly Report (MMWR)*, 72.

16 Hicks, B., Jones, S., Callaby, H., Bailey, D., Gordon, C., Rampling, T., et al. (2024). Evaluation of a multiplexed immunoassay for assessing long-term humoral immunity to orthopoxviruses. *Vaccine,* 42(26), 126453. DOI:10.1016/j.vaccine.2024.126453.

17 Jones, S., Hicks, B., Callaby, H., Bailey, D., Gordon, N.C., Rampling, T., et al. (2025). Assessment of MpoxPlex, a high-throughput and multiplexed immunoassay: A diagnostic accuracy study. *The Lancet Microbe,* 6(4), 100987. DOI:10.1016/j.lanmic.2024.100987.

18 Jones, S., Hicks, B., Callaby, H., Bailey, D., Gordon, C., Rampling, T., et al. (2024). MpoxPlex: A high-throughput and versatile multiplexed immunoassay for assessing and discriminating between IgG responses to mpox infection and vaccination. *medRxiv.* DOI:10.1101/2024.06.03.24308186.

19 Pettke, A., Keszei, M., Christ, W., Mayola Danés, N., Gredmark-Russ, S., Söderholm, S., et al. (2025). Serological differentiation between naturally acquired mpox and MVA-BN-vaccine induced antibody responses using ratios of MPXV and VACV antigen pairs in the MSD immunoassay. *Microbiology Spectrum*, 13, e00182-25. DOI:10.1128/spectrum.00182-25.

20 Nishmi, M., Niecikowski, H. (1963). Interaction of vaccinia virus and cells in primary and continuous culture. *Nature,* 199, 1117–1118. DOI:10.1038/1991117a0.

21 Subramanyam, P., Divakaran, S., Vinodraj, P. (1961). Cultivation of vaccinia virus in sheep kidney cell cultures. *Bulletin of the World Health Organization,* 25(1), 33–40.

22 Kroon, E.G., Abrahão, J.S., Trindade, G.S., Oliveira, G.P., Luiz, M.F., Costa, G.B., Lima, M.T., et al. (2018). Natural vaccinia virus infection: Diagnosis, isolation, and characterization. *Current Protocols in Microbiology*, 42(1), 14A.5.1–14A.5.43. DOI:10.1002/cpmc.13.

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Research article

Lameness in broiler chickens in Kazakhstan: prevalence and key risk factors from commercial farms

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Abstract

Background and Aim. Lameness in broiler chickens is a major welfare and economic concern affecting productivity and farm profitability worldwide. However, information regarding the prevalence and associated risk factors of broiler lameness in Kazakhstan remains limited. This study aimed to evaluate the prevalence of lameness in commercial broiler farms and identify the main contributing factors under local production conditions.

Materials and Methods. A cross-sectional survey was conducted from July to October 2025 on 10 commercial poultry farms located in different regions of Kazakhstan. Data were collected using a structured questionnaire covering housing conditions, litter management, stocking density, feeding practices, environmental control, health management, and the occurrence of lameness. Descriptive statistical analysis was performed to evaluate the collected data.

Results. Lameness was reported on mostfarms, although prevalence varied substantially among production systems. The proportion of lame birds at slaughter ranged from 0 to over 10%, with an average prevalence of 2.1 ± 3.2%. Severe lameness cases (Score ≥ 3) averaged 4.7 ± 6.9%. Poor litter quality, high stocking density, inadequate temperature and humidity control, and differences in feeding strategies were identified as major risk factors. Among infectious agents, *Staphylococcus* spp. was the most frequently reported pathogen. Although preventive measures were implemented on most farms, their implementationwas often inconsistent.

Conclusion. The findings demonstrate that broiler lameness in Kazakhstan is a multifactorial problem influenced by management, environmental, and infectious factors. This study provides one of the first comprehensive assessments of lameness in commercial broiler production in Kazakhstan and highlights the need for improved management practices, standardized monitoring systems, and more effective prevention strategies.

Keywords: broiler chickens; Kazakhstan; lameness; prevalence; poultry farms; risk factors.

Introduction

Poultry farming plays a key role in ensuring food security, economic development, and social stability both in Kazakhstan and worldwide. In Kazakhstan, the industry is characterized by dynamic growth, the adoption of innovative technologies, and government support, helping toreduce dependence on imports and create new jobs [1, 2]. These trends reflect the government's strategic focus on developing the agro-industrial complex. Globally, poultry farming provides an affordable source of protein; its products (meat and eggs) constitute an important component of the human diet, and increased domestic production reduces dependence on imports [2, 3].

Poultry meat production in Kazakhstan has shown steady growth over the past decade. According to data from the National Statistics Bureau of the Republic of Kazakhstan, production increased from approximately 220–230 thousand tons in 2020 to approximately 360 thousand tons in 2024, indicating a significant increase in domestic production. This trend reflects improved industry efficiency and expanded production capacity. This growth trend continued in 2025, increasing by approximately 2.6–2.7%, and data from early 2026 indicate that the industry continues to expand. The poultry population also grew, reaching 45.7 mln birds in 2025. These trends underscore the growing importance of broiler production in ensuring Kazakhstan's food security and economic stability [4].

Lameness in broiler chickens is one of the key challenges in modern poultry farming. It is directly linked to pain, deterioration in the birds' condition, and significant economic losses in industrial production. The importance of addressing this problem is increasing as production expands. The problem is exacerbated by the extremely rapid growth of modern broilers and intensive rearing technologies. Gait abnormalities are recognized as one of the most sensitive indicators of reduced bird welfare, as they limit mobility, consequently, access to feed and water, and are accompanied by pain. Lameness is often associated with metatarsal dermatitis and hock burns, reflecting poor litter quality and housing conditions [5, 6].

Lameness in poultry farming leads to significant economic losses due to reduced meat quality and increased costs for treatment and preventive measures [7]. In addition, lameness negatively affects both the welfare of the birds and their productivity. Collectively, these effects reduce production efficiency and increase production costs. For the industry, these consequences translate into substantial economic losses associated with mortality, culling, and reduced production performance.

The multifactorial nature of lameness in broiler chickens poses a serious problem both from an animal welfare perspective (pain, restricted movement) and from an economic standpoint (mortality, culling, reduced weight gain). Lameness, typically, does not have a single cause but is caused by a combination of factors. Growth, nutrition, infection, and management factors all play a role simultaneously. Bacterial chondronecrosis with osteomyelitis (BCO) is considered the leading cause of lameness: bacteria (primarily *Staphylococcus* spp.) colonize microcracks in bone tissue after penetrating from the gut or respiratory tract. Viral, bacterial, and mycotoxicosis-related diseases, as well as staphylococci, cause arthritis, osteomyelitis, deformities, and paralysis of the limbs [5, 8, 9]. Bone development is closely linked to the availability of IP6 (phytate), calcium, and phosphorus; mineral nutrition deficiencies impair bone quality. Deficiencies and imbalances in Ca, P, and vitamin D3, as well as other nutritional disorders, impair bone mineralization and increase the risk of lameness. Feed contaminated with mycotoxins (deoxynivalenol, fumonisin, aflatoxins) markedly increases the incidence of lameness and bone damage, and disrupts phosphorus metabolism and mineralization [10–12]. Lameness in broilers has a distinctly multifactorial nature: rapid growth, mineral imbalances and mycotoxins, mechanical stress, bacterial infection (BCO), and housing conditions act in concert. Therefore, the development of lameness in broilers is due to the combined effects of metabolic, infectious, and technological factors.

Surveys of farmers and experts complement the clinical assessment of lameness and provide data on the scale of the problem, risk factors, management characteristics, and economic aspects at the farm and industry levels. In poultry farming, this approach is used less frequently than direct clinical examinations, but where it is applied, it is evident that the farmers' attitudes and management practices often determine the severity of the problem and the success of prevention. Structured questionnaires are particularly valuable, as they allow the quantitative assessment of the influence of environmental and management factors. Questionnaire sections completed by farmers (feed type, duration of the dark period, stocking

density, slaughter age, mortality rate) enable statistical associations between lameness and management- and resource-related factors to be identified. In multifactorial studies of broilers, questionnaire data on housing conditions and management (lighting, litter quality, age) are combined with assessments of lameness, dermatitis, and fear, allowing for the identification of key risk factors (dark period duration, age, litter) and demonstrating that modifying just a few parameters can simultaneously improve several welfare indicators [13].

Despite the existence of numerous studies on lameness in broilers that detail the causes, prevalence, and preventive measures, most of them were conducted abroad (Europe, Australia, China, the USA, Pakistan, etc.). This limits the ability to generalize the results to conditions in Kazakhstan. Currently, no published scientific data reflecting the situation in Kazakhstan are available. Thus, the problem of lameness in broiler chickens in the context of domestic poultry farming remains insufficiently studied. The aim of this study was to assess the prevalence of lameness in broiler chickens and to identify key risk factors at the level of poultry farms in Kazakhstan using a structured questionnaire.

Materials and Methods

A cross-sectional study was conducted from September to November 2025. The questionnaire was developed using web-based software (Google Forms) and distributed to key stakeholders in Kazakhstan's broiler industry working in vertically integrated broiler production companies. Respondents included managers and executives of broiler farms, veterinary specialists at poultry farms, as well as executives of processing plants and employees of food quality control and safety departments.

The questionnaire was developed by the authors of this study. A preliminary version was tested by a group of researchers to assess the clarity and comprehensibility of the questions. In addition, the questionnaire was reviewed and approved by a research group in this field. Based on the comments and recommendations received, the questionnaire was revised and adjusted before being used in the main study. Participating farms were selected using purposive sampling to ensure representation of commercial broiler production systems from different regions of Kazakhstan. Farms were identified through professional industry networks, poultry production companies, and veterinary contacts. Invitations to participate were sent by email to managers, veterinarians, and technical specialists working in broiler production. Participation was voluntary, and only farms that completed the questionnaire were included in the final analysis. The surveyed farms represented both vertically integrated and independent broiler production systems.

A link to the online survey was distributed via email. Participation in the study was voluntary, and participants received no compensation for completing the questionnaire. The full version of the questionnaire in print format is available in the "Additional Materials" section.

All surveys were conducted anonymously, and no personal or corporate information was collected or processed. The questionnaire was structured and included six thematic sections. The first section collected basic descriptive data on poultry farms, including geographic location, type of production system (integrated or contract-based), farm capacity, number of production cycles per year, and annual production volume. Additional information included the presence of veterinary supervision on the farm, farm management experience, broiler breed/cross, and slaughter age. This section was used to characterize the epidemiological situation on the farms.

The second section assessed housing systems and microclimatic conditions affecting broiler welfare. Variables included temperature, humidity, ammonia ($NH_{3)}$ and carbon dioxide (CO_2) levels, ventilation type, lighting schedule, and stocking density. Particular attention was givento the type of litter, its quality, replacement frequency, and moisture content, as these factors are directly linked to foot health issues. General hygiene procedures and disinfection protocols between production cycles were also evaluated.

The third section focused on feed sources, feeding systems, and nutrient quality control. Information was collected on feed type (pelleted, mash, or mixed), feed origin (commercial or self-produced), and quality control methods (protein, energy, and mineral content, as well as mycotoxin testing). The use of feed additives intended to improveskeletal health was also recorded, including calcium, phosphorus, vitamin D_3, trace minerals, probiotics, enzymes, and toxin binders. Water sources, water treatment methods, and drinking system hygiene were included as additional indicators of proper nutrition.

The fourth section assessed the epidemiology and clinical manifestations of lameness in broiler chickens. Data were collected on the age at onset, severity, and frequency of lameness cases, as well as the proportion of affected birds at slaughter. Lameness severity was assessed using the Bristol Gait Scoring System, a widely accepted method for evaluating walking ability in broiler chickens. The system uses a 0–5 scale, where score 0 represents normal locomotion and score 5 indicates severe locomotor impairment with an inability to walk normally. Higher scores reflect increasing degrees of gait abnormality and reduced welfare. Where available, farms reported lameness prevalence using this scoring system. General clinical signs, such as gait abnormalities, joint swelling, pododermatitis, deformities, and reduced mobility, were also documented.

The fifth section assessed the involvement of infectious agents associated with lameness. Participants provided information on bacterial, viral, mycoplasmal, and fungal pathogens commonlydetected in their flocks, including *Staphylococcus* spp., Escherichia coli, reoviruses, infectious bursal disease virus, Mycoplasma synoviae, and Aspergillus spp. The frequency of laboratory diagnostics and the use of surveillance programs on breeding farms and in broiler flocks were also recorded.

The final section assessed disease prevention strategies and control measures implemented on farms. Data were collected on vaccination programs, the use of antibiotics (prophylactic and therapeutic), and alternative control measures, such as probiotics, organic acids, and herbal supplements. Management practices related to stocking density regulation, biosecurity measures, and welfare monitoring systems were also included. In addition, participants identified the main suspected causes of lamen ess and reported on economic losses and production problems associated with its occurrence.

Statistical Analysis

The data were analyzed using descriptive and non-parametric statistical methods. Continuous variables are presented as mean ± standard deviation (SD), along with median and range where appropriate, while categorical variables are summarized as frequencies and percentages.

Because the sample size was relatively small (n = 10 farms) and several variables were ordinal, non-parametric approaches were used throughout the analysis. Associations between lameness prevalence and selected management, environmental, and diagnostic factors were evaluated using Spearman's rank correlation.

To further explore differences between farms, they were grouped according to lameness levels, and comparisons between groups were performed using the Mann–Whitney U test where appropriate. Associations between categorical variables were assessed using Fisher's exact test.

Statistical significance was defined as $p < 0.05$. Given the exploratory nature of the study, the results were interpreted with appropriate caution.

Results

The study involved 10 commercial poultry farms from various regions of the Republic of Kazakhstan (Abai, Akmola, and Almaty regions, among others). Respondents included farm managers, veterinary specialists, and production staff representing both vertically integrated and independent broiler production systems.

Most farms operated under intensive production conditions. Most farms completed 6–7 production cycles per year. The majority of enterprises were characterized by large production capacities, with annual production volumes ranging from 1 to over 10 mln birds. 90% of the farms had a full-time veterinarian on staff, and all respondents had more than 5 years of experience in poultry farming.

The summarized production characteristics of the surveyed farms are presented in Table1.

Table 1. Production characteristics of poultry farms (n = 10)

Parameter	Value
Presence of a veterinarian (yes/no)	9 / 1
Type of production (% vertically integrated)	40%
Ross 308 strain (% of farms)	80%
Average slaughter age (days)	41.2 ± 4.1
Production cycles per year (predominant)	6–7

Most farms used enclosed poultry houses with controlled housing conditions and mechanical ventilation (including tunnel and cross-ventilation). However, variability in the effectiveness of microclimate control was noted, particularly regarding the maintenance of optimal humidity and air quality. This fact indicates differences in the level of technological implementation even in the presence of similar infrastructure.

Bedding management practices also varied significantly. Wood shavings and straw were the most commonly used materials. A number of respondents pointed to problems with high bedding moisture and condensation, which is considered a risk factor for the development of musculoskeletal disorders. Cleaning and disinfection of the premises were carried out between production cycles, but their frequency and effectiveness varied. This may contribute to the accumulation of pathogenic microflora and a deterioration in the sanitary condition of the poultry houses.

All farms used compound feed (either produced on-site or purchased). Regular feed quality control was carried out at 90% of the facilities. Analysis of feed additive useshowed that the most common were sources of calcium and phosphorus, mycotoxin adsorbents, and trace elements. The frequency of use of various types of additives is presented in Table 2. At the same time, inconsistencies were identified in approaches to the use of modern feeding strategies.

Table 2. Frequency of use of feed additives (n = 10)

Type of feed additive	Number of farms	% Of total
Bioavailable sources of Ca and P (dicalcium phosphate, etc.)	9	90%
Trace elements (Zn, Mn, Cu, Se) in organic form	4	40%
Mycotoxin binders	7	70%
Vitamin D3 (high doses)	1	10%
Enzymes (phytase, xylanase)	4	40%
Probiotics / prebiotics	3	30%

Most farms used nipple drinkers, with water supplied by centralized systems or wells. Bacteriological testing of water was conducted on 70% of farms, while pH and hardness testing was performed on 60%.

Regular gait assessment of broilers was performed on 70% of farms, while 30% did not conduct such assessments. Visual inspection of birds was performed on all farms; however, standardized scales (e.g., the Bristol Gait Score) were used in only 37.5% of cases. The frequency of monitoring varied: weekly – 50%, before slaughter – 25%, twice per cycle – 25%.

The average percentage of birds with marked lameness (Score ≥ 3) was 4.7 ± 6.9%, whereas the subjective assessment of lameness levels at the time of slaughter ranged from 0 to over 10% (average 2.1 ± 3.2%). Lameness most commonly developed between 29 and 42 days of rearing. In 20% of farms, no cases of lameness were recorded. Such variability in indicators may reflect differences in housing conditions and diagnostic approaches.

The impact of lameness on production performancewas assessed on a 4-point scale. The most pronounced impact was observed on a decrease in carcass quality and live weight gain. Detailed indicators are presented in Table 3.

Table 3. Indicators associated with lameness (n = 10)

Parameter	M ± SD	Median	Range	n
% Of birds with Score ≥ 3 (Bristol)	4.7 ± 6.9	3,5%	0–20%	8
% Of lameness at slaughter (assessment)	2.1 ± 3.2	1,0%	0–>10%	9
Reduction in body weight gain (score)	2.4 ± 1,3	2,0	1–4	10
Increase in feed conversion ratio (score)	2.1 ± 1,1	2,0	1–3	9
Increase in culling (score)	1.8 ± 1,1	1,0	1–4	10
Increase in mortality (score)	1.6 ± 1.0	1,0	1–3	10
Reduction in carcass quality (score)	2.5 ± 1,2	2,0	1–4	10

Laboratory testing for lameness was conducted on 60% of farms. Staphylococcus spp. was the most commonly identified pathogen, followed by Escherichia coli and adenoviruses. Pathogen

Laboratory investigations oflameness was conducted on 60% of farms. Staphylococcus spp. was the most commonly identified pathogen, followed by Escherichia coli and adenoviruses. Pathogen monitoring was carried out in 80% of farms for the parent flock and in 40% of farms for broilers.

Chicks were vaccinated primarily at an early age (1–10 days), and the vaccination schedule was followed in 90% of farms. Vaccines against infectious bronchitis and infectious bursal disease were the most widely used.

Antibiotic prophylaxis was used on 80% of farms, with half of the operations using antibiotics specifically to control lameness. However, 70% of farms used alternative agents, including organic acids and probiotics.

Poultry welfare monitoring programs were in place at 80% of farms (formal – 40%, informal – 40%), while 20% lacked such programs.

The respondents' perceptions of the main causes of lameness and the perceived effectiveness of control measures are presentedin Table 4.

Table 4. Perceived causes of lameness and effectiveness of measures (n = 10)

Parameter	Category	n	%
Main causes of lameness (multiple responses)	Multifactorial	6	60%
	Feeding	5	50%
	Housing conditions	5	50%
	Infection	4	40%
	Genetics	3	30%
Effectiveness of control measures (5-point scale)	5 (very effective)	4	40%
	4	1	10%
	3	1	10%
	2	2	20%
	1 (ineffective)	1	10%
	Not assessed	1	10%
Economic losses due to lameness	Yes	6	60%
	No	3	30%
	Not sure	1	10%
Willingness to participate in future studies	Need more information	7	70%
	No	1	10%
	No response	2	20%

The analysis identified the most important factors asthe quality of feed ingredients, staff qualifications, climatic conditions, and stocking density. Economic losses due to lameness were reported in 60% of farms and included reduced live weight, increased culling, and higher mortality rates.

To better understand the factors associated with lameness, a Spearman correlation analysis was conducted (Table 5). A significant positive relationship was found between lameness at slaughter and reported feed quality problems ($r = 0.737$, $p = 0.015$), suggesting that farms experiencing issues with feed quality tend to report higher levels of lameness. In contrast, severe lameness (Score ≥ 3) was strongly negatively associated with the perceived effectiveness of preventive measures ($r = -0.877$, $p = 0.004$). This indicates that farms implementing more effective prevention strategies generally have substantially lower levels of severe lameness.

Diagnostic limitations also appeared to be important. A moderate positive association was observed between diagnostic difficulty and both overall lameness prevalence ($r = 0.701$, $p < 0.05$) and severe lameness ($r = 0.722$, $p < 0.05$), implying that farms facing greater challenges in diagnosis tend to report higher levels of lameness.

Conversely, the use of laboratory diagnostics was negatively associated with lameness (r = -0.641 to -0.662, $p < 0.05$), suggesting that farms with more developed diagnostic capacity may be better equipped to manage and control lameness.

Table 5. Spearman correlation between lameness indicators and selected factors

Variable	Lameness at slaughter (r)	Score ≥ 3 (r)	p-value	Interpretation
Feed quality problems	0.737	-	0.015	Significant positive
Prevention effectiveness	-	-0.877	0.004	Strong negative
Diagnostic difficulty	0.701	0.722	<0.05	Moderate positive
Laboratory diagnostics	-0.641	-0.662	<0.05	Moderate negative

"–" indicates that the correlation coefficient was not calculated for the respective variable

Among preventive measures, respondents most frequently cited improving housing conditions, optimizing feeding, and adhering to production protocols. Thus, priority is given to management and technological solutions aimed at reducing the cumulative impact of risk factors.

Discussion

The results of this study confirm the multifactorial nature of lameness in broilers and demonstrate that, in commercial poultry farming, this problem is primarily influenced by management, technological, and economic factors, rather than by infectious causes alone. Thus, lameness in broilers should be viewed as a comprehensive indicator of overall production system performance.

The findings indicate that housing conditions, primarily stocking density, microclimate, and litter quality, play a key role in the development of lameness. Despite the widespread use of closed poultry houses with controlled environmental parameters, significant variability was observedin the management of humidity and air quality, indicating a gap between the availability of technological solutions and their actual implementation. This gap can significantly reduce the effectivenessof even modern production systems. International studies also confirm a strong link between lameness and wet or poor litter, high growth rates, short dark periods, and environmental stress (related to temperature and stocking density). The higher the average gait score, the greater the culling of carcasses at slaughter and the poorer the overall herd welfare index [5, 6, 14].

Most farms operate under an intensive production regime (6–7 cycles per year), which may limit opportunities for thorough disinfection of facilities and stabilization of the microclimate. Under these conditions, the technological burdenon production systems increases, raising the likelihood of poultry welfare issues. In this context, lameness serves not only as a veterinary problem but also as an indicator of overloading of the production system. An additional risk factor is injury occurring during the transport and handling of poultry, the frequency of which depends on the condition of the broilers prior to loading (their health and physical condition), as well as on external conditions—temperature and humidity. Adverse transport conditions can cause hyperthermia or hypothermia, which increases the risk of injury and mortality. Consequently, ensuring proper transport conditions is a key element of prevention. Reducing these risks requires improving transport conditions (temperature and humidity control) and optimizing handling procedures [14, 15].

The importance of feeding-related factors (feed quality, use of additives) is consistent with the fact that mineral metabolism disorders and deficiencies in bioavailable trace elements directly affect bone formation and the stability of the musculoskeletal system. Although most farms conduct feed quality control, the composition of the additives used indicates a lack of uniformity in approaches. This suggests a lack of standardized feed management practices at the farm level. In particular, the relatively limited use of organic trace elements and probioticsmay indicate limited adoption of modern nutritional strategies. Deficiencies or excesses of vitamin D, calcium, phosphorus, and trace elements (Zn, Mn, Cu, etc.) exacerbate the development of rickets, bone deformities, and limb pathologies in broilers. Organic trace elements, mycotoxin control, moderate restriction of early growth, and the use of whole grains or combined diets can significantly improve the condition of the musculoskeletal system.

However, the isolated application of these measures is insufficient without a comprehensive approach. A sustained reduction in lameness rates requires a combination of balanced nutrition with optimal housing conditions and consideration of the birds' genetic characteristics [8 ,11, 12].

Lameness in broilers is widespread and significantly compromises bird welfare, but its assessment and monitoring remain methodologically challenging. Although visual assessment of lameness is conducted on all farms, the use of standardized scales remains limited. This limitsthe comparability of data across farms and hinders an objective assessment of trends. Additionally, laboratory diagnostics are performed in only 60% of farms, and pathogen monitoring in broilers in 40%. This indicates insufficient integration of epizootiological surveillanceinto the flock health management system. Current diagnostic reviews recommend combining laboratory methods, visual examinations, radiography, thermography, and new automated tools to improve the accuracy and early detection of lameness [16].

The multifactorial nature of lameness observed in this study is summarized in Figure 1, which illustrates the interaction between nutritional, environmental, infectious, and management-related factors contributing to the development of musculoskeletal disorders in broiler chickens.

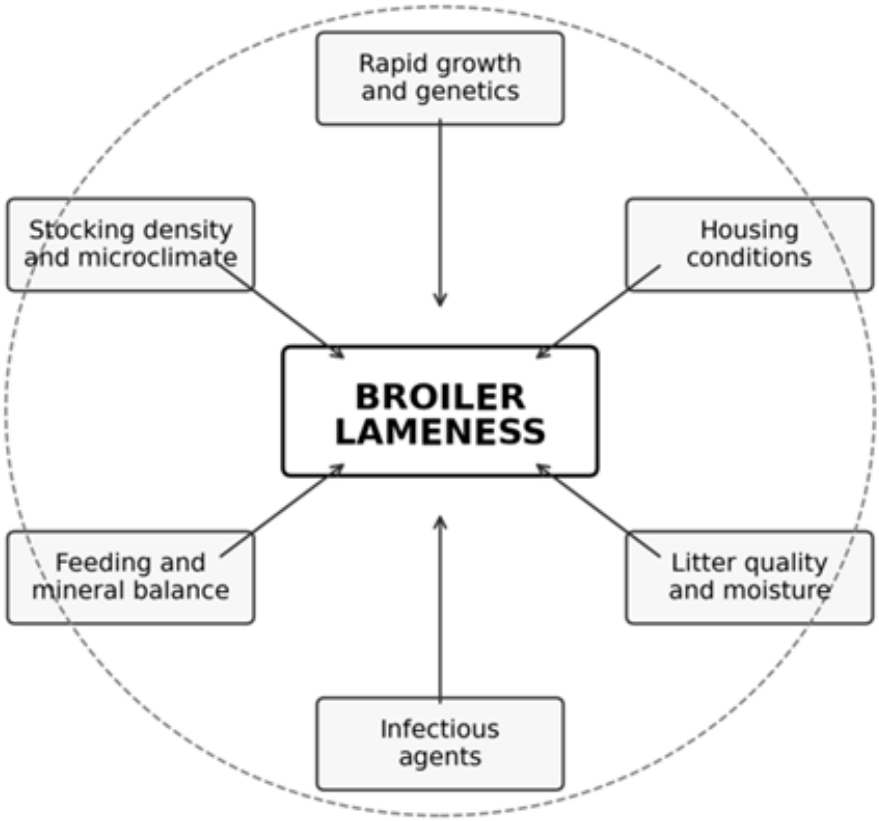

Figure 1. Conceptual model illustrates the multifactorial nature of lameness in broiler chickens

Lameness in broilers is a complex systemic problem resulting from the interaction of genetic factors, growth rate, infections, mycotoxins, feeding, housing conditions, litter quality, and management decisions. In other words, it is the result of the combined effects of many interrelated factors. Analysis of respondents' answers shows that in most cases, lameness is viewed as the result of a combination of factors (60%) rather than the consequence of a single cause. This aligns with the current understanding of the complex nature of this pathology. Furthermore, the proposed preventive measures (improving housing conditions, optimizing feeding, adhering to production protocols) indicate that industry participants themselves associate solving the problem primarily with the management of production processes. Consequently, effective prevention of lameness requires a systematic approach that includes optimizing breeding programs, rational feeding, microclimate control, and improving broiler production technologies.

Conclusions

This study demonstrated that lameness is a widespread and multifactorial problem in the commercial broiler industryin Kazakhstan. The results indicate that the development of lameness is associated not only with infectious agents but also with a combination of management, technological, dietary, and environmental factors. The main causes contributing to musculoskeletal disorders in broilers were identified as poor-quality litter, high stocking density, inadequate microclimate control, and differences in feeding practices.

The results also showed that preventive and diagnostic measures on poultry farms are not always implementedconsistently and systematically. Limited use of standardized lameness assessment scales and insufficient use of laboratory diagnostics can reduce the effectiveness of monitoring and early detection of the problem. This underscores the need to implement more standardized approaches to management and to improve veterinary control systems in the poultry industry.

Overall, lameness should be considered an important indicator of poultry welfare and the efficiency of the production system. Effective prevention requires a comprehensive approach, including optimization of housing conditions, balanced feeding, control of litter quality and microclimate, enhanced biosecurity measures, and continuous monitoring of the birds' condition. The present study is one of the first comprehensive assessments.

Authors 'Contributions

GA: conducted a comprehensive literature search, analyzed the gathered data, and drafted the manuscript. AZh, AA, DS: contributed to data interpretation, manuscript editing, and methodological support. ST, YB, AT, AK: assisted in data collection and preliminary analysis. BB: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript.

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Statement on Competing Interests

The authors declare that they have no competing interests.

References

1 Gabbassova, Zh., Dossanova, A., Lim, S.S. (2024). Current problems and prospects of poultry industry: Innovative vector. *Problems of AgriMarket,* 1, 113–124. DOI: 10.46666/2024-1.2708-9991.10.

2 Kozhabaeva, S., Baktymbet, A. (2021). Poultry farming in Kazakhstan: Problems and solutions. Problems of AgriMarket, 1, 115–121. DOI:10.46666/2021-1-2708-9991.14.

3 Abildaev, S., Amalbekova, G., Muratova, D.B., (2023). Poultry meat market: World experience, import, export. *Problems of AgriMarket,* 2, 188–197. DOI:10.46666/2023-2.2708-9991.18.

4 Byuro nacional'noi statistiki Agentstva po strategicheskomu planirovaniyu i reformam Respubliki Kazahstan [Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan]. (2026). https://stat.gov.kz/ru/ https://stat.gov.kz/en/. [*In Russian*].

5 Anthney, A., Do, A.D.T., Alrubaye, A.A.K. (2024). Bacterial chondronecrosis with osteomyelitis lameness in broiler chickens and its implications for welfare, meat safety, and quality: A review. *Frontiers in Physiology,* 15, 1452318. DOI:10.3389/fphys.2024.1452318.

6 Wideman, R.F. (2016). Bacterial chondronecrosis with osteomyelitis and lameness in broilers: A review. *Poultry Science*, 95(2), 325–344. DOI:10.3382/ps/pev320.

7 Alrubaye, A.A.K., Ekesi, N., Hasan, A., Elkins, E., Ojha, S., Zaki, S., Dridi, S., Wideman, R.F., Rebollo, M.A., Rhoads, D.D. (2020). Chondronecrosis with osteomyelitis in broilers: Further defining

lameness-inducing models with wire or litter flooring to evaluate protection with organic trace minerals. *Poultry Science,* 99(11), 5422–5429. DOI:10.1016/j.psj.2020.08.027.

8 Asnayanti, A., Do, A.D.T., Alharbi, K., Alrubaye, A. (2024). Inducing experimental bacterial chondronecrosis with osteomyelitis lameness in broiler chickens using aerosol transmission model. *Poultry Science,* 103(3), 103460. DOI: 10.1016/j.psj.2024.103460.

9 Alharbi, K., Ekesi, N., Hasan, A., Asnayanti, A., Liu, J., Murugesan, R., Ramirez, S., Rochell, S., Kidd, M.T., Alrubaye, A. (2024). Deoxynivalenol and fumonisin predispose broilers to bacterial chondronecrosis with osteomyelitis lameness. *Poultry Science,* 103(5), 103598. DOI:10.1016/j.psj.2024.103598.

10 Alharbi, K., Asnayanti, A., Hasan, A., Vaught, W. J., Buehler, K., Van Der Klis, J. D., Gonzalez, J., Kidd, M.T., Alrubaye, A. (2025). Investigating the effect of 1,25 dihydroxycholecalciferol-glycosides and phytogenic antioxidants against bacterial chondronecrosis induced by aerosol transmission model. *Journal of Applied Poultry Research,* 34(1), 100507. DOI:10.1016/j.japr.2024.100507.

11 Alharbi, K., Asnayanti, A., Do, A.D.T., Perera, R., Al-Mitib, L., Shwani, A., Rebollo, M.A., Kidd, M.T., Alrubaye, A.A.K. (2024). Identifying Dietary Timing of Organic Trace Minerals to Reduce the Incidence of Osteomyelitis Lameness in Broiler Chickens Using the Aerosol Transmission Model. *Animals,* 14(11), 1526. DOI:10.3390/ani14111526.

12 Wideman, R.F., Blankenship, J., Pevzner, I.Y., Turner, B.J. (2015). Efficacy of 25-OH Vitamin D3 prophylactic administration for reducing lameness in broilers grown on wire flooring. *Poultry Science*, 94(8), 1821–1827. DOI:10.3382/ps/pev160.

13 Bassler, A.W., Arnould, C., Butterworth, A., Colin, L., De Jong, I.C., Ferrante, V., Ferrari, P., Haslam, S., Wemelsfelder, F., Blokhuis, H.J. (2013). Potential risk factors associated with contact dermatitis, lameness, negative emotional state, and fear of humans in broiler chicken flocks. *Poultry Science*, 92(11), 2811–2826. DOI:10.3382/ps.2013-03208.

14 Kierończyk, B., Rawski, M., Józefiak, D., Świątkiewicz, S. (2017). Infectious and non-infectious factors associated with leg disorders in poultry – a review. *Annals of Animal Science*, 17(3), 645–669. DOI:10.1515/aoas-2016-0098.

15 Cockram, M.S., Dulal, K.J. (2018). Injury and mortality in broilers during handling and transport to slaughter. *Canadian Journal of Animal Science,* 98(3), 416–432. DOI:10.1139/cjas-2017-0076.

16 Weimer, S.L., Wideman, R.F., Scanes, C.G., Mauromoustakos, A., Christensen, K.D., Vizzier-Thaxton, Y. (2019). The utility of infrared thermography for evaluating lameness attributable to bacterial chondronecrosis with osteomyelitis. *Poultry Science*, 98(4), 1575–1588. DOI:10.3382/ps/pey538.

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Research article

Development of a dashboard for monitoring and risk assessment of highly pathogenic avian influenza in Kazakhstan

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Abstract

Background and Aim. Highly pathogenic avian influenza (HPAI) remains one of the most significant transboundary avian diseases, affecting affecting farmed, synanthropic, and wild bird populations. Migratory wild birds play a crucial role in the long-distance spread of avian influenza viruses across continents. The aim of this study is to develop an interactive geospatial dashboard for monitoring and assessing risk factors associated with HPAI along migration routes in Kazakhstan.

Materials and Methods. Relevant spatial datasets related to the risk of avian influenza spread were integrated into an interactive geospatial dashboard. The analysis included bird migration and ringing data from EURING and the Institute of Zoology, georeferenced wild bird observations from the eBird platform (25,828 records representing 137 species), and outbreak data from the FAO EMPRES-i and WOAH WAHIS databases. Spatial analysis and data processing were performed using ArcGIS Pro, ArcGIS Online, and the R statistical environment, while species spatial distribution modeling was performed using the MaxEnt algorithm.

Results. Through the integration and analysis of heterogeneous spatial datasets, an interactive geospatial dashboard was developed to assess the spatial distribution and potential spread of highly pathogenic avian influenza (HPAI) in Kazakhstan. The platform combines wild bird observation data from the eBird database, bird ringing and migration data, water body and wetland data, poultry density information, and officially recorded disease outbreaks. Interactive filtering tools allow data to be analyzed by geographic region, taxonomic group, and risk level. Spatial overlay analysis revealed overlap between areas of high wild bird activity and regions with intensive poultry farming, indicating potential ecological interfaces for virus transmission. The developed dashboard provides multi-layered visualization of epizootological data and supports rapid analysis of potential HPAI risk zones in Kazakhstan.

Conclusion. The developed geospatial dashboard is an effective decision-support tool for veterinary surveillance and epidemiological risk assessment. The integration of environmental and epidemiological data enables the rapid identification of potential high-risk areas for the introduction and spread of highly pathogenic avian influenza, facilitating the development of evidence-based surveillance strategies.

Keywords: Highly pathogenic avian influenza; geographic information systems; migratory birds; epidemiological surveillance; spatial analysis; Kazakhstan.

Introduction

Poultry farming is an important sector of Kazakhstan's agricultural economy, ensuring domestic supplies of poultry meat and eggs and contributing significantlyto the country's export revenues. In recent years, substantial investment and government development programs have development the growth of the poultry industry. According to official data, poultry production in Kazakhstan from January to November 2024 reached 326.3 thousand tons, an increase of 9.4% compared to the same period in 2023 [1, 2]. Currently, 67 industrial poultry farms operate throughout the country, including enterprises specializing in broiler, egg, and breeding production [3]. At the same time, the poultry industry is constantly exposed to various risks that can potentially negatively impact the economic performance of the enterprise and issues of ensuring biological safety. One of these risks is the emergence and spread of especially dangerous avian diseases, includinghighly pathogenic avian influenza [4].

Highly pathogenic avian influenza (HPAI) is a highly contagious transboundary avian disease with zoonotic potential caused by the influenza A virus, which meets the criteria for high pathogenicity [5]. The disease affects both domestic and wild birds and can be episodically transmitted to mammals, including humans [6, 7, 8]. In addition to affecting domestic and wild birds, the virus is increasingly detected in mammals, including foxes, mink, cats, and cattle. The international organizations FAO and WOAH call for strengthened epidemiological surveillance, improved early warning systems, and coordinated international control measures [9].

At the same time, the geographic spread of highly pathogenic avian influenza A(H5N5) viruses among wild birds, whose numbers have increased in greylag geese in recent years, should continue to be closely monitored worldwide [10]. Migratory wild birds, particularly aquatic species, are widely recognized as natural reservoirs of avian influenza viruses and play a crucial role in their intercontinental and continental spread [11].

Kazakhstan occupies a strategically important geographic position at the crossroads of several major migration routes. Specifically, the country lies at the crossroads of three major migration routes: the West Asian-East African, Black Sea-Mediterranean, and Central Asian [12]. These migration routes include important regional corridors such as the West Siberian and Caspian-Black Sea routes. Millions of migratory birds, including geese, ducks, swans, flamingos, herons, and waders, travel along these routes annually. The Central Asian Flyway alone is home to at least 279 populations of migratory waterfowl, representing 182 species [13].

In this regard, spatial epidemiological modelling tools in the form of dashboards integrating data on wild bird migration and environmental factors, as well as expanded international collaboration with organisations such as the Food and Agriculture Organization of the United Nations (FAO) and EURING [14], are needed to strengthen early warning capabilities for the emergence and spread of HPAI. Understanding the spatiotemporal dynamics of wild bird species that may act as reservoirs or vectors of highly pathogenic avian influenza is important for predicting outbreak risks and improving early detection systems [15, 16].

Despite growing interest in spatial avian influenza risk assessment, integrated analytical platforms for veterinary surveillance in Central Asia remain limited. Therefore, developing tools that integrate wildlife observations, environmental data, and disease outbreak information is essential for enhancing regional surveillance capacity. The aim of this study was to develop an interactive geospatial dashboard integrating multiple environmental and epidemiological datasets to assess spatial risk factors associated with highly pathogenic avian influenza along migration routes in Kazakhstan.

Materials and Methods

To develop an interactive geospatial analytical dashboard, relevant spatial data directly related to the assessment of the epizootic risk of HPAI infection was integrated.

The integrated database included information on migration activity and key ornithological locations, including migration routes, ringing sites, stopover sites, and breeding areas of migratory birds whose migration routes pass through the territory of Kazakhstan. The information was provided by the Institute of Zoology of the Ministry of Education and Science of the Republic of Kazakhstan and was also extracted from the international FAO EMPRES-i and EURING databases [17]. Georeferenced observations of species composition aggregated from the eBird citizen science crowdsourcing platform were also used [18]. The total sample size was 25,828 georeferenced records representing 137 wild bird species. These species were classified as "risk taxa" based on an analysis of global data on the spread of highly pathogenic avian influenza (HPAI), a set of ecological features, and taxonomic similarity to documented vectors of the infection.

The integration of these heterogeneous spatial data within a single user interface paved the way for the consolidation of spatial visualization tools and subsequent analytical modeling to assess the seasonality of migration processes and associated epizootic risks. Bird ringing data were used to reconstruct migration routes connecting Kazakhstan with other regions of Eurasia. The EURING database (EDB) contains a significant portion of the ringing return data [19].

Information on HPAI outbreaks (H5N1, H5N8) in wild and domestic birds was obtained from the FAO EMPRES-i and WOAH (WAHIS) databases [20]. These data provide insight into long-range ecological relationships and potential routes of virus spread.

Analytical data processing was performed using ArcGIS Pro (ESRI, USA), ArcGIS Online [21], and the R statistical environment [22]. The MaxEnt environment was used to model species distribution. MaxEnt is based on georeferenced points of species locations and raster layers of environmental factors significant for the species [23]. The following environmental covariates were used as predictor raster layers: bioclimatic variables (BIO1–BIO19 from WorldClim v2.1), proximity to open water bodies and wetlands (derived from the Global Surface Water dataset), normalized difference vegetation index (NDVI), and spatial density of poultry farming enterprises. The dataset was partitioned into training (75%) and testing (25%) subsets using random spatial blocking to minimize spatial autocorrelation. Model performance was evaluated using the area under the receiver operating characteristic curve (AUC); the mean AUC across 10 replicate model runs was 0.89 (SD = 0.02), indicating high discriminatory capacity.

All datasets were georeferenced and standardized within a single spatial coordinate system. Duplicate and incomplete records were removed, and spatial verification of observation coordinates was performed.

Results

As a result of the research, an interactive geospatial dashboard was developed through the integration, visualization, and analysis of existing data into a single user interface. This dashboard supports multilayered display and assessment of the spatial distribution and potential spread of highly pathogenic avian influenza (HPAI) in Kazakhstan. Interactive filtering tools allow data exploration by geographic area, taxonomic group, and risk level.

Specifically, the developed unified geospatial analytical platform integrates multiple heterogeneous spatial datasets. These include wild bird observation data from the eBird database, bird ringing and migration data, waterbody and wetland data, and poultry density data obtained from databases, enabling the visualization, exploration, and assessment of risks in near real time.

The platform is designed as a multi-layered geospatial system that allows users to combine and visualize multiple data sets relevant to HPAI transmission dynamics (Figure 1).

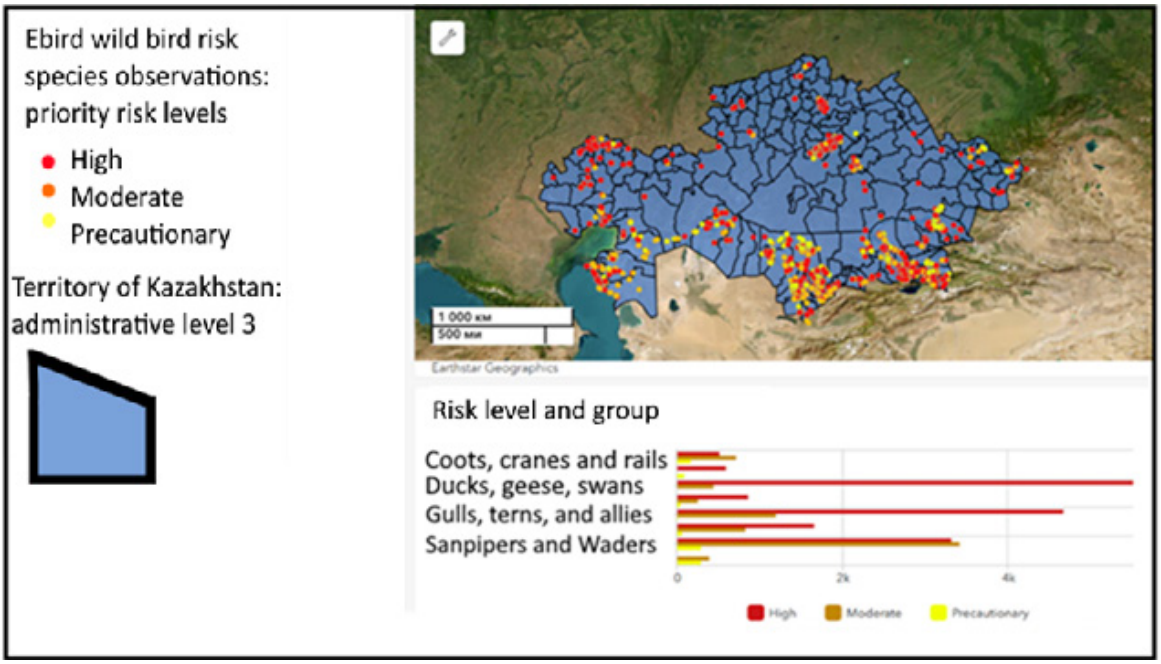

Figure 1. Distribution of wild bird observations by avian influenza risk groups in Kazakhstan, obtained from the eBird database

The dashboard includes several interactive components presenting key categories of wild bird observation risk data, derived from the eBird database. Observations are grouped according to taxonomic and ecological risk categories, including waterfowl, shorebirds, gulls, and other species known to play a role in maintaining and spreading the virus.

Data on bird ringing sites and migration routes make it possible to track the connections and routes of cross-border movement of potential carriers of the avian influenza virus. Thus, migration routes reconstructed based on bird ringing data demonstrate extensive ecological interconnections between Kazakhstan and many regions of Eurasia, indicating potential routes for the long-distance spread of avian influenza viruses (Figure 2).

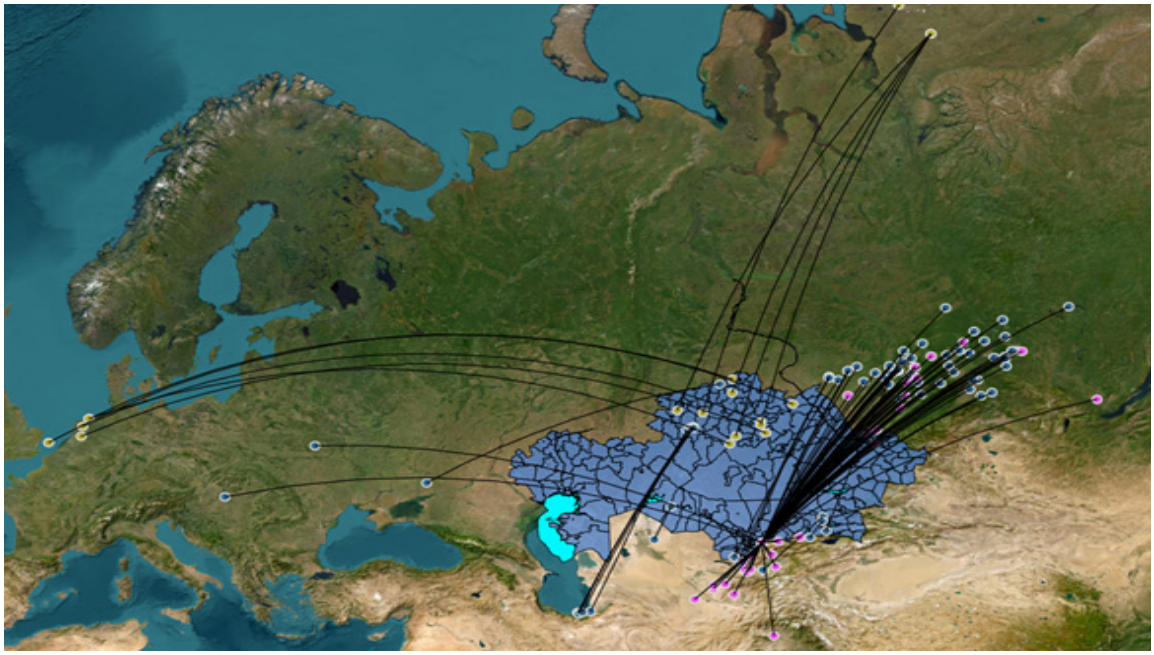

Figure 2. Migratory connection of wild birds with Kazakhstan, obtained on the basis of bird ringing data

The platform also allows for comparison of observed disease outbreaks, i.e., the current epizootic situation, with spatial patterns of predicted disease spread risk. This is additionally accomplished by using data on officially registered cases of highly pathogenic avian influenza outbreaks among wild and domestic birds (Figure 3).

Overlap analysis revealed partial spatial overlap between areas with high wild bird observation densities and regions characterized by intensive poultry farming. These ecological boundaries may represent potential hotspots for virus spread between wild and domestic bird populations.

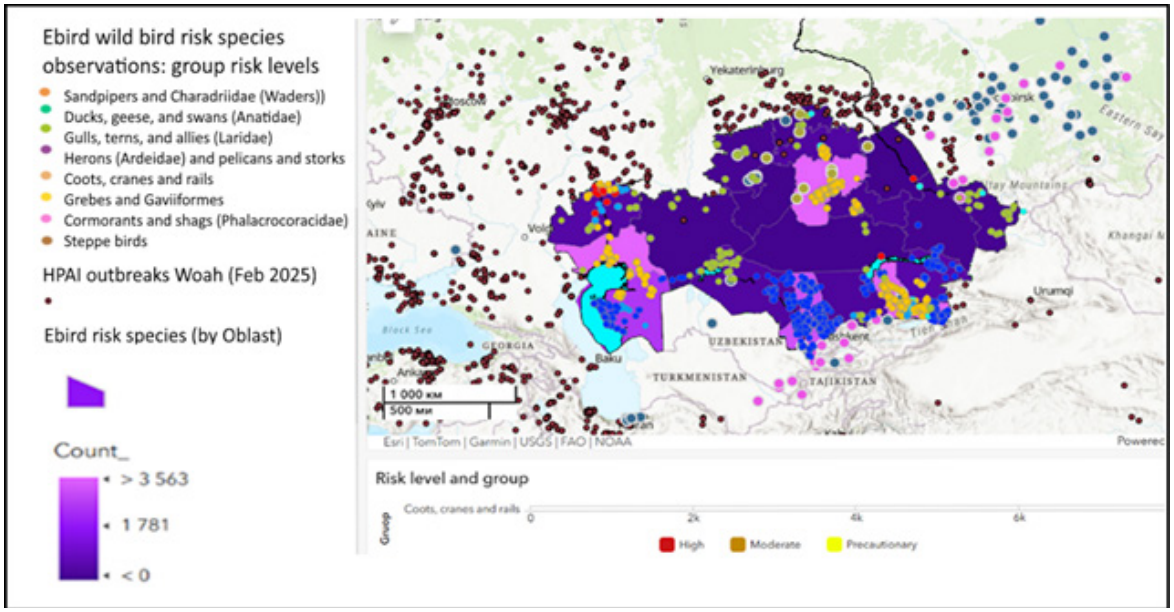

Figure 3. Spatial relationship between wild bird observations, poultry density and reported outbreaks of highly pathogenic avian influenza (HPAI)

Overall, the developed geospatial information dashboard represents an effective digital tool for risk monitoring and early warning of the emergence and spread of HPAI in Kazakhstan, contributing to a better understanding of the spatial interactions between wild bird reservoirs and poultry farming systems.

Discussion

The dominant factor determining the epizootic risk of the emergence and spatial spread of highly pathogenic avian influenza (HPAI) in the Republic of Kazakhstan is the large number and taxonomic diversity of migratory ornithocomplexes that annually cross the country's territory [24]. According to existing estimates, approximately 50 million individuals of waterfowl and semi-aquatic birds stopover in Kazakhstan's water bodies during the spring and autumn migration periods, confirming the country's status as one of the key and natural continental hubs for the concentration of migration flows [25, 26].

A key functional element of the developed geospatial analytical panel is the ability to differentiate risk assessment based on the species of birds present in a specific geographic region. Of fundamental importance here is the fact that different species demonstrate varying degrees of involvement in the circulation and transmission of the HPAI virus. In the context of this study, based on an analysis of global epizootological data, a set of ecological characteristics, and taxonomic similarity with verified carriers of the infection, 137 avifauna species found in Kazakhstan were identified as potentially dangerous taxa (risk species) [27].

The integration of heterogeneous ecological and epizootological datasets within a single interactive geospatial monitoring platform represents a methodologically promising approach aimed at improving the epidemiological surveillance system for highly pathogenic avian influenza. The spatial patterns identified during the study correlate with known migratory routes and the habitats of wetland ecosystems that support large populations of waterfowl. The spatial overlap of wild avifauna habitats and industrial poultry farming sites forms an epizootically significant ecological cluster within which HPAI virus transmission events from wild reservoir hosts to poultry are highly likely to occur. The implementation

of interactive monitoring systems based on geographic information systems (GIS) technologies creates the preconditions for increasing situational awareness and information and analytical support for epizootological surveillance strategies for transboundary animal and bird infections based on risk assessment principles.

From a practical standpoint, the developed dashboard can be actively operationalized within the targeted active surveillance frameworks of state veterinary inspectorates. Specifically, risk zone maps generated by the platform can serve as the basis for scheduling preventive inspections of commercial poultry farms located within high-risk buffer zones identified by spatial overlay analysis. During peak spring and autumn migration periods, inspectorates may use the dashboard's real-time filtering tools to prioritize biosecurity audits and intensify clinical and virological monitoring in farms situated within 10–15 km of major stopover wetlands or ringing recovery hotspots. Integration of the platform's outputs into national animal health information systems (e.g., the electronic veterinary reporting module of the Ministry of Agriculture of the Republic of Kazakhstan) would further enhance the timeliness and evidence-based nature of surveillance responses to emerging HPAI threats.

Conclusion

The developed geospatial dashboard represents an effective analytical and decision-making support tool for monitoring the risk of highly pathogenic avian influenza in Kazakhstan. By integrating multiple spatial datasets related to wild bird ecology, environmental conditions, and poultry farming, the system enables the rapid identification of areas where conditions may favor the introduction and spread of the virus. The approach presented in this study can contribute to the development of advanced veterinary surveillance systems and can be adapted for monitoring other transboundary animal and avian diseases.

Authors' Contributions

SA developed the concept and design of the study. II, PP, AM, SR, YM, AK and BK: conducted a comprehensive literature search, analyzed the collected data, and drafted the manuscript. SA and YM: Performed 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 conflicts of interest.

References

1 Bekshin, Z., Temirbekova, A., Nurbekova, Z., Amirkhanova, N., Satenova, A., Askarov, A., Zakarya, K., Abduraimov, Y., Rsaliyev, A. (2025). Epidemiology, Virology, and Control of Highly Pathogenic Avian Influenza in Kazakhstan. *Pathogens,* 14(11), 1084. DOI: 10.3390/pathogens14111084.

2 Sultankulova, K.T., Argimbayeva, T.U., Aubakir, N.A., Bopi, A., Omarova, Z.D., Melisbek, A.M., Karamendin, K., Kydyrmanov A., Chervyakova, O.V., Kerimbayev, A.A., Burashev, Y.D., Kasymbekov, Y.T., Orynbayev, M.B. (2024). Reassortants of the Highly Pathogenic Influenza Virus A/H5N1 Causing Mass Swan Mortality in Kazakhstan from 2023 to 2024. *Animals (Basel)*, 14(22), 3211. DOI: 10.3390/ani14223211.

3 Abenova, A.Z., Mukhanbetkaliyev, Y.Y., Kadyrov, A.S., Sytnik, I.I., Shevtsov, A.B., Korennoy, F.I., Martin, I.I., Perez, A.M., Abdrakhmanov, S.K. (2025). Environmental Suitability of Kazakhstan to Highly Pathogenic Avian Influenza Using Data on Eurasian Outbreaks, 2020-2024. *Viruses,* 17(4), 574. DOI: 10.3390/v17040574.

4 Zikibayeva, K.B., Svanbayev, A.A., Akhmetsadykov, N.N., Kudaibergenova, K.N., Akhmetsadykova, S.N., Nurolda, E.N., Kydyrmanov, A.I. (2025). Epidemiological investigation of poultry infectious in Kazakhstan (2021–2024). *Front. Vet. Sci.,* 11, 1520606. DOI: 10.3389/fvets.2024.1520606.

5 Alexakis, L., Buczkowski, H., Ducatez, M., Fusaro, A., Gonzales, J. L., Kuiken, T., Ståhl, K., Staubach, C., Svartström, O., Terregino, C., Willgert, K., Melo, M., Kohnle, L. (2025). Avian influenza overview December 2024–March 2025. *EFSA Journal,* 23(4). DOI: 10.2903/j.efsa.2025.9352.

6 Su, Y., Wu, R., Liu, P., Li, Z., Pu, J., Wang, L. (2026). Integrated region-specific modeling of H5 avian influenza in Asia using ENSO-based forecasts. *One Health*, 22, 101322. DOI: 10.1016/j.onehlt.2026.101322.

7 Behravesh, C.B., Charron, D.F., Liew, A., Becerra, N.C., Machalaba, C., Hayman, D.T., Mettenleiter, T.C. (2024). An integrated inventory of One Health tools: Mapping and analysis of globally available tools to advance One Health. *CABI One Health,* 3(1).

8 Burdakov, A.V., Ukharov, A.O., Wahl, T.G. (2013). One Health Surveillance with Electronic Integrated Disease Surveillance System. *Online Journal of Public Health Informatics,* 5(1), e199.

9 WHO/OIE/FAO H5N1 Evolution Working Group. (2012). Continued evolution of highly pathogenic avian influenza A (H5N1): updated nomenclature. *Influenza and Other Respiratory Viruses,* 6, 1–5. DOI:10.1111/j.1750-2659.2011.00298.x.

10 Abdrakhmanov, S.K., Mukhanbetkaliyev, Y.Y., Ruzmatov, S.I., Mukhanbetkaliyeva, A. A.. (2025). Spread of highly pathogenic avian influenza in Kazakhstan: investigating the role of bird migration and threats to poultry farming. *Herald of science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences,* 3(011), 52–60. DOI:10.51452/kazatuvc.2025.3(011).2013.

11 Dupas, M.C., Vincenti-Gonzalez, M.F., Dhingra, M., Guinat, C., Vergne, T., Wint, W., Dellicour, S. (2024). Global risk mapping of highly pathogenic avian influenza H5N1 and H5Nx in the light of epidemic episodes occurring from 2020 onward. *bioRxiv,* 2024–11. DOI:10.7554/eLife.104748.4.

12 Iglesias, I., Ruzmatov, S., Ibañez-Porras, P., Kadyrov, A., Bakishev, T., Korennoy, F., Abdrakhmanov, S. (2025). Risk assessment of highly pathogenic Avian Influenza (HPAI) in wild bird species in Kazakhstan. *German Journal of Veterinary Research,* 5(3), 76–93.

13 Bakishev, T.G., Bakisheva, Z.S., Yessembekova, G.N., Shirobokova, D.S., Kairzhanova, A.D., Syzdykova, A.S., Akzhunussova, I. (2025). Epizootic situation and comprehensive diagnosis of equine salmonellosis-associated abortion in the Republic of Kazakhstan. *Herald of science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences: Veterinary Sciences,* 2(010), 93–100. DOI:10.51452/kazatuvc.2025.2(010).1952.

14 Nisaa, Z., Häsler, B., Bennani, H., Jorquera, R., Scolamacchia, F., Alarcon, P. (2025). Global Economic Impacts of High Pathogenic Avian Inf luenza: A Systematic Review and Impact Framework. DOI:10.2139/ssrn.5641855.

15 Rehman, S., Batool, K., Lokapirnasari, W.P., Ullah, S., Khan, M.S., Abubakar, A.A., Jamal, M. (2024). The evolution of H5N1 influenza viruses in Indonesia to mammalian hosts: a review of molecular markers. *World's Poultry Science Journal*, 80(2), 349–370. DOI:10.1080/00439339.2023.2294268.

16 Pak, S.-I., Jheong, W.-H., Lee, K.-N. (2019). A GIS-Based Spatial Analysis for Enhancing Classification of the Vulnerable Geographical Region of Highly Pathogenic Avian Influenza Outbreak in Korea. *Journal of Veterinary Clinics. The Korean Society of Veterinary Clinics,* 36(1), 15.22. DOI:10.17555/jvc.2019.02.36.1.15.

17 Davis, C.T., Chen, L.M., Pappas, C., Stevens, J., Tumpey, T.M., Gubareva, L.V., Katz, J.M., Villanueva, J.M., Donis, R.O., Cox, N.J. (2014). Use of highly pathogenic avian influenza A(H5N1) gain-of-function studies for molecular-based surveillance and pandemic preparedness. *mBio,* 5(6), e02431-14. DOI:10.1128/mBio.02431-14.

18 Sayseng, J.O., Chuang, T.-W. (2025). Integrating citizen science and remote sensing data to identify key environmental factors influencing H5N1 avian influenza virus potential spillover risk in the Philippines. *GeoHealth,* 9, e2025GH001405. DOI:10.1029/2025GH001405.

19 Orkara, Sh., Khamzina, A., Sandybayev, N., Sattarova, R., Davletova, A., Khamzin, K., Pokharel, K., Ibragimov, P., Burambayeva, N., Smagulov, D. (2025). Epidemiological landscape and

genetic prospects for marker-assisted selection in Kazakh sheep. *Frontiers in Veterinary Science,* 12. DOI:10.3389/fvets.2025.1647959.

20 Alexakis, L., Buczkowski, H., Ducatez, M., Fusaro, A., Gonzales, J.L., Kuiken, T., Mirinavičiūtė, G., Ståhl, K., Staubach, C., Svartström, O., Terregino, C., Willgert, K., Melo, M., Kohnle, L (2025). Scientific report: Avian influenza overview March–June 2025. *EFSA Journal*, 23(7): 9520, 65. DOI: 10.2903/j.efsa.2025.9520.

21 Turner, A. (2025). Environmental Risk Factors for Highly Pathogenic Avian Influenza in Pennsylvania's Commercial Poultry Industry. West Chester University Graduate Theses, Dissertations, and Final Projects. 3. https://digitalcommons.wcupa.edu/all_capstones/3

22 Plaza, P., Santangeli, A., Rosciano, N., et al. (2025). Wild Birds Affected by Highly Pathogenic Avian Influenza A (H5N1) Worldwide: Epidemiological Insights Into the Recent Panzootic. *Global Change Biology*, 31: 10, e70523. DOI:10.1111/gcb.70523.

23 Martelli, L., Fornasiero, D., Martínez-Lanfranco, J.A., Spada, A., Scarton, F., Scolamacchia, F., Manca, G., Mulatti, P. (2025). Exploring the Role of Wild Bird Species in the Transmission of Avian Influenza to Poultry. *Transboundary and Emerging Diseases,* 2025: 2288535, 15. DOI:10.1155/tbed/2288535.

24 Hay, J., Yeh, K.B., Dasgupta, D., Shapieva, Z., Omasheva, G., Deryabin, P., Richards, A.L. (2016). Biosurveillance in Central Asia: Successes and challenges of tick-borne disease research in Kazakhstan and Kyrgyzstan. *Frontiers in Public Health,* 4, 4. DOI:10.3389/fpubh.2016.00004.

25 Biyashev, K.B., Makbuz, A.Z., Biyashev, B.K. (2016). Occurrence of enteroinfectious pathogens in agricultural animals and poultry. *Biol. Med,* 8(2).

26 Tabynov, K., Kuanyshbek, A., Zharmambet, K., Yelchibayeva, L., Karibayev, T., Berdikulov, M., Tabynov, K. (2025). Evaluation of commercial vaccines for efficacy and transmission control against the emergent H5N8 (clade 2.3. 4.4 b) avian influenza virus in Kazakhstan. *Virology,* 610. DOI: 10.1016/j.virol.2025.110601.

27 Zikibayeva, K.B., Svanbayev, A.A., Akhmetsadykov, N.N., Kudaibergenova, K.N., Akhmetsadykova, S.N., Nurolda, E.N, Kydyrmanov, A.I. (2025). Epidemiological investigation of poultry infectious in Kazakhstan (2021–2024). *Front. Vet. Sci.*, 11, 1520606. DOI: 10.3389/fvets.2024.1520606.

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Research article

Comparative evaluation of the structural and tenderness properties of minced beef from angus and akbas cattle using a penetration-based approach

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Abstract

Background and Aim. Meat tenderness is a key determinant of consumer acceptance and product quality; however, its evaluation in minced meat systems remains challenging due to the disruption of muscle fiber structure. The present study aimed to assess the structural and tenderness properties of minced beef derived from Angus and Akbas cattle using a penetration-based approach combined with the Rebinder equation.

Materials and Methods. A comparative analysis was performed on minced beef samples obtained from Angus and Akbas cattle (12 samples per breed). The structural and mechanical properties were determined using a Structurometer ST-2 (Radius, Russia) fitted with a 60° cone indenter at 4–6 °C. Structural resistance values were calculated using the Rebinder equation using penetration force and depth parameters.

Results. Angus samples exhibited significantly lower force values (282.19 ± 42.24 N) compared to Akbas (308.68 ± 56.82 N), indicating reduced structural resistance and improved tenderness ($p = 0.028$). Distribution analysis revealed greater variability in Akbas samples, suggesting more heterogeneous structural properties. The application of the Rebinder model enabled normalization of mechanical resistance and facilitated interpretation of structural behavior. These findings demonstrate that penetration-based methods are suitable for minced meat systems and reveal significant breed- related differences relevant for meat quality optimization.

Conclusion. Penetration-based analysis using the Structurometer ST-2 was effective for evaluating the structural and mechanical properties of minced beef. Angus beef demonstrated lower structural resistance and greater tenderness compared to Akbas beef. The results confirm the usefulness of the Rebinder equation for interpreting mechanical properties and support the application of this approach in meat quality assessment and industrial quality control. The study also highlights the potential to improve beef processing efficiency and promoting high-quality domestic meat products in Kazakhstan.

Keywords: Akbas cattle; Angus cattle; meat quality; minced beef; penetration test; tenderness.

Introduction

Beef quality is a critical determinant of consumer acceptance, market competitiveness, and export potential, particularly in countries seeking to expand their presence in the global meat industry. As international trade becomes increasingly competitive, the demand for consistently high-quality meat products continues to grow, making the evaluation of quality attributes an essential component of

modern meat science. Among these attributes, tenderness and structural integrity are widely recognized as key sensory and technological parameters that directly influence both consumer satisfaction and processing efficiency. Tenderness is closely associated with the overall eating experience, while structural integrity determines the behavior of meat during handling, processing, and storage. These properties are interrelated; rather, they are governed by complex interactions involving muscle fiber composition, the amount and organization of connective tissue, and a series of postmortem biochemical processes, including proteolysis, water redistribution, and changes in muscle ultrastructure [1–4].

In recent years, increasing attention has been directed toward the evaluation of meat texture using objective instrumental methods. This shift is largely driven by the limitations of traditional sensory assessments, which, although valuable, are inherently subjective and influenced by panelist variability, training, and environmental conditions. In contrast, instrumental techniques provide reproducible, quantifiable, and standardized data, allowing for more accurate comparisons across studies, production systems, and processing conditions. These methods are particularly important in research and industrial settings, where measurement consistency and reliability are critical [5, 6].

Among the available instrumental approaches, penetration-based analysis has become a practical and informative technique for assessing the structural resistance and mechanical strength of meat systems. Unlike conventional shear force methods, such as Warner-Bratzler shear force, which are primarily designed for intact muscle cuts, penetration methods offer distinct advantages when applied to minced or ground meat products. In such systems, the disruption of muscle fiber orientation limits the applicability of shear-based measurements, making penetration analysis a more suitable alternative. By measuring the force required for a probe to penetrate a sample, this technique provides insight into the resistance of the meat matrix to deformation and fracture [7, 8].

Parameters derived from penetration testing, including penetration resistance and structural strength (e.g., PNS), serve as valuable indicators of the internal organization and stability of meat matrices. These parameters reflect the combined effects of protein network formation, fat distribution, moisture retention, and interactions between structural components within the product. As a result, penetration-based measurements can be used not only to assess texture but also to monitor changes in product quality during processing, storage, and formulation adjustments. Overall, the application of such objective methods enhances the ability to characterize meat quality in a precise and scientifically robust manner, supporting both research advancements and practical improvements in the meat industry.

The aim of this study was to compare the structural and mechanical properties of minced beef obtained from Angus and Akbas cattle using a penetration-based method combined with the Rebinder equation in order to objectively evaluate meat tenderness and structural resistance.

The novelty of the study lies in the application of penetration analysis combined with the Rebinder equation for the evaluation of minced beef systems, where traditional shear force methods are less informative due to the disruption of muscle fiber orientation. In addition, this is one of the first comparative studies conducted in Kazakhstan using the Structurometer ST-2 to assess breed-related differences between Angus and Akbas beef. The findings demonstrate the potential of this approach for objective meat quality evaluation, industrial quality control, and optimization of beef processing.

Materials and Methods

A controlled comparative study was conducted using meat samples from Angus and Akbas cattle. A total of twelve independent samples per breed were analyzed. The *Longissimus dorsi* muscle was excised 24 hours post-mortem, vacuum-packaged, and stored at −20 °C. Prior to analysis, samples were thawed at 4 °C for 24 hours to minimize structural alterations.

The samples were minced using a standardized meat grinder to obtain a homogeneous minced structure. The minced meat was then placed into cylindrical containers of uniform dimensions to ensure consistent geometry and boundary conditions during testing (Figure 1).

Figure 1. Minced meat samples used in the study

Structural analysis was performed using a Structurometer ST-2 texture analyzer (Radius, Russia) equipped with a 60° conical indenter. The penetration test was conducted at a controlled temperature of approximately 4–6 °C. During the test, the indenter penetrated vertically into the sample while force and penetration depth were continuously recorded. The cone constant was set to K = 0.334.

Structural resistance was calculated using the Rebinder equation:

$$\theta = K \times \frac{F}{h^2} \quad (1)$$

where:

F-load value, H;

h is the penetration depth of the cone, m;

K is the cone constant, which depends on the cone apex angle (α).

The stability applied to the cone of the device with an angle of height α was calculated using the following equation:

$$K = \frac{cos^2(a/2)}{\pi * tg(a/2)} \quad (2)$$

where:

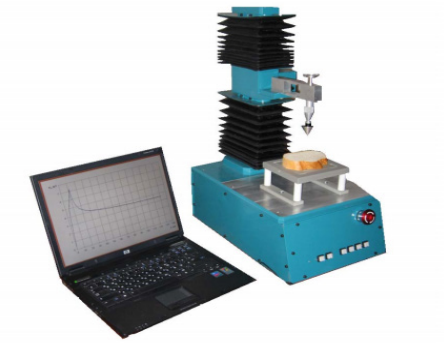
α is the cone apex angle.

For a 45° cone: K ≈ 0.612

For a 60° cone: K ≈ 0.334

This equation enables normalization of force relative to deformation and provides a more accurate representation of the internal structural strength of the minced meat system.

Statistical analysis was performed using R software. Data were tested for normality using the Shapiro–Wilk test, and differences between groups were assessed using an independent t-test. Statistical significance was defined as $p < 0.05$. Descriptive statistics were calculated, and data distribution was visualized using boxplots and mean comparison charts.



a) b)

Figure 2. Experimental setup for the Structurometer ST-2:
a) laptop used for data acquisition; b) Structrometer ST-2

Results

The penetration test revealed clear differences in the structural properties between the two cattle breeds. The mean penetration force for Akbas samples was 308.68 ± 56.82 N, whereas Angus samples exhibited a lower mean value of 282.19 ± 42.24 N. This corresponds to an approximately 8.6% reduction in structural resistance in Angus samples. The range of values was wider in Akbas samples (197.1–411.1 N) compared to Angus (220.0–380.0 N), indicating greater variability.

The difference between the groups was statistically significant ($p = 0.028$), confirming that breed has a measurable effect on the structural properties of minced beef. Structural resistance values calculated using the Rebinder equation followed the same pattern, with Akbas exhibiting higher values (2.58) compared to Angus (2.36), indicating a denser and more cohesive internal structure.

The distribution of penetration force values is illustrated in Figure 3. The boxplot shows that Akbas samples had a higher median and a wider interquartile range, reflecting greater variability. In contrast, Angus samples were more tightly clustered, indicating more uniform structural behavior.

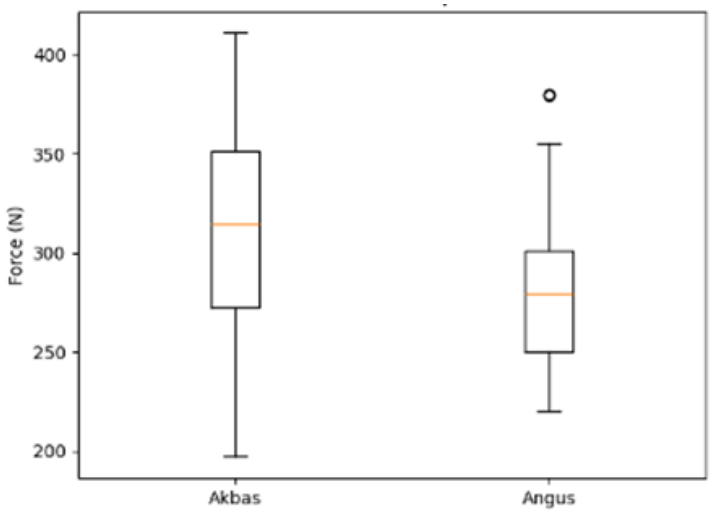


Figure 3. Distribution of penetration force values

The comparison of mean values is presented in Figure 4, which clearly demonstrates higher average force values in Akbas samples compared to Angus.

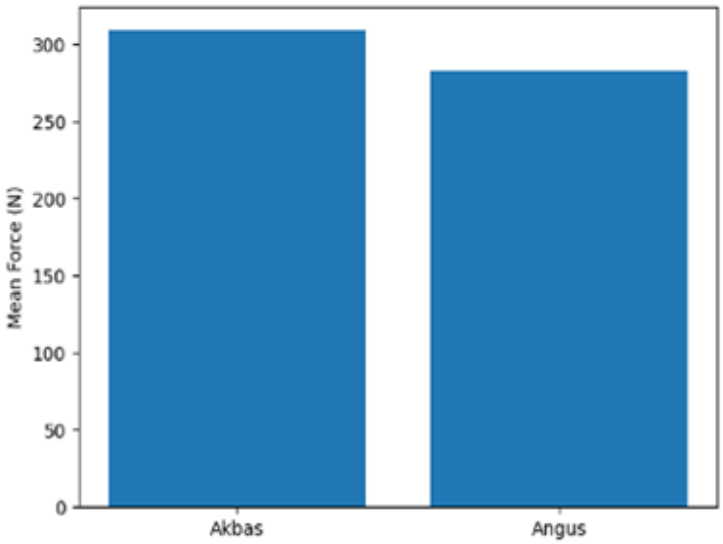


Figure 4. Mean values of penetration force

The lower penetration force values observed in Angus indicate reduced resistance to deformation and improved tenderness, whereas higher values in Akbas reflect stronger structural integrity and lower tenderness.

Table 1.The results of measuring the force during deformation of minced meat without adding fat

Sample	Akbas Hardness, g	Angus Hardness, g	Akbas (PNS, gPa)	Angus (PNS, gPa)
1	357.1	289.3	3.06	2.74
2	331.5	363.6	2.98	3.21
3	348.2	262.1	3.12	2.65
4	365.4	347.1	3.20	3.05
5	342.7	288.9	3.05	2.80
6	338.9	289.8	3.01	2.77
7	329.6	266.5	2.97	2.69
8	310.2	243.2	2.88	2.60
9	322.5	260.9	2.94	2.66
10	335.8	262.2	3.00	2.70
11	305.4	233.3	2.85	2.55
12	340.1	279.4	3.03	2.73

As shown in Table 1, significant differences were observed in the structural and mechanical properties of the Akbas and Angus minced meat samples.

In the Akbas samples, the force values ranged from 305.4 to 365.4 g, showing generally higher values than in the Angus samples. This suggests that Akbas minced meat has a denser texture and greater hardness.

In the Angus samples, the force values ranged from 233.3 to 363.6 g. Although higher values were observed in some samples (for example, 2 and 4), they tended to be lower than in Akbas.

The PNS indicators also showed a similar trend: the Akbas samples had 2.85–3.20 gPa, and the Angus samples had 2.55–3.21 gPa. Overall, the Akbas samples demonstrated greater structural stability and elasticity. Thus, the Akbas minced beef samples exhibited greater structural strength than the Angus samples.

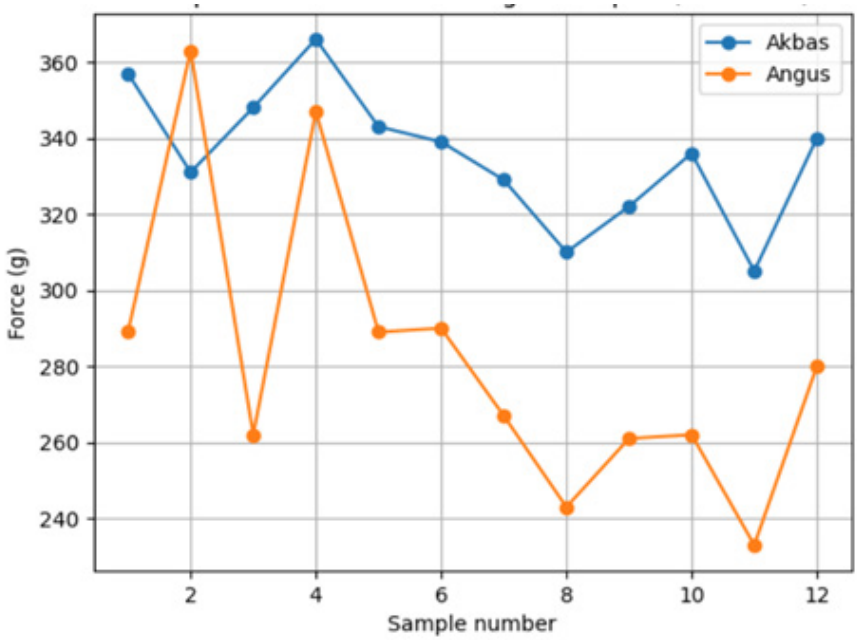


Figure 5. Comparison of Meat Hardness Between Akbas and Angus Beef Samples

The Figure 5 shows a comparison of the hardness (usilie) indicators of minced meat samples Akbas and Angus. It has been shown that the Akbas samples have higher overall values, which indicates that their structure is denser and more durable.

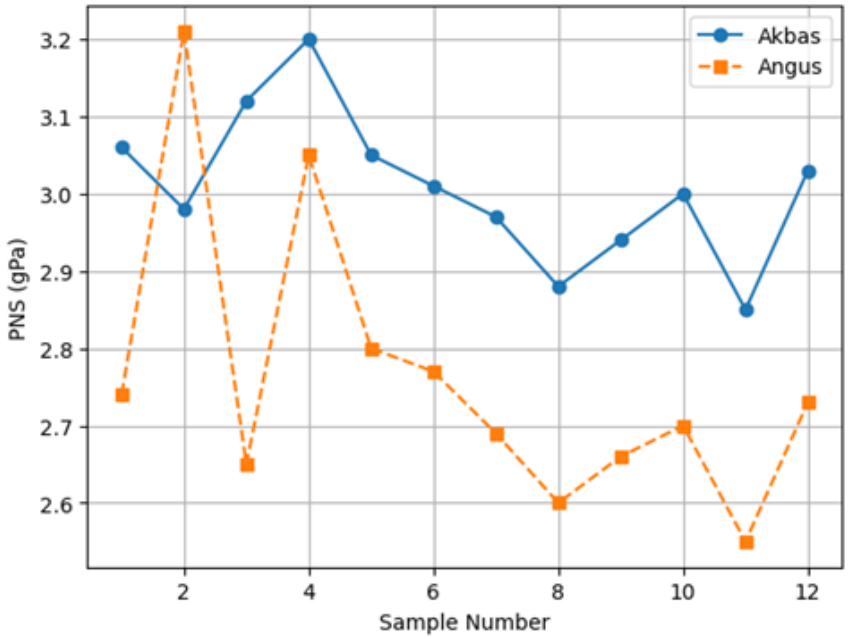


Figure 6. Comparison of Texture Strength (PNS) in Beef Samples from Akbas and Angus Breeds
As shown in Figure 6, the Akbas samples demonstrate relatively high PNS values,
indicating enhanced structural stability and mechanical strength

Discussion

The results of this study demonstrate that beef from the Angus breed exhibits significantly lower structural resistance compared to Akbas, indicating greatertenderness. This finding is consistent with the general understanding in meat science that tenderness is one of the most important determinants of consumer acceptance and overall eating quality. The statistically significant difference observed between the two groups ($p = 0.028$) confirms that breed-related factors have a measurable effect on the mechanical behavior of minced beef systems.

A key factor underlyingthese differences is likely the variation in intramuscular fat (IMF) content between breeds. Angus cattle are known for higher marbling, which has been consistently associated with improved tenderness and palatability. Intramuscular fat disrupts the continuity of the muscle protein network, reducing mechanical resistance and increasing deformability. As a result, less force is required to penetrate the meat matrix, which explains the lower penetration force and PNS values observed in Angus samples. Previous studies have demonstrated that IMF contributes positively to tenderness by weakening structural cohesion and improving juiciness and mouthfeel [9]. In contrast, the higher resistance observed in Akbas samples suggests a denser protein structure, potentially linked to lower fat content and a relatively greater contribution of connective tissue.

The structural differences observed in this study can also be explained by fundamental mechanisms governing meat texture. Tenderness is influenced by the interaction between myofibrillar proteins, connective tissue (particularly collagen cross-linking), and postmortem proteolysis processes. Increased connective tissue content and stronger collagen cross-links typically lead to higher resistance to deformation and reduced tenderness, which may explain the higher penetration values recorded in Akbas meat [4, 10].

An important methodological aspectis the use of minced meat systems. Grinding disrupts muscle fiber orientation and eliminates anisotropic properties that are typically measured using shear force techniques such as Warner-Bratzler analysis. Under these conditions, penetration testing is more suitable, as it reflects the bulk mechanical resistance of the product rather than directional fiber strength. The

consistency between penetration force and PNS values observed in this study supports the reliability of this approach for evaluating minced meat structure. Similar conclusions have been reported in texture research, where penetration methods are considered more appropriate for heterogeneous or processed systems [11].

The greater variability observed in Akbas samples is another important finding. The wider range and dispersion of penetration force values indicate a less uniform internal structure. This heterogeneity may be associated with uneven distribution of connective tissue or variability in muscle composition. In contrast, Angus samples showed a more consistent pattern, which may reflect more uniform fat distribution within the meat matrix. From an industrial perspective, such uniformity is highly desirable, as it ensures consistent processing performance and product quality. Variability in raw material properties can negatively affect technological processes such as grinding, mixing, and cooking, ultimately leading to inconsistent texture in the final products [12].

The application of the Rebinder equation to calculate structural strength (PNS) provides an additional level of interpretation beyond simple force measurements. While penetration force reflects resistance to deformation, PNS represents the intrinsic mechanical stability of the material. The fact that both parameters showed the same trend, with higher values in Akbas and lower values in Angus, confirms the robustness of the results. Although this approach is not widely used in meat science, it offers a valuable tool for standardizing mechanical measurements and improving comparability across studies.

Overall, the findings highlight clear breed-related differences in the structural and mechanical properties of minced beef. Angus meat appears more suitable for products where tenderness and uniformity are key quality attributes, whereas Akbas meat demonstrates higher structural strength, which may be advantageous in products requiring greater mechanical stability. However, it is important to note that meat texture is influenced by multiple factors beyond breed, including feeding system, animal age, and postmortem handling conditions. Future research combining instrumental analysis with microstructural and compositional studies would provide a more comprehensive understanding of the mechanisms underlying these differences.

Conclusions

Penetration-based analysis using the Structurometer ST-2 provides a reliable method for evaluating the structural properties of minced meat. Angus beef exhibited significantly lower structural resistance compared to Akbas, indicating superior tenderness. The integration of the Rebinder equation enhances the interpretation of mechanical data and supports its application in both scientific research and industrial quality control.

This study provides a comprehensive evaluation of the structural and mechanical properties of minced beef. The mass of minced meat affects its density, elasticity, and strength. The comparative evaluation of minced beef from Angus and Akbas cattle suggests that products from these breeds may be recommended for different applications according to their structural and mechanical characteristics. The work was performed at the Shakarim University in Semey, Abai region. The results of this study may contribute to future comprehensive research on improving the structural and mechanical properties of beef, thereby supporting agricultural development and providing consumers with high-quality domestically produced beef products.

Authors 'Contributions

GZh: conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. GA, GA: contributed to data interpretation, manuscript editing, and overall study supervision. BB: conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript".

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Statement on Competing Interests

The authors declare that they have no competing interests.

References

1 Geletu, U.S., Usmael, M.A., Mummed, Y.Y., Ibrahim, A.M. (2021). Quality of cattle meat and its compositional constituents. *Veterinary Medicine International*, 7340495,1–9. DOI:10.1155/2021/7340495.

2 Gagaoua, M., Terlouw, C.E.M., Mullen, A.M., Franco, D., Warner, R.D., Lorenzo, J.M., Purslow, P.P., Gerrard, D., Hopkins, D.L., Troy, D., Picard, B. (2021). Molecular signatures of beef tenderness: Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. *Meat Science,* 172, 108311. DOI: 10.1016/j.meatsci.2020.108311.

3 Felderhoff, C., Lyford, C., Malaga, J., Polkinghorne, R., Brooks, C., Garmyn, A., Miller, M. (2020). Beef quality preferences: Factors driving consumer satisfaction. *Foods*, 9(3), 289. DOI:10.3390/foods9030289.

4 Warner, R., Miller, R., Ha, M., Wheeler, T.L., Dunshea, F., Li, X., Vaskoska, R., Purslow, P. (2021). Meat tenderness: Underlying mechanisms, instrumental measurement, and sensory assessment. *Meat and Muscle Biology,* 4(2): 17, 1–25. DOI:10.22175/mmb.10489.

5 Maggiolino, A.A., Forte, L., Hopkins, D.L., Tomasevic, I., De Palo, P. (2026). A comparative analysis of volatile organic compound profiles and detection methodologies across beef, sheep, goat, pig, camel, donkey, horse and alpaca meats. *Meat Sci*., 232, 109982. DOI: 10.1016/j.meatsci.2025.109982.

6 Liu, C., Li, Y., Sun, W., Ma, F., Wang, X., Yang, Z. (2025). New Techniques of Meat Quality Assessment for Detecting Meat Texture. *Processes,* 13(3), 640. DOI: 10.3390/pr13030640.

7 Schlangen, M., Kyriakopoulou, K., Boom, R.M., van der Goot, A. Jan. (2021). Texture methods for evaluating meat and meat analogue structures: A review. *Food Control,* 127, 108103. DOI: 10.1016/j.foodcont.2021.108103.

8 Abdul M., Kumar, P., Bin Mohammad Rashedi, I-F., Mohd Zaki, N.N., Arshad, S., Ishak, I. (2024). Impact of ground size on meat quality and meat products: a review. *Cogent Food and Agriculture,* 11(1). DOI: 10.1080/23311932.2025.2557336.

9 Tornberg, E. (2005). Effects of heat on meat proteins - Implications on structure and quality of meat products. *Meat Science*, 70(3), 493–508. DOI: 10.1016/j.meatsci.2004.11.021

10 Starkey, C.P., Geesink , G.H., Collins, D., Oddy , V.H., Hopkins, D.L. (2016). Do sarcomere length, collagen content, pH, intramuscular fat and desmin degradation explain variation in the tenderness of three ovine muscles? *Meat Sci.*, 113, 51–8. DOI: 10.1016/j.meatsci.2015.11.013.

11 Mitchell, J. (2003). Food Texture and Viscosity: Concept and Measurement. *Int. J. Food Sci. Technol*., 38, 7.

12 Toldrá, F. (2022). *Lawrie's Meat Science.* Woodhead Publishing, 9.

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Research article

Phenotypic antimicrobial susceptibility of Escherichia coli isolated from bovine superficial cervical (prescapular) lymph nodes from market-derived bovine products in Semey, Kazakhstan

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Abstract

Background and Aim. Bovine lymph nodes are relevant to veterinary diagnostics and beef safety because bacteria within lymphatic tissue may evade carcass surface decontamination and enter the food chain. The aim of this study was to describe the phenotypic antimicrobial susceptibility profile of *E. coli* isolated from bovine superficial cervical (prescapular) lymph nodes collected from market-derived bovine products in Semey, Kazakhstan.

Materials and Methods. Thirty-two *E. coli* isolates recovered in autumn from bovine prescapular lymph nodes during pre-sale veterinary-sanitary inspection were tested by disk diffusion on Mueller-Hinton agar. Eighteen antimicrobial agents were evaluated. Final susceptible/intermediate/resistant (S/I/R) categories were determined using CLSI breakpoints where applicable, and Wilson 95% confidence intervals were calculated for susceptible proportions. Isolate-level MDR profiles were summarized from the available phenotypic susceptibility profiles.

Results. The highest susceptibility was observed for gentamicin and amikacin (32/32 each; 100%), followed by enrofloxacin and ceftriaxone (31/32 each; 97%), and ciprofloxacin, cefotaxime, and amoxicillin/clavulanate (30/32 each; 94%). High susceptibility was also observed for azithromycin and tetracycline (29/32 each; 91%), streptomycin (28/32; 88%), and kanamycin and neomycin (27/32 each; 84%). Lower susceptibility was observed for imipenem (18/32; 56%), amoxicillin (14/32; 44%), and doxycycline (4/32; 12%). The highest resistance rates were detected for benzylpenicillin (32/32; 100% R), tylosin (30/32; 94% R), doxycycline (15/32; 47% R), and amoxicillin (10/32; 31% R). Isolate-level analysis classified 17/32 isolates (53.1%) as MDR; the dominant resistant classes in MDR isolates were penicillins/beta-lactams, macrolides, and tetracyclines.

Conclusion. *E. coli* isolated from bovine prescapular lymph nodes showed a heterogeneous susceptibility profile. Gentamicin and amikacin showed complete in vitro susceptibility, and fluoroquinolones and third-generation cephalosporins retained high activity against most isolates. In contrast, benzylpenicillin and tylosin showed poor activity based ondescriptive phenotypic observations, whereas doxycycline and aminopenicillin-related agents showed mixed or reduced activity. MDR was detected in 17/32 isolates (53.1%), with isolate-level profiles dominated by combinations involving penicillins/beta-lactams, macrolides, and tetracyclines. These data support continued phenotypic surveillance of carcass-associated tissues and should be interpreted together with approved indications, food-animal regulations, and antimicrobial stewardship principles rather than as empirical treatment recommendations.

Keywords: antimicrobial susceptibility; beef safety; *Escherichia coli*; market-derived bovine product; prescapular lymph node.

Introduction

Antimicrobial resistance (AMR) in cattle-associated *E. coli* remains important for both veterinary medicine and food safety. *E. coli* is commonly used as an indicator organism in AMR surveillance because it is widespread in food-producing animals, clinically relevant, and able to acquire and disseminate resistance determinants [1].

Peripheral lymph nodes have attracted increasing attention in beef safety because bacteria located inside these tissues may escape surface decontamination and later enter trimmings used for ground products. While subiliac and popliteal lymph nodes are studied more often, data on superficial cervical (prescapular) lymph nodes remain relatively limited [2, 3].

Published studies from cattle lymph nodes, slaughterhouse environments, and dairy systems indicate heterogeneous susceptibility patterns. Resistance commonly persists against tetracyclines, aminopenicillins, and some older agents, whereas selected aminoglycosides, fluoroquinolones, and third-generation cephalosporins may retain *in vitro* activity depending on region and production context [4–9].

Such variability makes local phenotypic monitoring essential, particularly when isolates originate from products intended for human consumption. In the present case, the isolates were obtained during pre-sale inspection at markets in Semey, Kazakhstan, giving the results direct relevance for veterinary-sanitary control and food-chain surveillance.

The aim of this study was to describe the phenotypic antimicrobial susceptibility profile of *E. coli* isolated from bovine superficial cervical (prescapular) lymph nodes collected from market-derived bovine products in Semey, Kazakhstan. This manuscript is intentionally limited to conventional phenotypic susceptibility data and does not include PCR, sequencing, virulence profiling, or phylogenetic characterization. Therefore, the results should be interpreted as a preliminary local descriptive dataset rather than as a comprehensive molecular epidemiological investigation.

Materials and Methods

Ethical approval

The study used products sampled during routine pre-sale veterinary-sanitary inspection and did not involve experimental procedures on live animals; therefore, specific animal ethics approval was not required according to local practice. No personal data or farm identifiers were used in the analytical dataset.

Study design and sample origin

This work is a descriptive laboratory study based on the antimicrobial susceptibility component of an existing diagnostic dataset. Thirty-two *E. coli* isolates recovered from bovine superficial cervical (prescapular) lymph nodes were included. The lymph nodes obtained from cattle products presented for sale at markets in Semey, Kazakhstan, and were sampled in autumn during routine pre-sale veterinary-sanitary inspection. The study did not involve experimental infection or manipulation of live animals. Because the samples were obtained from market-derived products rather than a prospective farm-level sampling design, animal age, production type (beef or dairy), farm of origin, geographic origin before marketing, and previous antimicrobial exposure history were not available in the analytical dataset.

Phenotypic confirmation by Gram staining

Before antimicrobial susceptibility testing, presumptive *E. coli* isolates were examined by Gram staining as a phenotypic confirmation method. Smears were prepared from fresh pure cultures, stained using the conventional Gram-staining procedure, and examined microscopically. Isolates showing Gram-negative, rod-shaped morphology consistent with *E. coli* were included in the antimicrobial susceptibility analysis.

Disk diffusion testing

The disk-diffusion workflow was described based on the available laboratory records and routine laboratory procedures for antimicrobial susceptibility testing. For each isolate, bacterial material from a fresh pure culture was used to prepare a homogeneous suspension following routine laboratory practice. Mueller-Hinton agar was prepared according to the manufacturer's instructions and poured into Petri dishes to an approximate depth of 4 mm. The bacterial suspension was applied to the agar surface with a sterile cotton swab by evenly streaking the plate. After the surface had dried briefly, antibiotic disks were placed at appropriate intervalson the inoculated agar surface. Plates were incubated aerobically

under routine laboratory conditions, after which inhibition zone diameters were measured in millimeters using a ruler or caliper. Representative stages of sample handling, lymph-node preparation, and disk-diffusion testing are shown in Figure 1.

Figure 1. Main laboratory stages: A – pathological material; B – lymph-node separation; C – agar plate with antibiotic disks; D – incubated plates

Antimicrobial panel and interpretation

The antimicrobial panel included doxycycline, tylosin, kanamycin, streptomycin, tetracycline, neomycin, ampicillin, azithromycin, ciprofloxacin, enrofloxacin, benzylpenicillin, amoxicillin/clavulanate, amoxicillin, ceftriaxone, cefotaxime, imipenem, gentamicin, and amikacin. Final susceptible/intermediate/resistant (S/I/R) categories were determined using CLSI breakpoints where applicable. Azithromycin was interpreted using the following criteria: S >= 13.5 mm, I = 12.5-13 mm, and R <= 12 mm. The present study focuses oninhibition-zone diameters and overall phenotypic susceptibility patterns. Benzylpenicillin and tylosin were retained because they were included in the original local laboratory testing panel; however, these agents are not intended to serve as preferred clinical indicators for Enterobacterales. Agent-organism combinations with limited or non-harmonized interpretive criteria are discussed cautiously and presented as phenotypic monitoring data rather than direct therapeutic recommendations [10]. For isolate-level MDR evaluation, multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial classes [11]. Intermediate results were not considered resistant in this classification. Resistant classes were determined from the available phenotypic susceptibility profiles, and the resistant agents contributing to each MDR profile were summarized at isolate level.

Statistical analysis

Descriptive statistics were used. For each antimicrobial agent, the mean inhibition-zone diameter and the percentage of isolates categorized as S, I, and R were recorded. Wilson 95% confidence intervals (CIs) were calculated for susceptible proportions to reflect the uncertainty associated with the limited sample size. In addition to agent-level descriptive analysis, isolate-level MDR profiles were summarized by listing resistant antimicrobial agents and antimicrobial classes for each MDR-positive isolate. The MDR proportion was calculated by dividing the number of MDR isolatesby the total number of examined isolates. Inferential statistical testing and animal- or farm-level risk-factor analysis were not performed because the available metadata were limited.

Results

All isolates included in the susceptibility analysis showed Gram-negative, rod-shaped morphology on Gram staining, supporting their phenotypic consistency with E. coli. Representative stages of sample

handling and disk-diffusion testing are shown in Figure 1. Susceptibility results and isolate-level MDR profiles are summarized in Table 1, Figure 2, and Table 2.

Table 1. Phenotypic antimicrobial susceptibility profile of E. coli isolates according to CLSI breakpoints (n = 32)

Antimicrobial	Mean zone, mm	S, n (%)	I, n (%)	R, n (%)	S 95% CI, %
Doxycycline	12.9	4 (12)	13 (41)	15 (47)	5.0-28.1
Tylosin	9.1	0 (0)	2 (6)	30 (94)	0.0-10.7
Kanamycin	19.4	27 (84)	5 (16)	0 (0)	68.2-93.1
Streptomycin	19.1	28 (88)	3 (9)	1 (3)	71.9-95.0
Tetracycline	17.7	29 (91)	2 (6)	1 (3)	75.8-96.8
Neomycin	18.7	27 (84)	3 (9)	2 (6)	68.2-93.1
Ampicillin	18.3	22 (69)	4 (12)	6 (19)	51.4-82.0
Azithromycin	24.0	29 (91)	0 (0)	3 (9)	75.8-96.8
Ciprofloxacin	32.4	30 (94)	1 (3)	1 (3)	79.9-98.3
Enrofloxacin	29.7	31 (97)	0 (0)	1 (3)	84.3-99.4
Benzylpenicillin	6.5	0 (0)	0 (0)	32 (100)	0.0-10.7
Amoxicillin/clavulanate	21.3	30 (94)	0 (0)	2 (6)	79.9-98.3
Amoxicillin	15.6	14 (44)	8 (25)	10 (31)	28.2-60.7
Ceftriaxone	32.1	31 (97)	0 (0)	1 (3)	84.3-99.4
Cefotaxime	31.7	30 (94)	1 (3)	1 (3)	79.9-98.3
Imipenem	23.2	18 (56)	9 (28)	5 (16)	39.3-71.8
Gentamicin	23.4	32 (100)	0 (0)	0 (0)	89.3-100.0
Amikacin	22.6	32 (100)	0 (0)	0 (0)	89.3-100.0

Note: S/I/R, susceptible/intermediate/resistant; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute. S/I/R categories were assigned using the provided CLSI breakpoints where applicable. Azithromycin was interpreted as S ≥ 13.5 mm, I = 12.5–13 mm, and R ≤ 12 mm. "–" entries were treated as 0 mm/no inhibition zone so that n = 32 for every antimicrobial. Wilson 95% CI is shown for the susceptible proportion. Selected agent–organism combinations with limited interpretive relevance for Enterobacterales should be regarded as descriptive phenotypic observations rather than treatment recommendations.

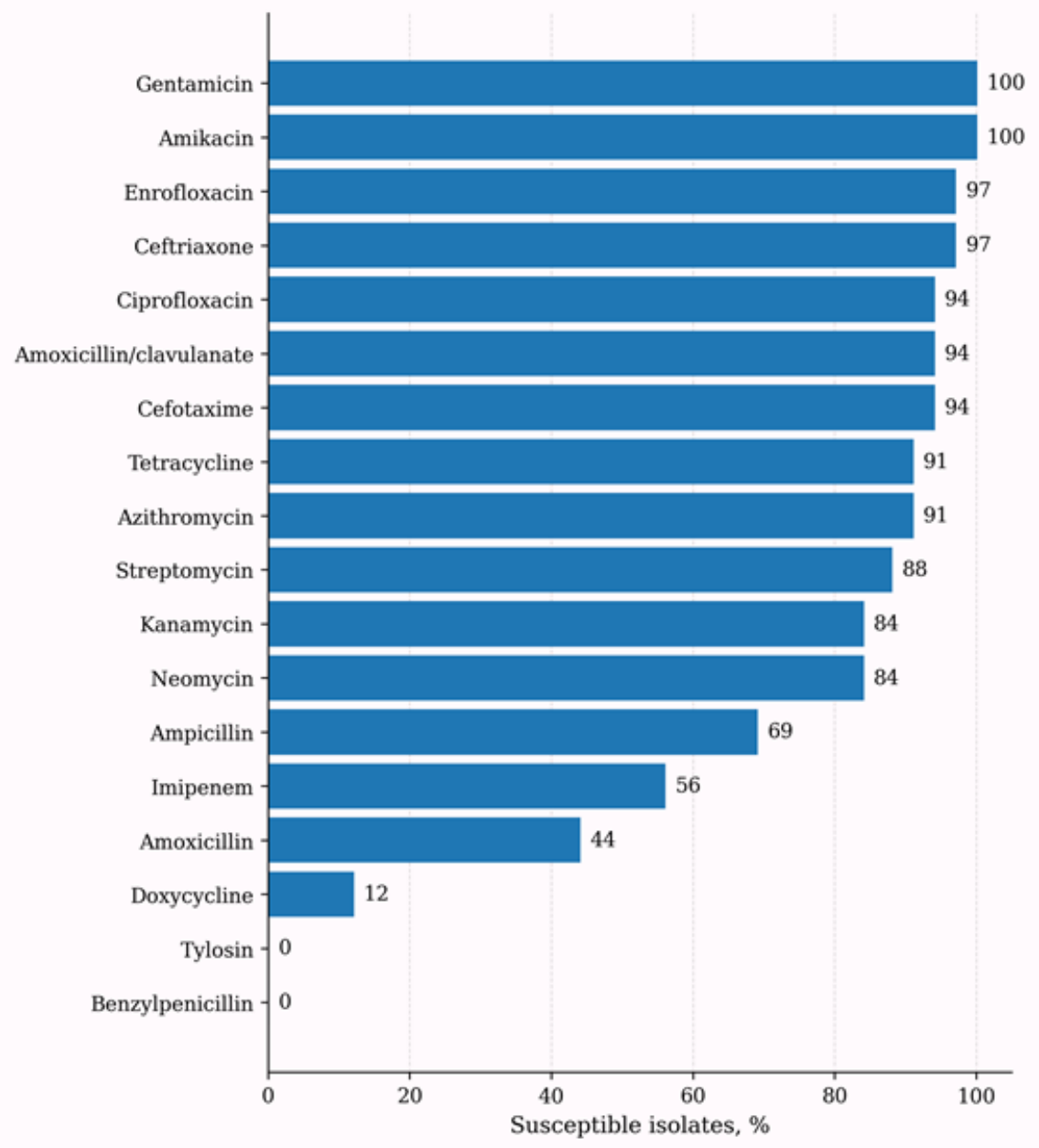

Figure 2. Percentage of susceptible isolates for the tested antimicrobials (n = 32; ordered by susceptible proportion)

Based on the updated CLSI interpretation, the highest susceptible proportions were observed for gentamicin and amikacin (32/32 each; 100%; Wilson 95% CI 89.3-100.0%). Very high susceptibility was also observed for enrofloxacin and ceftriaxone (31/32 each; 97%), followed by ciprofloxacin, cefotaxime, and amoxicillin/clavulanate (30/32 each; 94%), azithromycin and tetracycline (29/32 each; 91%), streptomycin (28/32; 88%), and kanamycin and neomycin (27/32 each; 84%).

Lower or variable susceptibility was observed for ampicillin (22/32; 69%), imipenem (18/32; 56%), and amoxicillin (14/32; 44%). Excluding benzylpenicillin and tylosin, which were retained as descriptive phenotypic observations, doxycycline showed the lowest susceptible proportion among the remaining agents (4/32; 12%) and also showed a large intermediate fraction (13/32; 41%) and high resistance (15/32; 47%).

The highest resistance rates were recorded for benzylpenicillin (32/32; 100%), tylosin (30/32; 94%), doxycycline (15/32; 47%), amoxicillin (10/32; 31%), ampicillin (6/32; 19%), and imipenem (5/32; 16%). Resistance to aminoglycosides, fluoroquinolones, and third-generation cephalosporins was

rare or absent, with no gentamicin or amikacin resistance and only one resistant isolate for ciprofloxacin, enrofloxacin, ceftriaxone, and cefotaxime.

Seventeen isolates were classified as MDR (17/32; 53.1%). Isolate-level MDR profiles are summarized in Table 2. Among MDR isolates, resistance most frequently involved penicillins/beta-lactams (17/17), macrolides (16/17), and tetracyclines (14/17), whereas fewer MDR isolates showed resistance involving carbapenems (5/17), aminoglycosides (2/17), and third-generation cephalosporins (1/17).

Table 2. Isolate-level MDR profiles of *E. coli* isolates

Isolate No.	No. of resistant classes	Resistant antimicrobial classes	Resistant antimicrobials
2	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
4	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (AMX)	DOX (Doxycycline), TYL (Tylosin), AMX (Amoxicillin)
6	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
7	4	Tetracyclines (DOX); Macrolides (TYL, AZM); Aminoglycosides (NEO); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), NEO (Neomycin), AZM (Azithromycin), PEN (Benzylpenicillin)
8	3	Tetracyclines (DOX); Macrolides (TYL, AZM); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), AZM (Azithromycin), PEN (Benzylpenicillin)
10	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
14	4	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN); Carbapenems (IPM)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin), IPM (Imipenem)
16	4	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN, AMX); Carbapenems (IPM)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin), AMX (Amoxicillin), IPM (Imipenem)
17	3	Tetracyclines (DOX, TET); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), TET (Tetracycline), PEN (Benzylpenicillin)
21	3	Macrolides (TYL); Aminoglycosides (STR); Penicillins/beta-lactams (PEN)	TYL (Tylosin), STR (Streptomycin), PEN (Benzylpenicillin)
23	3	Macrolides (TYL); Penicillins/beta-lactams (PEN, AMX); Third-generation cephalosporins (CRO, CTX)	TYL (Tylosin), PEN (Benzylpenicillin), AMX (Amoxicillin), CRO (Ceftriaxone), CTX (Cefotaxime)

Continuation of Table 2

25	4	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (AMP, PEN); Carbapenems (IPM)	DOX (Doxycycline), TYL (Tylosin), AMP (Ampicillin), PEN (Benzylpenicillin), IPM (Imipenem)
26	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
27	3	Tetracyclines (DOX); Penicillins/beta-lactams (PEN); Carbapenems (IPM)	DOX (Doxycycline), PEN (Benzylpenicillin), IPM (Imipenem)
28	3	Macrolides (TYL); Penicillins/beta-lactams (PEN, AMX); Carbapenems (IPM)	TYL (Tylosin), PEN (Benzylpenicillin), AMX (Amoxicillin), IPM (Imipenem)
31	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
32	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)

Note: Antimicrobial abbreviations: DOX, doxycycline; TYL, tylosin; STR, streptomycin; TET, tetracycline; NEO, neomycin; AMP, ampicillin; AZM, azithromycin; PEN, benzylpenicillin; AMX, amoxicillin; CRO, ceftriaxone; CTX, cefotaxime; IPM, imipenem. Only resistant antimicrobials recorded in MDR isolates are listed.

Discussion

This dataset from prescapular lymph nodes associated with market-derived bovine products showed a heterogeneous phenotype rather than generalized resistance. Gentamicin and amikacin showed complete *in vitro* susceptibility, and susceptibility to fluoroquinolones and third-generation cephalosporins remained high, although not absolute. In contrast, benzylpenicillin and tylosin showed the lowest in vitro activity, while doxycycline, amoxicillin, ampicillin, and imipenem showed mixed or reduced susceptibility. The isolate-level MDR table further showed that MDR was present in more than half of the examined isolates and was mainly driven by combinations involving penicillins/beta-lactams, macrolides, and tetracyclines.

The generally high susceptibility to ceftriaxone, cefotaxime, ciprofloxacin, and gentamicin was broadly consistent with previous cattle-associated studies. Grispoldi et al. reported high susceptibility of bovine lymph-node *E. coli* isolates to ceftriaxone, ciprofloxacin, cefotaxime, and gentamicin [2]. Similarly, slaughterhouse- and red-meat-production studies by Vázquez-Villanueva et al. and Guragain et al. showed that *E. coli* resistance patterns can vary by processing environment and production context, with some higher-importance agents retaining activity in specific datasets [5, 9].

The reduced or variable susceptibility todoxycycline, amoxicillin, and ampicillin agree with broader evidence that tetracycline and aminopenicillin-related resistance remains common in cattle-associated *E. coli.* Comparable patterns have been described in Canadian dairy herds, Bavarian dairy farms, Australian pasture-based dairy systems, and cattle isolates carrying diverse resistance genes [4, 6-8]. In the present dataset, doxycycline resistance was more prominent than tetracycline resistance, which may reflect differences in local breakpoint interpretation, disk performance, or isolate-level phenotype rather than a uniform class-wide pattern.

The added MDR table providesepidemiological detail beyond the agent-level susceptibility percentages. The most frequent resistant components among MDR isolates were benzylpenicillin and tylosin (16/17 MDR isolates each) and doxycycline (14/17), followed by imipenem (5/17) and amoxicillin

(4/17). Less frequent MDR components included azithromycin, neomycin, tetracycline, streptomycin, ceftriaxone, cefotaxime, and ampicillin. This pattern suggests that MDR in the present dataset was driven mainly by specific older or locally tested agents, whereas resistance to most fluoroquinolones and aminoglycosides remained uncommon.

Direct comparison with surveillance data from Central Asiaremains limited because available studies differ in animal category, sample source, and laboratory methods. Nevertheless, recent data from Kazakhstan demonstrate that bovine *E. coli* can carry clinically relevant resistance determinants; Alexyuk et al. reported a multidrug-resistant bovine *E. coli* isolate from Kazakhstan with multiple antimicrobial-resistance genes and phenotypic resistance to several drug classes [13]. A global geospatial analysis of food-animal AMR identified parts of Central Asia as predicted AMR hotspots; it also reported high average resistance prevalence to tetracycline in E. coli across low- and middle-income countries and found that many Asian locations were associated with penicillins or sulphonamides crossing critical resistance levels in future projections [14]. At the national level, Kazakhstan is developing AMR containment and veterinary-sector surveillance activities under a One Health framework [15].

The poor activity of benzylpenicillin and tylosin should not be interpreted as a direct therapeutic ranking because selected agent-organism combinations in the local panel have limited clinical relevance for Enterobacterales. Therefore, the MDR combinations shown in Table 2 are best understood as descriptive phenotypic patterns based onthe available panel. They should not be used as empirical treatment recommendations, especially for critically important agents or combinations lacking harmonized interpretive criteria. This interpretation is consistent with antimicrobialstewardship principles and with guidance emphasizing responsible and prudent antimicrobial use in veterinary medicine [10, 12].

This study has several limitations. First, this study included 32 *E. coli* isolates collected from market-derived bovine products in Semey during one season; therefore, the findings should be interpreted as preliminary local phenotypic data rather than as regional prevalence estimates. Second, although isolate-level MDR classification and Table 2 were added, complete animal- and farm-level metadata, including cattle age, production type, geographic origin before marketing, and previous antimicrobial exposure history, were not available. Third, complete disk lot metadata and quality-control records were not fully available in the laboratory documentation, and selected agent-organism combinations have limited interpretive relevance for *E. coli*. Fourth, PCR, sequencing, virulence-factor detection, and phylogenetic characterization were outside the scope of this work. Future studies should include larger numbersof samples, include prospective multi-season and multi-site sampling, retain isolate-level raw measurements and quality-control records, and combine phenotypic testing with molecular characterization of resistance determinants.

From a food-safety standpoint, monitoring peripheral lymph nodes may complement routine carcass-surface hygiene assessment because microorganisms located within lymphatic tissue are less accessible to external decontamination and may persist until trimming or further processing. For veterinary-sanitary control in Semey, continued seasonal sampling of such tissues could improve local risk assessment and support evidence-based surveillance of beef products offered for sale.

Conclusion

E. coli isolated from bovine superficial cervical (prescapular) lymph nodes sampled from market-derived bovine products in Semey showed a heterogeneous phenotypic susceptibility profile. Gentamicin and amikacin showed complete in vitro susceptibility, and fluoroquinolones and third-generation cephalosporins retained high activity against most isolates. By contrast, benzylpenicillin and tylosin showed poor activity based ondescriptive phenotypic observations, and doxycycline, amoxicillin, and imipenem showed reduced or mixed susceptibility. Seventeen *E. coli* isolates were classified as MDR, corresponding to an MDR frequency of 17/32 isolates (53.1%). The MDR profiles mainly involved resistance topenicillins/beta-lactams, macrolides, and tetracyclines, as shown in the added isolate-level table. Given the limited sample size, incomplete animal-level information, incomplete quality-control metadata, and absence of molecular characterization, these findings should be interpreted as preliminary local phenotypic surveillance data. These findingssupport continued monitoring of carcass-associated tissues and should be considered in conjunction with approved indications, food-animal regulations, and antimicrobial stewardship principles.

Authors' Contributions

ST: contributed to the study conception and design, sample processing, laboratory testing, data organization, descriptive analysis, visualization, and preparation of the original manuscript draft. BB: supervised the study, contributed to methodology validation and interpretation of results, and critically revised the manuscript. Both authors read and approved the final version of the manuscript.

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Statement on Competing Interests

The authors declare that they have no competing interests.

References

1 Mihailovskaya, V.S., Starcic Erjavec, M., Kuznetsova, M.V. (2024). Escherichia coli from healthy farm animals: Antimicrobial resistance, resistance genes and mobile genetic elements. *Acta Veterinaria Hungarica,* 72(4), 225234. DOI:10.1556/004.2024.01102.

2 Grispoldi, L., Karama, M., Hadjicharalambous, C., de Stefani, F., Ventura, G., Ceccarelli, M., Revoltella, M., Sechi, P., Crotti, C., D'Innocenzo, A., Couto-Contreras, G., Cenci-Goga, B. (2020). Bovine lymph nodes as a source of Escherichia coli contamination of the meat. *International Journal of Food Microbiology*, 331, 108715. DOI: 10.1016/j.ijfoodmicro.2020.108715.

3 Zhang, P., Wang, H., Klima, C., Yang, X. (2024). Microbiota in lymph nodes of cattle harvested in a Canadian meat processing plant. *Food Research International,* 191, 114693. DOI: 10.1016/j.foodres.2024.114693.

4 Fonseca, M., Heider, L.C., Stryhn, H., McClure, J.T., Leger, D., Rizzo, D., Warder, L., Dufour, S., Roy, J-P., Kelton, D.F., Renaud, D., Barkema, H.W., Sanchez, J. (2023). Intramammary and systemic use of antimicrobials and their association with resistance in generic Escherichia coli recovered from fecal samples from Canadian dairy herds: A cross-sectional study. *Preventive Veterinary Medicine*, 216, 105948. DOI: 10.1016/j.prevetmed.2023.105948.

5 Vazquez-Villanueva, J., Vazquez, K., Martinez-Vazquez, A.V., Wong-Gonzalez, A., Hernandez-Escareno, J., Cabrero-Martinez, O., Cruz-Pulido, W.L., Guerrero, A., Rivera, G., Bocanegra-Garcia, V. (2023). Molecular and antimicrobial susceptibility characterization of Escherichia coli isolates from bovine slaughterhouse process. *Antibiotics*, 12(2), 291. DOI:10.3390/antibiotics12020291.

6 Pirner, L.H., Petzl, W., Gangl, A., Huber-Schlenstedt, R., Sorge, U.S. (2024). In vitro antimicrobial resistance of Escherichia coli, Serratia marcescens, Klebsiella oxytoca, and Klebsiella pneumoniae on Bavarian dairy farms between 2014 and 2022. *Journal of Dairy Science*, 107(10), 8402–8412. DOI:10.3168/jds.2023-24536.

7 Tree, M., Lam, T.J.G.M., McDougall, S., Beggs, D.S., Robertson, I.D., Barnes, A.L., Chopra, A., Ram, R., Stockman, C.A., Kent, T.C., Aleri, J.W. (2025). Epidemiology of antimicrobial resistance in commensal Escherichia coli from healthy dairy cattle on a Mediterranean pasture-based system of Australia: A cross-sectional study. *Journal of Dairy Science*, 108(1), 803–820. DOI:10.3168/jds.2024-25157.

8 Tabaran, A., Soulageon, V., Chirila, F., Reget, O.L., Mihaiu, M., Borzan, M., Dan, S.D. (2022). Pathogenic E. coli from cattle as a reservoir of resistance genes to various groups of antibiotics. *Antibiotics,* 11(3), 404. DOI:10.3390/antibiotics11030404.

9 Guragain, M., Schmidt, J.W., Bagi, L.K., Paoli, G.C., Kalchayanand, N., Bosilevac, J.M. (2024). Antibiotic resistance and disinfectant resistance among Escherichia coli isolated during red meat production. *Journal of Food Protection,* 87(6), 100288. DOI: 10.1016/j.jfp.2024.100288.

10 European Committee on Antimicrobial Susceptibility Testing (EUCAST). (2024). *When there are no breakpoints? Guidance document*. https://www.eucast.org/bacteria/clinical-breakpoints-and-interpretation/when-there-are-no-breakpoints/

11 Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, 18(3), 268–281. DOI:10.1111/j.1469-0691.2011.03570.x.

12 World Organisation for Animal Health (WOAH). (2024). *Terrestrial Animal Health Code. Chapter 6.10. Responsible and prudent use of antimicrobial agents in veterinary medicine.* https://www.woah.org/fileadmin/Home/eng/Health_standards/tahc/2024/en_chapitre_antibio_use.htm

13 Alexyuk, P.G., Bogoyavlenskiy, A.P., Moldakhanov, Y.S., Akanova, K.S., Manakbayeva, A.N., Kerimov, T., Berezin, V.E., Alexyuk, M.S. (2025). Genomic and drug resistance profile of bovine multidrug-resistant Escherichia coli isolated in Kazakhstan. *Pathogens,* 14(1), 90. DOI:10.3390/pathogens14010090.

14 Zhao, C., Wang, Y., Mulchandani, R., Van Boeckel, T.P. (2024). Global surveillance of antimicrobial resistance in food animals using priority drugs maps. *Nature Communications*, 15, 763. DOI:10.1038/s41467-024-45111-7.

15 Food and Agriculture Organization of the United Nations (FAO). (2025). FAO Kazakhstan holds roundtable on tackling antimicrobial resistance in Kazakhstan. https://www.fao.org/countryprofiles/news-archive/detail-news/en/c/1735515/

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Research article

Determinants of the epizootic situation in the middle east: the interaction of historical, climatic, and socio-economic factors

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Abstract

Background and Aim. The Middle East is one of the most complex epizootic regions in the world due to the interaction of historical, natural, climatic, and socioeconomic factors that influence the spread of infectious animal diseases. Traditional epidemiological approaches cannot fully explain the persistence of epizootic threats in the region. The aim of this study is to conduct a comprehensive analysis of the epizootic architecture of the Middle East as a complex metasystem operating at the intersection of historical, environmental, and political-economic determinants.

Materials and Methods. The study was conducted as a meta-analysis and retrospective modeling for the period 2020–2024. Data from the international monitoring systems WOAH WAHIS and Handistatus II, FAO and ECDC materials, as well as scientific publications and analytical reports for 2005–2024 were used. A systematization, comparative analysis, and qualitative predictive modeling of epizootological processes were conducted, taking into account spatiotemporal, climatic, and socioeconomic parameters.

Results. It has been established that the epizootic situation in the Middle East is determined by three interrelated groups of factors: historical-institutional, natural-climatic, and socio-economic. The region is a heterogeneous megasystem, encompassing four types of epizootic regimes: high-tech, crisis, transit, and isolationist. Crisis areas form persistent infection hotspots, while transit countries increase the spread of pathogens through migration and animal trade. Climate change contributes to the concentration of livestock and an increased risk of disease outbreaks.

Conclusion. Effective management of epizootological risks requires a shift from universal veterinary measures to differentiated strategies that take into account the historical, environmental, and socio-economic characteristics of individual countries and subregions of the Middle East.

Keywords: Biosecurity; climatic determinants; epizootic regimes; epizootiology; historical gap; Middle East.

Introduction

The Middle East, recognized as the cradle of animal domestication and the locus where early veterinary practices emerged, currently represents one of the world's most complex and multifaceted epizootiological regions [2–4]. Despite substantial investments in biosecurity and disease control programs in the region, the region characterized by the persistence and periodic large-scale outbreaks of socio-economically significant infections such as foot-and-mouth disease (FMD), peste des petits

ruminants (PPR), Middle East respiratory syndrome (MERS), and rabies [1, 8, 12, 15, 21].

Traditional epidemiological approaches, which primarily focus on the host-agent-environment triad, prove insufficient to explain the resilience of these threats and the frequent failure of standardized interventions. Paradoxically, intensive control measures in technologically advanced enclaves coexist with a complete institutional collapse in conflict zones, while isolation efforts are undermined by flourishing informal transboundary livestock trade networks. This apparent inconsistency suggests that the region's epizootiological picture is not a random aggregation of national statistics, but rather an emergent property of a deeper, systemic reality [7, 13, 14, 18, 20].

Existing research in the field of veterinary epidemiology in the Middle East is generally limited to the analysis of specific nosologies or the evaluation of technical control aspects within individual countries. At the same time, the fundamental determinants shaping the very architecture of regional epizootiological risks remain poorly understood. These include: historical trajectories that have created a gap between indigenous practices and imported institutions; climatic dynamics acting as an active agent in the spatial reorganization of animal husbandry; and, most crucially, the structural politico-economic heterogeneity that generates fundamentally different regimes of production and management of bio-threat [10, 11, 16, 17, 22]. The lack of an integrative view considering these layers in their synergetic interaction leads to the inadequacy of strategies based on universal "recipes" and direct technology transfer [6, 9, 17].

In this regard, there is an urgent need for a new analytical framework capable of describing the Middle East as an integrated but internally heterogeneous meta-system. This approach involves moving from the study of outbreaks to the analysis of stable "epizootological regimes", from the search for technical solutions to the political and economic diagnosis of systemic causes of vulnerability. Understanding the region as a complex dissipative system where pathogen circulation patterns arise from non-linear relationships between historically established institutions, transformable ecological niches, and contrasting socio-economic orders is key to developing effective and sustainable risk management strategies.

Research Objective: Conducting a comprehensive analysis of the epizootological architecture of the Middle East as a complex metasystem operating at the intersection of historical, environmental and political-economic determinants.

Materials and Methods

The present study is a comprehensive meta-analysis and retrospective modeling conducted between 2020 and 2024 in order to integrate disparate epizootological data on the Middle East into a single socio-ecological model of the meta-system. The work was based on the systematization and critical synthesis of data from multiple sources, which made it possible to move from the analysis of individual outbreaks to the study of the architecture of regional epizootological regimes.

For the retrospective analysis, structured data from open international systems were used: official reports and the database of the World Organization for Animal Health (WOAH) – the WAHIS (World Animal Health Information System) system and the Handistatus II archive (for the period 2005-2024); statistical and reporting materials of the Food and Agriculture Organization of the United Nations (FAO), joint bulletins FAO-WOAH, as well as reports from the European Center for Disease Prevention and Control (ECDC) and relevant regional networks. For political economic and historical analysis, national legislative acts, reports on the development of the agro-industrial complex, historical research, publications in peer-reviewed journals on veterinary epidemiology and social sciences, field research materials and expert interviews were used. All quantitative data (on outbreaks, livestock numbers, vaccinations, livestock movements) have been cross-checked from several sources to minimize errors related to incomplete or contradictory official reporting, especially in areas of instability.

Disparate data was systematized into a single relational database with spatio-temporal reference. Key parameters included: geographic location (country, region), time period (year, season), nosological entity (FMD, PPR, rabies, etc.), animal species, epidemiological indices (number of outbreaks, cases, mortality), and intervention data (vaccination and diagnostic volumes). A comparative systems analysis was conducted by integrating quantitative data and qualitative descriptors (economic basis, animal status, risk configuration, control type). This resulted in the development of a conceptual politico-

economic typology identifying four ideal types of the Middle East metasystem: "Purchased Security," "Collapse Zone," "Transit Hub," and "Bio-Fortress." Qualitative predictive modeling was used to assess the sustainability of regimes and the effectiveness of control strategies. Scenarios were constructed taking into account key drivers identified in the meta-analysis: the density of susceptible populations, the permeability of administrative and ecological boundaries, the intensity of economic and trade links, and the adaptability of formal and informal control institutions. The meta-analysis results are presented in a figure and table.

Statistical Analysis and Operationalization of Epizootic Regimes

To transition from a purely conceptual framework to a verifiable quantitative model and establish objective criteria for the proposed country classification, a ROC (Receiver Operating Characteristic) curve analysis was executed. Countries of the Middle East meta-system were divided into two operational cohorts based on epidemiological data: "Favorable" (pathogen prevalence below the calculated regional baseline average) and "Unfavorable" (prevalence strictly exceeding the regional average).

The core evaluation variable utilized to differentiate these regimes was the estimated national livestock vaccination coverage. Through iterative assessment of varying coverage thresholds, diagnostic sensitivity (the probability of accurately identifying epidemiologically unfavorable territories) and specificity (the probability of correctly identifying favorable, high-tech, or isolated enclaves) were calculated. The optimal operational cut-off point was determined using the maximum trade-off balance between sensitivity and specificity, thereby formalizing the boundary conditions separating "Crisis-Driven/ Collapse Zone", " Transit Hub", and "High-Tech/Isolationist (Bio-Fortress)" regimes.

Results

The results of the meta-analysis show that the epizootic situation in the Middle East is shaped by three interconnected groups of factors that not only overlap but actively interact sometimes reinforcing, sometimes weakening each other This synergy leads to the emergence of complex, often unpredictable pathogen circulation patterns specific to this region (Figure 1).

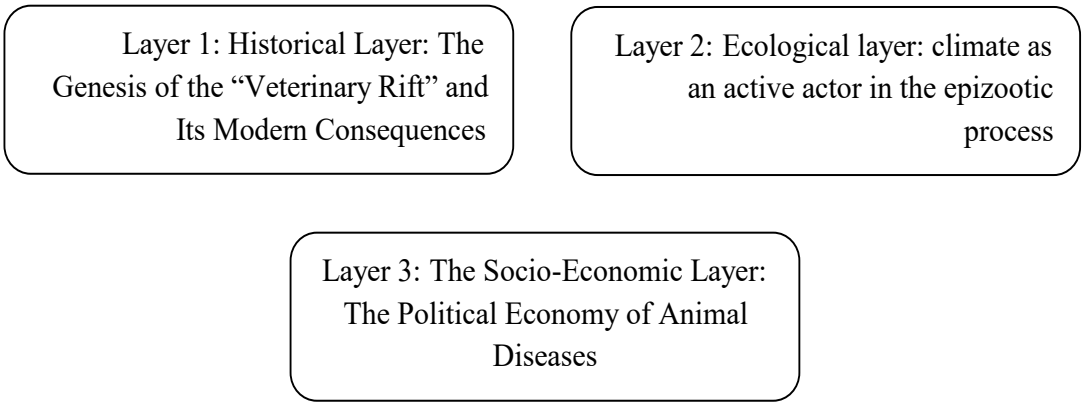

Figure 1. A conceptual model of the interaction of determinants of the epizootological landscape of the Middle East

Layer 1. Historical and institutional factors: In the region, there remains a gap between traditional animal husbandry practices and modern veterinary control systems, which creates conditions for the preservation and spread of infections.

Layer 2. Climatic conditions: Climate determines the structure of livestock farming and livestock migration routes, contributing to the formation of stable disease outbreaks in areas with a high concentration of animals.

Layer 3. Socio-Economic and political factors: Significant differences in the level of economic development, the state of veterinary services and political stability between the countries of the region lead to the formation of different types of epizootic regimes - from zones with effective control to territories with uncontrolled spread of infections.

An analysis of historical data reveals that veterinary knowledge in the Middle East developed discontinuously rather than linearly. The region's ancient civilizations (Egypt, Mesopotamia, Persia)

developed a coherent system of knowledge on animal care, in which practical experience was closely linked to the religious, economic, and social functions of animals. This system, reflected in sources such as the Avesta and Hippiatria, represented a closed body of knowledge transmitted within narrow circles of priests and specialists.

A key turning point occurred during the formation of modern science. In the 18th and 19th centuries, veterinary knowledge in Europe was secularized, systematized, and incorporated into university curricula and government services. In the Middle East, a similar process failed to develop due to specific historical conditions: prolonged Ottoman rule, the colonial period, and subsequent political instability. As a result, local veterinary traditions were supplanted, and the adoption of Western models was largely formal, without addressing the fundamentals of veterinary service organization. This led to a persistent institutional deficit: the absence of a training system, scientific schools, and an independent diagnostic base. Two parallel systems developed in the region: a formal, weak one dependent on international aid, and an informal one based on traditional practices and commercial interests.

The climate of the Middle East actively influences the epizootic situation in the region. Increased aridity leads to a reduction in areas suitable for animal husbandry and the concentration of livestock around limited water sources and irrigation systems. Such places become stable foci of pathogen circulation, where the risk of infection outbreaks is particularly high. The established system of seasonal cattle runs forms a network of routes along which diseases spread. Analysis of these pathways makes it possible to predict outbreaks and plan preventive measures, such as vaccination, taking into account real animal flows rather than administrative boundaries. Extreme temperatures and water scarcity not only weaken animals, but can also alter the course of diseases. For example, with foot and-mouth disease, dehydration increases mucosal lesions, and heat stress disrupts the immune response, making diagnosis difficult. Thus, the climate not only creates conditions for the transmission of infections, but also affects their clinical manifestation and the effectiveness of treatment.

Contemporary socio-economic differentiation among Middle Eastern countries has led to the emergence of distinct types of epizootiological risks. Divergences in economic development, political stability, and the organization of veterinary services dictate the patterns of spread and control of infectious animal diseases across the region. The classification of these Systems is detailed in Table 1.

Table 1. The Political Economy of Epizootiological Systems in the Middle East

System Type	Characteristics		
	Economic Basis	Primary Epizootiological Risk	Nature of Veterinary Control
High-Tech (Qatar, UAE, Israel)	Oil and Gas Sector, High Technologies	Infections brought in from outside due to logistical failures	High-tech, but dependent on imported equipment and drugs
Crisis-Driven/Fragile (Yemen, Syria, Gaza)	Survival economy, destruction of state institutions	Establishment of stable endemic foci (FMD, rabies)	Absent or declarative in nature
Transit/Hub-Based (Türkiye, Jordan)	Intermediation and semi-legal livestock trade	Amplification and dissemination of infections through livestock flows	Adaptive, often prone to corrupt practices
Isolationist/Enclosed (Cyprus, Bahrain)	Isolation as an asset (tourism, finance)	The infection breach across borders as a threat to the economic model	Strict preventive control, which requires significant investment

The presented table reflects not just a classification of countries, but structural differences in the formation of epizootic threats in the Middle East. Each type of system creates specific risks, and the means of protection against them are derived from the same socio-economic system. At the same time, high-tech import-dependent countries (Qatar, the United Arab Emirates, Israel) are transferring

biosafety functions to external suppliers of technologies and standards. Their vulnerability lies in a critical dependence on global logistical chains, which themselves serve as conduits for the introduction of infections. Effective border control does not protect against new threats emerging in neighboring regions. It should be noted that, within conditions of institutional vacuum, crisis zones (Yemen, Syria, the Gaza Strip) become persistent reservoirs of infection. Here, diseases such as FMD or rabies become endemic.

Uncontrolled use of antibiotics leads to the formation of foci of antibiotic resistance. Veterinary control in such conditions is practically impossible due to the lack of accounting systems and government regulation.

It has been demonstrated that transit countries (Turkey, Jordan) act as amplifiers of epizootiological risks. Their informal livestock markets and adaptive, often corrupt, control systems contribute to the mixing of pathogens from different foci and their further spread, making threats unpredictable. At the same time, isolationist states (Cyprus, Bahrain) rely on strict preventive border control, as their economies directly depend on veterinary and sanitary status. This strategy is effective, yet resource-intensive and highly vulnerable to isolated security failures.

Consequently, the primary threats originate within crisis zones and are subsequently amplified in transit countries. Rich and isolated States are trying to insulate themselves from these risks, but their security remains illusory due to the continued economic and trade ties in the region. Therefore, epizootic risk management in the Middle East requires coordination of actions between countries with different socio-economic conditions rather than unification of veterinary protocols. Overcoming epizootic cycles is impossible without transforming the structural causes that give rise to these threats.

The Middle East is a region where climate change, social inequality and political instability create unique conditions for the emergence and spread of epizootics. The study of these processes makes it possible to predict global biological threats in the context of modern human impact on the environment.

In crisis zones (Syria, Yemen), the uncontrolled use of veterinary drugs contributes to the formation of foci of antibiotic resistance. The disruption of traditional ecological boundaries leads to the emergence of hybrid disease reservoirs, creating a significant risk for the genesis of novel pathogens. At the same time, modern epidemiological surveillance systems using big data and satellite monitoring are being introduced in the developed countries of the region (Gulf States, Israel). In parallel, the proliferation of disinformation regarding diseases and vaccines across social platforms complicates outbreak management and response efforts.

The current epizootic situation in the region is the result of the interaction of three groups of factors: historical and cultural (institutional gaps in the veterinary system); natural and climatic (impact on animal migration and population concentration); socio-economic (formation of various epizootological regimes).

The analysis shows that the region consists of four interconnected, but structurally different subsystems. Disease control strategies that are effective in one subsystem (for example, strict border controls in isolated states) may be not only useless but also harmful in another (in humanitarian crisis zones). Therefore, the management of epizootological risks requires a differentiated approach that takes into account the specific socio-economic and political conditions of each territory.

In conclusion, ensuring veterinary and epidemiological security in the Middle East necessitates a paradigm shift from reactive outbreak response toward proactive strategic planning. This does not imply a unification of measures, but the development of targeted programs taking into account the historical context, environmental characteristics, and economic structure of each subregion.

Quantitative Validation of the Typology via ROC Analysis

To objectively justify the demarcation between the identified epizootic regimes, the relation between vaccination coverage thresholds and regional epidemiological stability was mathematically modeled. The simplified distribution of threshold metrics across the analyzed 17 territories demonstrates a clear non-linear correlation between systemic coverage and pathogen containment capability. The operational outputs of the ROC-curve analysis are structured in Table 2.

Table 2. ROC-Analysis Parameters for Vaccine Coverage Thresholds in Epizootic Risk Control

Vaccination coverage threshold	Sensitivity (TPR)	Specificity (TNR)
<20%	100% (all are crisis)	0% (No high-tech case was identified)
<30%	90%	40%
<40%	75%	65%
<50%	60%	80%
<60%	40%	95%
<70%	10%	100%

The calculated optimal mathematical cut-off point ensuring sustained baseline control over the transboundary epizootic process stands at 45%–50% vaccination coverage. Dropping below this specific threshold causes the model's sensitivity in identifying epidemiological crisis reservoirs to surge past 80%. Conversely, when national vaccination programs achieve stable thresholds exceeding 70%, the specificity of isolating favorable, bio-secure environments reaches up to 95%.

This quantitative stratification confirms that a minimum threshold of 50% coverage is strictly required to avert uncontrolled pathogen replication, while a comprehensive target of 70%–80% immunization coverage is mathematically necessary for robust disease elimination within regional livestock networks, directly correlating with standard WOAH guidelines.

Discussion

The results of this study demonstrate that the epizootic situation in the Middle East cannot be adequately described or predicted using conventional epidemiological models based solely on the host – agent–environment triad. Instead, our meta-analysis reveals a more complex, emergent phenomenon shaped by the synergistic interaction of historical, climatic, and socio-economic determinants. These findings align with recent calls for a systemic, transdisciplinary approach to veterinary epidemiology, particularly in regions characterized by high heterogeneity and rapid socio-environmental change [5, 6, 9, 16].

Our identification of a “veterinary rift” – a persistent institutional gap between formal, imported veterinary systems and informal, traditional practices – is consistent with historical analyses of post-colonial veterinary medicine in the Middle East and North Africa [9, 16]. Unlike previous studies that focused on individual countries or specific diseases, our research provides a region-wide conceptual model that links this institutional deficit directly to the failure of standardized disease control programmes. This finding is also supported by recent work on transboundary livestock networks in Africa and Asia, where informal trade routes often bypass official veterinary checks, creating parallel pathogen circulation pathways [8, 18, 20].

The role of climate as an active driver of epizootic processes has been increasingly recognized in the literature [6, 7, 22]. Our study contributes to this body of knowledge by specifically demonstrating how increasing aridity and seasonal water concentration transform specific ecological zones into persistent pathogen reservoirs. Moreover, we show that traditional seasonal migration routes (transhumance) not only persist but have become major corridors for the transboundary spread of diseases such as foot-and-mouth disease and peste des petits ruminants. This finding challenges the assumption that modernization of livestock production automatically reduces epizootic risks [10, 11].

The typology of four epizootic regimes – high-tech, crisis, transit, and isolationist – represents a novel contribution to veterinary political economy. While previous research has noted the correlation between economic development and disease control outcomes [1, 3, 12], our study goes further by demonstrating that these regimes are not isolated but are dynamically interconnected. For example, the amplification of pathogens in transit countries (Turkey, Jordan) and their spillover into high-tech enclaves (Israel, the Gulf states) has been documented for specific diseases such as rabies and avian influenza [1, 2, 13]. However, our work provides a unified explanatory framework applicable across multiple nosologies.

The integration of the ROC-curve analysis directly addresses the conceptual-quantitative gap within our regional model, granting objective mathematical validation to the proposed four-tier typology. Notably, the statistical identification of the optimal 45%–50% cut-off point clarifies why specific territories function inherently as "risk amplifiers". Transit hubs (such as Türkiye and Jordan), burdened by highly dynamic, semi-legal transboundary trade corridors, consistently operate with livestock immunization rates hovering around this critical 30%–50% margin.

Because their collective population immunity resides precisely at the mathematical tipping point identified by the ROC model, these transit systems possess insufficient immunological depth to interrupt pathogen transmission chains. Consequently, when highly vulnerable animal populations from intense crisis zones (vaccination coverage <30\%) pass through these under-immunized transit markets, the biological threats are amplified rather than contained. This statistical reality emphasizes that universal veterinary protocols will fail unless transit zones are incentivized to push their structural coverage ratios past the 70% specificity threshold.

The finding that universal veterinary protocols are ineffective across diverse regimes has profound implications for international organizations (WOAH, FAO) and donors. Rather than promoting one-size-fits-all solutions, our results advocate for asymmetric interventions tailored to the specific risk profile of each subsystem. For crisis zones, the priority should be rebuilding basic surveillance and cold-chain infrastructure, combined with conflict-sensitive vaccination campaigns. For transit countries, strengthening border controls and traceability systems is critical, alongside measures to reduce corruption in livestock markets. For high-tech and isolationist states, the main challenge is to maintain vigilance and invest in early warning systems that can detect pathogens emerging in neighbouring crisis or transit zones before they cross borders.

Furthermore, our analysis underscores the need to integrate climate adaptation strategies into veterinary planning. As aridity increases, the concentration of livestock around artificial water points will intensify, creating predictable hotspots for disease transmission. Targeted vaccination and vector control in these areas could yield high returns.

Several limitations must be acknowledged. First, the study relies on officially reported data from WOAH and national sources, which are known to be incomplete, particularly for crisis zones (Yemen, Syria, parts of Iraq). Underreporting of outbreaks may bias prevalence and mortality estimates downwards. To mitigate this, we cross-verified data from multiple sources and used qualitative information from expert interviews and field reports, but some degree of uncertainty remains.

Second, our typology of epizootic regimes is conceptual and qualitative. While it captures the essential structural differences, it does not provide quantitative thresholds (e.g., economic indicators or governance scores) for classifying countries. Future research could develop a composite index to allow dynamic tracking of regime shifts over time.

Third, the analysis of climatic determinants was based on general regional trends rather than high-resolution spatiotemporal modelling. Future studies using satellite data and species distribution modelling could refine our understanding of how specific climatic variables (temperature, precipitation extremes) drive pathogen transmission at local scales.

Fourth, the study did not explicitly model the impact of armed conflicts on veterinary infrastructure and reporting systems, although this was identified as a key driver in crisis zones. Detailed conflict-epizootiology studies are needed to quantify these effects.

Our findings open several avenues for future investigations. First, quantitative modeling of pathogen spillover between the four epizootic regimes could inform risk-based surveillance. Second, economic analyses of the cost-effectiveness of asymmetric interventions are urgently needed to justify investment in regionally coordinated control programmes. Third, the role of wildlife – both as reservoirs and as sentinels of environmental change – was beyond the scope of this study but should be integrated into future One Health frameworks.

In summary, this study challenges reductionist approaches to veterinary epidemiology in complex regions. By integrating historical, climatic, and socio-economic determinants into a single analytical framework, we demonstrate that the Middle East functions as a dissipative meta-system where traditional control strategies fail because they ignore the structural causes of vulnerability. Effective risk management requires a paradigm shift from reactive outbreak response to proactive, context-sensitive, and regionally coordinated strategic planning.

Conclusion

The conducted analysis demonstrates that the epizootic situation in the Middle East cannot be explained solely by biological factors or the quality of veterinary services in individual countries. Instead, it emerges from the non-linear interaction of three groups of determinants: historical-institutional, natural-climatic, and socio-economic. First, the historical-institutional rift – rooted in the incomplete secularization and systematization of veterinary knowledge during the 19th century – has resulted in a persistent institutional deficit. Formal veterinary systems remain weak and dependent on external aid, while informal traditional practices continue to dominate, creating a parallel structure that undermines effective disease control. Second, climatic conditions act as an active driver of the epizootic process. Increasing aridity concentrates livestock around scarce water sources, transforming these areas into stable pathogen reservoirs. Seasonal migrations of animals along traditional routes further facilitate the transboundary spread of infections, rendering administrative borders irrelevant. Third, the socio-political polarization of the region has given rise to four distinct epizootic regimes: high-tech (e.g., UAE, Israel), crisis-driven (Yemen, Syria, Gaza), transit (Turkey, Jordan), and isolationist (Cyprus, Bahrain). These regimes generate fundamentally different risks and responses, yet remain interconnected through trade, migration, and informal livestock movements. Consequently, threats originating in crisis zones are amplified in transit countries and only partially contained by high-tech or isolationist states, whose security is fragile due to persistent regional interdependencies. The key finding of this study is that universal veterinary protocols are insufficient and may even be counterproductive. Effective risk management requires a differentiated, regionally coordinated strategy that moves from reactive outbreak response to proactive, context-specific planning. This strategy must address the root causes of vulnerability, including institutional gaps, climate-induced ecological changes, and socio-economic inequalities. Future research should focus on quantifying the interactions between these determinants, developing predictive models for pathogen spread under climate change scenarios, and assessing the cost-effectiveness of targeted interventions in each epizootic regime.

Authors' Contributions

YK: data curation, formal analysis, visualization, writing – original draft; PR: conceptualization, methodology, supervision, writing – review & editing; YuV: methodology, investigation, writing – review & editing; VS: formal analysis, data curation; EK: investigation, visualization. All authors approved the final manuscript.

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Statement on Competing Interest

The authors declare that they have no competing interests.

References

1 Vatnikov, Yu.A., Rudenko, P.A., Rudenko, A.A., et al. (2021). Kliniko-terapevticheskoe znachenie mikrobioty pri gnojno-vospalitel'nyh processah u zhivotnyh [Clinical and therapeutic significance of microbiota in purulent-inflammatory processes in animals]. *Mezhdunarodnyi vestnik veterinarii,* 1, 286–291. DOI: 10.17238/issn2072-2419.2021.1.286. [*In Russian*].

2 Khatib, Ya, Rudenko, P.A. (2025). Rasprostranenie ptich'ego grippa na Blizhnem Vostoke [Spread of avian influenza in the Middle East]. *Veterinariya*, 1, 23–28. [*In Russian*].

3 Khatib, Ya., Rudenko, P.A. (2025). Chuma MRS, rasprostranenie na Blizhnem Vostoke i ekonomicheskoe znachenie [Peste des petits ruminants (PPR), its spread in the Middle East and economic significance]. *Veterinaria i kormlenie*, 1, 78–83. [*In Russian*].

4 Rudenko, A., Glamazdin, I., Lutsay, V., et al. (2022). Parasitocenoses in cattle and their circulation in small farms. *E3S Web of Conferences,* 363, 03029.

5 Abdrakhmanov, S.K., Mukhanbetkaliyev, Y.Y., Ruzmatov, S.I., Mukhanbetkaliyeva, A.A. (2025). Spread of highly pathogenic avian influenza in Kazakhstan: investigating the role of bird migration and threats to poultry farming. *Herald of science of s seifullin kazakh agro technical research university: veterinary sciences,* 3(011), 52–60. DOI: 10.51452/kazatuvc.2025.3(011).2013.

6 Aharonson-Raz, K., Steinman, A., Kavkovsky, A., et al. (2017). Analysis of the Association of Climate, Weather and Herd Immunity with the Spread of Equine Encephalosis Virus in Horses in Israel. *Transbound Emerg Dis.*, 64(2), 593–602. DOI: 10.1111/tbed.12424.

7 Al-Eitan, L., Khair, I., Shakhatreh, Z., et al. (2024). Epidemiology, biosafety, and biosecurity of Avian Influenza: Insights from the East Mediterranean region. *Rev Med Virol*., 34(4), e2559. DOI: 10.1002/rmv.2559.

8 Ali, H., Ali, A., Al Mawly, J., et al. (2024). Molecular characterization of rabies virus from wild and domestic animals in the Sultanate of Oman. *Zoonoses Public Health,* 71(7), 836–843. DOI: 10.1111/zph.13164.

9 Arzt, J., White, W.R., Thomsen, B.V., et al. (2010). Agricultural diseases on the move early in the third millennium. *Vet Pathol.,* 47(1), 15–27. DOI: 10.1177/0300985809354350.

10 Awaidy, S.A., Al Hashami, H. (2020). Zoonotic Diseases in Oman: Successes, Challenges, and Future Directions. *Vector Borne Zoonotic Dis.,* 20(1), 1–9. DOI: 10.1089/vbz.2019.2458.

11 Colina, S.E., Serena, M.S., Echeverría, M.G., et al. (2021). Clinical and molecular aspects of veterinary coronaviruses. *Virus Res.*, 297, 198382. DOI: 10.1016/j.virusres.2021.198382.

12 Hobbs, E.C., Colling, A., Gurung, R.B., et al. (2021). The potential of diagnostic point-of-care tests (POCTs) for infectious and zoonotic animal diseases in developing countries: Technical, regulatory and sociocultural considerations. *Transbound Emerg Dis.*, 68(4), 1835–1849. DOI: 10.1111/tbed.13880.

13 Houta, M.H., Hassan, K.E.., El-Sawah, A.A., et al. (2021). The emergence, evolution and spread of infectious bronchitis virus genotype GI-23. *Arch Virol*., 166(1), 9–26. DOI: 10.1007/s00705-020-04920-z.

14 Islam, M.M., Farag, E., Mahmoudi, A., et al. (2021). Rodent-Related Zoonotic Pathogens at the Human-Animal-Environment Interface in Qatar: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health.*, 18(11), 5928. DOI: 10.3390/ijerph18115928.

15 Lin, Q., Chiu, A.P., Zhao, S., et al. (2018). Modeling the spread of Middle East respiratory syndrome coronavirus in Saudi Arabia. *Stat Methods Med Res.*, 27(7), 1968–1978. DOI: 10.1177/0962280217746442.

16 Novick, T. (2022). On All Fours: Transient Laborers, the Threat of Movement, and the Aftermath of Disease. *Bull Hist Med.*, 96(3), 431–457. DOI: 10.1353/bhm.2022.0035.

17 Rabiee, M.H., Mahmoudi, A., Siahsarvie, R., et al. (2018). Rodent-borne diseases and their public health importance in Iran. *PLoS Negl Trop Dis.,* 12(4), e0006256. DOI: 10.1371/journal.pntd.0006256.

18 Rapoport, E., Shimshony, A. (1997). Health hazards to the small ruminant population of the Middle East posed by the trade of sheep and goat meat. *Rev Sci Tech.,* 16(1), 57–64.

19 Rosenberg, R. (2015). Detecting the emergence of novel, zoonotic viruses pathogenic to humans. Cell Mol Life Sci., 72(6), 1115–1125.

20 Seimenis, A., Tabbaa, D. (2014). Stray animal populations and public health in the South Mediterranean and the Middle East regions. *Vet Ital.*, 50(2), 131–136. DOI:10.12834/VetIt.48.134.3.

21 Sikkema, R.S.., Farag, EABA, Islam, M., et al. (2019). Global status of Middle East respiratory syndrome coronavirus in dromedary camels: a systematic review. *Epidemiol Infect.*, 147, e84. DOI: 10.1017/S095026881800345X.

22 Tabasi, M., Alesheikh, A.A., Sofizadeh, A., et al. (2020) A spatio-temporal agent-based approach for modeling the spread of zoonotic cutaneous leishmaniasis in northeast Iran. *Parasit Vectors.*, 13(1), 572. DOI: 10.1186/s13071-020-04447-x.

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Review article

Review of the prospects for the use of postbiotics in the growing of broiler chickens: from probiotics to postbiotics

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Abstract

Background and Aim. Systematization of current data on the impact of postbiotics on the health and productivity of poultry to justify their use in industrial poultry farming as an effective and safe alternative to antibiotic growth promoters.

Materials and Methods. This article examined review articles on the relevance and effectiveness of postbiotics in poultry farming, published between 2020 and 2026. The analysis was based on 106 review articles published in international and domestic scientific journals with varying impact factors.

Results. A review of scientific publications on the use of postbiotic preparations in poultry farming was conducted. The analysis of available sources demonstrated that postbiotics are considered a promising alternative to probiotics and antibiotic growth promoters due to their high stability, safety, and pronounced biological activity. The results of the review indicate that the use of postbiotic metabolites contributes to the improvement of gastrointestinal health in poultry, enhances feed digestibility, stimulates growth, and reduces the prevalence of pathogenic microorganisms. In addition, postbiotics have a positive effect on the formation of a balanced intestinal microbiota and the strengthening of the immune status of birds.

Conclusion. Thus, the findings of the literature analysis confirm the significant potential for the development and implementation of postbiotic preparations in poultry nutrition technologies, which may contribute to increased productivity and improved biosafety in poultry production.

Keywords: postbiotic; probiotic; eubiosis; microbiota; immune system; homeostasis.

Introduction

At the current stage of industrial poultry farming development, it is important not only to maintain the current level of poultry production but also to improve its quality and safety, including the creation of a domestic technological and breeding base, and, on this basis, to develop a healthy diet for the population.

Currently, there is a marked increase in global consumer demand for broiler meat produced without the use of antibiotic growth promoters. This trend has sparked growing interest in the microbial profiling of chickens raised under antibiotic-free protocols, although comprehensive research in this area remains limited [1]. Poultry meat is the most produced and consumed meat worldwide [2].

According to a report published by SAMMIT summarizing Professor O. Desouzart's presentation at the XIV European Conference on Poultry Farming (ECPP), global meat production is projected to reach 505.438 mln tons by 2050, while poultry production is expected to increase by 122.5% [3].

Analysis of the global poultry market shows that industrial poultry farming is the primary driver of global meat production, successfully maintaining its dominant position [4]. However, this expansion faces a critical economic bottleneck. Feed costs account for up to 80% of total production expenses, remaining the most pressing and persistent issue for the industry despite its intensive development [5].

Poultry farming in Kazakhstan is also one of the most progressive and dynamically developing sectors of agriculture, considered cost-effective and quickly profitable [6]. There are 70 large poultry farms operating in the country, including 34 egg and 36 meat farms, as well as 7 breeding farms. These enterprises not only supply the domestic market but also export. In this regard, it should be noted that today poultry farming in Kazakhstan is actively growing, striving for self-sufficiency in poultry meat (almost 80% by the end of 2025) and export to central Asian countries [7].

The dynamic growth in demand for poultry products is driven by global demographic and socioeconomic factors: population growth, urbanization, changing age structure, and rising affluence. These trends are leading to a significant increase in protein consumption, while poultry meat remains one of the most affordable and sought-after sources of high-quality animal protein [8, 9].

Figure 1. Role of Gut Health and Postbiotics in Enhancing Poultry Productivity, Product Quality, and Food Safety

Developing strategies to enhance poultry productivity in industrial settings remains a priority scientific and practical task. Gut health is a key factor here, determining the efficiency of nutrient absorption and the body's resistance. Targeted modulation of the microbiome during the early stages of development is particularly important, laying the foundation for long-term health and high productivity in chickens.

To solve this problem in industrial poultry farming, probiotics and postbiotics are used, which have a beneficial effect on the intestinal biocenosis by displacing pathogenic microorganisms, modulating immunity, producing antimicrobial compounds, and also maintaining the integrity of the intestinal barrier [10].

In this regard, we conducted an analytical review of the scientific literature dedicated to assessing the effectiveness of postbiotics in poultry farming. The data obtained formed the basis for the development of domestic postbiotic products with high potential for industrial implementation.

Materials and Methods

This study was conducted as a narrative literature review to summarize current knowledge on the use of postbiotics in poultry production. A total of 106 scientific publications published between 2020 and 2026 were identified and analyzed. The literature search was conducted using the scientific databases Scopus, Web of Science, PubMed, ScienceDirect, and Google Scholar.

The following keywords and their combinations were used: "postbiotics," "poultry," "broiler chickens," "layers," "gut health," "microbiota," "immune response," "growth performance," "antibiotic growth promoters," and "alternative feed additives." Publications that: (i) focused on postbiotics or postbiotic-derived compounds; (ii) assessed their impact on poultry health, performance, gut microbiota, immunity, or feed efficiency; and (iii) were published in peer-reviewed scientific journals. Studies not directly related to poultry production, duplicate publications, conference abstracts, and papers lacking sufficient methodological information were excluded.

The selected publications included review articles, original experimental studies, and research reports. Due to the heterogeneity of experimental designs, postbiotic formulations, dosages, and outcome measures, a quantitative meta-analysis was not conducted. Therefore, this work should be considered a narrative review with a qualitative synthesis of the available data. The collected information was grouped by postbiotic compound type, production methods, biological functions, mechanisms of action, and practical application in poultry production. Particular attention was paid to studies investigating growth performance, intestinal health, immunomodulation, feed conversion efficiency, and disease resistance in poultry.

Definition and classification of postbiotics

Postbiotics are preparations made from non-living beneficial microorganisms and their biologically active compounds that have therapeutic and functional potential. They consist primarily of microbial cell fractions, metabolites, enzymes, vitamins, polysaccharides, and short-chain fatty acids. These components can act directly or indirectly, but they all exert beneficial effects [11].

Postbiotics may contain intact, non-living microbial cells and/or microbial cell fragments/structures with or without metabolites/end products [12]. There are a number of microbial metabolites and/or microbial components that can be classified as postbiotics. Microbial metabolites can be very diverse, including enzymes, proteins/peptides, polysaccharides, organic acids, and lipids [13, 14].

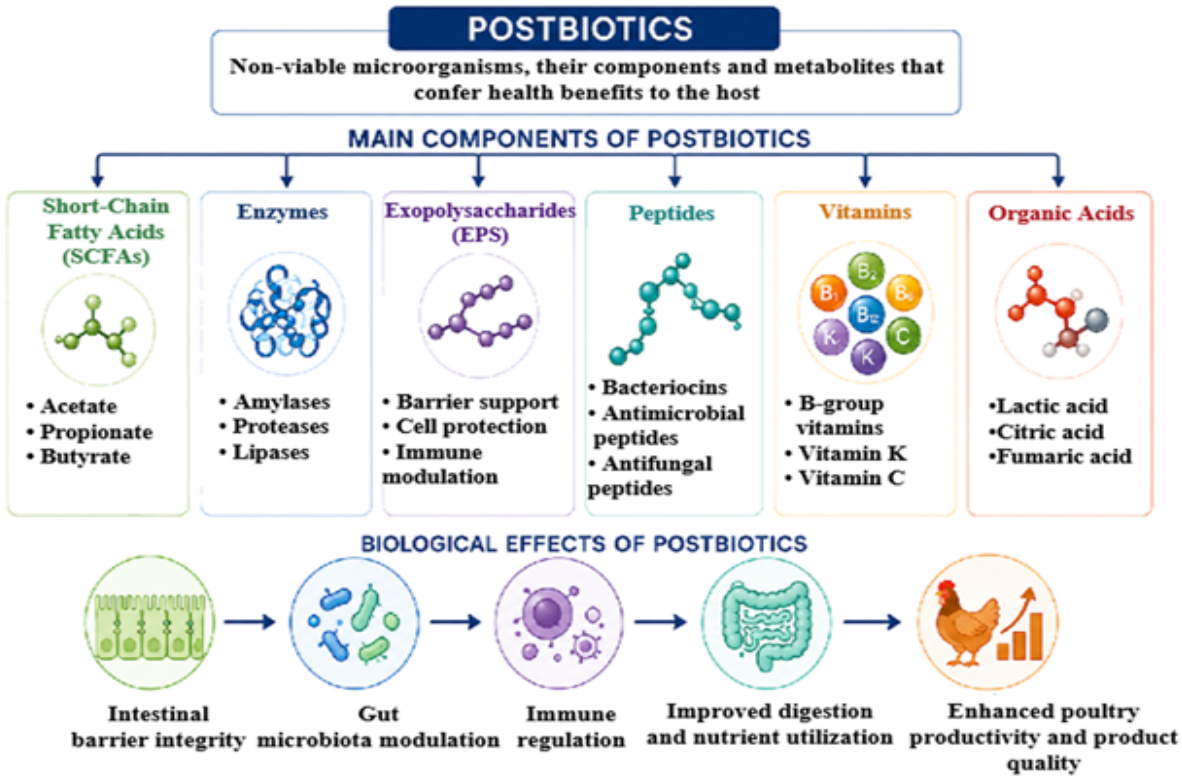

Figure 2. Main components of postbiotics and their biological functions

These low-molecular-weight biomolecules can provide a range of physiological health benefits and are the subject of revolutionary therapeutic strategies. Due to their diverse delivery methods and the ability to precisely dose, some postbiotics are promising therapeutic agents that can be used for both preventative and therapeutic purposes [15].

Short-chain fatty acids

Short-chain fatty acids (SCFAs) are produced by the intestinal microbiota as secondary metabolites during the fermentation of dietary fiber [16]. They serve as important chemical messengers in this axis, modulating hypothalamic feeding centers and systemic metabolism. The literature provides comprehensive data on the biosynthesis and kinetics of SCFAs in poultry and discusses their neural, humoral, and immune pathways to the brain [17].

Short-chain fatty acids are considered the most abundant postbiotic components formed during microbial fermentation and have been shown to be an important regulator of human gastrointestinal tract homeostasis [18].

Enzymes

Microbial enzymes, including lactase and antimicrobial proteins, can exert a positive impact on the body's health through their involvement in metabolic and protective processes. Depending on the nature of the reactions they catalyze, enzymes are divided into six main classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases [19].

Enzymes such as peptidases, lipases, amylases, proteases, ureases, and antioxidants catalyze various biological processes. These are active proteins present in all living organisms. Enzymes derived from microorganisms break down components such as carbohydrates, fats, and proteins into monomers, thereby facilitating human digestion and bioavailability, as well as improving the taste and texture of food [20, 21].

Exopolysaccharides

Exopolysaccharides are biopolymers produced by microorganisms and secreted outside the cell wall [22]. They receive more attention than other polysaccharides because they play a key role in microbial adaptation to environmental conditions. They are involved in bacterial adhesion to various surfaces and help protect cells from adverse factors and pathogens [23, 24].

The role of exopolysaccharides is described in the works of *X. Zhou* et al. The authors identified the function of exopolysaccharides in intestinal barrier function. Their data showed that exopolysaccharides inhibit intestinal inflammation by regulating the function of the intestinal epithelial barrier [25]. Also, studies by *Bleau* et al. have shown that EPS obtained from several species of lactobacilli have the ability to modulate systemic and mucosal immune responses and have direct beneficial effects on health [26].

Peptides

Many bacterial species produce antimicrobial peptides called bacteriocins, which are antagonists of specific microbes. Bacteriocins are produced by ribosomes and exported into the extracellular environment [27]. They are capable of inhibiting pathogens from Gram-negative and/or Gram-positive groups [28].

There are three classes of bacteriocins from lactic acid bacteria. Classes I and II bacteriocins are stable to pH and temperature and, therefore, can retain their antimicrobial function after exposure to heat [29]. Class III is the only heat-labile class of bacteriocins derived from lactic acid bacteria, and therefore, class III bacteriocins remain active only if the postbiotic preparation process does not include a thermal inactivation step [30].

Vitamins

Microorganisms also produce vitamins, leading to the consideration of fermented foods (FPs) as a potential means of improving vitamin intake [31].

Among the microorganisms responsible for fermentation, a number of strains possess the ability to biosynthesize vitamins, which are successfully used to enhance the vitamin content of raw materials [32].

Vitamins are a chemically diverse group of organic compounds that are required in trace amounts but are essential for maintaining cellular and systemic physiology in all areas of life. Unlike macronutrients, which serve as structural components or energy sources, vitamins function primarily as cofactors or precursors of coenzymes, mediating a wide range of enzymatic reactions involved in central metabolism, biosynthesis, regulation of redox processes, and cellular signaling [33, 34, 35, 36].

Organic Acids

The production of organic acids through microbial fermentation using less expensive raw materials is also a major advancement in industrial microbiology. A number of studies have focused on the microbial sources and methods for producing various organic acids (citric, gluconic, fumaric, and lactic acids), used primarily in the food industry [37].

Research by *I.V. Rozhkova* et al. demonstrated that organic acids are a component of metabolic processes occurring in postbiotic substances obtained using the studied probiotic cultures L. paracasei ABK and L. helveticus H9 [38].

Organic acids are produced by beneficial intestinal bacteria through the fermentation of carbohydrates, which have long been added to feed to minimize contamination and the growth of harmful bacteria and fungi, reduce spoilage, and extend the shelf life of feed products. Moreover, they are most often added to poultry feed in the form of mixtures to achieve maximum positive effect [39].

The Role of Microbiota in Chicken Production

The gut health of farm animals and poultry is fundamental to profitability, as it significantly impacts growth performance, disease resistance, and product quality. With the restriction of antibiotic use in livestock production, probiotics, prebiotics, synbiotics, and postbiotics (PPSPs) have emerged as promising alternatives [40, 41].

The gut is known to play a vital role in the healthy growth and development of chickens, serving as a barrier to the immune system. Approximately 70–80% of immune cells are associated with the gastrointestinal tract, which together form a unified defense against pathogens while maintaining the body's homeostasis. This balance is disrupted by various stresses, which negatively impact the immune system and the microbiota, leading to health problems, as the microbiota is involved in the synthesis of neurotransmitters and regulates the stress response [42].

Research by *Teame* et al. has shown that lactobacilli postbiotics, including pili and p40/p75 protein, protect the intestinal barrier, stimulate the production of aggregation factors, bacteriocins, and S-layer proteins, and promote pathogen elimination [43]. It was found that adding carriers consisting of lactobacilli in the form of a probiotic (Pro) and a postbiotic (Post) to the diet of broiler chickens resulted in accelerated growth up to day 35 and up to day 21, respectively. The postbiotic increased feed conversion after day 22, while the probiotic had no effect compared to the control group [44].

The gastrointestinal tract (GIT) is a complex microecosystem where billions of microorganisms (microbiota) interact closely with the host organism. This interaction is critical for digestion, immunity, and overall health, as the microbiota influences food absorption, protects against pathogens, and affects the development of the immune system, forming a dynamic symbiosis [45, 46, 47]. This complex structure plays a crucial role in maintaining the health and well-being of animals. The use of postbiotics helps to optimize the morphofunctional state of the intestine and improve the quality characteristics of broiler meat, while ensuring the intensification of nutrient absorption [48]. Their mechanism of action includes support of local immunity of the mucosa and effective modulation of the composition of the microbiome [49]. Possessing biological effects comparable to those of probiotics, postbiotics demonstrate significantly higher technological and biological stability [50].

Despite the proven benefits, a number of researchers report no significant changes in the qualitative and quantitative composition of the microbiome when postbiotics are included in standard poultry diets. However, these studies did document a significant effect of the supplements on the metabolic activity of the microflora, particularly on the concentration of short-chain fatty acids (SCFAs). Notably, in some experiments, regardless of the dietary composition, the addition of postbiotics did not cause statistically significant changes in the assessed immune status parameters [51].

Thus, the poultry gut microbiome plays a key role in nutrient digestion, immune system function, and overall health. It acts as a link between the external environment and the body, modulating intestinal morphology, immune responses, and even behavior [52, 53].

The antibiotic-probiotic-postbiotic concept in poultry farming

The concept of a sequential transition from antibiotics to postbiotics, through the probiotic stage, is currently recognized as the most promising. Postbiotics are the logical conclusion of this chain, as they lack the disadvantages of live microorganisms, providing consistent results and safety without sacrificing effectiveness in maintaining poultry health.

An analysis of historical and current data revealed a clear global trend: the poultry industry is undergoing a forced evolution of feed strategies driven by the need to overcome the antibiotic resistance crisis. Historically, the high stocking density of broiler chickens has predetermined the widespread use of in-feed antibiotics. However, a summary of recent findings [54, 55, 56] shows that the eradication of pathogens (antibiotics) has been replaced by a paradigm of modulating the indigenous microbiota (pro-, pre-, post-, and phytobiotics).

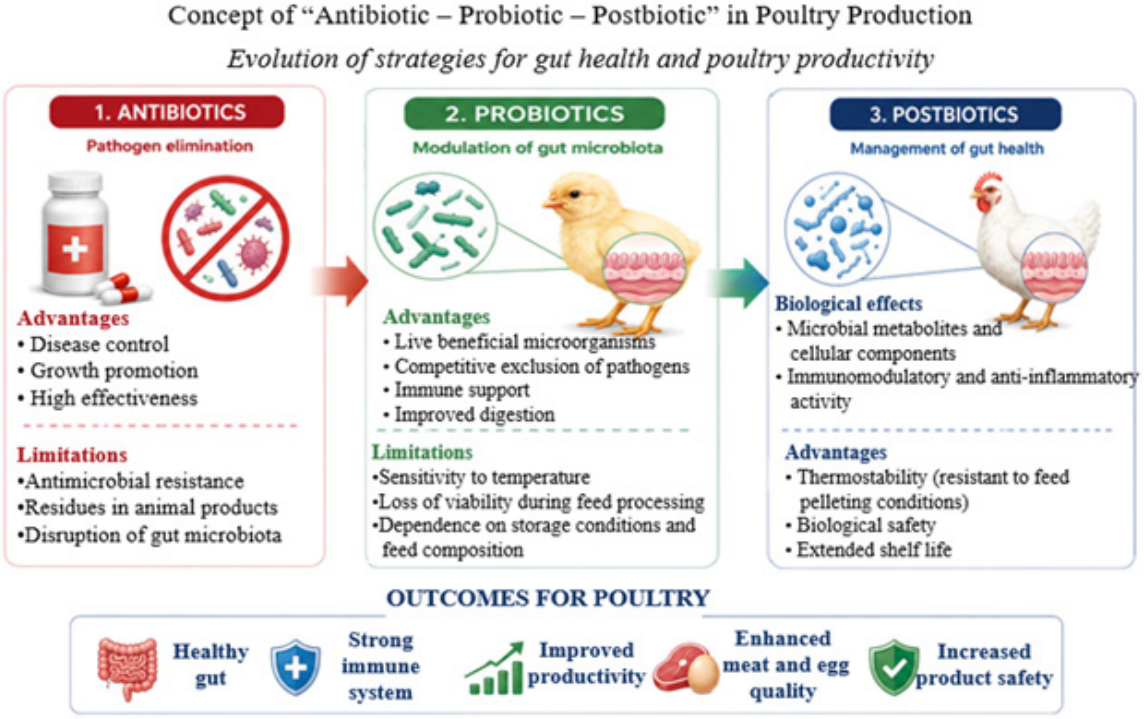

Figure 3. Evolution of nutritional strategies for gut health management in poultry: transition from antibiotics to probiotics and postbiotics

In 2001, a group of experts from the Food and Agriculture Organization of the United Nations and the World Health Organization officially defined probiotics as live bacteria that, when consumed in appropriate amounts, can confer a health benefit on consumers. However, despite the proven multifunctionality of live bacterial cultures, a detailed analysis of their use in industrial poultry farms revealed a systemic technological barrier associated with the viability of probiotics, which is critically dependent on feed pelleting conditions (high temperatures and pressure), as well as the pH of the poultry gastrointestinal tract [57].

The use of probiotics in poultry farms can ensure product safety and cost-effectiveness [58]. While the mechanisms of live probiotics for neutralizing mycotoxins [59, 60, 61] and removing heavy metals [62] have been well documented, the extrapolation of these properties to postbiotics remains controversial. There is virtually no data in the literature on whether inactivated cells (lysates, cell walls) are capable of maintaining similar enzymatic activity against toxins or whether their action is limited solely to passive physical sorption.

An analysis of accumulated data allows us to identify two key and complementary mechanisms of the protective effect of probiotics in broiler chickens. On the one hand, their biological role in the formation of a healthy microbiota is noted: lactic acid synthesized by lactic acid bacteria effectively suppresses pathogenic flora and stimulates early morphofunctional maturation of the poultry gastrointestinal tract during postnatal ontogenesis [63]. On the other hand, this effect is enhanced by chemical protection, expressed in the ability of probiotic cultures to reduce the overall toxic load on the chickens' bodies and accelerate the elimination of heavy metal salts [64].

The traditional paradigm for the use of probiotics is based on maintaining the target titer of viable microorganisms throughout the entire exposure period. However, in any native culture of the strain, a fraction of inactivated cells is inevitably present [65].

Scientific evidence indicates that these structural components and their metabolites possess independent biological activity and exert beneficial effects on the host organism, directly or indirectly [66]. The key advantage of postbiotics in industrial poultry farming is the combination of high technological resistance and guaranteed biological safety. Unlike live cultures, they maintain stable characteristics during aggressive feed processing and eliminate the risks associated with uncontrolled microbial behavior. Therefore, the use of inactivated microbial structures and their metabolites opens up broad prospects for the development of next-generation functional preparations with distinct competitive advantages over traditional live cultures.

Current trends in poultry farming indicate the high efficiency of postbiotics, both as standalone agents and as part of synergistic combinations. While postbiotics, when combined with prebiotics, are considered a direct alternative to feed antibiotics for stimulating the immune response and basic growth intensification in broiler chickens, their integration with phytobiotics provides a much more profound multifunctional effect. Complex diets based on post- and phytobiotic components enable targeted modulation of not only intestinal morphology but also macrostructural parameters: carcass characteristics, meat quality indicators, and tibia architecture in poultry.

Analysis of the available data revealed, postbiotics have been shown to have a predominant effect on innate immune mechanisms, possessing significant immunomodulatory potential. Under infectious stress, these drugs act to suppress proinflammatory reactions and promote a stable homeostatic response. In particular, studies by *C.N. Johnson* et al. confirmed the specific immunomodulatory effect of postbiotics directly on the lymphoid tissue of the small intestine of broilers [67].

A study of various dosages of a postbiotic based on Lactobacillus plantarum in broiler diets confirmed its pronounced multifunctional effectiveness. Including this feed supplement in the main diet has a comprehensive effect on the bird's body, optimizing the microbial profile and biodiversity of the cecum, activating the intestinal enzymatic system, which ultimately directly translates into improved growth and slaughter performance [68].

Analysis of efficacy data also shows that postbiotics improve intestinal villus health, increase lactic acid production, and reduce enterobacteria and fecal pH, which collectively leads to improved immune response and intestinal health, as well as increased growth performance. Postbiotics also reduce yolk and plasma cholesterol levels in laying hens and improve egg quality [69].

Postbiotic use has resulted in improved growth performance, meat quality, fecal lactic acid bacteria counts, villus height, and the ability to withstand heat stress in broilers, laying hens, and pigs [70]. Inclusion of postbiotics in feed and water has shown positive results in reducing the severity of nephrotic enteritis and improving liver and immune health in broiler chickens compared to commonly used probiotics and antibiotics [71].

In conclusion, it should be emphasized that the transition from antibiotics to probiotics and further to the use of postbiotics marks a fundamental evolution in poultry feeding strategies. This concept reflects a shift from attempts to quantitatively colonize the gut with live microflora to methods of targeted management of poultry health and homeostasis using heat-stable and technologically advanced biomolecules with predictable biological effects.

A comparative analysis of the data revealed a clear shift in research interest from probiotics to postbiotics. This is driven by a key technological trend: postbiotics, unlike live cultures, are heat-stable during feed pelleting and have a long shelf life, which solves the main problem with traditional probiotics.

Alternatives and the effectiveness of postbiotics in poultry farming

Postbiotics are the most rational alternative in poultry farming, as they combine the safety of antibiotics with the functional benefits of probiotics, while mitigating their production shortcomings.

Despite the steady growth in the number of publications on postbiotics, debate continues regarding their precise definition [72]. Over the past decade, probiotics have become the subject of intensive research due to their ability to improve gut health and modulate the microbiome, aiding in the treatment and prevention of disorders, strengthening the immune system, and participating in digestion, as confirmed by numerous clinical studies [73]. It should be noted, however, that preserving live, active bacteria with desired properties in probiotics is not always achievable. Therefore, the search for ways to utilize non-living probiotic bacterial cells, i.e., their metabolites-postbiotics-has become a pressing issue in the field of biotechnology.

The global literature contains extensive data confirming the effectiveness of postbiotics in poultry farming. Numerous publications attest to their positive impact on stimulating productivity and restoring the intestinal microbiome of poultry.

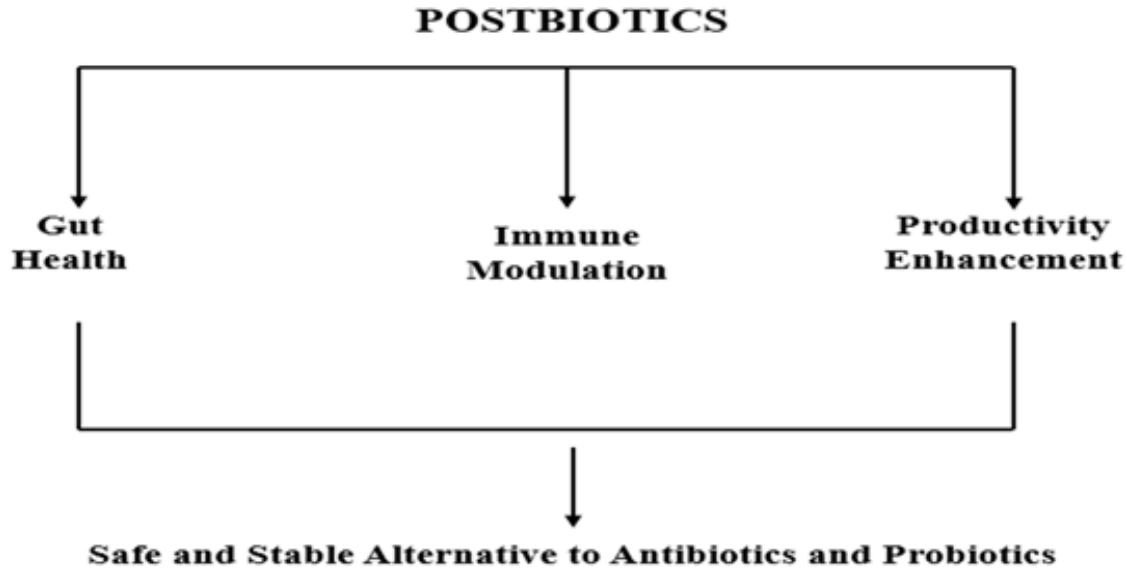

Figure 4. Postbiotics as a safe and stable alternative to antibiotics and probiotics in poultry production

In a study by *Kareem* et al. to determine the effect of postbiotics synthesized by Lactobacillus plantarum and inulin on the performance of broiler chickens, it was found that chickens fed a combination of postbiotics and inulin in starter and finisher diets demonstrated significantly higher body weight and weight gain compared to the control group. Postbiotics can reduce the number of pathogenic microorganisms in the intestine, improving intestinal health and growth efficiency [74].

Postbiotic use improves intestinal villi and intestinal barrier function, increases lactic acid production, and reduces enterobacteria and fecal pH, which contributes to improved immune response, intestinal health, and growth. Postbiotics also reduce plasma cholesterol and triglyceride levels in poultries [75].

Scientific studies by a number of authors confirm that introducing a 0.2% Lactobacillus acidophilus-based postbiotic into broiler diets effectively replaces feed-based antibiotic growth promoters. At this dosage, the preparation not only demonstrates high antibacterial activity but also eliminates the risks associated with the use of live probiotic cultures, such as excessive immune stimulation and competition between microflora and the host organism for nutrients [76].

Y. Chen et al. demonstrated the high therapeutic efficacy of Bifidobacterium bifidum (BbP)-based postbiotics as an alternative to antibacterial drugs for Salmonella Pullorum infection. The key mechanism of action of BbP is the suppression of pyroptosis, a specific form of programmed epithelial cell death induced by Salmonella. In parallel with this, the postbiotic ensures the regeneration of the intestinal barrier function through the expression of tight junction proteins and modulates the composition of the microbiota, creating unfavorable conditions for pathogen persistence [77].

The use of the postbiotic Culbac (a fermentation product of inactivated Lactobacillus acidophilus strains; TransAgra International Inc., USA) in chicken diets demonstrated its therapeutic potential, significantly reducing clinical morbidity and mortality, as well as minimizing pathological lesions of the intestinal mucosa, compared to an untreated infected control group [78].

All postbiotics available on the market represent a complex of biologically active metabolites of probiotic microorganisms that have significant potential for improving poultry productivity. Their effectiveness is due to their pronounced immunomodulatory effect, which is manifested by the activation of both innate and adaptive immune mechanisms in chickens [79].

A review by *R.C. Reuben,* et al. examined the concept, impact, and mechanisms of application of prebiotics, probiotics, and postbiotics in poultry farming and found that their use will undoubtedly lead to a more sustainable, safe, and cost-effective poultry industry, which will help feed the world [80].

The authors *Y. Danladi,* et al. investigated the effect of postbiotics and paraprobiotics from an active culture of *Lactiplantibacillus plantarum* instead of antibiotics and obtained positive effects on growth performance, small intestinal morphology, immune status, and liver growth gene expression in broiler chickens [81].

In the last decade, numerous scientific and clinical studies have been devoted to the study of the effect of probiotics, prebiotics, and symbiotics in diets, given their positive impact on microbial composition and normalization of intestinal balance [82].

The intestinal barrier is known to maintain intestinal integrity and provide protection against the penetration of intestinal pathogens [83]. The immune system can also influence the permeability of the intestinal barrier [84]. The literature reports the influence of pro- and postbiotics on immunity-related parameters [85].

Numerous studies examining postbiotics show that their effects on the immune system and intestinal barrier are comparable to those of probiotics, but they are also more stable and maintain their safety. This means that postbiotics are similar to probiotics in many ways and are more stable. However, optimal technologies for producing postbiotics, as well as their indications, have not yet been clearly developed. Therefore, this area requires in-depth and thorough research [86, 87].

Unlike probiotics, postbiotics do not require survival in the intestine, which makes them a promising option for poultry applications. Their mechanism of action is based on the direct interaction of bacterial metabolites with the intestinal epithelium and immunocompetent cells of the gastrointestinal tract. Postbiotics can modulate the immune response and enhance the integrity of the intestinal barrier. The latter, acting synergistically with the immune system and the microbiota, provides comprehensive protection against infections and helps maintain host homeostasis.

In this regard, Professor Mikhail Chikindas of Sechenov University noted in his presentation that postbiotics are unlikely to replace probiotics in the market, as probiotics can proliferate within the host and exert beneficial effects for a longer period than inactivated microorganisms. Despite the smaller size of the postbiotics market compared with the probiotics market, analysts report that the former is growing ten times faster than the latter; therefore, the health products market is expected to undergo significant changes in the near future.

Accordingly, an increase in the number of studies focused on postbiotics and the development of scientifically grounded approaches to their industrial production can be anticipated. Such advances may help mitigate the safety risks associated with live microbial cultures while ensuring high product stability and preserving biological activity.

Existing Limitations in the Standardization, Economic Aspects, and Prospects for the Application of Postbiotics

Despite the advantages of postbiotics highlighted in this review, further research is required to understand the more complex interactions between these compounds and their relationships within different poultry species and their immune systems [88].

Numerous studies have reported that, in addition to the positive effects of postbiotics on poultry health and growth performance, several challenges limit their large-scale implementation in production systems [89].

Studies evaluating the efficacy of postbiotics in broiler chickens have demonstrated no statistically significant improvements in feed conversion ratio (FCR) or average daily gain (ADG) compared with control groups. In this regard, strain specificity plays a significant role; that is, a postbiotic preparation derived from one particular bacterial strain may exhibit strong antimicrobial activity, whereas another strain of the same species may produce only negligible effects [90].

In most studies assessing the efficacy of postbiotics in animals, a specific dosage has not been established. In the study by *Y. Danladi,* et al. (2022), which investigated the use of postbiotics and paraprobiotics as alternatives to antibiotics in broiler chicken production, no statistically significant differences (($p > 0.05$)) were observed among the experimental groups with respect to final body weight, total body weight gain, or feed conversion ratio [81].

There are also reports indicating that the inclusion of various postbiotics in the diets of broiler chickens reared under heat stress conditions does not significantly affect carcass yield. This finding is consistent with the results reported by *Kareem* et al. (2016), who likewise observed that the combined administration of postbiotics and inulin had no effect on broiler dressing percentage [91].

The effects of postbiotics on growth performance and intestinal health remain inconsistent, despite their recognition as active growth-promoting agents. Therefore, researchers emphasize the need for additional studies to standardize postbiotic therapy and to investigate its long-term effects on poultry productivity [92].

The scientific literature describes numerous challenges associated with the standardization and industrial production of postbiotics, primarily due to the absence of a unified international regulatory framework and clearly defined quality control standards. *N. Piqué* et al. identified technological barriers, including the complexity of the chemical composition of postbiotic metabolites, which complicates the development of standardized quality control protocols for industrial production [93].

Consequently, postbiotics require a clearer classification system and nomenclature in both commercial and regulatory contexts [94].

It should also be noted that postbiotics present limitations for large-scale production from a commercial perspective. Their production costs remain substantially higher than those of traditional feed additives such as prebiotics and organic acids. This is attributable to the high costs associated with downstream processing required for cell inactivation, as well as spray-drying or freeze-drying procedures. Ultimately, these factors increase feed production costs, making the return on investment (ROI) unpredictable for large poultry enterprises.

The study by *T.H. Khayoon,* et al. (2024) experimentally confirmed that, despite significant improvements in feed conversion and the production index following postbiotic supplementation, the economic efficiency of feed expenditures declined compared with the unsupplemented control group due to the high cost of the postbiotic component itself [95].

In addition, the application of postbiotics in industrial poultry production presents operational challenges associated with high-temperature feed processing (80–90 °C) during pelleting. Although postbiotics are generally more thermostable than live probiotics, prolonged exposure to heat may still denature thermolabile enzymes and vitamins present within the matrix [96].

Thus, substantial heterogeneity in postbiotic characteristics, dosage regimens, and experimental designs limits the comparability of results across studies. Therefore, future research in this field should

prioritize the standardization of postbiotic composition, evaluation of dose response relationships, and large-scale commercial validation to ensure consistent application under industrial production conditions.

Conclusion

This review demonstrated improvements in the productivity and economic efficiency of poultry production.

The results of this review demonstrate the feasibility of using postbiotics in industrial poultry production. The use of postbiotics helps stabilize homeostasis and correct the physiological status of poultry, which translates into enhanced growth processes and optimized meat production profitability.

It is known that the traditional practice of extensively using antibiotics in poultry production for the prevention and promotion of growth has become unsustainable. The key negative consequences include the spread of antibiotic resistance and intestinal dysbiosis in poultry, which poses risks to food security.

One of the key factors driving the transition from antibiotics to probiotics in poultry production was the ban on their use, which forced the industry to seek safe biological alternatives. Officially, on January 1, 2006, the European Union imposed a complete ban on the use of antibiotics as growth promoters in livestock feed. The decision followed a long-standing debate about the risks associated with the widespread use of antimicrobials in industrial livestock production and reflected growing concern in the EU about the problem of bacterial resistance to antibiotics [97].

In this regard, the transition to the use of probiotics has become an important step towards greening poultry farming. While live cultures have the ability to competitively displace pathogenic microflora and stimulate the immune response, they nevertheless face significant technological challenges. Key challenges include low thermal stability, leading to loss of viability during feed expansion and pelleting at temperatures above 75–80 °C, as well as the additional time required for adhesion and colonization of the intestinal epithelium.

The limitations inherent in live cultures have led to the emergence of a new category of bioactive compounds – postbiotics. According to the ISAPP definition (2021), which remains valid in 2025, postbiotics are preparations of non-living microorganisms or their components that provide a health benefit to the host.

Thus, despite the proven effectiveness of probiotics in maintaining the microbiome and optimizing digestive processes in chickens, their widespread use is limited by their low technological stability. The main challenge remains maintaining the viability of vegetative forms of microorganisms under aggressive production conditions and during transit through the gastrointestinal tract. Postbiotics-preparations based on inactivated microbiotic structures and their metabolites-are being considered as a promising alternative that offsets these shortcomings.

The conducted theoretical analysis confirms the high potential of postbiotics in chicken rearing and opens up new prospects for industrial poultry farming. In this regard, as part of a grant project funded by the Ministry of Healthcare and Education of the Republic of Kazakhstan, we are conducting research to identify probiotic microorganisms that produce postbiotics, such as exopolysaccharides, organic acids, and bacteriocins. In conclusion, postbiotics and sustainable poultry farming will ensure food safety and security worldwide.

Authors' Contributions

The authors jointly developed the concept and study design. Literary source search and selection, data analysis – GK and all others; Critical text editing, scientific content verification - Zh T. Scientific supervision, author coordination, approval of the final version of the article – MB.

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References

1 Rama, E.N., Bailey, M., Kumar, S., Leone, C., den Bakker, H.C., Thippareddi, H., Singh, M. (2023). Characterizing the gut microbiome of broilers raised under conventional and no antibiotics ever practices. *Poultry Science*, 102(8), 102832.

2 OECD. (2021). OECD-FAO Agricultural Outlook 2021-2030. https://www.oecd.org/en/publications/oecd-fao-agricultural-outlook-2021-2030_19428846-en.html

3 SAMMIT. (2025). *Mirovoe i otechestvennoe pticevodstvo: realii i vyzovy budushhego [Global and Domestic Poultry Farming: Realities and Challenges of the Future].* https://mapsummit.ru/news/mirovoe_i_otechestvennoe_ptitsevodstvo_realii_i_vyzovybudushchego/

4 Khairullina, O.I. (2025). Mirovoi rynok myasa pticy: prognoz, analiz tendencii i perspektiv [World poultry meat market: forecast, analysis of trends and prospects]. *Prodovol'stvennaya politika i bezopasnost',* 12(2), 511–526. DOI:10.18334/ppib.12.2.121995.

5 Jaiyq press. (2025). *Zhivotnovodstvo Kazahstana uskoryaetsya: mjaso, moloko i ptica podderzhivajut stabil'nost' rynka [Kazakhstan`s livestock industry is accelerating: meat, milk, and poultry are supportimg market stability].* https://zhaikpress.kz/ru/news/zhivotnovodstvo-kazaxstana-uskoryaetsya-myaso-moloko-i-ptica-podderzhivayut-stabilnost-rynka/

6 Gabbassova, Z.H., Dossanova, A., Lim, S.S. (2024). Current problems and prospects of poultry industry: innovative vector. *Problems of AgriMarket*, 1, 113–124. DOI:10.46666/2024-1.2708-9991.10.

7 24KZ Live. (2025). *Kak razvivaetsya pticevodstvo v Kazahstane [How poultry farming is developming in Kazakhstan].* https://24.kz/ru/news/economyc/737868-kak-razvivaetsya-ptitsevodstvo-v-kazakhstane

8 Kleyn, F.J., Ciacciariello, M. (2021). Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies? *World's Poultry Science Journal,* 77(2), 267–278. DOI:10.1080/00439339.2021.1904314.

9 Erdaw, M.M., Beyene, W.T. (2022). Trends, prospects and the socio-economic contribution of poultry production in sub-Saharan Africa: a review. *World's Poultry Science Journal*, 78(3), 835–852. DOI:10.1080/00439339.2022.2092437.

10 Khomayezi, R., Adewole, D. (2022). Probiotics, prebiotics, and synbiotics: an overview of their delivery routes and effects on growth and health of broiler chickens. *World's Poultry Science Journal,* 78(1), 57–81. DOI:10.1080/00439339.2022.1988804.

11 Krivonogova, A.S., Donnik, I.M., Isaeva, A.G., Loginov, E.A., Petropavlovskiy, M.V., Bespamyatnykh, E.N. (2023). Antibiotic Resistance of Enterobacteriaceae in Microbiomes Associated with Poultry Farming. *Food Processing: Techniques and Technology*, 53(4), 710–717. DOI:10.21603/2074-9414-2023-4-2472.

12 Naeem, M., Bourassa, D. (2025). Probiotics in poultry: unlocking productivity through microbiome modulation and gut health. *Microorganisms*, 13(2), 257.

13 Sharma, S., Patel, B., Kumar, P., Lall, R. (2025). Postbiotics: Multifunctional Microbial Products Transforming Animal Health and Performance. *Veterinary Sciences*, 12(12), 1191. DOI: 10.3390/vetsci12121191.

14 Salminen, S., Collado, M.C., Endo, A., Hill, C., Lebeer, S., Quigley, E.M.M., Sanders, M.E., Shamir, R., Swann, J.R., Szajewska, H., et al. (2021). The International Scientific Association of Probiotics and Prebiotics (ISAPP) Consensus Statement on the Definition and Scope of Postbiotics. Nat. Rev. Gastroenterol. *Hepatol*, 18, 649–667.

15 Aguilar-Toalá, J.E., Garcia-Varela, R., Garcia, H.S., Mata-Haro, V., González-Córdova, A.F., Vallejo-Cordoba, B., Hernández-Mendoza, A. (2018). Postbiotics: An Evolving Term within the Functional Foods Field. *Trends Food Sci. Technol.,* 75, 105–114.

16 María José, H.-G., Elena, F.-R. (2020). Postbiotics in Human Health: Possible New Functional Ingredients? *Food Res. Int.,* 137, 109660.

17 Abbasi, A., Bazzaz, S., Sabahi, S., Yari, A.H., Maleki, L.A., Hosseini, H. (2026). Targeted delivery of postbiotics: oral, intradermal, and intravenous models. *Folia Microbiologica,* 1-23.

18 Yu, Y., Xiao, X., Zheng, B., Bai, D., Zhang, Y., Zhen, W., Ma, Y. (2026). Short-Chain Fatty Acids Regulate Poultry Feed Intake via the Hypothalamus: Receptor-Mediated and Epigenetic Mechanisms. *Animals*, 16(6), 954. DOI: 10.3390/ani16060954.

19 Farzana Beegum and Archana Chandran. Postbiotics: A developing notion to application. (2024). *Int. J. Adv. Biochem. Res.,* 8(9S), 148–153. DOI: 10.33545/26174693.2024.v8.i9Sb.2076.

20 Garcia-Cano, I., Rocha-Mendoza, D., Kosmerl, E., Zhang, L., Jimenez-Flores, R. (2020). Technically relevant enzymes and proteins produced by LAB suitable for industrial and biological activity. *Appl. Microbiol. Biot.,* 104, 1401–1422. DOI: 10.1007/s00253-019-10322-2.

21 Maske, B.L., Pereira, G.V.M., Vale, A.S., Neto, D.P.C., Karp, S.G., Viesser, J.A., Lindner, J.D., Pagnoncelli, M.G., Soccol, V.T., Soccol, C.R. (2021). A review on enzyme-producing lactobacilli associated with the human digestive process: From metabolism to application. *Enzyme Microb. Technol.,* 149, 109836. DOI: 10.1016/j.enzmictec.2021.109836.

22 Singh, P., Saini, P. (2017). Food and Health Potentials of Exopolysaccharides Derived from Lactobacilli. *Microbiol Res J Int.,* 22, 1–14. DOI: 10.9734/ mrji/2017/36935.

23 Castro-Bravo, N., Wells, J.M., Margolles, A., Ruas-Madiedo, P. (2018). Interactions of surface exopolysaccharides from Bifidobacterium and Lactobacillus within the intestinal environment. *Front Microbiol.,* 9, 2426. DOI: 10.3389/fmicb.2018.02426.

24 Kim, M.H., Choi, S.J., Choi, H., Choi, J.P., Park, H.K., Kim, E.K., et al. (2018) Lactobacillus plantarum-derived extracellular vesicles protect atopic dermatitis induced by Staphylococcus aureus-derived extracellular vesicles. *Allergy Asthma Immunol Res.*, 10, 516–32. DOI:10.4168/ aair.2018.10.5.516.

25 Zhou, X., Qi, W., Hong, T., Xiong, T., Gong, D., Xie, M., Nie, S. (2018). Exopolysaccharides from Lactobacillus plantarum NCU116 Regulate Intestinal Barrier Function via STAT3 Signaling Pathway. *Journal of Agricultural and Food Chemistry*, 66, 37. DOI: 10.1021/acs.jafc.8b03340.

26 Bleau, C., Monges, A., Rashidan, K., Laverdure, J.P., Lacroix, M., Van Calsteren, M.R., et al. (2010). Intermediate chains of exopolysaccharides from Lactobacillus rhamnosus RW-9595M increase IL-10 production by macrophages. *J Appl Microbiol.*, 108, 666–75. DOI: 10.1111/j.1365-2672.2009.04450.

27 Cotter, P., Hill, C., Ross, R. (2005). Bacteriocins: Developing innate immunity for food. N*at. Rev. Genet.,* 3, 777–788.

28 Kumariya, R., Garsa, A.K., Rajput, Y., Sood, S., Akhtar, N., Patel, S. (2019). Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. *Microb. Pathog.,* 128, 171–177.

29 Gálvez, A., Abriouel, H., López, R.L., Ben Omar, N. (2007). Bacteriocin-based strategies for food biopreservation. *Int. J. Food Microbiol*., 120, 51–70.

30 Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., Kuipers, O.P. (2016). Bacteriocins of lactic acid bacteria: Extending the family. *Appl. Microbiol. Biotechnol.,* 100, 2939–2951.

31 Keyvan, E., Adesemoye, E., Champomier-Vergès, M. C., Chanséaume-Bussiere, E., Mardon, J., Nikolovska Nedelkoska, D., Humblot, C. (2025). Vitamins formed by microorganisms in fermented foods: effects on human vitamin status – a systematic narrative review. *Frontiers in Nutrition,* 12, 1653666. DOI: 10.3389/fnut.2025.1653666.

32 Adesemoye, E.T., Sanni, A.I., Spano, G., Capozzi, V., Fragasso, M. (2025). Lactic acid bacteria diversity in fermented foods as potential bio-resources contributing to alleviate malnutrition in developing countries: Nigeria as a case study. *Fermentation*, 11, 103. DOI: 10.3390/fermentation11020103.

33 Kennedy, D.O. (2016). B Vitamins and the brain: mechanisms, dose and efficacy - a review. Nutrients, 8(2), 68. DOI: 10.3390/nu8020068.

34 Parra, M., Stahl, S., Hellmann, H. (2018). Vitamin B6 and its role in cell metabolism and physiology. *Cell*s, 7(7), 84. DOI: 10.3390/cells7070084.

35 Mukherjee, S., Banerjee, O., Singh, S. (2023). The role of B vitamins in protecting mitochondrial function. In Ostojic SM, editor. Molecular Nutrition and Mitochondria. *Elsevier*; 167-93.

36 Godoy-Parejo, C., Deng, C., Zhang, Y., Liu, W., Chen, G. (2020). Roles of vitamins in stem cells. *Cell Mol Life Sci*., 1771–91. DOI: 10.1007/s00018-019-03352-6.

37 Kumar, A., Gautam, A., Khan, H., Dutt, D. (2019). Organic Acids: Microbial Sources, Production and Applications. Microbial Enzymes and Additives for the Food Industry. *New York: Nova Science Publishers,* 9. DOI: 10.1016/B978-0-12-819815-5.00053.

38 Rozhkova, I.V., Yurova, E.A., Leonova, V.A. (2024). Evaluation of the Amino Acid Composition and Content of Organic Acids of Complex Postbiotic Substances Obtained on the Basis of Metabolites of Probiotic Bacteria Lacticaseibacillus paracasei ABK and Lactobacillus helveticus H9. *Foods and Raw Materials,* 12(2), 319–330. DOI: 10.21603/2308-4057-2024-2-319-330.

39 Abd El-Ghany, W.A. (2024). Applications of organic acids in poultry production: an updated and comprehensive review. *Agriculture*, 14(10), 1756.

40 Lautan, O., Cheng, Y.H., Pranata, R., Chen, Y.Y., Shi, Y.H., Chen, S.N., Chen, R.J. (2025). Antimicrobial-resistant (AMR) bacteria in the food chain: Current challenges and global mitigation strategies. *One Health*, 101273. DOI: 10.1016/j.onehlt.2025.101273.

41 Yue, T., Lu, Y., Ding, W., Xu, B., Zhang, C., Li, L., Huang, S. (2025). The role of probiotics, prebiotics, synbiotics, and postbiotics in livestock and poultry gut health: A review. *Metabolites*, 15(7), 478. DOI:10.3390/metabo15070478.

42 Roque, F., Lopes, M.H.S., Raffi, P., Oliveira, R., Caparroz, M., Longhini, G., Granghelli, C., Faria, D., Araujo, L., Araujo, C. (2025). Postbiotics: an alternative for improving health and performance of poultry production. *Microorganisms*, 13(7), 1472. DOI: 10.3390/microorganisms13071472.

43 Teame, T., Wang, A., Xie, M., Zhang, Z., Yang, Y., Ding, Q., Gao, C., Olsen, R.E., Ran, C., Zhou, Z. (2020). Paraprobiotics and postbiotics of probiotic Lactobacilli, their positive effects on the host and action mechanisms: A review. *Front Nutr.,* 7, 570344.

44 Jansseune, S.C., Hendriks, W.H., Pinard-van der Laan, MH., Blanc, F. (2025). Feed additive carriers affected the physiological effects of a lactobacilli-based pro-and postbiotic in broilers. *Animal Feed Science and Technology*, 329, 116485.

45 Skelly, A.N., Sato, Y., Kearney, S., Honda, K. (2019). Mining the microbiota for microbial and metabolite-based immunotherapies. *Nature Reviews Immunology*, 19(5), 305-323.

46 Wegh, C.A., Geerlings, S.Y., Knol, J., Roeselers, G., Belzer, C. (2019). Postbiotics and their potential applications in early life nutrition and beyond. *International journal of molecular sciences,* 20(19), 4673.

47 Leeming, E.R., Johnson, A.J., Spector, T.D., Le Roy, C.I. (2019). Effect of diet on the gut microbiota: rethinking intervention duration. *Nutrients,* 11(12), 2862.

48 Chen, X., Yuan, C., He, J., Li, W., Liao, C. (2025). Current research status and trends in the bioactivity of postbiotics. *Frontiers in Food Science and Technology*, 5, 1692683.

49 Nataraj, B.H., Ali, S.A., Behare, P.V., Yadav, H. (2020). Postbiotics-Parabiotics: The New Horizons in Microbial Biotherapy and Functional Foods. *Microb. Cell Factories*, 19, 168.

50 Kornienko, E.A. (2022). Probiotiki, postbiotiki i paraprobiotiki [Probiotics, postbiotics and paraprobiotics]. *Eksperimental'naya i klinicheskaya gastroienterologiya*, 96, 241-250. DOI:10.31146/1682-8658-ecg-205-9-240-250.

51 Jansseune, S.C., Blanc, F., Lammers, A., van Baal, J., Bruneau, N., Pinard-van der Laan, M.H., Hendriks, W.H., Calenge, F. (2024). Microbiota but not immune modulation by a pro- and postbiotic was associated with the diet-additive interaction in broilers. *Poult. Sci.,* 103, 104184.

52 Diaz Carrasco, J.M., Casanova, N.A., Fernández Miyakawa, M.E. (2019). Microbiota, gut health and chicken productivity: what is the connection? *Microorganisms*, 7, 374. DOI:10.3390/microorganisms7100374.

53 Cheng, Y.C., Krieger, M., Korves, A.M., Camarinha-Silva, A. (2025). The chicken gut microbiome in conventional and alternative production systems. *Journal of Animal Science and Biotechnology*, 16(1), 153.

54 Abd El-Ghany, W.A., (2020). Paraprobiotics and postbiotics: Contemporary and promising natural antibiotics alternatives and their applications in the poultry field. *Open Veterinary Journal,* 10, 323–330. DOI: 10.4314/ovj.v10i3.11.

55 Arain, M.A., Nabi, F., Shah, Q.A., Alagawany, M., Fazlani, S.A., Khalid, M., Soomro, F., Khand, F.M., Farag, M.R. (2022b). The role of early feeding in improving performance and health of poultry: Herbs and their derivatives. *Worlds Poult Sci J.*, 78, 499-513. DOI: 10.1080/00439339.2022.2043133.

56 Bognanni, A., Fiocchi, A., Arasi, S., Chu, D. K., Ansotegui, I., Assa'Ad, A. H., et al. (2024). World allergy organization (WAO) diagnosis and rationale for action against Cow's milk allergy

(DRACMA) guideline update - XII - Recommendations on milk formula supplements with and without probiotics for infants and toddlers with CMA. *World Allergy Organ J.,* 17, 100888. DOI: 10.1016/j.waojou.2024.100888.

57 Begdildayeva, N.ZH., Akhmetsadykova, SH.N., Kudaibergenova, A.K., Akhmetsadykov, N.N. (2020). Effektivnost' primeneniya probioticheskih preparatov v pticevodstve [Application efficiency of probiotics in poultry]. *Vestnik Almatinskogo tehnologicheskogo universiteta*, 3, 73-78. DOI: 10.48184/2304-568X-2020-3-73-78.

58 Chikov, A.E., Kononenko, S.I., Omelchenko, N.A., Pyshmantseva, N.A., Osepchuk, D.V. (2012). Effektivnost' probiotika pri povyshennom soderzhanii kletchatki v racione svinei [The effectiveness of the probiotic with increased fiber content in the diet of pigs]. *Kombikorma*, 7, 95-96.

59 Temiraev, R.B., Mildzikhov, T.Z., Kokaeva, M.G., Buzoeva, L.B., Plieva, Z.K. (2013). Ispol'zovanie biologicheski aktivnyh helatnyh dobavok v pitanii korov i broilerov dlya denitrifikacii [The use of biologically active chelate additives in the nutrition of cows and broilers for denitrification]. *Izvestiya Gorskogo gosudarstvennogo agrarnogo universiteta*, 50(3), 117-121.

60 Kononenko, S.I., Tedtova, V.V. Vityuk, L.A. Salbieva, F.T. (2012). Fiziologo-biohimicheskii status organizma cyplyat-broilerov pri sovershenstvovanii tehnologii obrabotki kormovogo zerna [Feeding efficiency of multienzymatic agent as a component for combined feeds]. *Politematicheskii setevoi elektronnyinauchnyi zhurnal Kubanskogo gosudarstvennogo agrarnogo universiteta,* 846, 482-491.

61 Ryabchik, I. (2021). Effektivnost' primeneniya molochnokislyh bakterii v sostave probiotika «Baktosel'» [The effectiveness of lactic acid bacteria in the composition of the probiotic "Bactosel"]. *Effektivnoe zhivotnovodstvo*, 168, 24-27.

62 Kvan, O.V., Sheida, E.V., Duskaev, G.K., Rakhmatullin, Sh.G. (2020). Vliyanie probioticheskogo shtamma bifidobacterium longum na soderzhanie himicheskih elementov v biologicheskih tkanjah cyplyat-broilerov pri mineraldeficitnoi diete [Effect of the probiotic strain Bifidobacterium longum on the content of chemical elements in biological tissues of broiler chickens with a mineral-deficient diet]. *Agrarnyi vestnik Urala*, S14, 28-34.

63 Vinderola, G., Sanders, M.E., Salminen, S. (2022). The Concept of Postbiotics. *Foods*, 11, 1077.

64 Loh, T.C. (2017). Animal Feed The Way Forward; Penerbit UPM: Seri Kembangan, Malaysia.

65 Barros, C.P., Guimaraes, J.T., Esmerino, E.A., Duarte, M.C.K.H., Silva, M.C., Silva, R., *et al.* (2020). Paraprobiotics and postbiotics: concepts and potential applications in dairy products. *Curr. Opin. Food Sci.,* 32, 1–8. DOI: 10.1016/j.cofs.2019.12.003.

66 Doski, JM.M., Kareem, K.Y. (2023). Effects of combinations of postbiotics and phytobiotics on growth performance, meat quality, gut morphology, and tibia bone characteristics in broiler chickens. I*ranian Journal of Applied Animal Science*, 13(4), 743-754.

67 Johnson, C. N., Kogut, M. H., Genovese, K., He, H., Kazemi, S., & Arsenault, R. J. (2019). Administration of a postbiotic causes immunomodulatory responses in broiler gut and reduces disease pathogenesis following challenge. *Microorganisms*, 7(8), 268.

68 Huang, J.G., Zhang, Y., Li, F.F., Zhang, R.Y., Yin, D. F., Li, X P., Yin, M.H. (2023). Effects of postbiotics Lactobacillus plantarum on growth performance, slaughter performance and intestinal health of broilers. *Animals (Basel),* 13(18), 2958. DOI: 10.3390/ani13182958.

69 Saeed, M., Afzal, Z., Afzal, F., Khan, R.U., Elnesr, S.S., Alagawany, M., Chen, H. (2023). Use of Postbiotic as Growth Promoter in Poultry Industry: A Review of Current Knowledge and Future Prospects. *Food Sci Anim Resour.*, 43(6), 1111-1127. DOI: 10.5851/kosfa.2023.e52.

70 Thu, T.V., Loh, T.C., Foo, H.L., Yaakub, H., Bejo, M.H. (2011). Effects of liquid metabolite combinations produced by Lactobacillus plantarum on growth performance, faeces characteristics, intestinal morphology and diarrhoea incidence in postweaning piglets. *Trop. Anim. Health Prod.*, 43, 69–75.

71 Choe, D.W., Loh, T.C., Foo, H.L., Hair-Bejo, M., Awis, Q.S. (2012). Egg production, faecal pH and microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced by Lactobacillus plantarum strains. *Br. Poult. Sci.*, 53, 106–115.

72 Żółkiewicz, J., Marzec, A., Ruszczyński, M., Feleszko, W. (2020). Postbiotics-A Step Beyond Pre- and Probiotics. *Nutrients*, 12(8), 2189. DOI: 10.3390/nu12082189.

73 Chambers, L., Avery, A., Dalrymple, J., Farrell, L., Gibson, G., Harrington, J., Rijkers, G., Rowland, I., Spiro, A., Varela-Moreiras, G., et al. (2019). Translating Probiotic Science into Practice. *Nutr. Bull.,* 44, 165–173.

74 Kareem, K.Y., Loh, T.C., Foo, H.L., Akit, H., Samsudin, A.A. (2016). Effects of Dietary Postbiotic and Inulin on Growth Performance, IGF1 and GHR MRNA Expression, Faecal Microbiota and Volatile Fatty Acids in Broilers. *BMC Vet. Res.,* 12, 163.

75 Waqas, M., Nastoh, N.A., Çinar, A.A., Farooq, M.Z., Salman, M. (2024). Advantages of the use of postbiotics in poultry production: A new concept. *Brazilian Journal of Poultry Science*, 26(3), eRBCA-2024.

76 Monika, M., Tyagi, J.S., Sonale, N. et al. (2024). Evaluating the efficacy of Lactobacillus acidophilus derived postbiotics on growth metrics, Health, and Gut Integrity in broiler chickens. *Sci Rep.,* 14, 24768. DOI: 10.1038/s41598-024-74078-0.

77 Chen, Y., Zhu, F., Yu, G., Peng, N., Li, X., Ge, M., Dong, W. (2025). Bifidobacterium bifidum postbiotics prevent Salmonella Pullorum infection in chickens by modulating pyroptosis and enhancing gut health. *Poultry Science,* 104(4), 104968.

78 Abd El-Ghany, W.A., Abdel-Latif, M.A., Hosny, F., Alatfeehy, N.M., Noreldin, A.E., Quesnell, R.R., Elbestawy, A.R. (2022). Comparative efficacy of postbiotic, probiotic, and antibiotic against necrotic enteritis in broiler chickens. *Poultry science,* 101(8), 10198854.

79 Karuvelan, M., Sumukhi, S.S., Rajakannu, S., Chelliah, R., Barathikannan, K., Vijayalakshmi, S., Oh, D.H. (2025). Immunomodulatory and growth-enhancing postbiotic effects in poultry. In Postbiotics. *Academic Press*. 575–587.

80 Reuben, R.C., Sarkar, S.L., Roy, P.C., Anwar, A., Hossain, M.A., Jahid, I.K. (2021). Prebiotics, probiotics and postbiotics for sustainable poultry production. *World's Poultry Science Journal*, 77(4), 825–882. DOI: 10.1080/00439339.2021.1960234.

81 Danladi, Y., Loh, T.C., Foo, H.L., Akit, H., Md Tamrin, N.A., Naeem Azizi, M. (2022). Effects of postbiotics and paraprobiotics as replacements for antibiotics on growth performance, carcass characteristics, small intestine histomorphology, immune status and hepatic growth gene expression in broiler chickens. *Animals,* 12(7), 917.

82 Li, H.-Y., Zhou, D.-D., Gan, R.-Y., Huang, S.-Y., Zhao, C.-N., Shang, A., Xu, X.-Y., Li, H.-B. (2021). Effects and Mechanisms of Probiotics, Prebiotics, Synbiotics, and Postbiotics on Metabolic Diseases Targeting Gut Microbiota: A Narrative Review. *Nutrients,* 13, 3211.

83 Alam, A., Neish, A. (2018). Role of Gut Microbiota in Intestinal Wound Healing and Barrier Function. Tissue Barriers 2018, 6, 1539595.

84 Yin, Y., Liao, J., Li, Z., Pei, L., Wang, Y., Shi, H., Peng, Y., Tan, C., Li, H., Bai, C., Ma, Y., Gong, T., Wei, H.P. (2023). Lactobacillus plantarum GX17 benefits growth performance and improves functions of intestinal barrier/intestinal flora among yellow-feathered broilers. *Front. Immunol.,* 14, 1195382.

85 Lebedeva, I.A., Novikova, M.V., Vershinina, I.Yu. (2022). Probiotics for poultry and livestock farming - an evolutionary and biological necessity. *Agrarian Science,* 1(7-8), 102-104.

86 Kommersant. (2025). *Na smenu probiotikam [To replace probiotics]*. https://www.kommersant.ru/doc/8138860.

87 Humam, A.M., Loh, T.C., Foo, H.L., Samsudin, A.A., Mustapha, N.M., Zulkifli, I., Izuddin, W.I. (2019). Effects of Feeding Different Postbiotics Produced by Lactobacillus plantarum on Growth Performance, Carcass Yield, Intestinal Morphology, Gut Microbiota Composition, Immune Status, and Growth Gene Expression in Broilers under Heat Stress. *Animals,* 9(9), 644. DOI: 10.3390/ani9090644.

88 Radhi, K.S., Arif, M., ur Rehman, A., Faizan, M., Almohmadi, N.H., Youssef, I.M., Mahrose, K.M. (2023). Growth performance of broiler chickens fed diets supplemented with amylase and protease enzymes individually or combined. *Open Vet. J.,* 13(11), 1425-1435. DOI: 10.5455/OVJ.2023.v13.i11.5.

89 Sugiharto, S., Setiaji, A., Adli, D.N., Sholikin, M.M. (2024). Effects of Enzyme and Probiotic on the Growth Performance of Broiler Chickens: A Meta-Analysis. J*urnal Agriculture Peternakan,* 24(2), 148-155. DOI:10.17969/agripet.v24i2.36492.

90 Nataraj, B.H., Ali, S.A., Behare, P.V., Yadav, H. (2020). Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. *Microb. Cell Factories*, 19(1), 168. DOI: 10.1186/s12934-020-01426-w.

91 Kareem, K.Y., Loh, T.C., Foo, H.L., Asmara, S.A., Akit, H., Abdulla, N.R., Ooi, M.F. (2015). Carcass, meat and bone quality of broiler chickens fed with postbiotic and prebiotic combinations. *Int. J. Probiotics Prebiotics.*, 10, 23.

92 Szabó, S., Kovács-Weber, M., Pap, T.I., Dolányi, Á., Ferenc, P., Ákos, B., Szabó, R. T. (2025). Effect of postbiotics on the production parameters of rearing goose. *Veterinary and Animal Science,* 100541. DOI: 10.1016/j.vas.2025.100541.

93 Piqué, N., Berlanga, M., Miñana-Galbis, D. (2025). Postbiotics: Technical aspects, quality control, and regulatory challenges in food and pharmaceutical industries. *Journal of Functional Foods,* 114, 106120.

94 Guglielmetti, S., Boyte, M.E., Smith, C.L., Ouwehand, A.C., Paraskevakos, G., Younes, J.A. (2025). Commercial and regulatory frameworks for postbiotics: an industry-oriented scientific perspective for non-viable microbial ingredients conferring beneficial physiological effects. *Trends in Food Science & Technology,* 163, 105130. DOI: 10.1016/j.tifs.2025.105130.

95 Khayoon, T.H., Abbas, R.J., Abdullah, F.A. (2024). Effects of feeding various levels of postbiotics produced by lactic acid bacteria on growth performance, gastrointestinal microbiota count, and digestibility of some nutrients in broiler chickens. *Mesopotamia Journal of Agriculture,* 52(2), 68-81. DOI:10.33899/mja.2024.145531.1329

96 Nuningtyas, Y.F., Natsir, M.H., Widodo, E., Rahman, M.M., Atole, A.F.F., Utami, M.M.D., Sjofjan, O. (2026). Postbiotics in broiler nutrition: a critical review of mechanisms, efficacy, and applications in antibiotic-free production systems. *Poultry Science,* 107199. DOI: 10.1016/j.psj.2026.107199.

97 National News Agency. (2026). *EU bans use of antibiotic growth promoters in livestock farming.* https://nia.eco/2026/01/05/110686/.

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Research article

The impact of helminth infections on the effectiveness of brucellosis immunoprophylaxis in cattle

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Abstract

Background and Aim. Brucellosis remains one of the most significant bacterial zoonoses affecting livestock productivity and public health worldwide. Vaccination is the cornerstone of bovine brucellosis control; however, its efficacy may be reduced by factors that affect the host immune response, including parasitic infections. Helminths are known to modulate immune and may suppress vaccine-induced antibody production. This study aimed to evaluate the effect of helminth infestation on antibody production following vaccination with Brucella abortus strain 82 and to assess the impact of prior anthelmintic treatment.

Materials and Methods. A total of 20 cattle were divided into two groups (n = 10 each). Helminth infections were confirmed in all animals using coproscopic and larvoscopic methods, including the Fulleborn flotation technique. The experimental group received anthelmintic treatment (Monizen forte, 1 µL/20 kg body weight, orally) before vaccination, whereas the control group was vaccinated without prior deworming. All animals were immunized with B. abortus strain 82 (5 µL, subcutaneously). Blood samples were collected 26 days after vaccination, and antibody responses were assessed using the indirect hemagglutination assay, agglutination test, and thecomplement fixation test.

Results. Parasitological analysis revealed mixed helminth infections *(Dictyocaulus* spp., strongylates, *Nematodirus* spp., and *Moniezia* spp.) in allanimals. Anthelmintic treatment demonstrated high efficacy, resulting in the complete elimination of strongylates and monieziosis. Serological results showed significantly stronger and more consistent antibody responses in the treated group compared with untreated controls, which exhibited lower titers and heterogeneous reactions.

Conclusion. Helminth infestation adversely affects post-vaccination humoral immunity against brucellosis in cattle. Pre-vaccination deworming enhances antibody production and may improve the effectiveness of brucellosis control programs.

Keywords: Brucellosis; *Brucella abortus;* helminth infestation; vaccination; anthelmintic treatment; cattle immunity.

Introduction

Brucellosis is one of the most widespread bacterial zoonosesaffecting both animals and humans worldwide. The disease is caused by Gram-negative bacteria of the genus Brucella, which are facultative intracellular pathogens capable of infecting a wide range of domestic and wild animal species. In cattle, the main etiological agent is *Brucella abortus*, which causes abortion, infertility, reduced milk production, and other reproductive disorders. These pathological effects lead to considerable economic losses in livestock production and pose a serious threat to food safety and public health [1–6].

Despite long-term control programs, involving vaccination, veterinary surveillance, and sanitary measures, brucellosis remains endemic in many regions of the world. In countries with well-developed livestock industries, the disease remains one of the major infectious constraints affecting cattle production and requires the continuous improvement of preventive strategies and vaccination approaches [7–9].

Vaccination remains the main method for the specific prevention of bovine brucellosis. Live attenuated vaccines, such as *Brucella abortus* strain S19 and RB51 are widely used in cattle to induce protective immunity and reduce the spread of infection within animal populations. These vaccines stimulate both humoral and cell-mediated immune responses, which together contribute to protection against infection. However, because *Brucella* spp. are facultative intracellular pathogens that replicate within macrophages, effective immunity largely depends on the activation of cellular immune mechanisms. In particular, T helper type 1 (Th1) cells play a critical role in protective immunity by producing interferon-gamma (IFN-γ), which activates macrophages and enhances their bactericidal activity against intracellular bacteria. Humoral immunity also contributes to host defense, particularly in the later stages of infection, when antibodies promote bacterial opsonization and facilitate phagocytosis [7, 8, 10–13].

However, the effectiveness of vaccination programs may vary considerably depending on several biological and environmental factors. The development of post-vaccination immunity may be influenced by the physiological status of animals, nutritional deficiencies, stress, management practices, and the presence of concurrent infections and other immunosuppressive factors [9, 14, 15]. Changes in the immunobiological reactivity of animals may therefore lead to reduced vaccine-induced immune responses and reduced effectiveness of preventive measures.

Parasitic infections are among the factors that can substantially influence the immune status of animals. Parasites have pronounced immunomodulatory properties and can significantly alter both innate and adaptive immune responses in the host. Numerous studies have demonstrated that chronic parasitic infections can lead to secondary immunodeficiency, reduce host resistance, and increase susceptibility to infectious diseases of various etiologies [14, 16].

Helminthiases comprise a large group of parasitic diseases caused by helminths, including nematodes, cestodes, and trematodes. These parasites are widely distributed among livestock worldwide. In cattle, the most common helminths include *Ostertagia, Haemonchus, Trichostrongylus, Fasciola,* and *Moniezia.* Helminths can persist in the host for long periods, causing chronic infections associated with metabolic disorders, decreased productivity, and modulation of the host immune system [16–18].

A characteristic feature of helminth infections is their ability to induce a T helper type 2 (Th2) immune response. This response is characterized by increased production of cytokines such as interleukin-4 (IL-4), IL-5, and IL-13, the activation of eosinophils and mast cells, and enhanced production of immunoglobulin E (IgE). The Th2-mediated immune response plays a crucial role in eliminating multicellular parasites from the host. However, this immune polarization may simultaneously suppress Th1-mediated immune responses that are required for effective protection against intracellular pathogens, including bacteria of the genus *Brucella* [16, 17, 19, 20].

In addition to inducing Th1/Th2 immune polarization, helminths can also activate regulatory mechanisms of the immune system. These include the expansion of regulatory T cells (Treg) and increased production of anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β). Such mechanisms reduce inflammatory responses and promote parasite survival, but may simultaneously suppress protective immune responses against unrelated pathogens or vaccines [18].

In recent years, increasing attention has been paid to the effects of helminth infections on vaccine-induced immunity. Experimental and epidemiological studies have demonstrated that chronic helminth infections can reduce the immunogenicity and protective efficacy of vaccines against various infectious diseases. These effects are mainly associated with parasite-induced immunomodulation and suppression of Th1-type immune responses required for effective vaccine-mediated protection [21, 22].

In livestock production systems, this interaction between parasitic infections and vaccination outcomes is of particular importance. Helminth infections are widespread in cattle populations and often occur as chronic subclinical infections that remain undetected during routine veterinary monitoring. Although such infections may not always cause obvious clinical signs, they can significantly alter the immunological reactivity of animals. Consequently, helminth infection in cattle may reduce the effectiveness of vaccination against infectious diseases, including brucellosis [6, 15].

A better understanding of the interactions between parasitic and bacterial infections may contribute to improving vaccination strategies, increasing the effectiveness of immunoprophylaxis, and optimizing disease control programs in livestock production.

Materials and Methods

Ethical approval

All procedures involving animals were carried out in accordance with generally accepted veterinary and ethical standards for the use of animals in scientific research.

Animals and study design

The study was conducted on 4-year-old red-steppe cows (n = 20) on a brucellosis-free farm in the Gunibsky District of the Republic of Dagestan. Due to limited material and technical resources, 20 animals were selected for the study. All animals were clinically examined before the experiment and serologically tested for brucellosis. Individual fecal samples were collected from each animal to determine the presence of helminth infections. According to the study design, the animals were divided into two groups of 10 animals each: an experimental group and a control group. Animals in the experimental group received anthelmintic treatment 7 days before vaccination., whereas animals in the control group were vaccinated without prior deworming.

Parasitological examination

Helminth infections were diagnosed using standard parasitological techniques, including coproscopic and larvoscopic examinations. Helminth eggs in fecal samples were detected using the Fulleborn flotation method. Parasitological analysis allowed the identification of helminths belonging to different taxonomic groups.

To evaluate the level of helminth infestation, the extent of the invasion (EI) was calculated as the percentage of infected animals in the examined population. After anthelmintic treatment, the extensive efficiency (EE) was determined to assess the therapeutic efficacy of the drug.

Anthelmintic treatment

Animals in the experimental group were treated with a broad-spectrum anthelmintic drug, Monizen forte. The drug was administered orally as a single dose at a dose of 1 μL per 20 kg of body weight according to the manufacturer's instructions. Treatment efficacy was evaluated based on the absence of helminth eggs in fecal samples collected after treatment.

Vaccination

Following the parasitological examination and anthelmintic treatment, animals in both groups were vaccinated against brucellosis using a vaccine prepared from the low-agglutinogenic Brucella abortus strain No. 82. The vaccine was administered subcutaneously at a dose of 5 μL into the posterior third of the neck according to the recommended vaccination protocol.

Serological testing

Blood samples were collected from the animals 26 days after vaccination to assess the formation of post-vaccination antibodies. Serum samples were obtained by centrifugation and used for serological testing. Three serological diagnostic methods were employed: indirect hemagglutination test (IHA), agglutination test (AT), and complement fixation test (CFT).

The agglutination test and the complement fixation test were performed according to the procedures described in the official "Guidelines for the Diagnosis of Animal Brucellosis." The indirect hemagglutination test was conducted according to the manufacturer's instructions.

Statistical analysis

The obtained data were analyzed using descriptive statistical methods. The prevalence of helminth infections and the efficacy of anthelmintic treatment were expressed as percentages. The serological responses of animals in the experimental and control groups were compared to evaluate the influence of helminth infestation on the development of the immune response following vaccination.

Results

The objective of the present study was to investigate antibody production following vaccination with the Brucella abortus strain 82 vaccine in cattle under conditions of helminth infestation.

For this purpose, two groups of 10 animals each were formed. Before the experiment, all animals were examined for the presence of helminth infections using standard parasitological diagnostic methods. The results of the examination showed that the prevalence of helminth infection among the studied animals was 100%. Parasitological analysis revealed the presence of several groups of helminths, including nematodes (*Dictyocaulus* spp., strongylates, *Nematodirus* spp.) and cestodes (*Moniezia* spp.).

Considering the highprevalence of mixed helminth infections in the studied animals, a broad-spectrum anthelmintic drug, Monizen forte, was selected for deworming. The drug was administered to animals of the experimental group according to the manufacturer's instructions at a dosage of 1 ml per 20 kg of body weight. The results of the treatment effectiveness are presented in Table 1.

Table 1. Efficacy of Monizen forte in the experimental group of animals

Names of diseases	Number of animals freed from parasites after treatment (heads)	Drug dosage (mL/kg)	Route of administration	Extent of the invasion (EI, %)	Extensive efficiency (EE, %)
Nematode infections					
Dictyocaulosis	7	1 mL/20 kg body weight	Single dose, individually, orally	70.0	79.3
Strongylid infection	10	1 mL/20 kg body weight	Single dose, individually, orally	100.0	100.0
Nematodirosis	8	1 mL/20 kg body weight	Single dose, individually, orally		
Cestode infections					
Monieziosis	10	1 mL/20 kg body weight	Single dose, individually, orally	100.0	100.0

The data presented in Table 1 demonstrate the high therapeutic effectiveness of the *Monizen* forte preparation against the helminth infections detected in the animals. In cases of strongylate infections and monieziosis, complete elimination of helminths from the animals' bodies was observed, with both the extent of the invasion (EI) and the extensive efficiency (EE) reaching 100%. High effectiveness was also observed against Nematodirus infections, where 8 out of 10 animals were freed from the parasites. Slightly lower but still significant effectiveness was recorded in the treatment of dictyocaulosis, where 7 animals recovered following treatment, corresponding to an EI of 70.0% and an EE of 79.3%.

Overall, the results indicate that *Monizen* forte exhibits a pronounced anthelmintic effect against the most common gastrointestinal and pulmonary helminths of cattle. The elimination of helminth infections prior to vaccination is particularly important, as parasitic infestations can negatively affect the immune system and alter the formation of post-vaccination immunity.

After evaluating the effectiveness of the anthelmintic treatment, the animals were vaccinated against brucellosis using a vaccine based on Brucella abortus strain 82. Serological examination of blood sera

was performed 26 days after vaccination using three diagnostic methods: the indirect hemagglutination test (IHA), the agglutination test (AT), and the complement fixation test (CFT). The results obtained in the experimental group are presented in Table 2.

Table 2. Results of serological testing of animal blood sera following treatment with the anthelmintic *Monizen* forte

Serological tests			
No	IHA	SAT, ME	CFT
1	1:200 #	100 #	1:20 #
2	1:200 #	100 #	1:20 +++
3	1:200 #	100 ++	1:20 #
4	1:200 +++	100 ++	1:20 ++
5	1:200 #	100 ++	1:20 #
6	1:200 #	100 #	1:20 +++
7	1:200 #	100 #	1:20 #
8	1:200 #	-	1:20 ++
9	1:200 #	100 +++	1:20 #
10	1:200 +++	100 ++	1:20 #

Note: IHA: 1:200 #, +++ high antibody titers
SAT, ME: 100 #, +++ high antibody titers
SAT, ME: 100 ++ low antibody titers
SAT, ME: - no antibody titers
CFT: 1:20 #, +++, ++ high antibody titers

Analysis of the data presented in Table 2 shows that animals of the experimental group, which received anthelmintic treatment prior to vaccination, developed a pronounced humoral immune response to the vaccine. Most animals demonstrated positive serological reactions in at least one of the diagnostic tests. In several animals (Nos. 4, 6, and 10), strong positive reactions were detected simultaneously in two or three tests, indicating a sufficiently high level of antibody production following vaccination.

For example, animal No. 4 demonstrated strong positive reactions (+++) in the IHA and AT tests and a positive reaction (++) in the complement fixation test. Animal No. 6 showed strong antibody titers (+++) in both the IHA and CFT tests. Similarly, animal No. 10 exhibited strong positive reactions (+++) in the IHA test and moderate positive reactions (++) in the AT test. These results indicate active formation of post-vaccination antibodies and confirm the development of a specific immune response to the brucellosis vaccine.

At the same time, several animals showed weaker or borderline reactions in individual tests. However, the presence of positive reactions in different serological assays suggests that the vaccinated animals developed detectable levels of antibodies against Brucella antigens.

In contrast, the results obtained in the control group (Table 3), where animals were vaccinated without prior anthelmintic treatment, showed a less pronounced immune response. Although some animals demonstrated positive serological reactions, the overall antibody response was weaker and more inconsistent compared to the experimental group.

Table 3. Results of serological testing of animals in the control group (without anthelmintic treatment)

Serological tests			
No	IHA	SAT, ME	CFT
1	1:200 +++	100 ++	-
2	1:200 #	100 +	1:20 +
3	1:200 +++	100 +++	1:20 +
4	1:200 #	100 #	1:20 #

Continuation of Table 3

5	1:200 +++	100 ++	-
6	-	100 ++++	1:20 +
7	-	-	1:20 +++
8	1:200 +++	-	-
9	1:200 +++	100 ++	1:20 #
10	1:200 +++	-	-

Note: IHA: 1:200 #, +++ high antibody titers
IHA: - no antibody titers
SAT, ME: 100 #, +++ high antibody titers
SAT, ME: 100 ++ low antibody titers
SAT, ME: - no antibody titers
CFT: 1:20 #, +++ high antibody titers
CFT: 1:20 + low antibody titers
CFT: - no antibody titers

In the control group, several animals demonstrated negative reactions in one or more serological tests. For example, animals Nos. 5, 7, 8, and 10 showed negative reactions in two diagnostic assays. In addition, antibody titers were generally lower, and strong positive reactions (+++) were observed less consistently across the different tests. The uneven distribution of serological responses suggests that helminth infections may have negatively influenced the development of post-vaccination immunity.

The comparison of the serological results between the experimental and control groups clearly demonstrates that animals receiving anthelmintic treatment prior to vaccination developed a more pronounced and consistent immune response. In the experimental group, positive reactions were detected more frequently and were characterized by higher antibody titers across multiple serological tests.

The observed differences in the immune response may be explained by the immunomodulatory effects of helminths. Helminth infections are known to induce a Th2-dominated immune response and activate regulatory immune mechanisms that suppress Th1-mediated immunity. Since protective immunity against Brucella relies largely on Th1-type immune responses, the presence of helminths may interfere with the development of effective post-vaccination immunity.

By eliminating helminths before vaccination, the immune system of animals in the experimental group was likely able to respond more efficiently to the vaccine antigen. This may have contributed to the more active production of antibodies and the stronger serological reactions observed in this group.

Thus, the results obtained in the present study demonstrate the importance of conducting deworming procedures prior to vaccination of cattle against brucellosis. The use of an effective anthelmintic drug allowed elimination of helminth infections and contributed to the development of a more pronounced post-vaccination immune response.

These findings confirm that helminth infections can negatively affect the immunological reactivity of animals and reduce the effectiveness of vaccination programs. Therefore, the integration of antiparasitic treatment into preventive veterinary programs may significantly improve the effectiveness of immunoprophylaxis against brucellosis in cattle.

Discussion

The results obtained in the present study clearly demonstrate that helminth infections may considerably influence the formation of immune responses following vaccination against brucellosis in cattle. Animals that underwent deworming before vaccination developed a more pronounced humoral immune response compared to animals that remained infected with helminths.

The observed differences between the experimental and control groups can be explained by the immunomodulatory properties of helminths. Numerous studies have shown that helminth infections are capable of altering the host immune system by inducing a Th2-dominated immune response and activating regulatory immune pathways. These mechanisms promote parasite survival but may

simultaneously suppress Th1-mediated immune responses, which are essential for protection against intracellular pathogens such as Brucella. Since effective immunity against brucellosis largely depends on the activation of cellular immune mechanisms and the production of interferon-gamma, helminth-induced immune modulation may interfere with the formation of protective post-vaccination immunity.

In the present study, animals treated with the anthelmintic drug Monizen forte demonstrated stronger and more consistent antibody responses following vaccination. The higher frequency of positive serological reactions and the presence of strong antibody titers in several diagnostic tests suggest that elimination of helminths prior to vaccination creates more favorable conditions for the development of immune responses to the vaccine antigen.

Conversely, animals in the control group, which remained infected with helminths, demonstrated weaker and more inconsistent serological reactions. The presence of negative reactions in several diagnostic assays and lower antibody titers indicate that helminth infections may reduce the immunogenicity of the vaccine. These findings are consistent with previously reported data indicating that parasitic infections may suppress immune responses to vaccines and reduce their effectiveness.

The results of this study therefore confirm the importance of considering parasitic infections as a significant factor influencing the outcome of vaccination programs in livestock. In regions where helminth infections are widespread, failure to eliminate parasites prior to vaccination may lead to inadequate development of post-vaccination immunity and consequently reduce the effectiveness of disease control measures.

The use of effective anthelmintic treatment prior to vaccination may therefore represent an important component of integrated veterinary preventive programs. Such an approach may improve the immunological responsiveness of animals and contribute to the formation of a stronger and more stable immune response following vaccination against brucellosis.

Conclusion

The results of the present study demonstrated that the administration of the anthelmintic drug Monizen forte did not produce any negative effects on the physiological condition of the animals during the experimental period. At the same time, the drug showed high anthelmintic efficacy against the helminth infections detected in the studied cattle, including nematodes and cestodes.

The obtained data indicate that helminth infestation may significantly influence the formation of the immune response following vaccination with the Brucella abortus strain 82 vaccine. Animals that received anthelmintic treatment prior to vaccination developed a more pronounced antibody response compared to the control group, which remained infected with helminths. This suggests that helminth infections can reduce the effectiveness of immunoprophylaxis by modulating the immune system and interfering with the development of vaccine-induced immunity.

Based on the results obtained, it is recommended to carry out deworming of cattle 10–14 days prior to vaccination against brucellosis. The implementation of this approach may contribute to the formation of a stronger and more stable immune response following vaccination and may therefore improve the overall effectiveness of brucellosis prevention programs in cattle.

Authors' Contributions

MM: concept development, visualization, and manuscript writing. ShG: formal analysis and resource provision. AKh: methodology development and research. EY: research and manuscript editing. PA: scientific supervision.

Conflicts of Interes

The authors declare that they have no conflicts of interest.

References

1 Corbel, M.J. (2006). Brucellosis in humans and animals. World Health Organization, Geneva.

2 Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., Tsianos, E.V. (2006). The new global map of human brucellosis. *The Lancet Infectious Diseases,* 6(2), 91–99.

3 McDermott, J., Grace, D., Zinsstag, J. (2013). Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique OIE,* 32(1), 249–261.

4 Deryabin, P.N. (2020). Vakcinoprofilaktika i biologicheskaya bezopasnost' strany [Vaccination and bio-security of the country]. *Farmaciya Kazahstana,* 7–8, 20–25.

5 Kulakov, Y.K., Ivanov-Pryanichnikov, I.A. (2022). Vydayushhiisya vklad akademika P.A. Vershilovoi v vakcinoprofilaktiku brucelleza [Outstanding contribution of academician p.a. vershilova to the vaccine prevention of brucellosis]. B*ulletin of Semashko National Research Institute of Public Health*, 1-2, 182-191. DOI: 10.25742/NRIPH.2022.01.024.

6 Berezkina, G.V., Starostina, O.Ya., Panyushkina, I.I., Romanova, S.N. (2013). Skriningovye issledovaniya v ocenke riska zarazhenija vozbuditelyami bakterial'nyh zoonoznyh infekcii i parazitarnyh invazii [Screening study in assessing risk of bacterial zoonotic infections and parasitic infestations]. *Pidemiologiya i vakcinoprofilaktika*, 3(70), 56-58.

7 Tsirelson, L.E., Zheludkov, M.M., Scliarov, O.D., Borovoi, V.N. (2011). Borovoi Sostojanie specificheskoi immunoprofilaktiki brucelleza v Rossiiskoi Federacii [Condition specific immunoprophylaxis of brucellosis in russia]. *Epidemiologiya i vakcinoprofilaktika,* 1(56), 59-63.

8 Arakelyan, P.K., Khristenko, N.V., Gaivoronskaya, YU.E., Dimova, A.S., Dimov, S.K., Yanchenko, T.A. (2023). Tehnologichnost' raznyh shem immunizacii krupnogo rogatogo skota protiv brucelleza s vozmozhnost'yu rannei postvakcinal'noi diagnostiki [Manufacturability of different schemes of immunization of cattle against brucellosis with the possibility of early post-vaccination diagnosis]. *Veterinariya*, 5, 11-16. DOI 10.30896/0042-4846.2023.26.5.11-15.

9 Baldwin, C.L., Goenka, R. (2006). Host immune responses to the intracellular bacteria Brucella: does the bacteria instruct the host to facilitate chronic infection? *Critical Reviews in Immunology*, 26(5), 407–442.

10 Skendros P., Boura P. (2013). Immunity to brucellosis. *Revue Scientifique et Technique de OIE*, 32(1), 137–147.

11 Vinokurov, N.V., Sleptsov, E.S., Alfero, I.V. (2025). Osobennosti provedenija specificheskih profilakticheskih meropriiatiy protiv brucelleza severnyh domashnih olenei [Features of specific preventive measures against brucellosis of reindeer]. *Veterinariya i kormlenie,* 5, 22-25. DOI: 10.30917/ATT-VK-1814-9588-2025-5-5.

12 Arakelyan, P.K., Tregubov, A.N., Vergun, A.A., Ilin, E.N., Yanchenko, T.A., Dimova, A.S., Borovoy, V.N., Sklyarov, O.D. (2020). Effektivnost' konjunktival'noi immunizacii krupnogo rogatogo skota vakcinoi iz shtamma B. abortus 19 pri brucelleze [Antiepizootic effectiveness of conjunctival immunization of cattle with a vaccine from the b. Abortus 19 strain in brucellosis]. *Veterinariya*, 10, 9-12. – DOI: 10.30896/0042-4846.2020.23.10.09-12.

13 Gaynelyanov, R.D., Nikolaeva, O.N., Mullayarova, I.R., Shaimukhametov, M.A., Razyapov, M.M., Savintsev, D.A. (2024). Monitoring epizooticheskoi situacii po infekcionnym i invazionnym boleznyam zhivotnyh v Respublike Bashkortostan [Monitoring of the epizootic situation on infectious and invasive diseases of animals in the Republic of Bashkortostan]. *Veterinariya, zootehniya i biotehnologiya*, 10, 48-54. DOI 10.36871/vet.zoo.bio.202410006.

14 Bogacheva, N.V., Okhapkina, V.Y., Pyatkova, N.V., Fedotov, A.K., Kucherenko, A.S. (2016). Eksperimental'noe izuchenie vliyaniya immunomoduljatorov na effektivnost' primeneniya vakciny brucelleznoi zhivoi suhoi [Experimental research of the influence of immunomodulators on efficiency using of brucellosis living dry vaccine]. *Epidemiologiya i vakcinoprofilaktika*, 2(87), 84-92.

15 Shevkoplyas, V.N., Lopatin, V.G. (2008). Vliyanie gel'mintozov na techenie immunologicheskih processov u zhivotnyh [The influence of helminthosis on current immunologic processes at animals]. *Rossiiskii parazitologicheskii zhurnal*, 4, 94-101.

16 McSorley, H.J., Maizels, R.M. (2012). Helminth infections and host immune regulation. *Clinical Microbiology Reviews*, 25(4), 585–608.

17 Maizels, R.M., Smits, H.H., McSorley, H.J. (2018). Modulation of host immunity by helminths: the expanding repertoire of parasite effector molecules. *Immunity*, 49, 801–818.

18 Maizels, R.M., McSorley, H.J. (2016). Regulation of the host immune system by helminth parasites. *Journal of Allergy and Clinical Immunology*, 138(3), 666–675.

19 Sleptsov, E.S., Fedorov, A.I., Vinokurov, N.V., Grigoriev, I.I., Plemyashov, K.V., Fedorova, P.N. (2020). Specificheskaya profilaktika brucelleza krupnogo rogatogo skota v Rossiiskoi Federacii [Specific prevention of bovine brucellosis in the russian federation]. *Veterinariya i kormlen*ie, 6, 47-50. DOI 10.30917/ATT-VK-1814-9588-2020-6-14.

20 Elias, D., Akuffo, H., Pawlowski, A., Haile, M., Schon, T., Britton, S. (2008). Schistosoma mansoni infection reduces the protective efficacy of BCG vaccination. *Vaccine,* 26, 1457–1463.

21 Ndeffo Mbah M.L., Skrip L.A., Greenhalgh S., Hotez P.J., Galvani A.P. (2013). Impact of Schistosoma mansoni infection on immune responses to vaccination. *PLoS Neglected Tropical Diseases,* 7(1), e2210.

22 Kokolova, L.M., Vinokurov, N.V., Gavrilyeva, L.Y., Sivtseva, E.V., Plemyashov, K.V. (2020). Rasprostranennost' associativnyh invazii u olenei i reakciya na Brucellez pri allergicheskih probah [Prevalence of associative infestations in deer and reaction to brucellosis in allergic tests]. *Veterinariya i kormlenie*, 7, 20-22. DOI 10.30917/ATT-VK-1814-9588-2020-7-5.

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• **Keywords:** Keywords should be listed in alphabetical order, separated by semicolons, in singular form, and should include 4–6 words or phrases.

• **Introduction:** Provide a brief description of the relevance of the topic or problem based on a review of current literature, substantiating the significance of the study objective and indicating the novelty of the work.

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• **Results:** Present an analysis of the findings using tables, graphs, and/or figures. All data must be subjected to statistical analysis.

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• **Conclusion:** Summarize the main conclusions and outline prospects for further research on the studied topic/problem.

• **Author contributions, acknowledgements, and funding:** Brief statements on each author's contribution, acknowledgements to colleagues or other individuals who assisted in the research, and information on funding sources must be included **ONLY IN THE REVISED**

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1 Ayuti, S.R., Khairullah, A.R., Lamid, M., Warsito, S.H., Al Arif, M.A., Kim, E.J., Moses, I.B., Shin, S., Wardhani, B.W.K., Wasito, W., Khalisa, A.T., Ahmad, R.Z. (2025). Bluetongue in ruminants: Global epidemiology, pathogenesis, and advances in diagnostic and control strategies within a One Health framework. *Vet World,* 18(10), 3070-3093. DOI:10.14202/vetworld.2025.3070-3093.

2 Hermosilla, C. (2022). Aqmola oblysy sharwashylyqtaryndagy qoy ishek biotsenozindagy eimeriyalardyn turlik quramy [Species composition of Eimeria in the intestinal biocenosis of sheep in farms of the Akmola region]. *Herald Sci. Seifullin Kazakh Agrotech. Univ*., 4(115), 16–26. DOI: 10.51452/kazatu.2022.4.1263. [*In Kazakh*].

3 Khusainov, D.M. (2022). Kontrol biologicheskoi aktivnosti virusa infektsionnoi anemii loshadei pri statsionarnom i suspenzionnom kultivirovanii [Control of the biological activity of equine infectious anemia virus during stationary and suspension cultivation]. *Herald Sci. Seifullin Kazakh Agrotech. Univ.,* 2(113), 156–164. DOI: 10.51452/kazatu.2022.2(113).1074. [*In Russian*].

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Abbreviations of veterinary, medical, and/or biological terms should be used only if they are widely recognized in the international scientific community. For all other terms, the full name must be provided in parentheses at the first mention, followed by the abbreviation to be used thereafter.

Terminology should follow the most recent recommendations of the relevant international nomenclature authorities.

Bacterial names must conform to the latest edition of Bergey's Manual of Determinative Bacteriology (The Williams & Wilkins Co., Baltimore). Viruses must be classified and named in accordance with the recommendations of the International Committee on Taxonomy of Viruses (ICTV).

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Research article

PCR-based identification of animal species in commercial sausage products using cytochrome b gene markers

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Abstract

Background and Aim.....
Materials and Methods.....
Results.....
Conclusion.....(max. 300 words)
Keywords:.....(4-6 words)

Introduction.....
Materials and Methods.....
Results.....
Discussion.....
Conclusion.....
Authors' Contributions.....
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SB, AB: Planned the study and drafted and revised the manuscript. KT, AS: Performed the extraction of DNA and PCR analysis. KM, AB: Performed the analysis date. All authors have read, reviewed, and approved the final manuscript.

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