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Research article

Mapping and analysis of the distribution of parasitic infections of cats in cities of Kazakhstan

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Abstract

Background and Aim. Endoparasitic infections in domestic and stray cats are of veterinary and public health importance due to their potential zoonotic transmission and environmental contamination. In Kazakhstan, systematic spatial data on the distribution of endoparasites in cats remain limited. This study aimed to develop an information database and a GIS-based cartographic model for collecting, analyzing, and spatially visualizing data on endoparasitic infections in cats and to identify areas with potentially increased infection risk.

Materials and Methods. Data from 1,301 cats from five major cities of Kazakhstan (Almaty, n=282; Astana, n=382; Shymkent, n=389; Uralsk, n=76; and Kostanay, n=192) were collected from veterinary clinics and animal shelters. Fecal samples from 1,301 cats were examined for helminth eggs and coccidian oocysts; 1,256 cats were tested for Giardia spp. coproantigen, and serum samples from 273 cats were tested for Toxoplasma gondii-specific IgG antibodies. The database included biological characteristics, sample types, laboratory results, and geographic coordinates of sampling sites. GIS-based spatial visualization was performed using ArcGIS to assess the distribution of *Toxocara* cati, *Giardia* spp., *Cystoisospora* spp., and *Cryptosporidium* spp. based on coprological examinations, as well as T. gondii seropositivity based on serological testing.

Results. GIS-based visualization demonstrated parasite-specific spatial variation among the studied cities. The distribution patterns differed according to parasite species; for example, higher proportions of *Toxocara cati*, *Cystoisospora* spp., and *Giardia* spp.-positive samples were observed in Almaty, whereas Cryptosporidium spp. showed the highest observed proportion in Uralsk. These descriptive spatial patterns should not be interpreted as evidence of specific climatic or anthropogenic determinants, which were not directly measured in the present study. The developed GIS model enabled visualization of parasite distribution and identification of areas with higher concentrations of positive samples.

Conclusion. The developed information database and GIS-based cartographic model provide useful tools for the systematic collection, spatial analysis, and visualization of data on endoparasitic infections in cats. Their application can support epidemiological surveillance, risk assessment, and the planning of targeted preventive measures in veterinary and public health programs in Kazakhstan.

Keywords: cat; geographic information system; Kazakhstan; spatial analysis; *Toxoplasma gondii*.

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Introduction

Parasitic zoonoses associated with domestic and stray animals remain a major concern for global public health, particularly in the context of rapid urbanization and increasing human–animal interactions. Among these, infections transmitted by cats are of particular importance due to their close contact with humans and their role as hosts of several clinically significant parasites. For instance, *Toxoplasma gondii*, whose definitive hosts are cats that excrete environmentally resistant oocysts in their feces, is estimated to infect up to 30–50% of the global human population, making it one of the most widespread parasitic infections worldwide [1].

Cats serve as hosts for a range of endoparasites, including *Toxocara cati*, *Giardia* spp., *Cryptosporidium* spp., *Cystoisospora* spp., and *Toxoplasma gondii* [2–4]. These pathogens are capable of persisting in the environment in the form of resistant eggs, oocysts, or cysts, contaminating soil, water, and urban surfaces. Protozoan parasites such as *Giardia* spp. and *Cryptosporidium* spp. are particularly important because of their low infectious dose and role in waterborne outbreaks [5], whereas *Toxoplasma gondii,* for which cats are the definitive hosts and a key component of transmission dynamics, poses a serious risk to pregnant women and immunocompromised individuals [6].

Large urban areas represent complex ecological systems in which high densities of domestic and stray animals, combined with variable sanitary conditions and anthropogenic factors, create favorable conditions for the maintenance and transmission of parasitic infections. In such environments, stable foci of parasite circulation may develop, contributing to sustained infection pressure and increasing the risk of zoonotic transmission [7].

Within the framework of the One Health concept, which integrates human, animal, and environmental health, the study of the spatial distribution of zoonotic infections is especially important [8]. Modern Geographic Information Systems (GIS) provide powerful tools for integrating epidemiological, veterinary, and environmental data, enabling spatial analysis, visualization of infection distribution, and identification of clustering patterns [9].

Despite the growing importance of this field, comprehensive studies combining parasitological data with spatial analysis remain limited in Central Asia, including Kazakhstan. The development of an information database containing epidemiological characteristics of feline parasitic zoonoses allows for the systematization and analysis of data, identification of high-risk foci, and visualization of parasite distribution using cartographic models. This approach improves the efficiency of monitoring and prevention programs and supports the development of strategies aimed at reducing the risk of human infection [10].

Therefore, the aim of the present study was to map and analyze the spatial distribution of parasitic infections in cats in major cities of Kazakhstan using a GIS-based approach, with emphasis on visualization of parasite-specific spatial patterns and areas with higher concentrations of positive samples.

Materials and Methods

A structured geospatial database integrating parasitological and epidemiological data was developed to assess the distribution of zoonotic parasitic infections in cats across major cities of Kazakhstan, including Astana, Almaty, Shymkent, Uralsk, and Kostanay.

All primary data, including information on sampling locations (precise geographic coordinates), biological characteristics of the animals (owned or stray cats, sex, age), type of biological material (feces, blood serum), sampling and laboratory examination dates, as well as results of parasitological analyses, were initially structured in tabular format using Microsoft Excel. Each record was assigned a unique identification code to facilitate database management and prevent duplication.

The prepared dataset was imported into ArcGIS 10.3 using the Display XY Data function, with the coordinate system set to GCS_Krasovsky_1940, which is considered the most suitable for cartographic analyses within Kazakhstan. Based on the input coordinates, a separate shapefile layer containing georeferenced information for each sampling case was created [11].

Spatial data analysis and visualization were performed in ArcGIS using built-in geoprocessing tools. The Definition Query function was applied to extract data subsets according to specific criteria (e.g., positive parasite detection, sampling by owned or stray cats, or administrative regions).

Graphical visualization was carried out using the Symbology module, in which parasitological results were represented as point features with varying color and size attributes reflecting parasite species, prevalence, or infection intensity. For improved spatial interpretation, these point layers were overlaid with raster maps of cities and administrative regions of Kazakhstan, allowing the identification of distribution patterns in relation to geographic and landscape factors.

Data sources included statistical records from veterinary clinics and animal shelters, encompassing information on animal health status, case frequency, and population size, as well as laboratory results obtained at the Institute of Animal Science and Veterinary Medicine of Saken Seifullin Kazakh Agrotechnical University and the Laboratory of Biodiversity and Genetic Resources of the National Center for Biotechnology. These analyses were aimed at detecting pathogens, determining infection prevalence, and assessing the overall epidemiological situation.

In total, the database included records for 1,301 urban cats: Almaty (n=282), Astana (n=382), Shymkent (n=389), Uralsk (n=76), and Kostanay (n=192), including both owned and stray (shelter-based) animals. Samples were collected from August 2023 to January 2025. Data were obtained from 20 veterinary clinics and 10 animal shelters that voluntarily participated in the study. The cats were older than one month of age. All animals were either presented by owners for routine or clinical examinations at veterinary clinics or housed in shelters. Cats that had received antiparasitic treatment within the previous four weeks were excluded from the study.

Fecal samples from 1,301 cats were examined for helminth eggs and coccidian oocysts using the standardized Sheather's sugar flotation method. *Giardia* spp. coproantigen was assessed in 1,256 cats using a commercial immunochromatographic assay. Blood serum samples from 273 cats were analyzed for *Toxoplasma gondii*-specific immunoglobulin G (IgG) antibodies using a commercially available enzyme-linked immunosorbent assay. Detection of *T. gondii*-specific IgG was interpreted as evidence of previous exposure to the parasite and not as proof of active infection at the time of sampling.

Results

The characteristics of the studied cities, including geographic location, climate zone, human population size, and number of registered cats, are presented in Table 1.

Gastro-intestinal parasites were detected in cats in all five cities (Figure 1). Both helminths and protozoa were identified, with *Toxocara cati* representing the predominant helminth species, whereas protozoan findings included *Giardia* spp., *Cystoisospora* spp., and *Cryptosporidium* spp. The observed proportions varied among cities and parasite species:

- In Almaty (n=282), *Toxocara cati* was detected in 15.2% of samples, *Cystoisospora* spp. in 19.1%, *Giardia* spp. in 12.4%, and *Cryptosporidium* spp. in 2.9%.
- In Shymkent (n=389), *Toxocara cati* was detected in 5.4% of samples, *Giardia* spp. in 2.7%, *Cystoisospora* spp. in 1.6%, and *Cryptosporidium* spp. in 1.1%.
- In Uralsk (n=76), Toxocara cati was detected in 1.3% of samples, *Giardia* spp. in 8.7%, *Cystoisospora* spp. in 9.2%, and *Cryptosporidium* spp. in 6.6%. Other helminths were detected at low frequencies in the overall dataset, including *Capillaria* spp. (0.4%), taeniid eggs (0.4%), *Dipylidium* spp. (0.08%), and *Diphyllobothrium/Spirometra* spp. (0.08%).
- In Kostanay (n=192), *Toxocara cati* was detected in 4.2% of samples, *Giardia* spp. in 2.3%, *Cystoisospora* spp. in 1.6%, and *Cryptosporidium* spp. in 1.6%.
- In Astana (n=382), *Toxocara cati* was detected in 2.4% of samples, *Giardia* spp. in 6.4%, *Cystoisospora* spp. in 6.3%, and *Cryptosporidium* spp. in 2.9%.

Table 1. Geographic location, climatic zone, population size, and number of registered cats in the surveyed cities of Kazakhstan (from: *Lider* et al. 2025, *Veterinary World* 18(6), 1748–1758)

City	Coordinates[1]	Climate zone[2]	Population	Number of registered cats
Almaty	43.25°N 76.9167°E	Dfb – warm-summer humid continental climate with relatively constant precipitation throughout the year	2,290,000	43,450

Continuation of Table 1

Astana	51.1801°N 71.446°E	Dfb – humid continental climate with cold winters and warm summers	1,529,000	19,750
Uralsk	51.2333°N 51.3667°E	Dfa – hot-summer humid continental climate	369,000	ND
Kostanay	53.2144°N 63.6246°E	Dfb – humid continental climate with cold winters and warm summers	273,000	ND
Shymkent	42.3°N 69.6°E	Csa – Mediterranean climate with hot summers	1,125,000	ND

Notes: ND = No data available.
[1] Source: Geodata.
[2] Climate zones classified according to the Köppen–Geiger climate classification.

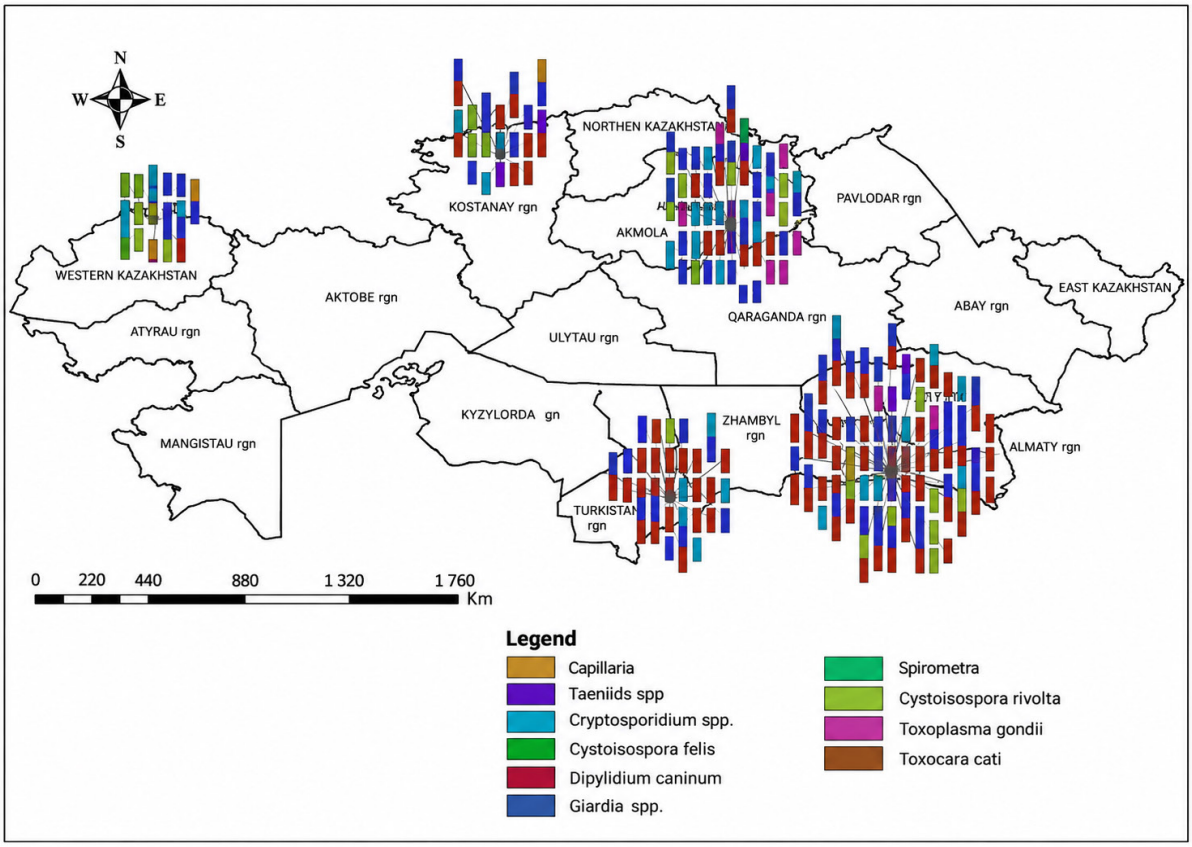

Figure 1. Cartographic model of the distribution of gastro-intestinal parasites of cats in five cities of Kazakhstan

City-specific spatial distributions of parasites are presented in Figures 2–4. Mapping of the sampling locations revealed an uneven distribution of positive cases within each city.

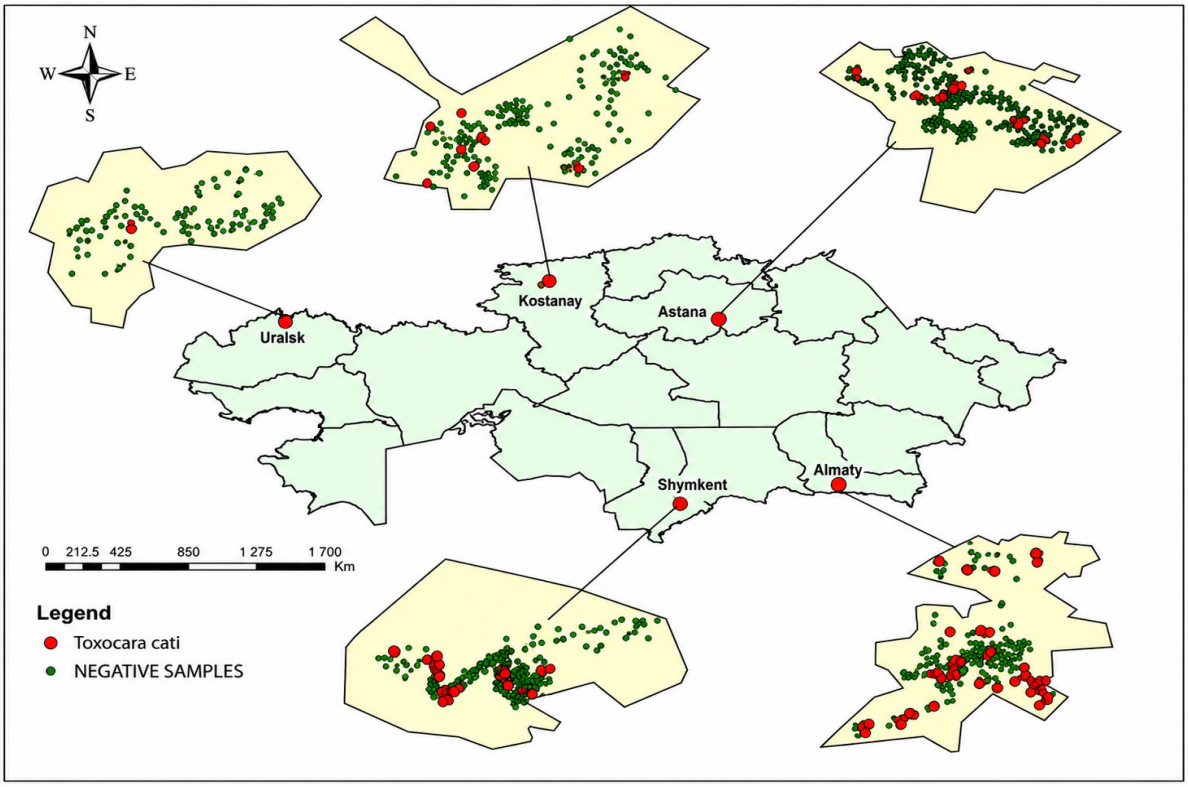

Figure 2. Distribution of *Toxocara cati* egg-positive fecal samples in cats

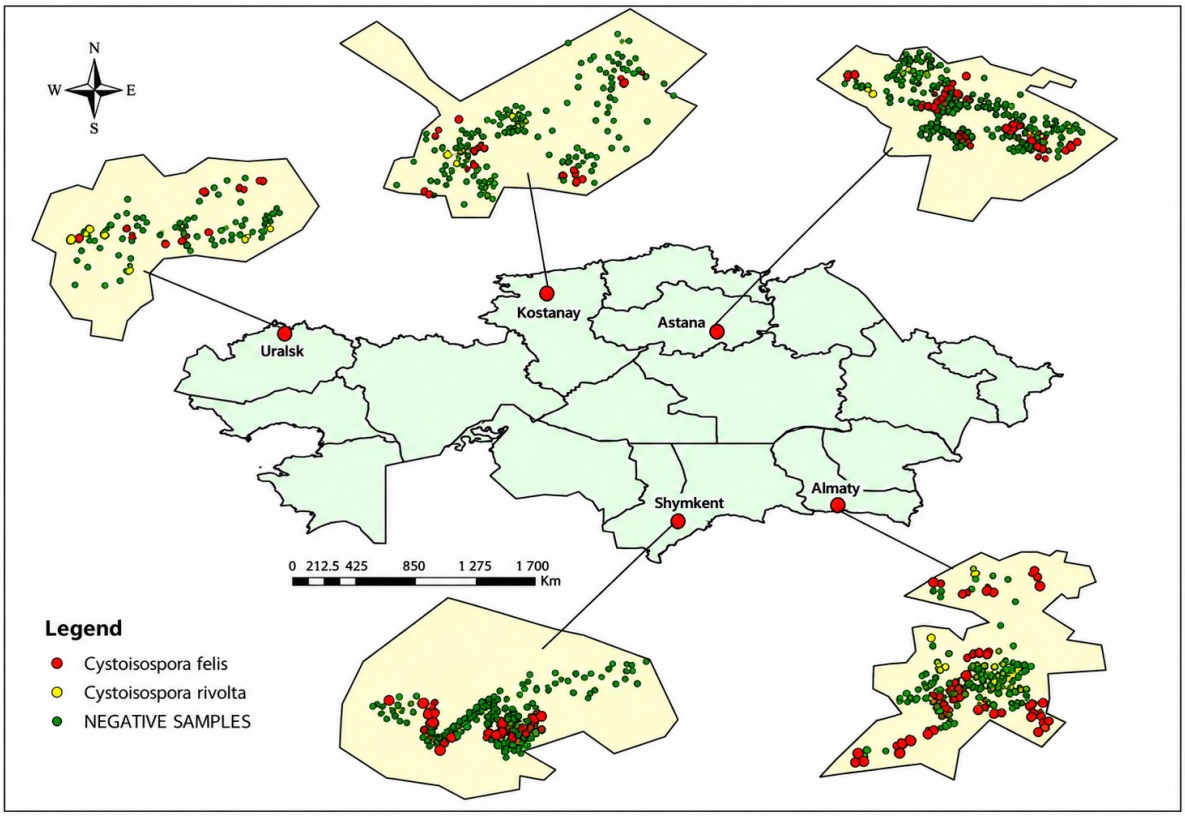

Figure 3. Distribution of *Cystoisospora* spp. oocyst-positive fecal samples in cats

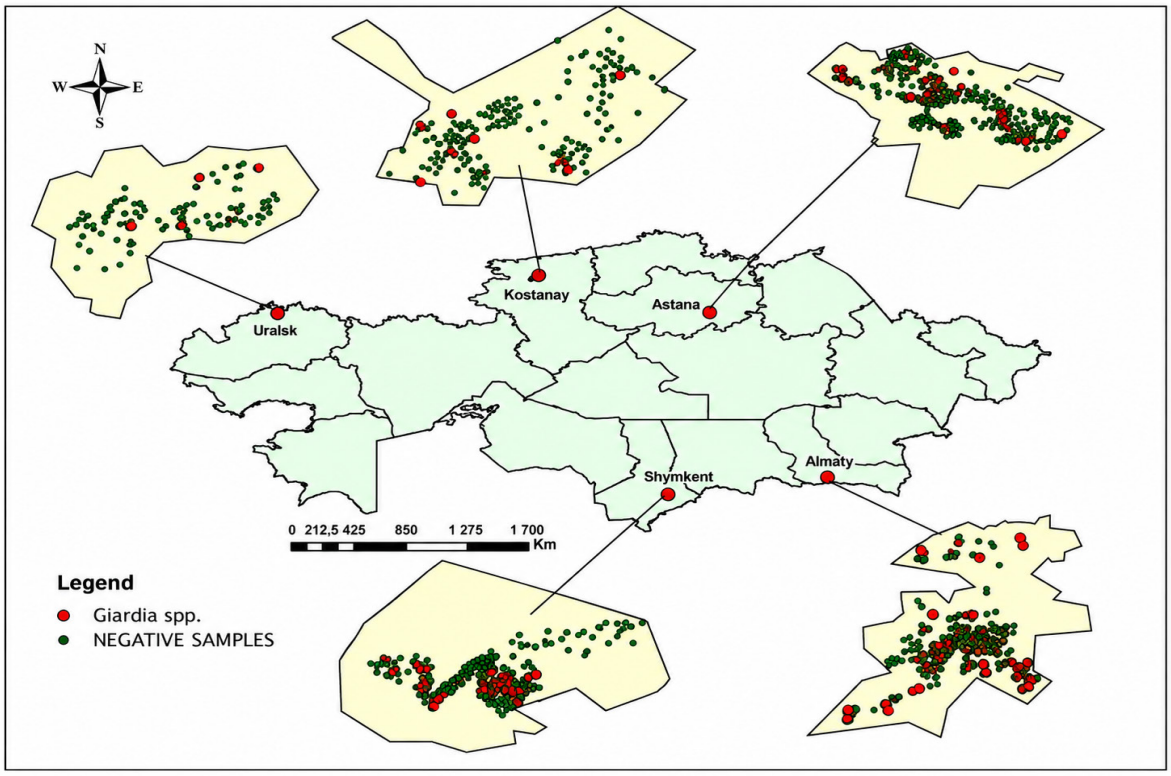

Figure 4. Distribution of *Giardia* spp. coproantigen-positive fecal samples in cats

Serological analysis of Toxoplasma gondii-specific IgG demonstrated variability in seropositivity among the cities (Figure 5). Seropositive animals were recorded in Astana (21.5%), Almaty (20.5%), Uralsk (15.2%), Shymkent (10.5%), and Kostanay (8.0%). These results indicate previous exposure to *T. gondii* and should not be interpreted as evidence of active infection at the time of sampling.

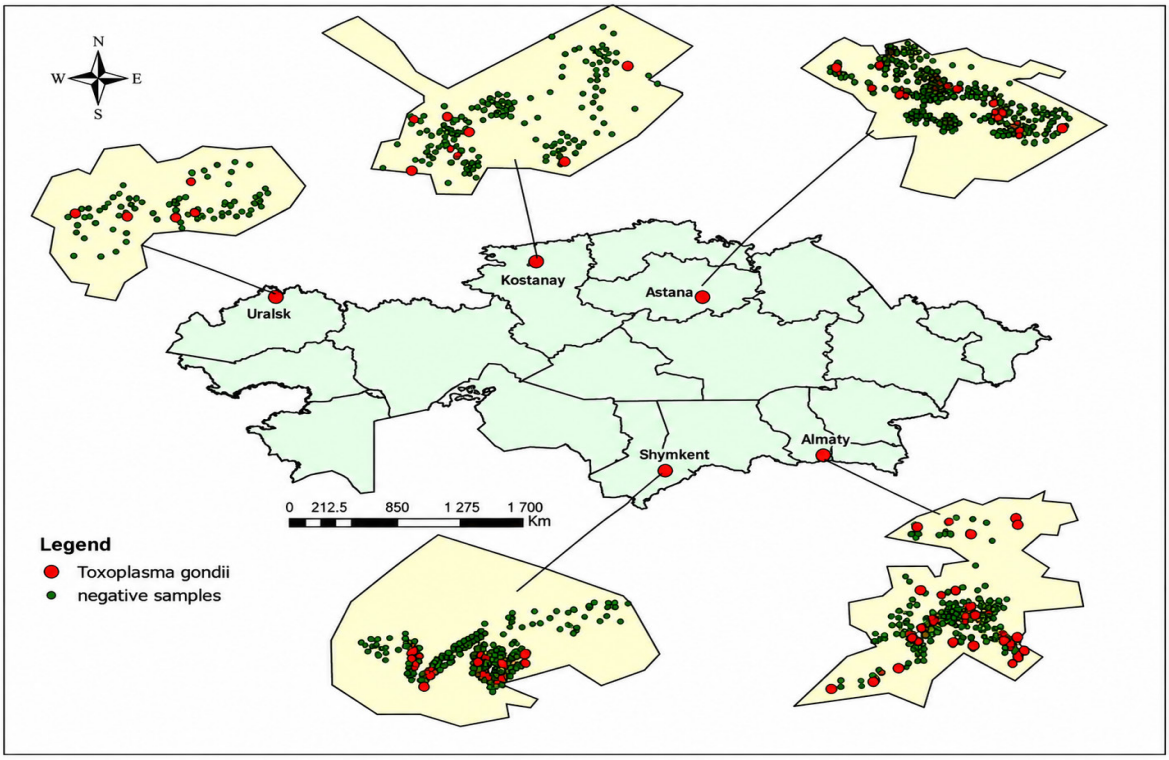

Figure 5. Distribution of *Toxoplasma gondii* antibody-positive blood samples in cats

Discussion

The present study demonstrated spatial variation in the distribution of feline parasites across five major cities of Kazakhstan. The observed patterns were parasite-specific and should be interpreted descriptively. Environmental, climatic, and anthropogenic factors may plausibly contribute to spatial variation; however, these variables were not directly measured or statistically evaluated in the present study and therefore cannot be considered established determinants of the observed patterns.

In Almaty, relatively high observed proportions were recorded for *Toxocara cati, Cystoisospora* spp., and *Giardia* spp. Previous studies have shown that temperature, humidity, host density, and animal management can influence the persistence and transmission of parasitic stages [2, 10]. However, the present study did not directly measure these factors; therefore, they should be regarded only as possible explanations for the observed spatial pattern.

Astana showed lower observed proportions for some parasites, including *Toxocara cati*, whereas the proportions of *Giardia* spp., *Cystoisospora* spp., and *Cryptosporidium* spp. followed different patterns. Although climatic conditions may influence the survival of parasite developmental stages in the environment [11], no direct association between climate and parasite occurrence was tested in the present study.

The distribution patterns of protozoan parasites, especially *Giardia* spp. and *Cryptosporidium* spp., varied among cities. *Cryptosporidium* spp. showed the highest observed proportion in Uralsk (6.6%), compared with lower proportions in the other surveyed cities. Because environmental and host-related determinants were not directly assessed here, the reasons for this pattern cannot be established from the present data. *Giardia* spp. and *Cryptosporidium* spp. include taxa of recognized zoonotic importance; however, species- or genotype-specific zoonotic transmission was not evaluated in this study [5, 12].

Geospatial mapping of sampling points was used to visualize the distribution of *Toxoplasma gondii*-seropositive cats across the surveyed cities. The proportions of seropositive animals were 21.5% in Astana, 20.5% in Almaty, 15.2% in Uralsk, 10.5% in Shymkent, and 8.0% in Kostanay. These findings do not support a simple south-to-north or central-to-western geographic gradient. Moreover, *T. gondii*-specific IgG indicates previous exposure and does not demonstrate active infection or current oocyst shedding.

The application of GIS-based methods provided an effective approach for integrating epidemiological and spatial data and visualizing the geographic distribution of positive samples. In the present study, the maps were used for descriptive spatial visualization rather than formal statistical cluster detection. Accordingly, areas with a higher concentration of positive samples are interpreted as descriptive spatial patterns and not as statistically validated hotspots. Similar GIS-based approaches are widely used in spatial epidemiology to support visualization and subsequent hypothesis-driven analyses [9, 13, 14].

From a One Health perspective, the detection of parasites with recognized zoonotic potential in urban cats highlights the relevance of integrated veterinary, environmental, and public health surveillance. However, the present study did not investigate human infections or directly assess transmission from cats to humans. Therefore, the findings should be interpreted as indicating potential zoonotic relevance rather than demonstrating zoonotic transmission.

Conclusion

A GIS-based spatial analysis and cartographic visualization of feline endoparasites, including *Toxocara cati, Giardia* spp., *Cystoisospora* spp., and *Cryptosporidium* spp., together with *Toxoplasma gondii* seropositivity, was developed for five cities of Kazakhstan using ArcGIS.

The spatial visualization demonstrated parasite-specific heterogeneity among and within the five surveyed cities. Local areas with higher concentrations of positive samples were observed on the maps; these represent descriptive spatial patterns rather than statistically validated hotspots. The GIS-based approach can support veterinary surveillance and help prioritize locations for further targeted epidemiological investigation. Because environmental determinants and human infection were not directly assessed, causal and zoonotic transmission inferences should not be drawn from these spatial patterns alone.

Author's Contributions

LL and CB: Conceptualized and designed the study, conducted a comprehensive literature search. VK, MYe and IS: Analyzed the gathered data and drafted the manuscript. DS, RU and CB: Conducted

the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript

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Conflicts of Interest

The authors declare that they have no conflicts of interest related to this study. No financial or non-financial competing interests exist that could have influenced the results or interpretation of the data presented in this manuscript.

References

1 World Population Review. (2024). *Cat population by country 2024.* https://worldpopulationreview.com/country-rankings/cat-population-by-country

2 TANBA. (2025). *Animal Accounting Information.* https://tanba.kezekte.kz/en/tanba-public/stat

3 Deplazes, P., Eckert, J., Mathis, A., von Samson-Himmelstjerna, G., Zahner, H. (2016). *Parasitology in Veterinary Medicine.* Wageningen: Wageningen Academic Publishers: 650.

4 Foreman-Worsley, R., Finka, L.R., Ward, S.J., Farnworth, M.J. (2021). Indoors or outdoors? An international exploration of owner demographics and decision making associated with lifestyle of pet cats. *Animals*, 11(2), 253.

5 Maciag, L., Morgan, E.R., Holland, C. (2022). Toxocara: Time to let cati 'out of the bag'. *Trends Parasitol*., 38(4), 280–289.

6 Lider, L.A., Akibekov, O.S., Tokpanov, S.S., Ibraev, N.E., Borovikov, S.N. (2010). Helminths of domestic carnivores in the Akmola region. *Bull. Sci. S. Seifullin Kazakh Agrotech. Univ.,* 48–52.

7 Lider, L.A., Akmambayeva, B.Y., Seitkamzina, D.M., Kiyan, V.S., Mannapova, N.E., Oleks, I.A., Sytnik, I.I. (2023). Stepen' zarazhennosti koshek parazitozami v nekotoryh megapolisah Kazahstana [The degree of infection with cat parasites in some megacities of Kazakhstan]. *Nauka i obrazovanie*, 1(2(75), 95–106. DOI: 10.52578/2305-9397-2024-2-1-95-106. [*In Russian*].

8 Lider, L., Ussenbayev, A., Kiyan, V., Kurenkeyeva, D., Seitkamzina, D., Akmambayeva, B. et al. (2024). Prevalence of Giardia duodenalis in household and shelter cats in Almaty, south-eastern Kazakhstan. *Am. J Anim. Vet. Sci.,* 19(3), 273–279. DOI:10.3844/ajavsp.2024.273.279.

9 Lider, L.A., Seitkamzina, D.M., Ussenbayev, A.E., Kiyan, V.S., Zhanabayev, A.A., Sakhariya, L. (2025). Prevalence of zoonotic intestinal protozoa infections of cats in Central Asia and border regions. *Herald Sci. S. Seifullin Kazakh Agrotech. Res. Univ: Vet. Sci.,* 2(010), 80–92. DOI:10.51452/kazatuvc.2025.2(010).1942.

10 Layman, N.C., Basinski, A.J., Zhang, B., Eskew, E.A., Bird, B.H., Ghersi, B.M. et al. (2023). Predicting the fine-scale spatial distribution of zoonotic reservoirs using computer vision. *Ecol. Lett.,* 26(11), 1974–1986. DOI:10.1111/ele.14307.

11 Esri. Geographic Coordinate System: GCS_Krasovsky_1940. In: ArcGIS Desktop 10.3 Coordinate System Database. Redlands (CA): Environmental Systems Research Institute; 2014.

12 Feng, Y., Xiao, L. (2011). Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. *Clin. Microbiol. Rev.,* 24(1), 110–140. DOI:10.1128/CMR.00033-10.

13 Lider, L.A., Kiyan, V., Mannapova, N.E., Olex, I.A., Bauer, C. (2025). Seroprevalence of Toxoplasma gondii infection in urban client-owned cats in Kazakhstan. *Vet. Res. Commun.*, 49, 291. DOI:10.1007/s11259-025-10862-1.

14 Lider, L.A., Kiyan, V., Seitkamzina, D.M., Ussenbayev, A., Akmambaeva, B.E., Uakhit, R.S., Mannapova, N.E., Sytnik, I., Bauer, C. (2025). Prevalence, risk factors, and zoonotic implications of gastrointestinal parasites in urban cats in Kazakhstan: A cross-sectional multicity study. *Vet. World*., 18(6), 1748–1758. DOI:10.14202/vetworld.2025.1748-1758.

Review article

Wheat bran-derived xylo- and maltooligosaccharides as potential synergistic prebiotics for animal nutrition

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Abstract

Background and Aim. Xylooligosaccharides (XOS) and maltooligosaccharides (MalOS) are promising prebiotic candidates for functional feed additives in animal nutrition. Wheat bran, containing arabinoxylan-rich hemicellulose and residual starch, is a potential source of both oligosaccharide fractions. This review analyzes their structural-functional properties, possible prebiotic synergy, and prospects for use in animal feeding.

Materials and Methods. Wheat bran was considered a plant-derived biomatrix containing arabinoxylans as a source of XOS and residual starch as a potential source of maltooligosaccharides (MalOS). This review analyzed English-language peer-reviewed publications from 2004–2025 on wheat bran composition, XOS and maltooligosaccharide structure, digestive stability, gut microbiota modulation, short-chain fatty acid production, and use in animal nutrition. Searches were conducted in Web of Science, PubMed, and Google Scholar using keywords including wheat bran, xylooligosaccharides, maltooligosaccharides, prebiotic properties, animal nutrition, gut microbiota, fermentation kinetics, and short-chain fatty acids.

Results. The reviewed data indicate that wheat bran is a promising substrate for obtaining two functional oligosaccharide groups: XOS from the arabinoxylan fraction and maltooligosaccharides from residual starch. XOS are resistant to digestive enzymes and can reach the lower intestine, where they serve as substrates for saccharolytic bacteria, whereas maltooligosaccharides may be fermented more rapidly if partially preserved after digestion. The prebiotic effects of XOS are supported by *in vitro* and animal studies, particularly in poultry, whereas evidence for MalOS is more limited and is derived mainly from in vitro studies. Available data indicate the potential of MalOS to modulate gut microbiota and promote short-chain fatty acid production, but these effects require further validation in animals. The combined use of XOS and MalOS may provide complementary prebiotic effects due to differences in their structure, digestive stability, and fermentation rate; however, direct evidence for synergistic action between XOS and MalOS is currently lacking.

Conclusion. Wheat bran is a promising source of XOS- and maltooligosaccharide-containing fractions for functional feed additives. Their combination may support animal gut microbiota, enhance short-chain fatty acid production, strengthen intestinal barrier function, and improve feeding efficiency. Further studies should focus on standardizing fraction composition, optimizing production conditions, and validating XOS-maltooligosaccharide efficacy in *in vitro, in vivo*, and productive animal trials.

Keywords: animal nutrition; maltooligosaccharides; xylooligosaccharides; wheat bran.

Introduction

Gastrointestinal diseases and functional disorders in farm animals remain among the key factors contributing to reduced productivity, impaired feed conversion, increased treatment costs, and higher mortality rates in young animals [1, 2]. The most vulnerable periods include the early postnatal stage in calves, the weaning period in piglets, and the intensive growth phase in poultry, when the intestinal

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microbiota, mucosal barrier function, and digestive enzyme system undergo active restructuring. For example, in a study of dairy calves up to six months of age, diarrhea was the most common disease, with a risk of 25.2%, and accounted for 33.3% of all mortality cases [3].

In pig production, one of the major problems is post-weaning diarrhea, which is associated with abrupt dietary changes, restructuring of the intestinal microbiota, reduced intestinal barrier function, and an increased risk of colonization by enterotoxigenic E. coli strains. This leads to reduced body weight gain, impaired feed conversion, and economic losses [4]. In this regard, current studies emphasize the need for feeding strategies aimed at supporting the gut microbiota, strengthening the intestinal barrier, and reducing dependence on antibiotics and pharmacological doses of zinc oxide [5, 6].

In poultry production, necrotic enteritis associated with *Clostridium perfringens* is a significant example of intestinal pathology. This disease disrupts intestinal integrity, impairs nutrient absorption, reduces body weight gain, and increases the feed conversion ratio [7]. According to recent reviews, necrotic enteritis is among the most economically important intestinal diseases in poultry, with global annual losses estimated at approximately USD 6 billion [8].

In the light of this, there is a growing need for functional feed components capable of supporting the intestinal microbiota of animals without the direct use of antibiotic growth promoters [9]. These components include prebiotics - substrates selectively utilized by the microbiota in both monogastric and ruminant animals [10, 11]. Such substrates promote the growth of beneficial bacteria, stimulate the production of short-chain fatty acids, reduce intestinal pH, strengthen the epithelial barrier, improve the stability of the intestinal ecosystem, and may contribute to improved animal performance [12].

Functional oligosaccharides, including xylooligosaccharides (XOS) and maltooligosaccharides, are of particular interest among feed prebiotics [13]. XOS are non-digestible xylan-derived substrates capable of reaching the lower intestinal tract, where they are utilized by saccharolytic bacteria, including *Bifidobacterium* and *Lactobacillus* [14]. Evidence on the use of XOS in animal production has already accumulated, particularly in poultry. A meta-analysis of broiler studies, including 36 peer-reviewed papers published between 2000 and 2024, showed that XOS supplementation increased average daily gain by 6.445% and average daily feed intake by 1.544%, while reducing the feed conversion ratio by 7.147% compared with the control [15]. In laying hens, dietary inclusion of XOS for 24 weeks was associated with improved intestinal morphology, increased cecal concentrations of butyric and isovaleric acids, greater bacterial diversity, and an increased relative abundance of *Lactobacillus* and *Bifidobacteriaceae [*16].

Maltooligosac charides have been studied in animal nutrition to a much lesser extent than XOS, fructooligosaccharides, galactooligosaccharides, or mannan-oligosaccharides; therefore, their feed-related potential should be considered cautiously – as a promising but still insufficiently validated direction [17, 18]. From a scientific perspective, interest in maltooligosaccharides (hereafter referred to as MalOS to avoid confusion with mannan-oligosaccharides) is associated with their potential role as starch-derived substrates with faster fermentative availability, whereas XOS may provide a more prolonged effect on the intestinal microbiota. These differences in structure and fermentation kinetics form the basis for the hypothesis of complementary effects of XOS and MalOS in feed compositions [19, 20].

Wheat bran is a promising raw material for obtaining such oligosaccharide fractions. It is an accessible by-product of flour milling that contains an arabinoxylan-rich hemicellulosic fraction and residual starch. Arabinoxylans can serve as a source of XOS, whereas the residual starch fraction can be used to obtain MalOS [21]. Experimental data confirm the high potential of wheat bran as a source of XOS: after enzymatic treatment of destarched and deproteinized wheat bran, *Wang* et al. obtained XOS with a purity of approximately 95%, a degree of polymerization of DP2–DP7, and a yield of about 31.2% based on the mass of wheat bran insoluble dietary fiber [22]. Similar data were obtained by *Kumari* et al., who reported a maximum XOS yield of 374 mg/g from raw wheat bran and 419 mg/g from wheat bran-extracted xylan [23]. These values indicate that wheat bran should be considered not only as a conventional feed component but also as a promising bioresource for producing concentrated XOS fractions potentially suitable for functional feed additives.

The practical interest in XOS is supported not only by experimental studies but also by the development of the feed prebiotic market. A recent review by *Ali* et al. indicates that XOS are used

in various sectors, including the feed industry, and commercial catalogues already offer feed-grade XOS products, such as powdered forms containing 35% XOS for livestock, poultry, pet food, and aquaculture [24]. However, in most commercial descriptions, the raw material source of XOS is not disclosed; therefore, such products cannot automatically be considered wheat bran-derived.

In contrast to XOS, quantitative data and commercial feed solutions for MalOS specifically obtained from the residual starch of wheat bran are much more limited. Nevertheless, experimental data on MalOS as functional starch-derived oligosaccharides indicate their potential significance. In the study by *Jang* et al., enzymatic treatment of starch-containing raw material resulted in a MalOS content of 52.34%, and the fraction contained 21.74% maltotriose, 18.84% maltotetraose, and 11.76% maltopentaose. The addition of 1% MalOS stimulated the growth of *Bifidobacterium breve,* while 2% MalOS during fecal fermentation promoted increased production of short-chain fatty acids and altered the microbial profile [19]. A comparative study by *Cheon* et al. also showed that MalOS have moderate digestibility: after *in vitro* digestion, the reducing sugar content was 17.55%, and released glucose accounted for 10.3%, indicating partial preservation of the fraction for subsequent microbial fermentation [25]. Therefore, with respect to wheat bran, it is most appropriate to state that XOS already have confirmed technological and feed-related potential, whereas MalOS derived from the residual starch of wheat bran represent a promising additional fraction requiring further standardization, yield assessment, and validation in trials with productive animals.

Thus, wheat bran has a dual raw-material potential: its arabinoxylan-rich fraction can be used to obtain XOS, while its residual starch fraction can serve as a source of MalOS. The combination of these oligosaccharides is of scientific interest from the perspective of feed biotechnology, since differences in their chemical structure, digestive stability, and rate of microbial fermentation may provide complementary prebiotic effects. A potential XOS–MalOS composition may be directed toward supporting the intestinal microbiota of animals, stimulating beneficial bacteria, enhancing short-chain fatty acid production, maintaining intestinal barrier function, and improving feeding efficiency. However, the integrated use of wheat bran as a single raw material source of XOS and MalOS, as well as the evaluation of their combined effects in animal production, remains insufficiently studied.

This review aims to analyse wheat bran as a source of xylo- and maltooligosaccharides, to discuss their structural – functional properties, potential complementary prebiotic effects, and prospects for application in animal nutrition.

Materials and Methods

Source Material

Wheat bran, a by-product of the flour-milling industry, was considered in this review as a complex plant-derived biomatrix. Its composition includes an arabinoxylan-rich hemicellulosic fraction and a residual starch fraction retained after grain milling. The presence of these biopolymers provides a basis for the targeted transformation of wheat bran into functional oligosaccharide fractions.

Wheat bran was evaluated as an accessible and promising bioresource for obtaining value-added oligosaccharide products, including xylooligosaccharides (XOS) and maltooligosaccharides (MalOS). The arabinoxylan fraction represents the main substrate for XOS formation, whereas residual starch may serve as a source of MalOS through the action of amylolytic enzymes.

Data Sources and Search Strategy

This study was designed as a narrative literature review. Scientific publications related to the biotechnological processing of wheat bran, the production of xylo- and maltooligosaccharides, and the evaluation of their functional and prebiotic properties were analysed. Particular attention was given to studies addressing the structure-dependent properties of XOS and MalOS, including degree of polymerization, type of glycosidic linkages, presence of structural substituents, resistance to digestive enzymes, and microbial fermentation patterns.

The review included studies describing the effects of oligosaccharides on gut microbiota, the growth of beneficial bacteria such as Bifidobacterium and Lactobacillus, the formation of short-chain fatty acids, and differences in fermentation kinetics among individual oligosaccharide fractions. Experimental *in vitro* and *in vivo* studies, data from animal nutrition trials, review articles, and book chapters were considered. The main time frame of the analysed literature covered publications from 2004 to 2025.

Search Tools and Keywords

The literature search was conducted using the international databases Web of Science, PubMed, and Google Scholar. The following keywords and their combinations were used: wheat bran, xylooligosaccharides (XOS), maltooligosaccharides, malto-oligosaccharides, MalOS, prebiotic properties, animal nutrition, feed additives, gut microbiota, fermentation kinetics, short-chain fatty acids, enzymatic hydrolysis, arabinoxylan, starch hydrolysis, and functional oligosaccharides.

The search was mainly limited to English-language peer-reviewed publications. Priority was given to studies containing experimental data on the production of oligosaccharides from plant-derived raw materials, characterization of their structure and degree of polymerization, and assessment of their functional effects. Additional attention was paid to studies discussing the possible combined use of XOS and MalOS as a basis for complementary or potentially synergistic prebiotic effects on the gut microbiota of farm animals.

The reliability of the selected studies was assessed based on the type of publication, the presence of clearly described experimental methods and controls, and the relevance of the reported data to the objectives of this review. Greater weight was given to peer-reviewed experimental studies and recent systematic or comprehensive reviews. Where evidence was limited or indirect, the corresponding conclusions were interpreted cautiously and identified as requiring further experimental validation.

Results

Chemical Composition and Biopolymeric Structure of Wheat Bran

Wheat bran (Triticum aestivum) is a multilayered plant structure separated from the endosperm during grain milling [26]. Its morphological and chemical composition is determined by the structural characteristics of the outer layers of the wheat kernel, including the pericarp, seed coat, hyaline layer, and aleurone layer. These anatomical fractions are characterized by dense cell walls, a high dietary fiber content, and a complex spatial organization of biopolymers, which determine both the technological resistance of wheat bran and its value as a raw material for biotechnological processing [27].

From a chemical perspective, wheat bran is a multicomponent plant biomatrix dominated by non-starch polysaccharides, primarily arabinoxylans, cellulose, and other cell-wall components [28]. In addition, wheat bran contains residual starch, proteins, lipids, minerals, B vitamins, phytic acid, and phenolic compounds. This combination of components makes wheat bran not only a source of dietary fiber but also a promising substrate for the production of value-added products, including functional oligosaccharides [29].

According to review studies, wheat bran usually contains a substantial proportion of non-starch polysaccharides, residual starch, proteinaceous compounds, lipids, and mineral components. The quantitative ratio of these fractions may vary depending on wheat cultivar, growing conditions, degree of grain milling, and processing technology [30, 31]. Therefore, wheat bran should not be regarded as a homogeneous by-product, but rather as a variable biopolymeric system whose properties are determined by both the original grain material and the processing method [32].

The biopolymeric structure of wheat bran is formed through close interactions among polysaccharides, proteins, and phenolic compounds within the cell wall. Arabinoxylans and cellulose constitute the structural framework of the grain outer layers, whereas proteins, lignin-like components, and phenolic acids further reinforce the cell-wall matrix [33]. This organization provides high mechanical strength to wheat bran, but at the same time limits enzymatic access to the target polysaccharide substrates. Therefore, efficient release of oligosaccharide fractions requires pretreatment and controlled enzymatic hydrolysis [34].

Of particular importance for the production of xylo- and maltooligosaccharides is the presence of two distinct carbohydrate fractions in wheat bran: the arabinoxylan fraction and the starch fraction. Arabinoxylans are the major hemicellulosic components of the outer grain layers and serve as potential substrates for the formation of xylooligosaccharides, whereas residual starch retained in bran due to incomplete separation of the endosperm can be used to obtain maltooligosaccharides. Thus, wheat bran has dual raw-material potential, serving as a source of both hemicellulose-derived and starch-derived oligosaccharides [29, 34].

Therefore, wheat bran should be considered a multicomponent plant biomatrix in which structural polysaccharides, residual starch, proteins, lipids, minerals, and phenolic compounds form a complex cell-wall system. The combined presence of arabinoxylan and starch fractions determines its value as a raw material for obtaining functional oligosaccharides, including xylooligosaccharides (XOS) and maltooligosaccharides (MalOS), and justifies further analysis of individual wheat bran fractions in the following subsections.

Chemical Structure of Xylooligosaccharides

Xylooligosaccharides (XOS) are short-chain oligosaccharides composed predominantly of D-xylose residues linked by β-(1→4)-glycosidic bonds. The basic monomeric unit of XOS is D-xylose, with the molecular formula $C_5H_{10}O_5$. Condensation of several xylose residues results in the formation of linear XOS with different chain lengths, among which the most common are xylobiose ($C_{10}H_{18}O_9$), xylotriose ($C_{15}H_{26}O_{13}$), xylotetraose ($C_{20}H_{34}O_{17}$), xylopentaose ($C_{25}H_{42}O_{21}$), and xylohexaose ($C_{30}H_{50}O_{25}$). In general, linear XOS can be represented by the formula $C_{5n}H_{8n}+2O_{4n}+1$, where n corresponds to the degree of polymerization (DP). For the most extensively studied XOS fractions, the DP usually ranges from DP2 to DP6 [24, 35].

Structurally, XOS may occur either as linear xylooligosaccharides or as substituted forms, primarily arabino-xylooligosaccharides (AXOS), which are formed during the partial hydrolysis of arabinoxylans. Enzymatic hydrolysis of xylan using xylanases is considered an effective approach for the production of XOS, as it enables selective cleavage of the β-(1→4)-xylosidic linkages in the xylan backbone under mild reaction conditions [36, 37]. In AXOS, side substituents are attached to the xylan backbone, most commonly α-L-arabinofuranose residues located at the O-2 and/or O-3 positions of xylose units. The presence of these substituents, as well as possible acetyl or phenolic moieties, substantially influences the physicochemical properties of the oligosaccharides, including their solubility, molecular weight, hydration capacity, and accessibility for microbial fermentation [38].

As shown in Figure 1, the structural organization of XOS includes the D-xylose monomeric unit, linear oligomers with different degrees of polymerization ranging from xylobiose to xylohexaose, their formation through partial hydrolysis of xylan/arabinoxylan, and substituted AXOS forms containing lateral arabinose residues. The same figure schematically illustrates that XOS are not hydrolysed during digestion in the upper gastrointestinal tract, whereas their main transformation occurs during microbial fermentation in the large intestine.

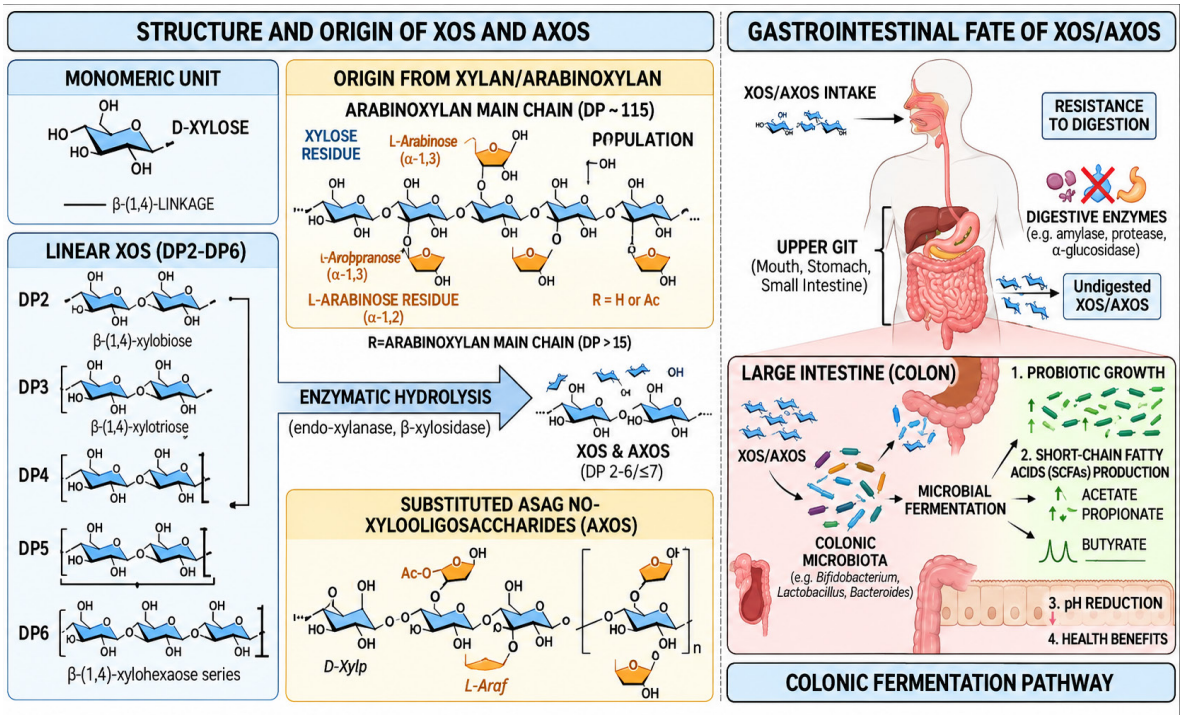

Figure 1. Structure, origin, and gastrointestinal transformation of xylo- and arabino-xylooligosaccharides in animals

From a chemical perspective, the key parameters determining the properties of XOS are the type of glycosidic linkage, degree of polymerization, and degree of substitution. Linear XOS, represented by the repeating fragment [β-D-Xylp-(1→4)] n, differ from substituted AXOS in that the latter contain additional side groups capable of altering the spatial conformation of the molecule and its interactions with water and enzymes. In general, short-chain XOS fractions with DP2–DP4 are characterized by higher solubility and greater accessibility for microbial utilization, whereas longer or more highly substituted forms may exhibit slower fermentation and a more prolonged effect in the intestinal environment [39].

A fundamental feature of XOS is that β-(1→4)-xylosidic linkages are not hydrolysed by digestive enzymes in the upper gastrointestinal tract of animals. Unlike α-glucans, which are susceptible to amylase-mediated hydrolysis, XOS retain their structure during passage through the stomach and small intestine and are therefore classified as non-digestible oligosaccharides. This property enables their delivery to the lower gastrointestinal tract, where they become substrates for the fermentative activity of the intestinal microbiota. Consequently, XOS are regarded as promising prebiotic compounds, while their structural parameters – primarily DP and the presence of substituents – are key factors determining the selectivity and intensity of microbial fermentation [35, 38].

Thus, xylooligosaccharides represent a structurally heterogeneous group of non-digestible oligosaccharides whose functional properties are determined by chain length, the presence of β-(1→4)-glycosidic linkages, and the degree of substitution of the xylan backbone. The occurrence of both linear XOS and substituted AXOS expands their physicochemical and biological characteristics by influencing solubility, resistance to digestive enzymes, and availability to the intestinal microbiota. Owing to their stability in the upper gastrointestinal tract and their ability to undergo microbial fermentation in the lower intestine, XOS are considered promising prebiotic compounds, the effectiveness of which largely depends on the degree of polymerization and molecular organization.

Chemical Structure of Maltooligosaccharides

Maltooligosaccharides (malto-oligosaccharides, MalOS) are starch-derived homooligosaccharides composed of D-glucose residues linked predominantly by α-(1→4)-glycosidic bonds. The basic monomeric unit of these compounds is D-glucose, with the molecular formula $C_6H_{12}O_6$, while linear MalOS can generally be represented by the formula $C_{6n}H_{10n}+_2O_{5n}+1$, where n corresponds to the degree of polymerization. Typical representatives of this group include maltose ($C_{12}H_{22}O_{11}$; DP2), maltotriose ($C_{18}H_{32}O_{16}$; DP3), maltotetraose ($C_{24}H_{42}O_{21}$; DP4), maltopentaose ($C_{30}H_{52}O_{26}$; DP5), and maltohexaose ($C_{36}H_{62}O_{31}$; DP6). In recent reviews, MalOS are generally described as compounds containing approximately 3–10 glucose residues, although in technological schemes maltose is often also included in maltooligosaccharide fractions as a DP2 component [18].

Unlike XOS, which are formed from the xylan backbone of hemicellulose, MalOS are produced through partial hydrolysis of starch, a polysaccharide composed of amylose and amylopectin. Amylose is a predominantly linear α-(1→4)-linked glucan, whereas amylopectin has a branched structure due to α-(1→6) linkages at branching points. Therefore, the profile of the resulting MalOS depends not only on the degree of hydrolysis but also on the amylose-to-amylopectin ratio in the original starch, the accessibility of starch granules, and the presence of branched dextrins. In the case of incomplete amylopectin degradation, limit dextrins or fractions containing residual α-(1→6) linkages may remain in the hydrolysate, affecting the molecular-weight distribution and functional properties of the final product (Figure 2) [40].

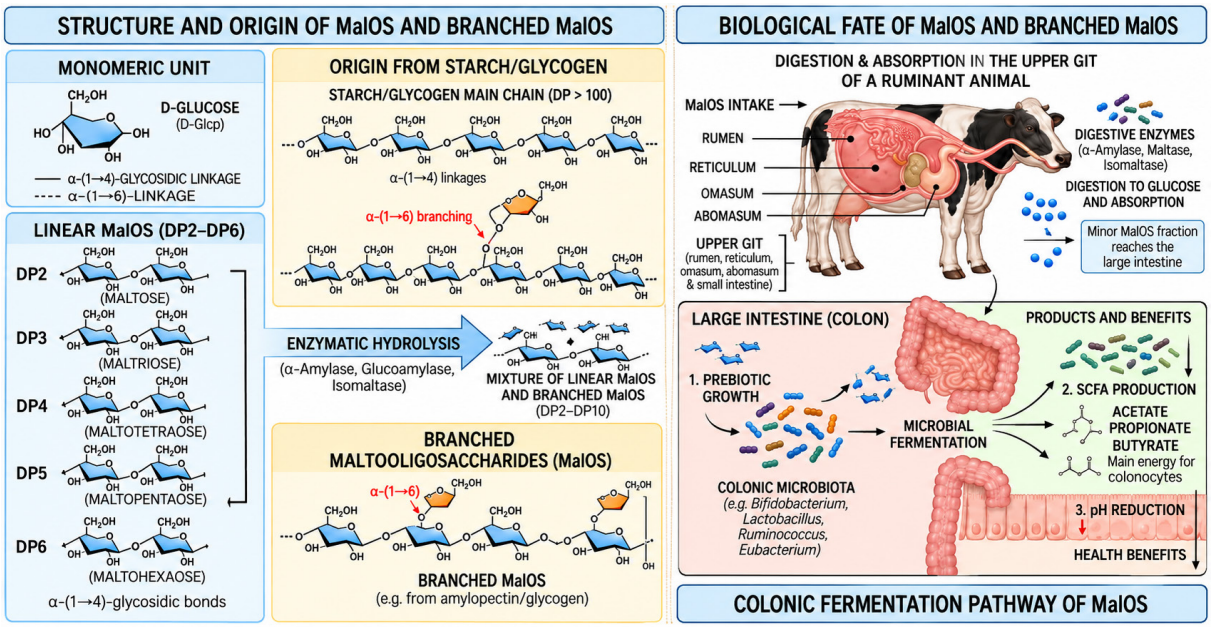

Figure 2. Structure, origin, and biological transformation of maltooligosaccharides (MalOS) in the gastrointestinal tract of farm animals

The formation of MalOS is determined by the specificity of amylolytic enzymes. α-amylases catalyse the endohydrolysis of internal α-(1→4) linkages in starch, resulting in the formation of dextrins, maltose, maltotriose, and other short glucose oligomers [41, 42]. In addition to conventional α-amylases, maltooligosaccharide-forming amylases are of particular importance, as they can selectively generate products with a defined degree of polymerization, such as maltotriose, maltotetraose, maltopentaose, or maltohexaose. This is essential for obtaining standardized MalOS fractions, since the physiological and technological properties of maltooligosaccharides depend not only on their general glucose-based nature but also on the distribution of individual DP fractions [18, 43].

From the perspective of food chemistry, MalOS occupy an intermediate position between readily digestible starch hydrolysis products and potentially functional oligosaccharide fractions. Since α-(1→4)-glycosidic linkages are accessible to human digestive enzymes, part of the MalOS fraction may be hydrolysed in the upper gastrointestinal tract by salivary and pancreatic α-amylases, as well as by brush-border enzymes of the small intestine. However, their degree of digestibility depends on chain length, fraction structure, food matrix, and associated components. Therefore, MalOS should not automatically be classified as completely non-digestible compounds: their prebiotic potential requires evaluation of digestive stability and the ability of preserved fractions to reach the large intestine [25, 40].

Experimental data confirm that certain MalOS-containing fractions may exhibit prebiotic-like effects. In the study by *Jang* et al., MalOS produced with the involvement of amylase contained substantial proportions of maltotriose, maltotetraose, and maltopentaose; these fractions were evaluated for their resistance to *in vitro* digestion, their ability to stimulate the growth of *Bifidobacterium breve*, and their capacity to alter the fermentation profile of human faecal microbiota [19]. In a comparative study by *Cheon* et al., MalOS were evaluated together with other commercially available prebiotic oligosaccharides; the authors showed that MalOS demonstrated moderate enzymatic digestibility but, during subsequent faecal fermentation, were able to influence microbiota composition and the metabolomic profile [25].

In a broader context, MalOS belong to the group of gluco-oligosaccharides, for which structure, synthesis methods, purification, digestive stability, and potential prebiotic effects have been actively studied in recent years. The review by *Zeng* et al. emphasizes that the prebiotic properties of gluco-oligosaccharides depend on the number of glucose residues, the type of O-glycosidic linkages, the degree of polymerization, and selective fermentability by beneficial bacteria; however, their effects remain more variable compared with classical non-digestible prebiotics [43]. This point is particularly

important for MalOS, since their functional activity is determined not only by the presence of α-(1→4) linkages but also by the proportion of the fraction that remains after digestion and becomes available for microbial fermentation.

Thus, the chemical structure of MalOS is determined by the glucose nature of the monomeric units, the predominance of α-(1→4)-glycosidic linkages, the degree of polymerization, and the characteristics of enzymatic production from starch. Unlike XOS, maltooligosaccharides are more susceptible to digestive enzymes in humans; however, certain MalOS fractions may persist after digestion and participate in microbial fermentation. Therefore, MalOS should be regarded as structure-dependent functional oligosaccharides whose prebiotic potential is determined by the balance between digestibility, degree of polymerization, fraction composition, and the ability to support the growth of beneficial members of the intestinal microbiota.

Prebiotic Effects and the Influence of XOS and MalOS on the Gut Microbiota

The prebiotic effect of oligosaccharides is associated with their ability to reach the large intestine in an unchanged or partially preserved form and serve as selective substrates for metabolically active members of the gut microbiota. The most important effects include stimulation of beneficial bacteria, primarily representatives of the genera *Bifidobacterium* and *Lactobacillus*, enhanced production of short-chain fatty acids (SCFA), reduction of intestinal pH, inhibition of opportunistic microorganisms, and support of intestinal epithelial barrier function [44, 45].

Effects of XOS on Bifidobacterium, Lactobacillus, and the Microbiota Profile

Xylooligosaccharides (XOS) are among the most extensively studied non-digestible oligosaccharides with pronounced bifidogenic potential. Their β-(1→4)-linked xylosyl backbone is not degraded by human digestive enzymes but can be utilized by specific groups of intestinal bacteria possessing the corresponding xylosidases, xylanases, and arabinofuranosidases. Recent review studies indicate that XOS can selectively stimulate the growth of beneficial members of the gut microbiota, primarily *Bifidobacterium* and certain lactic acid bacteria, enhance the production of short-chain fatty acids, and limit the development of opportunistic microorganisms; however, the magnitude of these effects depends on the degree of polymerization, raw material source, production method, and presence of structural substituents [13, 20, 46].

Of particular importance is that different bacterial species utilize XOS and AXOS unevenly. For example, *Bifidobacterium adolescentis* is able to utilize both unsubstituted XOS and arabinose-substituted AXOS, whereas certain representatives of *Lactobacillus* may ferment branched forms less efficiently. This is explained by differences in the enzymatic machinery of microorganisms: bifidobacteria can produce enzymes capable of removing arabinose substituents, thereby increasing the accessibility of the xylan backbone for further degradation. Such structure-dependent differences create prerequisites for selective modulation of the microbiota and the formation of cross-feeding interactions among different bacterial groups [36, 47].

Data from *in vitro* and clinical studies confirm that XOS can increase the relative abundance of *Bifidobacterium* and certain lactic acid bacteria, as well as shift the microbiota composition toward a profile associated with improved intestinal homeostasis. Review data indicate that in studies using individual strains and fecal cultures, XOS promoted the growth of *Bifidobacterium, Lactobacillus, Bacteroides*, and other saccharolytic microorganisms, while in some cases limiting the growth of *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus,* and other opportunistic bacteria [13, 38].

Prebiotic Potential of MalOS and Features of Their Microbial Fermentation

Maltooligosaccharides (MalOS) have been studied as prebiotic compounds less extensively than XOS, fructooligosaccharides, or inulin; however, the available data indicate their functional potential. Unlike XOS, MalOS consist of α-(1→4)-linked glucose residues, and therefore part of these fractions may be accessible to human digestive enzymes. Nevertheless, the stability of certain MOS-containing fractions in the in vitro digestion models and their ability to be utilized by fecal microbiota indicate their possible involvement in prebiotic fermentation [19].

In the study by *Jang* et al., MalOS fractions obtained using amylase and containing substantial proportions of maltotriose, maltotetraose, and maltopentaose stimulated the growth of *Bifidobacterium breve* when used as a carbon substrate. In addition, fermentation involving human fecal microbiota was associated with changes in the microbial metabolic profile and SCFA formation. These data

suggest that MOS can be considered not only as intermediate products of starch hydrolysis but also as potential functional substrates for specific members of the intestinal microbiota [19].

Comparative evaluation of commercial prebiotic oligosaccharides in an *in vitro* gut model also indicates that MOS can influence the microbial ecosystem, although their effects differ from those of classical non-digestible substrates. In the study by *Cheon* et al., MalOS were compared with fructooligosaccharides, agar-derived fructooligosaccharides, and resistant maltodextrin; the authors showed that MOS exhibited moderate digestibility by digestive enzymes but, during subsequent fecal fermentation, contributed to changes in *Bifidobacterium* abundance and in the metabolomic profile of the intestinal model [25].

Formation of Short-Chain Fatty Acids

A key functional outcome of microbial fermentation of XOS and MalOS is the formation of short-chain fatty acids, primarily acetate, propionate, and butyrate. SCFA are formed in the large intestine during the fermentation of dietary components that have not been digested or absorbed in the small intestine; acetate, propionate, and butyrate are the most abundant SCFA and constitute the major acid pool in the colon [48].

XOS are typically associated with pronounced acetate production as one of the main products of bifidobacterial metabolism. Acetate can then serve as a substrate for other bacteria, including butyrate-producing microorganisms such as *Faecalibacterium prausnitzii, Anaerostipes, Eubacterium,* and *Roseburia*. This cross-feeding mechanism explains why stimulation of bifidobacteria by prebiotics may indirectly enhance butyrate formation and support the metabolic stability of the intestinal ecosystem [47].

MalOS, in turn, may be incorporated more rapidly into fermentative metabolism, provided that part of the fraction remains after digestion. In *in vitro* studies, MalOS-containing fractions were associated with increased production of acetate, propionate, and butyrate, indicating their ability to contribute to the energetic and metabolic support of the intestinal microbiota [19, 25].

Support of the Intestinal Barrier and Immunometabolic Significance

Short-chain fatty acids are not only end-products of fermentation but also important signaling molecules that influence the state of the intestinal barrier. Butyrate serves as an energy substrate for colonocytes, participates in the regulation of tight-junction protein expression, reduces inflammatory activity, and supports the integrity of the epithelial layer. Acetate and propionate are also involved in the regulation of pH, metabolic pathways, and immune responses, making SCFA an important link between diet, microbiota, and the state of the intestinal mucosa [48, 49].

From this perspective, the prebiotic action of XOS and MalOS is realized not only through an increase in the abundance of individual beneficial bacteria but also through changes in the metabolic activity of the entire microbial ecosystem. Increased SCFA production contributes to a reduction in intestinal pH, which may limit the growth of pH-sensitive pathobionts, while also supporting the epithelial barrier and local immune homeostasis. Therefore, evaluation of XOS and MalOS should include not only analysis of *Bifidobacterium* and *Lactobacillus* growth, but also determination of the SCFA profile, indicators of barrier function, and markers of the inflammatory response.

The combined study of XOS and MalOS is of particular interest because these oligosaccharides differ in chemical structure, resistance to digestive enzymes, and probable rate of microbial fermentation. XOS, as β-linked non-digestible xylan-derived oligosaccharides, may provide a more selective and prolonged stimulation of bifidobacteria and other saccharolytic bacteria. MalOS, as α-(1→4)-linked starch-derived oligosaccharides, may potentially be incorporated more rapidly into microbial fermentation, provided that they are partially preserved after digestion. This difference creates a basis for complementary effects: XOS may support selective bifidogenic activity, whereas MalOS may contribute to an earlier fermentative response and SCFA production [19, 38].

Thus, XOS and MalOS may be regarded as functionally distinct and potentially complementary substrates. The prebiotic effects of XOS are supported by substantially broader experimental evidence, whereas the functional effects of MalOS have been demonstrated mainly in vitro and require further validation. Their potential complementary action should therefore be considered a hypothesis requiring direct experimental confirmation.

Potential Synergy between XOS and MalOS

The potential synergy between xylooligosaccharides (XOS) and maltooligosaccharides (MalOS) is associated with the fact that these compounds differ in chemical nature, resistance to digestive enzymes, and, presumably, the rate of microbial fermentation in the large intestine. XOS are β-(1→4)-linked xylan-derived oligosaccharides and more closely meet the criteria for non-digestible prebiotic substrates, whereas MalOS are α-(1→4)-linked glucose oligomers, some of which may undergo digestive hydrolysis, while certain fractions can persist and become involved in microbial fermentation. Therefore, their joint consideration makes it possible to formulate a hypothesis of complementary action: MalOS may provide an earlier fermentative response, whereas XOS may exert a more selective and prolonged effect on the intestinal microbiota [19, 20].

From the perspective of fermentation kinetics, XOS and MalOS may perform different functions within a single prebiotic composition. MalOS, as starch-derived oligosaccharides, are more accessible to amylolytic and microbial metabolism; therefore, if part of the fraction remains after digestion, they may be incorporated into fermentation processes more rapidly. In the study by *Jang* et al., a MalOS-containing fraction composed of maltotriose, maltotetraose, and maltopentaose stimulated the growth of *Bifidobacterium breve* and altered the fermentation profile of human fecal microbiota *in vitro* [19]. In a comparative in vitro gut model, MalOS were also evaluated among commercially available prebiotic oligosaccharides and demonstrated the ability to influence the microbiome and metabolomic profile after fecal fermentation [25].

In contrast, XOS are characterized by greater resistance to human digestive enzymes and can reach the large intestine as substrates for bacteria possessing enzymes capable of degrading the xylan backbone. Their fermentation largely depends on the degree of polymerization, the presence of arabinose substituents, and the structure of the original oligosaccharide fraction. Recent review studies show that XOS can selectively stimulate the growth of *Bifidobacterium* and other saccharolytic bacteria, increase the formation of short-chain fatty acids, and limit the development of opportunistic microorganisms [13, 20, 46].

The concept of XOS–MalOS synergy is consistent with the broader principle of combining carbohydrate substrates with different fermentation rates. *Canfora* et al. demonstrated that the combination of rapidly and slowly fermentable dietary fibers can be considered an approach to enhance distal colonic fermentation and increase acetate production in the large intestine [50]. By analogy with this approach, it can be assumed that MalOS may provide a more rapid onset of microbial fermentation, whereas XOS may support more prolonged substrate utilization and more sustained stimulation of saccharolytic bacteria.

An important mechanism underlying the potential complementary action of XOS and MalOS is the formation of short-chain fatty acids, including acetate, propionate, and butyrate. These metabolites participate in the energy supply of colonocytes, pH regulation, maintenance of the intestinal barrier, and immunometabolic regulation [48] In addition, acetate and lactate produced during bifidobacterial metabolism can be utilized by butyrate-producing bacteria, including representatives of *Faecalibacterium, Anaerostipes, Eubacterium*, and *Roseburia*. This cross-feeding mechanism explains why stimulation of bifidobacteria may indirectly enhance butyrate formation and support the metabolic balance of the intestinal ecosystem [47].

From this perspective, the combination of XOS and MalOS should not be regarded as a simple sum of two oligosaccharides, but rather as a potential substrate system acting at different levels. MalOS may more rapidly provide an available carbon substrate for part of the microbial community, whereas XOS may maintain more selective bifidogenic activity and create conditions for subsequent cross-feeding of butyrate-producing bacteria. As a result, more balanced SCFA formation, a reduction in intestinal pH, and maintenance of the functional stability of the microbiota may be achieved. However, this assumption should be regarded as a scientifically grounded hypothesis, since direct studies of the specific combination of XOS and MalOS obtained from a single raw material source remain insufficient.

The use of wheat bran as a single raw material source for such a combination is of particular interest. The arabinoxylan fraction of wheat bran can serve as a basis for obtaining XOS and AXOS, whereas residual starch can be directionally transformed into MalOS. This creates a technological prerequisite for producing a complex oligosaccharide composition from one accessible agro-industrial

resource. In contrast to approaches in which different prebiotics are obtained from different raw materials and subsequently mixed, the use of wheat bran makes it possible to consider XOS and MalOS as interrelated products of the deep enzymatic processing of a single biomatrix.

Thus, the potential synergy between XOS and MalOS may be determined by three main factors: differences in resistance to digestion, differences in the rate of microbial fermentation, and the possibility of establishing cross-feeding between primary oligosaccharide degraders and secondary producers of short-chain fatty acids. Experimental confirmation of this hypothesis requires studies involving in vitro digestion modelling, fecal fermentation, dynamic assessment of *Bifidobacterium* and *Lactobacillus* growth, determination of SCFA profiles, and comparison of the individual and combined effects of XOS, MalOS, and XOS–MalOS compositions.

Discussion

Wheat bran is of considerable interest as an accessible raw material for obtaining functional oligosaccharide ingredients, since its biopolymeric matrix contains both arabinoxylan fractions suitable for the production of xylooligosaccharides (XOS) and residual starch that can be used for the formation of maltooligosaccharides (MalOS). This approach is consistent with the modern concept of deep processing of cereal by-products, in which low-cost raw materials are converted into value-added products. XOS- and MalOS-containing fractions may be used in the development of functional foods, nutraceuticals, specialized nutrition products, and microbiome-oriented ingredients aimed at supporting the intestinal microbiota and the metabolic activity of the large intestine [34, 44].

The practical application of XOS and MalOS may cover several areas. In the food industry, such ingredients can be incorporated into bakery and cereal products, fermented dairy beverages, functional snacks, powdered mixtures, and specialized nutrition products. XOS are of interest as prebiotic oligosaccharides that are more resistant to digestive enzymes, capable of reaching the large intestine and being utilized by saccharolytic bacteria. MalOS, in turn, may be considered starch-derived oligosaccharides with potentially faster fermentative availability, provided that part of the fraction is preserved after digestion. In this regard, their combination may be promising for the development of compositions with different fermentation rates and a more balanced effect on the microbiota [13, 19].

A separate applied direction is the use of oligosaccharides in livestock and poultry production. In farm animal nutrition, prebiotics are considered functional feed additives aimed at maintaining intestinal microbiota, enhancing intestinal barrier function, improving productivity, and reducing dependence on antibiotic growth promoters. Reviews on feed prebiotics indicate that various non-digestible carbohydrates are used in animal diets, including fructooligosaccharides, galactooligosaccharides, isomaltooligosaccharides, xylooligosaccharides, and other substrates capable of modulating the gut microbiota [10]. This review emphasizes that prebiotics and synbiotics are used primarily to maintain the balance of the intestinal microbiota in animals and as alternatives to antibiotic growth promoters.

The most convincing recent data in the animal nutrition field have been obtained for XOS, particularly in poultry production. A meta-analytical study on broilers, which included 36 peer-reviewed studies published between 2000 and 2024, showed that XOS supplementation increased average daily gain and feed intake and reduced the feed conversion ratio compared with the control [15]. According to this study, XOS increased ADG by 6.445% and ADFI by 1.544%, while reducing FCR by 7.147%, indicating their potential to improve broiler productivity.

In laying hens, dietary XOS supplementation for 24 weeks was also associated with positive changes in productivity and intestinal status. In the study by *Xiong* et al., XOS increased egg production during the early experimental period, improved small intestinal morphology, increased butyric and isovaleric acid concentrations in the cecum, enhanced bacterial diversity, and was associated with an increased proportion of *Firmicutes, Bifidobacteriaceae,* and *Lactobacillus* [16]. These data confirm that XOS may act not only as a source of fermentable substrate but also as a factor influencing intestinal morphology and the profile of microbial metabolites.

Studies of XOS under infectious or inflammatory stress are of particular interest. Ren et al. showed that XOS supplementation attenuated growth retardation and intestinal damage in broiler chickens challenged with avian pathogenic *Escherichia col*i; XOS also reduced intestinal APEC colonization, increased *Lactobacillus levels*, and elevated acetate, butyrate, and valerate concentrations in the

cecum [51]. These findings suggest that XOS may be considered not only as growth- and productivity-oriented additives but also as potential tools for supporting intestinal barrier function under pathogenic challenge.

Maltooligosaccharides require more cautious consideration in the context of animal nutrition. In feed literature, the abbreviation MOS is often used to denote mannan-oligosaccharides; therefore, when discussing maltooligosaccharides specifically, it is advisable to write the term in full or use the abbreviation MalOS. Unlike XOS, direct recent studies on the use of maltooligosaccharides in farm animal diets are considerably fewer. Therefore, their feed-related potential should be regarded as promising but not yet sufficiently validated. The most reasonable approach is to study maltooligosaccharides not as an already established independent feed technology, but as an additional starch-derived fraction within complex XOS–MalOS compositions obtained from wheat bran.

At the same time, existing data on the combined effects of XOS and maltooligosaccharides remain limited in both food and feed biotechnology. Most studies consider these oligosaccharides separately: XOS mainly as non-digestible xylan-derived prebiotics, and maltooligosaccharides as starch-derived fractions potentially capable of participating in microbial fermentation. Therefore, the hypothesis of a synergistic effect between XOS and maltooligosaccharides requires further experimental confirmation. Comparative studies evaluating individual XOS, individual maltooligosaccharides, and their combined compositions under identical digestion and fermentation conditions are especially important.

One of the key future directions is the standardization of oligosaccharide fraction composition. For XOS, it is necessary to consider the degree of polymerization, the proportion of xylobiose, xylotriose, and longer fractions, the level of arabinose substitution, and the presence of phenolic residues. For maltooligosaccharides, the distribution of DP fractions, the ratio of maltose, maltotriose, maltotetraose, and longer oligomers, as well as the presence of residual dextrins, are important. Without such standardization, it is difficult to correctly compare the results of different studies and assess the real contribution of each fraction to the prebiotic effect [18].

Another important direction is the optimization of enzymatic hydrolysis of wheat bran. Since the raw material contains both arabinoxylans and residual starch, the technological scheme must take into account the sequence of action of xylanolytic and amylolytic enzymes, pretreatment conditions, pH, temperature, hydrolysis duration, and enzyme-to-substrate ratio. A promising approach may involve stepwise processing: first, controlled treatment of the starch fraction to obtain maltooligosaccharides, followed by targeted hydrolysis of the arabinoxylan matrix to form XOS/AXOS. Alternatively, a combined enzyme system may be used; however, it requires strict control to avoid excessive monosaccharide formation and loss of the target oligosaccharide fractions.

Multilevel studies are required to confirm the functional efficacy of XOS–maltooligosaccharide compositions. At the first stage, *in vitro* models, including simulated digestion and fecal fermentation, should be used to assess fraction stability, growth dynamics of *Bifidobacterium* and *Lactobacillus*, pH changes, and short-chain fatty acid profiles. At the next stage, in vivo studies are needed, not only in laboratory animals but also in productive species such as poultry, pigs, and ruminants. For food applications, the final stage should involve clinical studies confirming safety, tolerability, effective dosage, and the actual effects of XOS–maltooligosaccharide compositions on human health [25, 44].

Thus, further development of this field should move from the general evaluation of wheat bran as a source of oligosaccharides toward the creation of standardized XOS–maltooligosaccharide fractions with defined structural and functional characteristics. The scientific and practical significance of this approach lies in the possibility of integrating deep processing technologies for cereal raw materials with the development of functional ingredients for the food industry and feed additives for animal husbandry. The potential value of such compositions is determined by their possible complementary effects on the gut microbiota, their ability to enhance short-chain fatty acid production, support the intestinal barrier, and their application in functional foods, nutraceuticals, specialized nutrition products, and prebiotic feed compositions.

Conclusion

Wheat bran represents a promising biopolymeric matrix for obtaining functional oligosaccharides, as it contains both an arabinoxylan fraction, which serves as a source of xylooligosaccharides (XOS),

and residual starch, which may be used as a source of maltooligosaccharides (MalOS). This combination allows wheat bran to be considered not only as a by-product of flour milling but also as a raw material for deep enzymatic processing aimed at producing value-added functional ingredients.

XOS and MalOS differ in their chemical structure, type of glycosidic linkages, and resistance to digestive enzymes, which determines differences in their fermentation kinetics and effects on the intestinal microbiota. XOS exhibit higher non-digestibility and may provide a more prolonged prebiotic effect, whereas MalOS may be incorporated into microbial fermentation more rapidly, provided that part of the fraction remains after digestion.

Thus, the available evidence supports the prebiotic effects of XOS, including their ability to modulate the gut microbiota and promote short-chain fatty acid production, with supporting data from animal studies. In contrast, the prebiotic potential of MalOS is supported mainly by *in vitro* evidence and requires further validation in productive animals. Therefore, the proposed complementary action of XOS and MalOS should currently be regarded as a scientifically grounded hypothesis rather than an experimentally confirmed synergistic effect. Further studies should focus on standardizing oligosaccharide fraction composition, optimizing enzymatic hydrolysis, and directly comparing XOS, MalOS, and their combinations using in vitro and in vivo models.

Author Contributions

Conceptualization: AK: formal analysis: AK, YS: resources: AK: writing-original draft preparation: AK, YS: writing-review and editing: AK, AK: funding acquisition: AK.

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Conflict of Interest

The authors declare that they have no known financial interests or personal relationships that could have influenced the results presented in this work.

References

1 Tharwat, M., Alkheraif, A.A., Oikawa, S. (2025). Production diseases in farm animals: A comprehensive and illustrated clinical, laboratory, and pathological overview. *Open Vet J.,* 15(1), 18–34. DOI:10.5455/OVJ.2025.v15.i1.3.

2 Ihuț, A., Răducu, C., Ranta, M., Andrecan, A., Uiuiu, P. (2026). Gut microbiome health in farm animals and fish: implications for human health and the risk of gastrointestinal diseases. *Microorganisms*, 14(2), 447. DOI:10.3390/microorganisms14020447.

3 Alemu, Y.F., Jemberu, W.T., Mekuriaw, Z., Abdi, R.D. (2022). Incidence and predictors of calf morbidity and mortality from birth to 6-months of age in dairy farms of Northwestern Ethiopia. *Front Vet Sci.,* 9, 859401. DOI:10.3389/fvets.2022.859401.

4 Han, X., Hu, X., Jin, W., Liu, G. (2024). Dietary nutrition, intestinal microbiota dysbiosis and post-weaning diarrhea in piglets. *Anim Nutr.,* 17, 188–207. DOI: 10.1016/j.aninu.2023.12.010.

5 Canibe, N., Højberg, O., Kongsted, H., Vodolazska, D., Lauridsen, C., Nielsen, T.S. et al. (2022). Review on Preventive Measures to Reduce Post-Weaning Diarrhoea in Piglets. *Animals*, 12(19), 2585. DOI:10.3390/ani12192585.

6 Tang, Q., Lan, T., Zhou, C., Gao, J., Wu, L., Wei, H. et al. (2024). Nutrition Strategies to Control Post-Weaning Diarrhea of Piglets: From the Perspective of Feeds. *Anim Nutr.*, 17, 297–311. DOI: 10.1016/j.aninu.2024.03.006.

7 Abd El-Hack, M.E., El-Saadony, M.T., Elbestawy, A.R., El-Shall, N.A., Saad, A.M., Salem, H.M., El-Tahan, A.M., Khafaga, A.F., Taha, A.E., AbuQamar, S.F., El-Tarabily, K.A. (2022). Necrotic enteritis in broiler chickens: Disease characteristics and prevention using organic antibiotic alternatives - a comprehensive review. *Poult. Sci.,* 101(2), 101590. DOI: 10.1016/j.psj.2021.101590.

8 Emami, N.K., Dalloul, R.A. (2021). Centennial Review: Recent Developments in Host-Pathogen Interactions during Necrotic Enteritis in Poultry. *Poult. Sci,* 100, 101330. DOI: 10.1016/j.psj.2021.101330.

9 Idowu, A., Idowu, P., Mbambalala, L. (2025). Probiotics as sustainable alternatives to antibiotic growth promoters: Mechanisms, applications, and future perspectives in livestock production. *Lett. Anim. Biol.*, 5, 129–142. DOI:10.62310/liab.v5i1.258.

10 Markowiak, P., Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathog.,* 10, 21. DOI:10.1186/s13099-018-0250-0.

11 Ravanal, M.C. et al. (2025). Prebiotics in Animal Nutrition: Harnessing Agro-Industrial Waste for Improved Gut Health and Performance. *Anim. Nutr*i., 21, 179–192. DOI: 10.1016/j.aninu.2024.11.025.

12 Kezer, G., Paramithiotis, S., Khwaldia, K., Harahap, I.A., Čagalj, M., Šimat, V., Smaoui, S., Elfalleh, W., Ozogul, F., Esatbeyoglu, T. (2025). A comprehensive overview of the effects of probiotics, prebiotics and synbiotics on the gut-brain axis. *Front. Microbiol.*, 16, 1651965. DOI:10.3389/fmicb.2025.1651965.

13 Valladares-Diestra, K.K., de Souza Vandenberghe, L.P., Vieira, S., Goyzueta-Mamani, L.D., de Mattos, P.B.G., Manzoki, M.C., Soccol, V.T., Soccol, C.R. (2023). The Potential of Xylooligosaccharides as Prebiotics and Their Sustainable Production from Agro-Industrial By-Products. *Foods*, 12(14), 2681. DOI:10.3390/foods12142681.

14 Huang, C., Yu, Y., Li, Z., Yan, B., Pei, W., Wu, H. (2022). The preparation technology and application of xylo-oligosaccharide as prebiotics in different fields: A review. *Front. Nutr.*, 9, 996811. DOI:10.3389/fnut.2022.996811.

15 Naeem, M., Bourassa, D., Rochell, S., Bedford, M. (2025). Evaluating the Impacts of Xylo-Oligosaccharides on Growth Performance of Broiler Chickens: A Meta-Analytical Study. *J. Appl. Poult. Res.,* 34(3), 100546. DOI: 10.1016/j.japr.2025.100546.

16 Xiong, S., Zhang, K., Wang, J., Bai, S., Zeng, Q., Liu, Y. (2024). Effects of Xylo-Oligosaccharide Supplementation on the Production Performance, Intestinal Morphology, Cecal Short-Chain Fatty Acid Levels, and Gut Microbiota of Laying Hens. *Poult. Sci.,* 103(12), 104371. DOI: 10.1016/j.psj.2024.104371.

17 Cheon, S., Kim, G., Bae, J.H., Lee, D.H., Seong, H., Kim, D.H., Han, J.S., Lim, S.Y., Han, N.S. (2023). Comparative analysis of prebiotic effects of four oligosaccharides using an in vitro gut model: Digestibility, microbiome, and metabolome changes. *FEMS Microbiol. Ecol.,* 99(2), fiad002. DOI:10.1093/femsec/fiad002.

18 Bláhová, M., Štefuca, V., Hronská, H., Rosenberg, M. (2023). Maltooligosaccharides: Properties, Production and Applications. *Molecules,* 28(7), 3281. DOI:10.3390/molecules28073281.

19 Jang, E.Y., Hong, K.-B., Chang, Y.B., Shin, J., Jung, E.Y., Jo, K., Suh, H.J. (2020). In vitro Prebiotic Effects of Malto-Oligosaccharides Containing Water-Soluble Dietary Fiber. *Molecules*, 25(21), 5201. DOI:10.3390/molecules25215201.

20 Nogueira-Prieto, N.M., Ansín-Vallejo, C., Becerra-Fernández, M., González-Siso, M.I. (2025). A Review of the Capacity of Xylooligosaccharides to Modulate Gut Microbiota and Promote Health. *Food Funct.,* 16(12), 4654–4672. DOI:10.1039/D5FO00169B.

21 Bilal, M., Li, D., Xie, C., Yang, R., Gu, Z., Jiang, D., Xu, X., Wang, P. (2024). Valorization of wheat bran arabinoxylan: A review on nutritional and materials perspectives. *Grain Oil Sci. Technol.*, 7(3), 196–208. DOI: 10.1016/j.gaost.2024.05.006.

22 Wang, J., Sun, B., Cao, Y., Tian, Y., Wang, C. (2009). Enzymatic Preparation of Wheat Bran Xylooligosaccharides and Their Stability during Pasteurization and Autoclave Sterilization at Low pH. *Carbohydr. Polym.*, 77(4), 816–821. DOI: 10.1016/j.carbpol.2009.03.005.

23 Ali, K., et al. (2025). Xylooligosaccharides: a comprehensive review of production, purification, characterization, quantification and applications. *Food Res. Int.,* 201, 115631. DOI: 10.1016/j.foodres.2024.115262.

24 Cheon, S., et al. (2023). Comparative analysis of prebiotic effects of four commercially available prebiotic oligosaccharides in an in vitro gut model. *FEMS Microbiol. Ecol.*, 99(2), fiad002. DOI:10.1093/femsec/fiad002.

25 Li, C., Wu, W. (2024). Wheat bran: A nutritional treasure or bread-making challenge? A mini-review. *Food Biomacromol.*, 1, 3–8. DOI:10.1002/fob2.12007.

26 Hemdane, S., Jacobs, P.J., Dornez, E., Verspreet, J., Delcour, J.A., Courtin, C.M. (2016). Wheat (Triticum aestivum L.) bran in bread making: A critical review. *Compr. Rev. Food Sci. Food Saf.,* 15(1), 28–42. DOI:10.1111/1541-4337.12176.

27 Sztupecki, W., Rhazi, L., Depeint, F., Aussenac, T. (2023). Functional and nutritional characteristics of natural or modified wheat bran non-starch polysaccharides: A literature review. *Foods,* 12(14), 2693. DOI:10.3390/foods12142693.

28 Onipe, O.O., Jideani, A.I.O., Beswa, D. (2015). Composition and functionality of wheat bran and its application in some cereal food products. *Int. J. Food. Sci. Technol.*, 50(12), 2509–2518. DOI:10.1111/ijfs.12935.

29 Ferreira, D.M., Coscueta, E.R., Brassesco, M.E., Pintado, M. (2026). Processing and valorization of wheat bran, germ and their fractions: An evidence-graded review of composition, technologies and applications. *Foods,* 15(8), 1455. DOI:10.3390/foods15081455.

30 Suanno, C., Marincich, L., Corneti, S., Aloisi, I., Pincigher, L., Papi, E., Parrotta, L., Antognoni, F., Del Duca, S. (2025). Biochemical analysis of wheat milling by-products for their valorization as potential food ingredients. *Int. J. Mol. Sci.,* 26(12), 5830. DOI:10.3390/ijms26125830.

31 Sztupecki, W., Dziki, D., Gawlik-Dziki, U., Różyło, R., Hassoon, W.H. (2023). Functional and nutritional characteristics of natural or modified wheat bran. *Foods*, 12(14), 2693. DOI:10.3390/foods12142693.

32 González-Alonso, V., Verce, M., De Vuyst, L., Leroy, F. (2026). Cereal arabinoxylans their enzymatic degradation and relevance for breadmaking and human health. *Compr. Rev. Food Sci. Food Saf.,* 25(1), e70391. DOI:10.1111/1541-4337.70391.

33 Prückler, M., Siebenhandl-Ehn, S., Apprich, S., Höltinger, S., Haas, C., Schmid, E., Kneifel, W. (2014). Wheat bran-based biorefinery 1: Composition of wheat bran and strategies of functionalization. *LWT Food Sci. Technol.,* 56(2), 211–221. DOI: 10.1016/j.lwt.2013.12.004.

34 Valladares-Diestra, K.K., et al. (2023). The Potential of Xylooligosaccharides as Prebiotics and Their Sustainable Production from Agro-Industrial Residues. *Foods*, 12, 2681. DOI:10.3390/foods12142681.

35 Nogueira-Prieto, N.M. et al. (2025). A review of the capacity of xylooligosaccharides to modulate gut microbiota and promote health. *Food & Funct.,* 16(12), 4654-4672. DOI:10.1039/D5FO00169B.

36 Kiribayeva, A., Mukanov, B., Silayev, D., Akishev, Z., Ramankulov, Y., Khassenov, B. (2022). Cloning, expression, and characterization of a recombinant xylanase from Bacillus sonorensis T6. *PLoS ONE*, 17(3), e0265647. DOI: 10.1371/journal.pone.0265647.

37 Shamsiyeva, Y., Silayev, D., Yermukhanov, A., Yakupov, B., Timochshuk, S., Abdishov, D., Kiribayeva, A. (2026). Comparative Enzymatic Production of Xylooligosaccharides from Wheat, Rice, Barley, and Oat Straw Using Xylanase from Bacillus sonorensis. *Fermentation*, 12, 299. DOI: 10.3390/fermentation12070299.

38 da Silva, M.F. et al. (2024). Carboxymethylated and sulfated Cyclocarya paliurus polysaccharides inhibited colon cancer cells growth via PI3K/AKT-MAPKs/NF-κB pathways and immunomodulation. *Food Biosci.,* 58, 103836. DOI: 10.1016/j.fbio.2024.103836.

39 Freitas, D. et al. (2025). Starch digestion: A comprehensive update on the mechanisms, models and physiological relevance. *Trends Food Sci. Technol.,* 159, 104969.

40 Zeng, M., van Pijkeren, J.-P., Pan, X. (2023). Gluco-oligosaccharides as potential prebiotics: Synthesis, purification, structural characterization, and evaluation of prebiotic effect. *Compr. Rev. Food Sci. Food Saf.,* 22(4), 2611–2651. DOI:10.1111/1541-4337.13156.

41 Kiribayeva, A., Silayev, D., Abdullayeva, A., Shamsiyeva, Y., Ramankulov, Y., Khassenov, B. (2022). Hydrolysis of plant biomass using recombinant alpha-amylase from *Bacillus licheniformis* and xylanase from *Bacillus sonorensis. EJAB,* 4, 31–39. DOI: 10.11134/btp.4.2022.4

42 Kiribayeva, A., Silayev, D., Akishev, Z., Baltin, K., Aktayeva, S., Ramankulov, Y, Khassenov, B. (2024). An impact of N-glycosylation on biochemical properties of a recombinant α-amylase from *Bacillus licheniformis. Heliyon,* 13:10(6), e28064. DOI: 10.1016/j.heliyon.2024.e28064.

43 Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K., Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.,* 14, 491–502. DOI:10.1038/nrgastro.2017.75.

44 Yang, S., Wu, C., Yan, Q., Li, X., Jiang, Z. (2023). Nondigestible Functional Oligosaccharides: Enzymatic Production and Food Applications for Intestinal Health. *Annu. Rev. Food Sci. Technol.,* 14, 297–322. DOI:10.1146/annurev-food-052720-114503.

45 Bouiche, C., Houfani, A.A., Azzouz, Z., Taouzinet, L., Medouni-Haroune, L., Amrane-Abider, M. (2023). Exploring the potential of xylooligosaccharides as prebiotics: Insights from CAZymes families and their emerging health benefits. *Biol.,* 78, 1789–1806. DOI:10.1007/s11756-023-01440-4.

46 Rivière, A., Selak, M., Lantin, D., Leroy, F., De Vuyst, L. (2016). Bifidobacteria and butyrate-producing colon bacteria: Importance and strategies for their stimulation in the human gut. *Front. Microbiol.,* 7, 979. DOI:10.3389/fmicb.2016.00979.

47 Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de los Reyes-Gavilán, C.G., Salazar, N. (2016). Intestinal short-chain fatty acids and their link with diet and human health. *Front. Microbiol.,* 185. DOI:10.3389/fmicb.2016.00185.

48 Hays, K.E. et al. (2024). The interplay between gut microbiota, short-chain fatty acids, and host health. *Gut Microbes*, 16(1), 2393270. DOI:10.1080/19490976.2024.2393270.

49 Canfora, E.E., Hermes, G.D.A., Müller, M., Bastings, J., Vaughan, E.E., van den Berg, M.A., Holst, J.J., Venema, K., Zoetendal, E.G., Blaak, E.E. (2022). Fiber mixture-specific effect on distal colonic fermentation and metabolic health in lean but not in prediabetic men. *Gut Microbes,* 14(1), 2009297. DOI:10.1080/19490976.2021.2009297.

50 Ren, L., Cao, Q., Ye, H., Dong, Z., Zhang, C., Feng, D., Zuo, J., Wang, W. (2024). Supplemental Xylooligosaccharide Attenuates Growth Retardation and Intestinal Damage in Broiler Chickens Challenged by Avian Pathogenic Escherichia coli. *Agriculture,* 14(10), 1684. DOI:10.3390/agriculture14101684.

51 Ríos-Ríos, K.L., Dejonghe, W., Vanbroekhoven, K., Rakotoarivonina, H., Rémond, C. (2021). Enzymatic Production of Xylo-oligosaccharides from Destarched Wheat Bran and the Impact of Their Degree of Polymerization and Substituents on Their Utilization as a Carbon Source by Probiotic Bacteria. *J. Agric. Food Chem.,* 69(44), 13217–13226. DOI: 10.1021/acs.jafc.1c02888.

Review article

Avian leukosis and reticuloendotheliosis viruses: biology, epidemiological risks, and the need for molecular surveillance in Kazakhstan

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Abstract

Background and Aim. Avian leukosis virus (ALV) and reticuloendotheliosis virus (REV) induce severe immunosuppression and neoplastic processes, increasing susceptibility to concurrent infections and causing substantial hidden economic losses. Despite active viral circulation in neighboring countries (e.g., China, Russia), epidemiological data for Kazakhstan remain scarce. This review aims to synthesize current knowledge on retroviral biology, raise awareness of this hidden threat among veterinary professionals, and justify the need for systematic control at poultry facilities.

Material and Methods. A comprehensive analysis of current scientific literature addressing the biology, classification, life cycle, and molecular mechanisms of retrovirus-host cell interactions was conducted. The role of exogenous and endogenous retroviruses in the development of infectious and oncological diseases was examined. Additionally, available information on the epizootiological situation in countries bordering Kazakhstan was reviewed, alongside contemporary approaches to the diagnosis, therapy, and prevention of retroviral infections.

Results. ALV and REV exhibit high genetic variability and establish persistent infections via host genome integration. These infections often progress subclinically: virus-induced lesions remain undetected, and secondary outbreaks are frequently misattributed to vaccine failure. The broad host range and active pathogen circulation in border regions pose a high risk of introduction into Kazakhstan, exacerbated by the current lack of systematic molecular epidemiology monitoring.

Conclusion. Implementing continuous molecular-genetic monitoring of avian retroviruses in Kazakhstan is critical to ensure poultry biosecurity, mitigate economic losses, and develop effective disease control strategies.

Keywords: Avian retroviruses; epidemiology; immune response; pathogenesis.

Introduction

Avian species, with a particular emphasis on the domestic chicken (*Gallus gallus domesticus*), have served as indispensable and historically monumental models in the realm of biomedical and oncological research for over a century. The unique developmental biology of the avian embryo, which develops in ovo and offers unparalleled accessibility for experimental manipulation, combined with the groundbreaking early discoveries of avian viral agents, positioned poultry at the very epicenter of virological science. Avian retroviruses were first identified as filterable, carcinogenic agents capable of inducing solid tumors in chickens at the dawn of the 20th century, largely stemming from the seminal work of Peyton Rous in 1911. However, avian retroviruses have persisted to evolve continuously since their initial discovery, adapting to modern intensive poultry farming practices and making them a crucial focus for continuous, highly prioritized global research. Studying these viruses has not only protected agricultural interests but has also led to a profoundly better understanding of the

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fundamental molecular mechanisms of viral replication, reverse transcription, oncogene capture, and carcin-ogenesis [1].

Retroviruses constitute a highly complex family of enveloped, single-stranded positivesense RNA viruses (*Retroviridae*). Structurally, the viral particle encompasses a lipid bilayer envelope derived from the host cell membrane, embedded with viral glycoproteins that mediate receptor recognition and entry. Upon entering a susceptible host cell, these viruses utilize the virally encoded, multifunctional enzyme reverse transcriptase which possesses both RNA-dependent DNA polymerase and RNase H activities to synthesize a doublestranded DNA provirus from their RNA template. Subsequently, the viral integrase enzyme facilitates the irreversible insertion of this synthesized DNA into the host cell's chromatin (a mechanism universally known as reverse transcription and integration). This specific type of infection establishes a permanent, persistent state, as the integrated provirus behaves essentially as a host gene and is transmitted vertically along with the host cell's genetic material during cellular division [2, 3]. In the avian virosphere, there are both exogenous retroviruses (which are actively transcribed, assembled into infectious virions, and transmitted horizontally between living organisms, or vertically to offspring via the egg albumen) and endogenous retroviruses (which represent ancient viral infections that have integrated into the germline genome over the course of millennia of evolution, currently existing as stable DNA sequences that are inherited in a strict Mendelian fashion) [4].

From a taxonomic and clinical perspective, birds are predominantly susceptible to two main groups of retroviruses, classified under the subfamily *Orthoretrovirinae*: alpharetroviruses (genus *Alpharetrovirus*) and gammaretroviruses (genus *Gammaretrovirus*). Both genera belong to the *Retroviridae* family and are fundamentally characterized by their shared ability to irreversibly integrate viral DNA into the host genome via the reverse transcription of the viral RNA blueprint [5].

Avian alpharetroviruses include an extensive group of pathogens historically referred to as the leukosis and sarcoma group. Prominent members include the avian leukosis virus (ALV), Rous sarcoma virus (RSV), avian myeloblastosis virus (AMV), and turkey lymphoproliferative disease virus (LPDV). The genomic architecture of a standard, replication-competent alpharetrovirus typically consists of three primary open reading frames: *gag* (encoding group-specific internal structural proteins such as the matrix, capsid, and nucleocapsid), *pol* (encoding reverse transcriptase and integrase), and *env* (encoding the envelope glycoproteins). These coding sequences are flanked by Long Terminal Repeats (LTRs) containing vital enhancer and promoter elements. These viruses are fundamentally characterized by high oncogenic activity and are capable of inducing various types of devastating neoplastic processes in infected birds, primarily through insertional mutagenesis where the viral LTR abnormally activates cellular proto-oncogenes.

Avian gammaretroviruses include the highly significant reticuloendotheliosis virus (REV), which exists in two distinct virological forms: a defective, acute transforming strain (REV-T) and a replicationcompetent helper virus (REV-A). Unlike ALV, REV-T carries a specialized viral oncogene (*v-rel*), which allows it to induce rapid and aggressive cellular transformation. Additionally, avian gammaretroviruses encompass the spleen necrosis virus (SNV), chick syncytial virus (CSV), and duck infectious anaemia virus (DIAV). These viruses are distinguished by a remarkably broad spectrum of pathogenic effects that extend far beyond simple tumor formation, critically including profound cellular immunosuppression and severe disruption of the normal developmental pathways of hematopoietic tissues [6].

Consequently, both taxonomic groups of these avian retroviruses play a highly significant and detrimental role in the overall epidemiology and pathogenesis of concurrent avian diseases, representing substantial scientific interest for veterinary virology, global food security, and strict infectious disease control protocols within the modern, high-density poultry industry.

Materials and Methods

To systematically prepare this comprehensive literature review, critically evaluate the epizootiological significance of avian retroviruses, and scientifically substantiate the urgent need for their systematic control and monitoring within the Republic of Kazakhstan, we analyzed a wide array of scientific publications indexed in primary biological databases, prominently including Medline (PubMed), Scopus, Web of Science, and Google Scholar. The systematic literature search

was meticulously conducted using the following specialized Boolean keyword combinations: (“avian retroviruses” or “avian retroviral infections”) and/or (“avian leukosis virus” or “ALV”) and/or (“reticuloen-dotheliosis virus” or “REV”) and/or (“retrovirus prevalence” or “molecular epidemiology” or “immu-nosuppression” or “phylogenetic analysis”).

The initial search yielded hundreds of preliminary results, which were subjected to rigorous inclusion and exclusion criteria. Articles were included if they were published in peer-reviewed journals, written in English, and provided high-quality genomic, diagnostic, or epidemiological data. In total, 40 distinct data sources were selected for the final, intensive analysis following a comprehensive thematic screening and the strict exclusion of duplicate, outdated, or non-relevant records. Based on the careful categorization of the retrieved literature, the final review incorporated 38 peer-reviewed journal articles consisting of foundational veterinary virology textbooks, viral encyclopedias, and official taxonomic reports from the International Committee on Taxonomy of Viruses (ICTV).

Geographically and thematically, the thoroughly analyzed literature encompasses 1 specific local study confirming the active circulation of avian leukosis virus within the borders of Kazakhstan, multiple high-resolution epidemiological reports from immediate neighboring countries (specifically the Russian Federation and the People's Republic of China) alongside other globally significant poultryproducing regions. Furthermore, the dataset includes foundational molecular studies detailing the genomic structure, receptor-binding mechanisms, and deep pathogenesis of alpharetroviruses and gammaretroviruses, as well as the latest advances in molecular diagnostic assays, explicitly focusing on multiplexing technologies and novel geneediting prevention strategies.

Results

Avian retroviruses, predominantly and persistently affecting various domesticated and wild bird species, are established and thoroughly documented etiological agents for a wide array of severe, lifethreatening pathologies. These encompass diverse neoplastic formations (ranging from lymphomas to sarcomas and hemangiomas), pronounced and debilitating immunosuppression, and other systemic morbidities that severely degrade flock health [7]. Among naturally occurring avian retrovi-ruses, ALV, REV, LPDV are historically and contemporarily the most prevalent. ALV and REV, classified molecularly as simple retroviruses (lacking complex accessory genes like *tat* or *rev* found in lentiviruses), represent highly significant oncogenic agents. Their epidemiology is highly dynamic, intricately dependent on the geographical region, biosecurity levels, and the specific genetic charac-teristics of the affected avian populations. These resilient pathogens are persistently detected across both wild, migratory avian reservoirs and highly concentrated commercial poultry production ecosys-tems [8, 9, 10].

ALV is an exceptionally widespread group of oncogenic alpharetroviruses within the *Retroviridae* family and serves as a primary, persistent cause of neoplastic diseases in poultry, especially in commercial meat and egg-laying chickens. Active infection is robustly associated with lymphoid leukosis, diffuse internal organ neoplasms, severe physiological growth retardation, drastically decreased reproductive capacity, and catastrophic drops in overall egg production metrics [11, 12, 13, 14]. Furthermore, ALV induces severe, generalized immunosuppression that fundamentally compromises the host's ability to resist secondary environmental pathogens. ALV is divided into several distinct subgroups determined precisely by genomic nucleotide sequences, which absolutely dictate host cell range, strain pathogenicity, and primary transmission routes. The specific pathogenicity and cellular tropism of ALV are intimately associated with its env gene, which encodes the vital viral envelope glycoprotein complex (comprising the surface gp85 and transmembrane gp37 subunits). The highly variable gp85 surface protein strictly dictates host cell receptor binding and ultimately determines subgroup specificity [15, 16].

ALV subgroups (A, B, C, D, J, and K) are classified as exogenous; they are highly capable of rapid horizontal transmission via direct contact or fomites, as well as vertical transmission, and typically exhibit pronounced pathogenicity. Conversely, subgroups E and F are strictly endogenous, existing purely as dormant or defective proviruses within the host genome, transmitting exclusively vertically through Mendelian inheritance, and generally demonstrating minimal to no direct pathogenicity. Subgroup ALV-J has emerged globally as the most virulent, economically devastating, and epidemiologically significant exogenous variant since its discovery in the late 1980s. Genetically

linked to earlier subgroups A, B, and C, ALV-J exhibits extraordinarily high adaptability, aggressively af-fecting *Galliformes, Anseriformes*, and *Passeriformes* [5, 17, 18]. It is widely suggested by molecular phylogenists that ALV subgroups J and K originally emerged through complex genetic recombination events between different exogenous and endogenous ALV variants within a single coinfected host. This high propensity for rapid genomic recombination significantly complicates infection control, confounds differential diagnosis, and continuously generates novel variants.

The molecular entry mechanism of ALV-J specifically relies on the chicken sodium-hydrogen exchanger type 1 (chNHE1) serving as its obligate cellular receptor. Recent investigations have demonstrated that the N123I mutation within the receptor-binding domain (RBD) of the ALV-J gp85 protein significantly enhances the binding affinity to the chNHE1 receptor, thereby profoundly intensifying viral replication capabilities and increasing in vivo viral shedding among infected commercial flocks [19]. Furthermore, the absolute necessity of the chNHE1 receptor for viral entry has spurred highly advanced genomic engineering efforts. Recent advancements utilizing the CRISPR/Cas9 system to precisely delete the critical tryptophan residue at position 38 (W38) of the chNHE1 gene in chicken primordial germ cells have successfully generated edited cell lines exhibiting complete and highly stable resistance to ALV-J infection, presenting a groundbreaking, futureproof strategy for breeding disease-resistant poultry lines [20].

While both are classified as retroviruses, the tumors induced by REV differ mechanistically and substantially from ALV-induced neoplasms. ALV typically induces B-cell lymphomas through slow insertional mutagenesis near the c-*myc* proto-oncogene [21], whereas REV-associated B-cell lymphomas are considerably rarer, exhibit a highly prolonged latent period, and are triggered by in-sertional mutagenesis affecting different regulatory pathways [22]. REV, a gammaretrovirus, successfully affects a remarkably broad spectrum of avian species, including commercial chickens, turkeys, ducks, geese, pigeons, and numerous wild migratory birds [9, 11]. One uniquely aggressive representative is the T-strain (REV-T), an acute transforming virus capable of rapidly transforming primary avian hematopoietic cells in a matter of days due to its evolutionary acquisition of the potent viral oncogene *v-rel* [23]. The complete genome of the replication-competent REV-A helper strain is a single-stranded positive-sense RNA (approximately 9,000 nucleotides in length) canonically structured as 5'-LTR-*gag-pol-env*-LTR-3'. The Long Terminal Repeats (LTRs) essentially regulate robust transcription and facilitate proviral integration into the host DNA [24].

REV infections in chickens present a remarkably broad clinical spectrum that varies drastical-ly depending on the specific viral genotype, the age of the host at exposure, and the baseline immune status of the flock. Clinical presentations range from severe neurological disorders closely mimicking Marek's disease to chronic, utterly silent subclinical infections. Stunting syndrome conditions charac-terized by severe weight loss, physiological paleness, and specific feathering abnormalities frequently referred to as Nakanuke disease – is also routinely observed in the field, though REV's precise, direct pathogenic role in the multi-factorial etiology of this syndrome requires further advanced elucidation [25, 26]. Similar to the pathogenic mechanisms of ALV, REV severely disrupts both cellular (T-cell mediated) and humoral (B-cell mediated) immunity, leading to lymphoid depletion in the bursa of Fabricius and the thymus [27].

Pathogenesis, Immunosuppression, and Viral Co-Infections

The most insidious and biologically destructive characteristic of both ALV and REV is their profound capacity to induce long-lasting immunosuppression. The pathogenesis of this immunodeficiency is driven by virus-induced apoptosis of premature lymphocytes, severe disruption of cytokine signaling pathways, and the functional impairment of macrophages. This biological state of immuno-suppression acts as an incredibly potent catalyst for secondary pathogens. When commercial flocks are afflicted with subclinical retroviral infections, they lose their ability to mount robust neutralizing antibody responses following routine vaccinations [28]. Consequently, this state paves the way for catastrophic coinfections. For instance, the pathogenesis and clinical severity of highly contagious respiratory pathogens, such as the Avian Infectious Bronchitis Virus (IBV) or Newcastle Disease Virus (NDV), are drastically exacerbated in retrovirus-compromised birds. Coinfection with IBV and ALV leads to significantly heightened respiratory distress, accelerated renal tissue damage (nephro-pathogenicity), and a much steeper drop in flock survivability compared to an IBV infection in a retrovirus-free, immunocompetent flock [29].

Advancements in Molecular Diagnostics

Traditional diagnostic protocols, such as standard Enzyme-Linked Immunosorbent Assays (ELISA) targeting the p27 group-specific antigen, possess a critical limitation: they are entirely incapable of differentiating between highly pathogenic exogenous subgroups and harmless endogenous retroviral proteins naturally expressed by the chicken genome. To overcome these critical diagnostic limitations and address the rapid emergence of complex recombinant strains, novel multiplex realtime quantitative PCR (qPCR) assays have been recently developed, enabling the highly sensitive, rapid, and simultaneous differential detection of the most epidemiologically significant subgroups: ALV-A, ALV-B, ALV-J, and ALV-K [30]. The implementation of such high-throughput, multiplexed molecular tools is strictly necessary for accurate epidemiological surveillance and the success-ful execution of rigorous eradication programs.

Prevalence and Epidemiological Distribution in Central Asia

Molecular genetic investigations and rigorous phylogenetic surveillance in countries immediately neighboring Kazakhstan, most notably the People's Republic of China [31–33] and the Russian Federation [34], reveal a widespread, deeply entrenched distribution and a highly concerning level of significant genetic diversity among ALV strains. Subtypes including ALV-A, ALV-B, ALV-J, and various novel recombinant forms are actively circulating, rapidly mutating, and relentlessly adapting to commercial poultry lines. Extensive epidemiological monitoring of native chicken lineages in China between 2022 and 2025 has revealed the concurrent circulation of highly adaptable ALV-J isolates, which predominantly cluster within Clades 1.2 and 1.3 and possess multiple specific functional point mutations and deletions in the 3' untranslated region, indicative of enhanced pathogenicity and rapid genetic evolution [35].

Preliminary and foundational data [36] definitively confirm the active circulation of ALV in at least the high-density agricultural regions of the Almaty region in Kazakhstan. Additionally, acute REV-induced outbreaks have been thoroughly documented in the border regions of China [37]. The broad, multi-species susceptibility to REV-A, combined seamlessly with its extremely high potential for interspecies transmission among migrating wild birds, actively maintains a robust, uncontrollable natural reservoir [38]. Despite this imminent and well-documented biological threat, comprehensive, systematic molecular epidemiological data regarding the true prevalence of gammaretroviruses and alpharetroviruses within the entirety of Kazakhstan remain critically and dangerously scarce.

Discussion

The diverse findings synthesized and meticulously detailed in this literature review highlight a critical, yet frequently underestimated and severely underfunded, threat to the modern global poultry industry. Although ALV and REV may not consistently induce direct, acute flock mortality as fre-quently or visibly as more aggressive retroviruses like the historically notorious RSV, their insidious capacity to induce delayed lymphoblastomas and subtle neoplastic lesions of various internal organs often remains completely undetected during standard, routine veterinary post-mortem examinations on the farm level.

The absolute most profound consequence of these persistent retroviral infections is the systemic induction of severe, generalized immunodeficiency. This long-term immunosuppression acts as a biological catalyst, heavily predisposing affected flocks to devastating, rapidly spreading concurrent infections and significantly undermining the calculated efficacy of standard, expensive immunoprophylaxis programs. Consequently, otherwise properly vaccinated flocks that are silently infected with ALV or REV become highly vulnerable, biologically defenseless targets for lethal endemic pathogens, such as various strains of the avian influenza virus, Newcastle disease virus, and infectious bronchitis virus.

From a strict industrial and macroeconomic perspective, this complex viral dynamic translates into profound, multi-layered, and largely hidden economic losses. Massive financial capital and labor resources heavily allocated for comprehensive vaccination programs completely fail to yield the expected protective flock immunity. The ensuing, highly destructive secondary infection outbreaks are frequently, yet entirely erroneously, attributed to compromised vaccine batch quality, broken coldchain logistics, or poor administration techniques, rather than accurately pointing to the true

underlying culprit: the subclinical, silent circulation of immunosuppressive retroviruses. Furthermore, REV poses an exceptional, highly specific virological hazard due to its highly unusual capacity to physically integrate its genetic material directly into the larger genomes of other unrelated avian DNA viruses (such as the Fowlpox virus or Marek's disease virus), a phenomenon which heavily complicates standard diagnostic procedures and severely limits traditional infection control strategies.

An additional, highly critical dimension to the economic and epidemiological impact of avian retroviruses is the severe risk of iatrogenic transmission through contaminated biological products. The global poultry industry relies heavily on live-attenuated viral vaccines (e.g., vaccines against Marek’s Disease, Infectious Bursal Disease, and Infectious Bronchitis) which are traditionally cultivated in Specific Pathogen Free (SPF) chicken embryos or primary avian cell cultures. If the breeding flocks producing these SPF eggs experience an undetected breach in biosecurity and become subclinically infected with exogenous ALV or REV, the resulting vaccine batches can become contaminated with live retroviruses. Administering these compromised biological products directly into day-old chicks essentially inoculates entire commercial flocks with immunosuppressive retroviruses, leading to catastrophic, widespread dissemination. This reality necessitates the implementation of ultrasensitive, multiplex molecular screening of all master seed viruses and final vaccine batches.

Currently, the Republic of Kazakhstan critically lacks a systematic, continuous, and statefunded molecular monitoring framework for retroviral infections in both domestic agricultural operations and wild, migrating avian populations; epidemiological surveillance remains strictly episodic and highly localized. The complete, systemic absence of robust, continuous national data regarding the true prevalence, exact subgroup distribution, and high-resolution genomic characterization of circulating ALV and REV strains represents a massive, glaring vulnerability in regional veterinary bi-osecurity and national food safety protocols.

This highly precarious epidemiological situation is further exacerbated by the inherent, everpresent risk of accidental retroviral transmission through imported live-attenuated vaccines manufactured for other severe avian diseases, as detailed above. Such undeniable biological risks dictate an absolute, urgent need for the rapid implementation of stringent, state-mandated quality control measures. These measures must demand the rigorous, mandatory retroviral molecular screening (utilizing advanced qPCR technologies) of both domestically produced and internationally imported biological products, strictly enforced alongside continuous, uncompromising biosecurity oversight of all commercial poultry breeding facilities operating within the nation.

The uncontrolled, silent circulation of immunosuppressive retroviruses is not merely a localized agricultural or economic issue; it is, fundamentally, a transnational biosecurity concern of the highest order. Poultry flocks suffering from severe retrovirusinduced immunodeficiency provide an optimal, highly unrestrictive biological environment for the prolonged replication, unchecked amplification, and rapid genetic adaptation of secondary viral and bacterial pathogens. In absolute essence, these immunocompromised birds function as massive, unregulated biological "bioreactors," actively facilitating accelerated viral mutation rates, dangerous antigenic drift, and the eventual, inevitable generation of novel, highly virulent pathogenic strains capable of crossing regional borders.

Finally, the undeniable transboundary dissemination of both ALV and REV via wild, migratory avian species constitutes a paramount, highly complex ecological threat. Geographically situated precisely in the very heart of the Eurasian continent, the vast territory of Kazakhstan is directly inter-sected by four of the eight major global migratory flyways, most notably the Central Asian and the East Asian-Australasian flyways. The extensive, highly biologically diverse network of freshwater lakes, wetlands, and river systems in Eastern and Northern Kazakhstan serves as crucial, densely populated staging, resting, and nesting grounds for hundreds of numerous migratory waterfowl species. This unique, undeniable geographical reality positions the country as a highly critical, high-risk hub for the potential intercontinental spread of avian retroviruses, rendering the immediate funding and execution of comprehensive, long-term molecular-epidemiological monitoring programs not just an academic pursuit, but an urgent scientific, economic, and practical imperative.

Conclusion

In summation, avian retroviral infections, particularly the highly adaptable ALV and the aggressive REV, remain a severe, critical, and continuously evolving issue at the very forefront of modern veterinary

virology, directly threatening the sustainability of the global poultry industry. The extraordinarily high genetic variability and rapid recombination rates of these viruses, combined with their fundamental, biological ability to irreversibly integrate directly into the host cell's chromatin and establish lifelong persistent infections, underscores the extreme complexity of attempting epizootiological control, rendering traditional disease prevention methods highly inadequate.

Numerous high-resolution genomic studies unequivocally indicate that various, highly mutated genetic variants of ALV (particularly Clades 1.2 and 1.3 of ALV-J, which boast mutations like N123I that heavily augment receptor binding) actively circulate and dominate widely in countries immediately neighboring Kazakhstan, most notably including China and Russia. This firmly confirms the active, relentless processes of viral evolution and the steady, cross-border dissemination of retro-viruses within regional domestic poultry populations. Of equal, distinct significance is REV, which is uniquely characterized by an exceptionally broad spectrum of devastating clinical manifestations, ranging from aggressive neoplastic processes and severe neurological disorders to profound, generalized cellular immunosuppression in infected birds of multiple species. The profound immunosuppressive effects dictated by both ALV and REV critically result in massively reduced avian resistance to other endemic infectious agents (such as IBV and AIV), severely deteriorated agricultural productivity, ruined vaccine efficacy, and exponentially increased, largely hidden economic losses throughout the poultry industry. Furthermore, the unusually wide range of susceptible natural hosts, comprehensively encompassing both highly concentrated commercial flocks and highly mobile wild migratory birds, massively contributes to the secure maintenance of natural, unreachable viral reservoirs and guarantees their further, unhindered dissemination across the continent.

Despite the existence of isolated, separate molecular data regarding the limited circulation of ALV specifically in Kazakhstan, cohesive, national-level information on the true prevalence, phylogenetic origins, and distribution density of avian retroviral infections within the broader country remains extremely, dangerously limited. The glaring lack of a systematic, statesponsored molecular epidemiological monitoring framework completely prevents a full, accurate assessment of the true scale of distribution and the complex genetic diversity of these stealthy pathogens. Concurrently, the official, documented registration of acute REV outbreaks in the immediate border regions of China firmly indicates an immediate, highly probable potential threat of the rapid introduction and silent spread of these highly pathogenic viruses deep into the sovereign territory of Kazakhstan via both trade routes and migratory flyways.

Consequently, initiating and consistently conducting highly comprehensive, state-of-the-art research aimed directly at the rapid identification, deep molecular-genetic characterization (including next-generation sequencing), and continuous geographic monitoring of avian retroviruses is of paramount scientific and practical importance. The highly actionable data gathered through such rigorous molecular surveillance can heavily facilitate a significantly better, clearer understanding of the regional epizootiological situation. Ultimately, this will directly drive the development of highly effective, targeted biosecurity protocols, the implementation of advanced diagnostic screening measures (such as multiplex qPCR), the exploration of novel prophylactic strategies (like CRISPR-mediated receptor editing), and the successful, permanent mitigation of the devastating economic damage inherently associated with retroviral infections in the modern poultry industry.

Authors' contributions

LM: original draft; KI: investigation, original draft; YO, YS: reviewing and editing.

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Conflicts of Interest

The authors declare that they have no conflicts of interest related to this study. No financial or non-financial competing interests exist that could have influenced the results or interpretation of the data presented in this manuscript.

References

1 Justice, J., Beemon, K.L. (2013). Avian retroviral replication. *Cur. Opin. Virol.*, 3(6), 664–669. DOI: 10.1016/j.coviro.2013.08.008.

2 Vorobiev, A.A., Bykov, A.S. (Eds.). (2023). Atlas po medicinskoi mikrobiologii, virusologii i immunologii [*Atlas of medical microbiology, virology and immunology*]. MIA, 132. [*In Russian*].

3 Coffin, J., Blomberg, J., Fan, H., Gifford, R., Hatziioannou, T., Lindemann, D., Mayer, J., Stoye, J., Tristem, M., Johnson, W., Ictv Report Consortium (2021). ICTV Virus Taxonomy Profile: Retroviridae 2021. *J Gen Virol.,* 102(12), 001712. DOI:10.1099/jgv.0.001712.

4 Sharp, P.M., Bailes, E., Gao, F., Beer, B.E., Hirsch, V.M., Hahn, B.H. (2000). Origins and evolution of AIDS viruses: estimating the time-scale. *Biochem. Soc Trans*., 28(2), 275–282. DOI:10.1042/bst0280275.

5 Souci, L., Denesvre, C. (2024). Interactions between avian viruses and skin in farm birds. *Vet Res*., 55(1), 54. DOI:10.1186/s13567-024-01310-0.

6 Fadly, A.M. (1997). Avian retroviruses. *Vet. Clin. N. A. Food Anim Pract,* 13(1), 71–85. DOI:10.1016/s0749-0720(15)30365-0.

7 Fandiño, S., Gomez-Lucia, E., Benítez, L., Doménech, A. (2023). Avian Leukosis: Will We Be Able to Get Rid of It? *Animals,* 13(14), 2358. DOI:10.3390/ani13142358.

8 Thomas, J.M., Allison, A.B., Holmes, E.C., Phillips, J.E., Bunting, E.M., Yabsley, M.J., Brown, J.D. (2015). Molecular Surveillance for Lymphoproliferative Disease Virus in Wild Turkeys (Meleagris gallopavo) from the Eastern United States. *PloS ONE*, 10(4), e0122644. DOI: 10.1371/journal.pone.0122644.

9 Caleiro, G.S., Nunes, C.F., Urbano, P.R., Kirchgatter, K., de Araujo, J., Durigon, E.L., Thomazelli, L.M., Stewart, B.M., Edwards, D.C., Romano, C.M. (2020). Detection of Reticuloendo-theliosis Virus in Muscovy Ducks, Wild Turkeys, and Chickens in Brazil. *J Wildl Dis.*, 56(3), 631–635. DOI:10.7589/2019-04-088.

10 Adcock, K.G., Berghaus, R.D., Goodwin, C.C., Ruder, M.G., Yabsley, M.J., Mead, D.G., Nemeth, N.M. (2024). Lymphoproliferative Disease Virus and Reticuloendotheliosis Virus Detection and Disease in Wild Turkeys (Meleagris gallopavo). *J Wildl Dis.,* 60(1), 139–150. DOI.10.7589/JWD-D-23-00012.

11 Mosad, S.M., El-Tholoth, M., El-Kenawy, A.A., Abdel-Hafez, L.J.M., El-Gohary, F.A., El-Sharkawy, H., Elsayed, M.M., Saleh, A.A., Elmahallawy, E.K. (2020). Molecular Detection of Reticu-loendotheliosis Virus 5' Long Terminal Repeat Integration in the Genome of Avipoxvirus Field Strains from Different Avian Species in Egypt. *Biology*, 9(9), 257. DOI.10.3390/biology9090257.

12 Gopal, S., Manoharan, P., Kathaperumal, K., Chidambaram, B., Divya, K.C. (2012). Differential detection of avian oncogenic viruses in poultry layer farms and Turkeys by use of multiplex PCR. *J Clin. Microbiol.*, 50(8), 2668–2673. DOI.10.1128/JCM.00457-12.

13 Zhang, J., Ma, L., Li, T., Li, L., Kan, Q., Yao, X., Xie, Q., Wan, Z., Shao, H., Qin, A., Ye, J. (2021). Synergistic pathogenesis of chicken infectious anemia virus and J subgroup of avian leukosis virus. *Poult Sci.*, 100(11), 101468. DOI.10.1016/j.psj.2021.101468.

14 Feng, W., Zhou, D., Meng, W., Li, G., Zhuang, P., Pan, Z., Wang, G., Cheng, Z. (2017). Growth retardation induced by avian leukosis virus subgroup J associated with down-regulated Wnt/β-catenin pathway. *Microb. Pathog.*, 104, 48–55. DOI.10.1016/j.micpath.2017.01.013.

15 Li, Q., Wang, P., Li, M., Lin, L., Shi, M., Li, H., Deng, Q., Teng, H., Mo, M., Wei, T., Wei, P. (2020). Recombinant subgroup B avian leukosis virus combined with the subgroup J env gene signifi-cantly increases its pathogenicity. *Vet. Microbiol.,* 250, 108862. DOI: 10.1016/j.vetmic.2020.108862.

16 Nair, V., Fadly, A.M. (2013). *Leukosis/Sarcoma Group.* In: Swayne, D.E, Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L., Nair, V., editors. Diseases of Poultry, 13th edition. Ames, IA: Wiley-Blackwell,553-592.

17 Smith, L.P., Petheridge, L., Nair, V., Wood, A., Welchman, D. (2018). Avian leukosis virus subgroup J-associated myelocytoma in a hobby chicken. *Vet Rec.*, 182(1), 23. DOI:10.1136/vr.104626.

18 Zhang, F., Li, H., Lin, C., Wei, Y., Zhang, W., Wu, Y., Kang, Z. (2024). Detection and genetic diversity of subgroup K avian leukosis virus in local chicken breeds in Jiangxi from 2021 to 2023. *Front Microbiol.*, 15, 1341201. DOI:10.3389/fmicb.2024.1341201.

19 Yu, M., Zhang, Y., Zhang, L., Wang, S., Liu, Y., Xu, Z., Liu, P., Chen, Y., Guo, R., Meng, L., Zhang, T., Fan, W., Qi, X., Gao, L., Zhang, Y., Cui, H., Gao, Y. (2024). N123I mutation in the ALV-J receptor-binding domain region enhances viral replication ability by increasing the binding affinity with chNHE1. *PLoS Pathog*., 20(2), e1011928. DOI: 10.1371/journal.ppat.1011928.

20 Zhou, X., Liao, R., Tan, M., Zhang, Y., Wang, H., Zhang, K., Wang, Q., Lan, X. (2025). Precise Editing of chNHE1 Gene via CRISPR/Cas9 Generates ALV-J-Resistant Chicken Primordial Germ Cell. *Animals*,15(14), 2018. DOI:10.3390/ani15142018.

21 Malhotra, S., Winans, S., Lam, G., Justice, J., Morgan, R., Beemon, K. (2017). Selection for avian leukosis virus integration sites determines the clonal progression of B-cell lymphomas. *PLoS Pathog.,* 13(11), e1006708. DOI: 10.1371/journal.ppat.1006708.

22 Maclachlan, N.J., Dubovi, E.J. (2017). *Retroviridae.* In Maclachlan N.J., Dubovi E.J. (Eds.), *Fenner's veterinary virology.* Academic Press, 5th ed., 269–297. DOI:10.1016/B978-0-12-800946-8.00014-3.

23 Niebora, J., Data, K., Domagała, D., Józkowiak, M., Barrett, S., Norizadeh Abbariki, T., Bryja, A., Kulus, M., Woźniak, S., Ziemak, H., Piotrowska-Kempisty, H., Antosik, P., Bukowska, D., Mozdziak, P., Dzięgiel, P., Kempisty, B. (2024). Avian Models for Human Carcinogenesis-Recent Findings from Molecular and Clinical Research. *Cells*, 13(21), 1797. DOI:10.3390/cells13211797.

24 Chacón, R.D., Sedano-Herrera, B., Alfaro-Espinoza, E.R., Quispe, W.U., Liñan-Torres, A., De la Torre, D., de Oliveira, A., Astolfi-Ferreira, C.S., Ferreira, A.J.P. (2022). Complete Genome Characterization of Reticuloendotheliosis Virus Detected in Chickens with Multiple Viral Coinfections. *Viruses,*14(4), 798. DOI:10.3390/v14040798.

25 Chacón, R.D., Astolfi-Ferreira, C.S., Valdeiglesias Ichillumpa, S., Lage Hagemann, H., Furlan Rocha, M., Fernandes Magalhães, L., Freitas Raso, T., Ferreira, A.J.P. (2025). First Complete Genome of Reticuloendotheliosis Virus in a Mallard Duck from Brazil: Phylogenetic Insights and Evolutionary Analysis. *Pathogens*, 14(2), 189. DOI:10.3390/pathogens14020189.

26 Shosha, E.A.E., Zanaty, A.M., Darwesh, M.M., Fotouh, A. (2024). Molecular characterization and immunopathological investigation of Avian reticuloendotheliosis virus in breeder flocks in Egypt. *Virol J.*, 21(1), 259. DOI:10.1186/s12985-024-02525-5.

27 Wu, W.J., Lv, X.P., Wang, X.Y., Gao, X.L., Liu, C.N., Zhao, C.H., Gu, X.Z., Zheng, S.M. (2023). Effects of Reticuloendotheliosis virus on TLR-3/IFN-B pathway in specific pathogen-free chickens. *Res Vet Sci.,* 156, 36–44. DOI: 10.1016/j.rvsc.2023.01.018.

28 Zhang, X., Zhou, D., Zhou, J., Liu, X., Ding, L., Yan, T., Shi, M., He, S., Cheng, Z. (2025). Avian leukosis virus subgroup J evades innate immunity by activating miR-155 to dually target TRAF3 and STAT1. *PLoS Pathog.,* 21(10), e1013552. DOI: 10.1371/journal.ppat.1013552.

29 Cui, Y., Dong, M., Wang, Z., Liu, Y., Liu, J., Zeng, H., Shao, R., Liu, R., Liu, Y., Xie, Q., Zhang, X. (2026). Co-infection with CAV facilitates ALV-K replication and induces severe immuno-suppression in chickens. *Vet Microbiol.*, 320, 111125. DOI: 10.1016/j.vetmic.2026.111125.

30 Dou, J., Wang, Z., Li, L., Lu, Q., Jin, X., Ling, X., Cheng, Z., Zhang, T., Shao, H., Zhai, X., Luo, Q. (2023). A Multiplex Quantitative Polymerase Chain Reaction for the Rapid Differential Detec-tion of Subgroups A, B, J, and K Avian Leukosis Viruses. *Viruses*, 15(9), 1789. DOI:10.3390/v15091789.

31 Fan, W., Chen, Y., Yu, M., Integrated ALV-J Research Group. (2024). Advances on ALV-J in China over the past two decades. *J. Integr. Agric.*, 24(2), 429–440. DOI: 10.1016/j.jia.2024.05.009.

32 Woźniakowski, G., Frant, M., Mamczur, A. (2018). Avian Reticuloendotheliosis in Chickens - An Update on Disease Occurrence and Clinical Course. *J Vet Res.,* 62(3), 257–260. DOI:10.2478/jvetres-2018-0036.

33 Bi, Y., Xu, L., Qiu, L., Wang, S., Liu, X., Zhang, Y., Chen, Y., Zhang, Y., Xu, Q., Chang, G., Chen, G. (2018). Reticuloendotheliosis Virus Inhibits the Immune Response Acting on Lymphocytes from Peripheral Blood of Chicken. *Front Physiol.*, 9, 4. DOI:10.3389/fphys.2018.00004.

34 Shi, M., Li, M., Wang, P., Wang, W., Li, H., Gao, Y., Lin, L., Huang, T., Wei, P. (2021). An outbreak in three-yellow chickens with clinical tumors of high mortality caused by the coinfection of reticuloendotheliosis virus and Marek's disease virus: a speculated reticuloendotheliosis virus contamination plays an important role in the case. *Poul Sci.,* 100(1), 19–25. DOI: 10.1016/j.psj.2020.09.034.

35 Liu, Q., Ji, S., Li, M., He, L., Ding, K., Yu, Z., Chen, J. (2025). Isolation and molecular characterization of subgroup J avian leukosis virus in native chicken breeds of China during 2022-2025. *Front Microbiol.,* 16, 1684812. DOI:10.3389/fmicb.2025.1684812.

36 Fandiño, S., Gomez-Lucia, E., Benítez, L., Doménech, A. (2023). Comparison of Endogenous Alpharetroviruses (ALV-like) across Galliform Species: New Distant Proviruses. *Microorganisms,* 12(1), 86. DOI:10.3390/microorganisms12010086.

37 Jiang, L., Wang, X., Li, Zh., Wang, Y., Jin, K., Feng, Zh., Xu, J., Bian, J., Liu, S., Meng, F., Liu, J. (2026). Prevalence and co-infection of infectious tumour viruses in chickens in Shandong Province, China. *Avian Pathol.,* 1–14. DOI:10.1080/03079457.2026.2662474.

38 Alfaki, S.H., Hussien, M.O., Elsheikh, F.M., Taha, K.M., Elbrissi, A.H., El Hussein, A.R.M. (2019). Serological and molecular identification of Reticuloendotheliosis virus (REV) in chickens in Sudan. *Vet Med Sci.,* 5(4), 508–511. DOI:10.1002/vms3.188.

Research article

Evaluation of DNA extracted from hair follicles of four types of Kazakh horses (Zhabe, Naiman, South Altai and Aday)

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Abstract

Background and Aim. This study aimed to assess the quality and concentration of genomic DNA isolated from hair follicles of four Kazakh horse types: Zhabe, Naiman, South Altai, and Aday. A total of 86 hair follicle samples were collected from Kazakh horses originating from four regions of Kazakhstan: Pavlodar, East Kazakhstan, Zhetisu, and Mangystau.

Materials and Methods. Genomic DNA was extracted from these samples using the PureLink Genomic DNA Mini Kit according to the manufacturer's recommended protocol with minor modifications, and the quantity and purity of the extracted DNA were evaluated using a NanoDrop spectrophotometer based on the A260/A280 absorbance ratio.

Results. The analysis revealed that the concentration of extracted DNA ranged from 93.7 to 247.2 ng/µl, while DNA purity values were predominantly within the range of 1.8–1.9, indicating high-quality DNA suitable for subsequent molecular genetic analyses.

Conclusion. The results highlight the potential of hair follicles as a practical and minimally invasive source of genomic DNA in Kazakh horses. The extraction approach provided DNA of sufficient quantity and quality for downstream molecular applications, supporting its use in future genomic studies. Further quality assessment will determine whether the obtained DNA meets the requirements for whole-genome sequencing and subsequent investigation of genetic variation among different types of Kazakh horses.

Keywords: DNA extraction; horse hair follicles; Kazakh horses; PureLink Genomic DNA Kit; Whole genome sequencing.

Introduction

The Kazakh horse is recognized as one of the most ancient horse breeds worldwide. Although several theories have been proposed concerning its origin, the prevailing view is that the contemporary Kazakh horse descended from the ancient Wusun horses, which inhabited Central Asia during the 2nd century BCE [1, 2].

The present-day Kazakh horse has been shaped through centuries of natural selection and selective breeding. Its historical development was closely linked to the lifestyle of nomadic populations, serving as a vital means of transportation along the Great Silk Road and playing an important role in military campaigns and the exchange of horses between Central Asia and China. Today, the breed is mainly found in Kazakhstan, western China, northeastern Kyrgyzstan, Mongolia, and the western Altai region of Russia [3].

According to *Yu. Barmintsev* [4] and *A. Belyaev* [5], the Kazakh horse breed includes several distinct types and local varieties. The emergence of these populations has been largely influenced by regional differences in environmental and climatic conditions, together with variations in livestock management

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and breeding strategies across Kazakhstan. Furthermore, the introduction of genetic material through crossbreeding with other horse breeds, particularly the Mongolian horse, has contributed to the diversification and formation of the breed's intra-population types and local varieties. For instance, the Aday variety of Kazakh horses, distributed in the Caspian desert zone, is characterized by a more pronounced riding-horse body conformation. In the eastern regions of Kazakhstan, local Kazakh horses demonstrate considerable similarity to Kyrgyz, Mongolian, and Altai horses.

The Aday variety was formed in ancient times under the influence of Turkmen horses and the harsh environmental conditions of desert and semi-desert regions. This variety is predominantly classified as a riding-type horse, exhibiting a lean body conformation, agile movements, and favorable speed characteristics. Its development has been strongly influenced by traditional horse husbandry and riding practices, with selective breeding emphasizing individuals capable of sustaining long-distance travel, agility, and high endurance. The desert climate contributed to the development of traits associated with a dry body constitution. Among the seven varieties described by *Yu. Barmintsev*, the Aday variety is the only one that has retained its distinctive specific characteristics. Currently, breeding of purebred Aday horses is carried out in five breeding farms in the Mangystau region [4].

One of the most valuable characteristics of the Aday horse is its exceptional adaptation to the harsh environmental and climatic conditions of the Mangystau desert zone. At the same time, modern representatives of the breed have not yet achieved sufficient uniformity in terms of body conformation and production characteristics. At various stages, attempts were made to increase live body weight through crossbreeding with established and specialized horse breeds. However, these breeding efforts did not produce the expected results [5].

The South Altai horse is regarded as the easternmost regional variety of the Kazakh breed and shares many morphological and conformational characteristics with the Altai horse, including similar body dimensions. In contrast, the Naiman variety, which is native to the Dzungarian Alatau mountain range, displays phenotypic features closely related to those of Mongolian horses. Among the regional varieties of the Kazakh horse, the Naiman type is the smallest in size, yet it is exceptionally well adapted to mountainous terrain and is recognized for its remarkable endurance and efficiency as a pack horse [6].

According to *M.Z. Dyussegaliyev* the Naiman represents the third recognized type and has long been maintained by local communities in the Dzungar Alatau. It is valued as a versatile horse capable of performing effectively under mountainous conditions. In terms of overall conformation, the Naiman type shows similarities to Mongolian horses and is regarded as one of the lighter and more refined types within the Kazakh horse population. The Naiman type of horse possesses a number of valuable economically important traits, including a strong body conformation, robust health, and high reproductive capacity. Under year-round herd management conditions, Naiman horses demonstrate a high foal production rate, which contributes to the economic efficiency of their breeding [6]. In addition to preserving the gene pool, the primary objective of breeding Naiman horses at the Tokzhailau production cooperative is the production of breeding stock. The developed breeding young stock is used to supply horse-breeding farms located in the mountainous regions of southern Kazakhstan [7].

Whole-genome sequencing (WGS) in horses enables the identification of genetic variations at the whole-genome level, including single nucleotide polymorphisms (SNPs) that cannot be detected using marker-based approaches (*Jagannathan* et al., 2019). WGS has also contributed to improving the quality of the horse reference genome, thereby increasing the accuracy of mutation detection and gene annotation (*Kalbfleisch* et al., 2018). These data are used for the identification of hereditary diseases, assessment of breed-specific genetic load, and optimization of breeding programs [8, 9].

Regardless of the objectives of genetic research, the quality of obtained data largely depends on the method used for the initial extraction of nucleic acids [10]. The extracted DNA must possess high quality to ensure successful application in genetic, population, genomic, and forensic studies, while the biological samples used should be easy to collect and process. Hair follicles are considered a non-invasive source of DNA suitable for subsequent genetic investigations [11].

According to *Tozaki* et.al, hair follicles collected from the mane or tail represent a practical source of genomic DNA in horses because sampling is minimally invasive compared with blood collection and the material can be readily preserved for extended periods. Consequently, archived hair-root samples

may provide a useful resource for subsequent genetic and genomic investigations. However, storage conditions have a substantial effect on DNA integrity. Long-term preservation of hair roots at room temperature was associated with considerable DNA degradation, whereas samples maintained under refrigerated conditions of 4–8 °C showed better preservation of DNA quality [12].

Previous studies on equine DNA sampling have emphasized several pre-analytical factors that can influence the suitability of hair-derived material for downstream genomic analyses. These include maintaining samples in a dry condition during prolonged storage, assessing the integrity of collected hairs, applying an appropriate washing procedure before DNA extraction, and preferentially selecting hairs with intact follicles and associated tissue sheaths.

The extraction and quantification of DNA concentration represent fundamental procedures that determine the reliability and efficiency of sample storage and subsequent utilization [13, 14].

Although hair follicles have previously been used as a source of genomic DNA in horses, comparative information regarding DNA quality among different indigenous Kazakh horse types remains limited. Therefore, this study aimed to evaluate the concentration and purity of genomic DNA extracted from hair follicles of four Kazakh horse types using a standardized extraction protocol as a basis for future population genomic investigations.

Materials and Methods

Ethical approval

Institutional Review Board Statement: The study was approved by the Bioethics Commission of Kazakh National Agrarian Research University, Kazakhstan (approval date: 02 November 2025, protocol number 15/09).

The sample collection was conducted between August and November 2025. Hair follicle samples were collected from clinically healthy Kazakh horses of both sexes aged 1.5–9 years that were maintained under traditional herd management systems in four breeding regions of Kazakhstan. The sampling sites represented four distinct horse types: Zhabe, South Altai, Naiman, and Aday. In August, samples from Zhabe-type horses were collected in the Pavlodar region, Maiskiy and Yereimentau districts, at the “Aqshiman-Agro” and “Ata-Zhurt” farms. In November, South Altai-type horses were sampled in Zaisan, East Kazakhstan, at the “Tuyusqan” farm; Naiman-type horses were sampled in Tekeli, Zhetisu, at the “Tekeli”farm; and Aday-type horses were sampled in Ushtagan, Mangystau, at the “Shomaq” and “Miras” farms.

A total of 86 horses were included in the study, comprising 22 Zhabe, 22 Naiman, 20 South Altai, and 22 Aday horses. One biological sample was collected from each animal. For DNA analysis, hair root samples were obtained from the tail by manually plucking approximately 40–50 hairs with intact follicles from each horse. Only hairs with clearly visible hair bulbs were selected to ensure the presence of sufficient biological material for subsequent DNA extraction. Each sample was placed individually in a sterile paper envelope, labeled with a unique identification number, and stored dry at 4°C until the DNA extraction. To minimize DNA degradation and ensure sample quality, DNA extraction was performed within seven days of sample collection.

Genomic DNA was extracted from hair follicles collected from the roots of horse hairs using the PureLink™ Genomic DNA Mini Kit (Invitrogen, Thermo Fisher Scientific, USA) according to the manufacturer's protocol with minor modifications [14]. Briefly, approximately 1–2 cm of hair containing 5–10 follicles was collected from each sample and transferred to sterile 1.5-ml microcentrifuge tubes after preliminary mechanical cleaning.

The hair follicle samples were subjected to enzymatic digestion using Digestion Buffer and Proteinase K. The samples were incubated at 55 °C until complete digestion was achieved.

Following digestion, the lysates were centrifuged, and the resulting supernatants were transferred to clean microcentrifuge tubes. RNase A treatment was performed according to the manufacturer's protocol. Genomic Lysis/Binding Buffer and ethanol were subsequently added to the lysates, and the resulting mixtures were applied to PureLink spin columns. The columns were centrifuged to allow binding of genomic DNA to the silica membrane.

The columns were washed sequentially with Wash Buffer 1 and Wash Buffer 2, followed by an additional centrifugation step to remove residual wash solution and ethanol. Purified genomic DNA was eluted from the spin columns using Elution Buffer. The extracted DNA was stored at −80 °C until further analysis.

DNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

Descriptive statistical analysis was performed using Microsoft Excel. DNA concentration and A260/A280 purity values were expressed as mean ± standard deviation (SD), minimum, and maximum for each horse type.

Results

In the present study, the concentration and purity of genomic DNA extracted from hair follicles of four Kazakh horse types (Zhabe, Naiman, South Altai, and Aday) were evaluated. The quantitative and qualitative parameters of DNA were determined using a NanoDrop spectrophotometer. The obtained results demonstrated variation in DNA concentration among all investigated horse types, which may be associated with individual characteristics of the samples, the number of hair follicles collected, and the efficiency of the extraction process. Nevertheless, all groups yielded DNA concentrations sufficient for further molecular genetic analyses.

In Zhabe-type horses, DNA concentration ranged from 95.4 to 247.2 ng/µl, while the A260/A280 purity ratios varied from 1.76 to 1.94. The majority of samples demonstrated values within the range of 1.8–1.9, indicating good quality of the extracted DNA and minimal contamination with protein impurities (Figure 1).

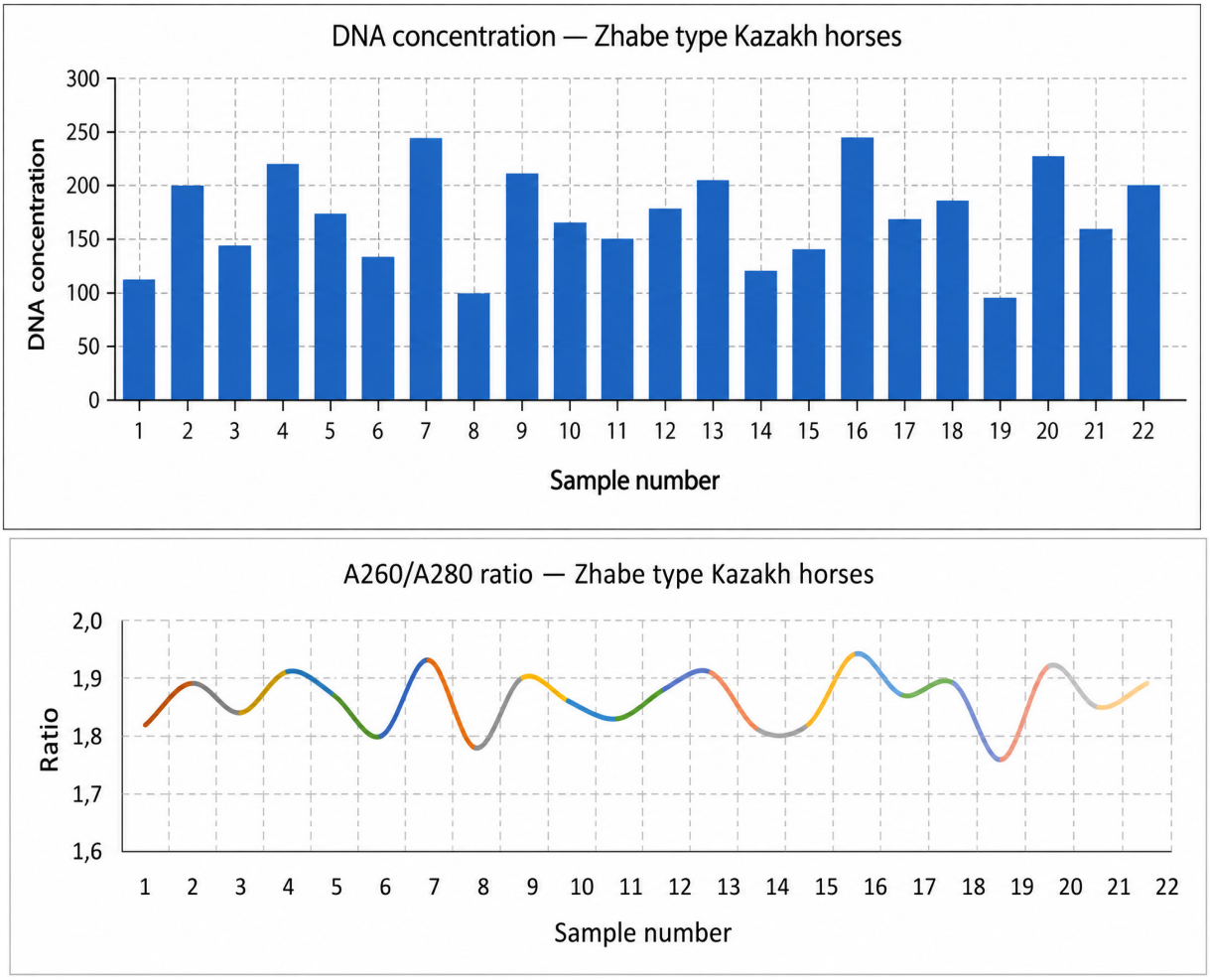

Figure 1. DNA concentration and purity parameters of genomic DNA extracted from hair follicle samples of Zhabe type Kazakh horses, measured using a NanoDrop spectrophotometer

For the Naiman type, DNA concentration ranged from 104.9 to 241.9 ng/µl, while the A260/A280 ratios were within the range of 1.79–1.94. These results also indicate a high degree of DNA purity and confirm the efficiency of the applied extraction method (Figure 2).

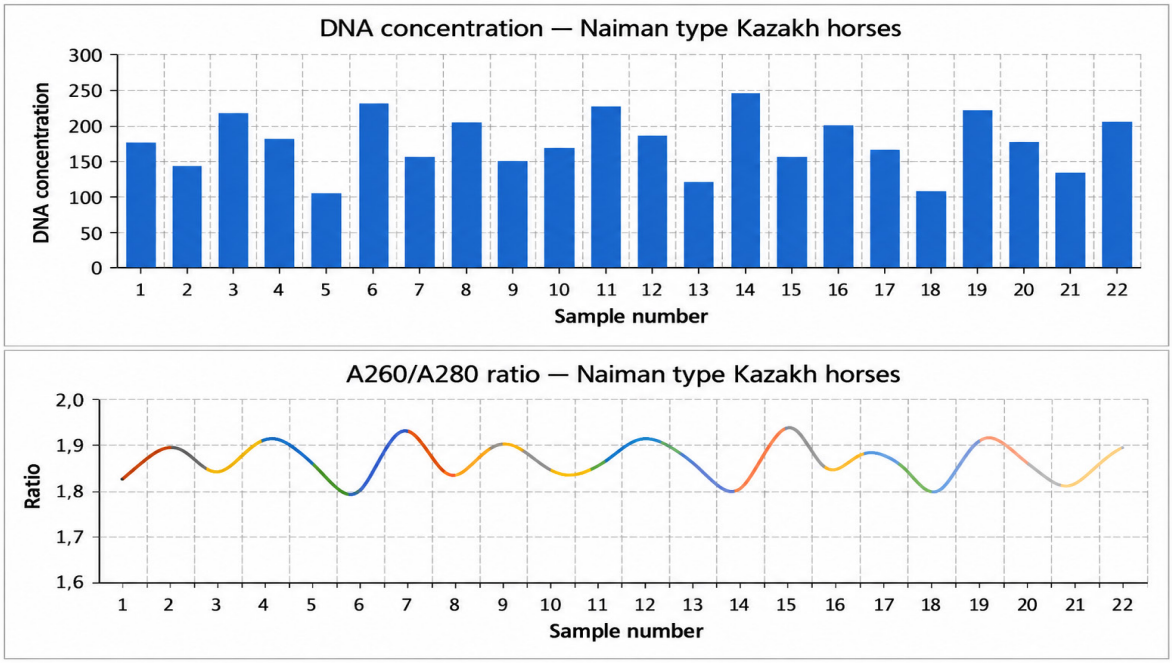

Figure 2. DNA concentration and purity parameters of genomic DNA extracted from hair follicle samples of Naiman type Kazakh horses, measured using a NanoDrop spectrophotometer

For the South Altai type samples, DNA concentration ranged from 93.7 to 243.5 ng/µl. The A260/A280 values ranged from 1.72 to 1.94. Slightly reduced purity values were observed in some individual samples (samples No. 9 and No. 19), which may indicate the presence of minor protein contaminants or residual reagents. However, the majority of samples corresponded to the recommended quality parameters for molecular genetic studies (Figure 3).

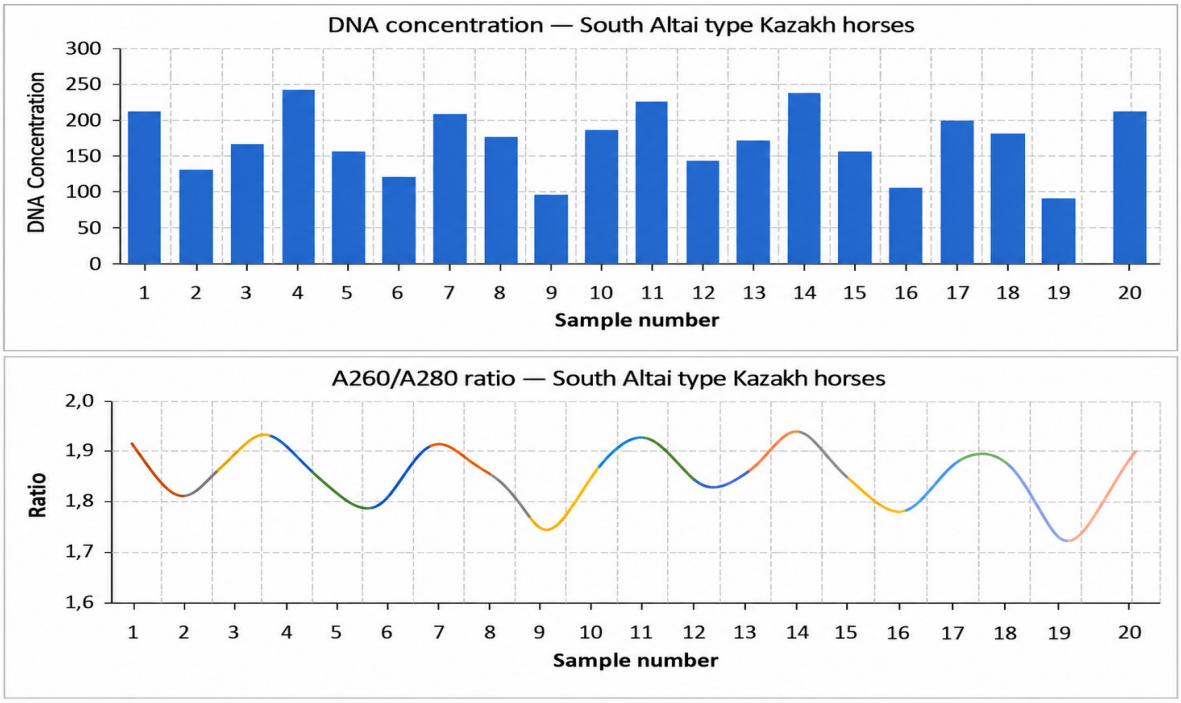

Figure 3. DNA concentration and purity parameters of genomic DNA extracted from hair follicle samples of South Altai type Kazakh horses, measured using a NanoDrop spectrophotometer

In Aday horses, the DNA concentration ranged from 97.3 to 245.3 ng/µl, and the purity values were 1.77–1.94. High values of the A260/A280 ratio confirm the receipt of high-quality genetic material suitable for subsequent sequencing and genomic analysis (Figure 4, Table 1).

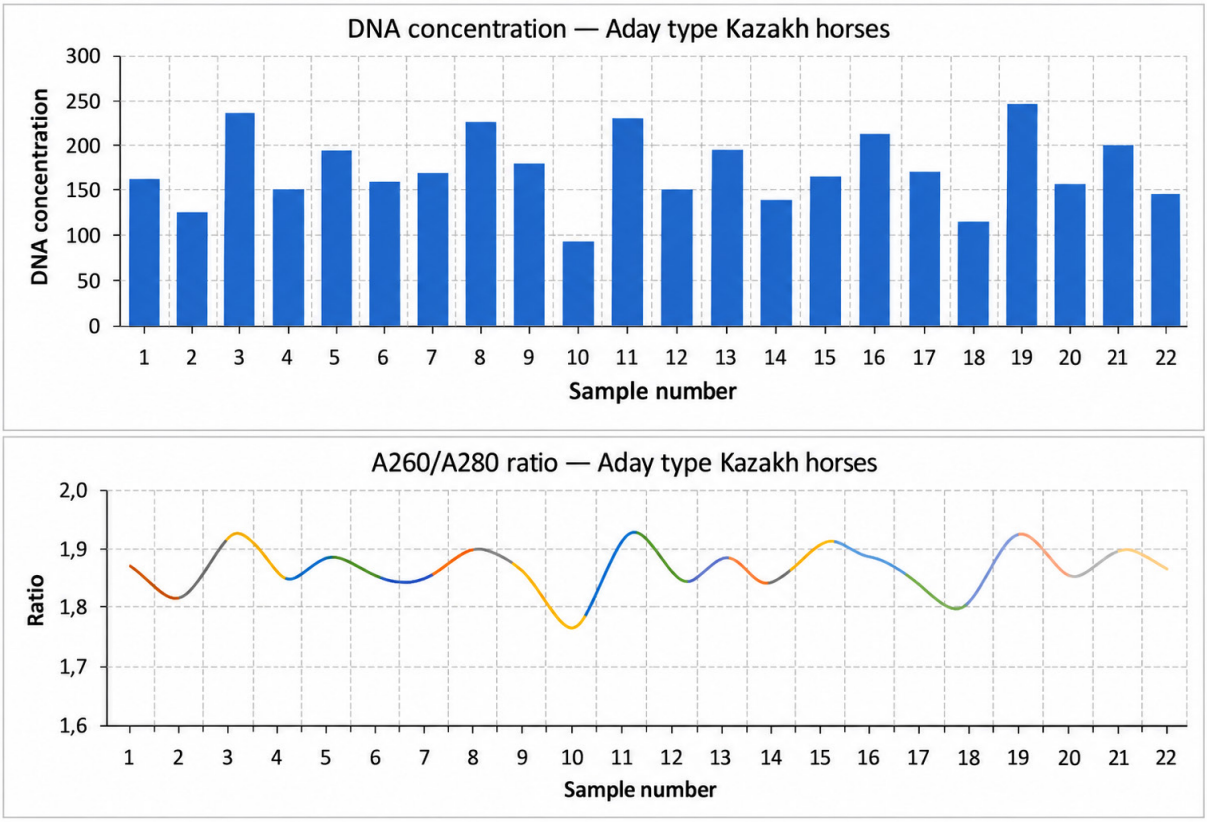

Figure 4. DNA concentration and purity parameters of genomic DNA extracted from hair follicle samples of Aday type Kazakh horses, measured using a NanoDrop spectrophotometer

Table 1. Descriptive statistics of DNA concentration and A260/A280 purity ratio in four Kazakh horse types

Horse type	Zhabe	Naiman	South Altai	Aday
N	22	22	20	22
DNA concentration, mean ± SD (ng/µL)	172.8 ± 43.3	176.9 ± 40.6	171.8 ± 44.9	175.3 ± 40.3
Min–Max	95.4–247.2	104.9–241.9	93.7–243.5	97.3–245.3
A260/A280, mean ± SD	1.86 ± 0.05	1.87 ± 0.04	1.85 ± 0.06	1.87 ± 0.04
Min–Max	1.76–1.94	1.79–1.94	1.72–1.94	1.77–1.94

The mean DNA concentration was 172.8 ± 43.3 ng/µl in Zhabe horses, 176.9 ± 40.6 ng/µl in Naiman horses, 171.8 ± 44.9 ng/µl in South Altai horses, and 175.3 ± 40.3 ng/µl in Aday horses. The corresponding concentration ranges were 95.4–247.2, 104.9–241.9, 93.7–243.5, and 97.3–245.3 ng/µl, respectively.

The mean A260/A280 ratios were 1.86 ± 0.05 in Zhabe horses, 1.87 ± 0.04 in Naiman horses, 1.85 ± 0.06 in South Altai horses, and 1.87 ± 0.04 in Aday horses. The corresponding ranges were 1.76–1.94, 1.79–1.94, 1.72–1.94, and 1.77–1.94, respectively. Overall, the obtained values indicated a generally satisfactory level of DNA purity in all four horse types.

Discussion

In the present study, the PureLink Genomic DNA Mini Kit demonstrated reliable and efficient performance for DNA extraction from hair follicles of different Kazakh horse types. The mean A260/A280 ratios ranged from 1.85 to 1.87 across the four horse types. This indicates that the extracted DNA was of sufficient quality for subsequent molecular analyses. Together with previous reports,

our findings confirm that hair follicles represent a practical and minimally invasive biological material for obtaining genomic DNA, while commercial extraction kits can provide reproducible DNA quality suitable for downstream molecular applications. *Kabylbekova* et al. compared three methods of DNA extraction from the hair follicles of Kazakh horses of the Zhabe type: a commercial kit, CTAB, and the phenol-chloroform method. Although the methods produced DNA of comparable quality, the authors recommended CTAB as the most suitable method for genotyping, considering its safety, cost-effectiveness, and efficiency [15, 16]. These findings highlight the importance of selecting an extraction method according to the intended downstream application and the characteristics of the biological material. *Ibadullayeva* et al. demonstrated that the PureLink Microbiome Purification Kit enables efficient DNA extraction from microbiome samples of Kazakh horses of the Jabe and Aday types, yielding DNA suitable for subsequent 16S rRNA sequencing [17]. *Similarly, Villacrez-Arce* et al. showed that high-quality genomic DNA suitable for genomic studies and genotyping-by-sequencing (GBS) can be obtained non-invasively from horse tail and mane hair. The highest DNA yield was obtained using 80 hair follicles (22.99 ng/µl), with DNA quality parameters within the requirements for sequencing platforms [18]. These studies support the suitability of hair-derived material as a convenient and minimally invasive source of genomic DNA for molecular and genomic analyses in horses.

A limitation of the present study is that DNA quality was evaluated using the A260/A280 absorbance ratio only. Electrophoretic assessments of DNA integrity were not performed and should be included in future investigations.

Conclusion

The obtained DNA was of satisfactory concentration and purity for subsequent molecular genetic analyses. Its suitability for whole-genome sequencing requires further confirmation through additional assessment of DNA integrity and purity. In future studies, sequencing is planned to study genetic diversity, identify genetic variants, and compare four types of Kazakh horses.

Authors' Contributions

AI, and AKh: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript. TA, TSh, AK: conducted final editing and proofreading of the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

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Conflict of Interest

The authors declare that they have no known financial interests or personal relationships that could have influenced the results presented in this work.

References

1 Kalinin, V.I., Yakovlev, A.A. (1966). *Konevodstvo* [*Horse breeding*]. Moskva: Kolos, 17–18. [*In Russian*].

2 Vitt, V.O. (1937). *Konskie porody Srednei Azii* [*Horse breeds of Central Asia*]. M.: VASHNIL, 16. [*In Russian*].

3 Zhao, T.Z. (1991). The Kazakh horses. *Xinjiang Agricultural Sciences*, 5, 222–223.

4 Barmintsev, Yu.N. (1963). *Myasnoe i molochnoe konevodstvo* [*Meat and dairy horse breeding*]. Moscow: Sel'khozgiz, 224. [*In Russian*].

5 Belyaev, A.I. (1973). *Kazakhskaya loshad' zhabe* [*Kazakh Zhabe horse*]. Alma-Ata: 133. [*In Russian*].

6 Dyussegaliyev, M.Z. (2022). Genotipy tabunnyh loshadei Zapadnogo regiona Kazahstana [The genotypes of herd horses of the West Region of Kazakhstan]. *Sel'skoe hozyaistvo i ekosistemy v sovremennom mire: regional'nye i mezhstranovye issledovaniya,* 33–43.

7 Sydykov, D.A., Baktybaev, G.T. (2012). Malochislennaya i unikal'naya populyatsiya naimanskikh loshadey [A small and unique population of Naiman horses]. *Konevodstvo i Konny Sport*, 6, 12–14. [*In Russian*].

8 Jagannathan, V., Gerber, V., Rieder, S., Tetens, J., Thaller, G., Drögemüller, C., Leeb, T. (2019). Comprehensive characterization of horse genome variation by whole-genome sequencing of 88 horses. *Anim. Genet.,* 50(1), 74–77.

9 Kalbfleisch, T.S., Rice, E.S., DePriest Jr, M.S., Walenz, B.P., Hestand, M. S., Vermeesch, J. R., MacLeod, J.N. (2018). Improved reference genome for the domestic horse increases assembly contiguity and composition. *Comm. Biol.,* 1(1), 197.

10 Leigh Greathouse, K., Sinha, R., Vogtmann, E. (2019). DNA extraction for human microbiome studies: The issue of standardization. *Genome Biol.*, 20(1), 212.

11 Ortega Torres, M.J., Almeida Braga, J., Torres, C., Shaker, A.S. (2021). Alkaline lysis as an efficient and economical alternative for DNA extraction from horsehair samples: Comparison between different methods. *Int. J. Adv. Res.,* 9(2), 836–841. DOI: 10.21474/IJAR01/12522.

12 Tozaki, T., Ohnuma, A., Kikuchi, M., Ishige, T., Kakoi, H., Hirota, K., Hamilton, N., Kusano, K., Nagata, S. (2020). Whole-genome resequencing using genomic DNA extracted from horsehair roots for gene-doping control in horse sports. *J. Equine Sci.,* 31, 75–83. DOI: 10.1294/jes.31.75.

13 Balanovskiy, O., Kagazezheva, Zh., Ol'kova, M. (2019). Metody izmereniya kontsentratsii DNK: sovpadenie otnositel'nykh velichin i razlichiya absolyutnykh [Methods for measuring DNA concentration: Agreement of relative values and differences in absolute values]. *Vestnik RGMU*, 27, 33. [*In Russian*].

14 Thermo Fisher Scientific. (n.d.). *PureLink Genomic DNA Mini Kit Handbook* [Manual]. Available online.

15 Pozharskiy, A., Khamzina, A., Gritsenko, D., Khamzina, Z., Kassymbekova, S., Karimov, N., et al. (2020). SNP genotyping and population analysis of five indigenous Kazakh sheep breeds. *Livest. Sci.,* 241, 104252.

16 Kabylbekova, D., Khamzina, Z., Aubakirova, K., Ibadullayeva, A. (2023). Evaluation and comparison of three methods of DNA extraction from Kazakh horse of the type Zhabe. *Fundam. Exp. Biol.*, 28(1), 69–75. DOI: 10.31489/2023bmg1/69-75.

17 Ibadullayeva, A., Asanbayev, T., Khamzina, A., Kassymbekova, Sh. (2023). Otsenka DNK iz obraztsov kazakhskikh porod loshadey tipa Zhabe i Adayskogo tipa dlya 16S rRNK sekvenirovaniya [Evaluation of DNA samples of Kazakh horse breeds of the zhabe and adai types for 16s rRNA sequencing]. *Nauka i obrazovanie*, 2(71), 70–79. [*In Russian*].

18 Villacrez-Arce, A., Ccatamayo, D.S., Torres, H.F., Murga Valderrama, N.L., Saldaña Serrano, C.L., Escalante, W.B. (2026). High-quality DNA extraction protocol for genomic studies in Peruvian horses. *Rev. Inv. Vet. Perú.*, 37(2), e27320. DOI: 10.15381/rivep.v3.

Research article

Evaluation of the effectiveness of DNA extraction methods from sheep ear tissue samples for WGS

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Abstract

Background and Aim. Whole-genome sequencing is a powerful tool for studying the genetic structure of livestock populations. Effective implementation of WGS is largely determined by the quality of the source genomic DNA. Sheep ear notches obtained in the field are a convenient source of biomaterial; however, their high connective tissue density makes DNA extraction challenging. The aim of this study was to compare the CTAB method and the commercial PureLink Genomic DNA Kit for DNA extraction from ear notches of Kazakh fat-tailed sheep.

Materials and Methods. The study was conducted in two stages. CTAB lysis buffer was used in the first stage, and the commercial PureLink Genomic DNA Kit (Thermo Fisher Scientific) was used in the second stage. Quality control was performed using a NanoDrop2000 spectrophotometer and gel electrophoresis.

Results. The average DNA concentration isolated by the CTAB method was 293 ng/µl, and the A260/280 ratio ranged from 1.70 to 1.86 (mean 1.78 ± 0.04), indicating heterogeneous purity. The PureLink kit demonstrated consistently high quality: concentration 350.2 ± 96.9 ng/µl, A260/280 1.86 ± 0.01, and A260/230 2.13 ± 0.08.

Conclusion. The commercial PureLink Genomic DNA Kit is recommended for DNA extraction from sheep ear notches, as it provides consistent quantitative and qualitative data that meet WGS requirements.

Keywords: DNA extraction; Kazakh fat-tailed sheep breed; PureLink Genomic DNA Kit; sheep ear notches; CTAB; whole genome sequencing.

Introduction

Whole-genome sequencing (WGS) occupies a key position in modern agricultural genetics, as it offers unique opportunities for the comprehensive analysis of genomic variability and the identification of genes associated with economically significant traits [1]. With the advancement of Illumina and other platform technologies, WGS of farm animals has become accessible even to relatively small laboratories [2].

Efficient implementation of WGS is largely determined by the quality of the original genomic DNA [3]. High-throughput sequencing technologies require DNA of sufficient concentration (typically at least 10 ng/µL), high purity (A260/280 ratio in the range of 1.8–2.0 and A260/230 in the range of 2.0–2.2), and preserved molecular integrity [4]. The presence of impurities, inhibitors, or DNA fragmentation can lead to incomplete library preparation and reduced sequencing data quality [4].

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One common source of biomaterial for molecular genetic studies of sheep is ear pinna tissue, obtained by collecting ear notches. This type of biomaterial is convenient for field collection and does not require invasive procedures. However, the ear pinna is characterized by a high density of connective tissue and such components as collagen, which can reduce the efficiency of nucleic acid extraction and inhibit PCR, requiring optimization of extraction protocols. Therefore, choosing the optimal method for DNA extraction from ear notches remains a pressing issue.

Currently, various approaches are used to isolate genomic DNA: classical laboratory protocols, in particular the CTAB-based (cetyltrimethylammonium bromide, Sigma-Aldrich, St. Louis, MO, USA), method [5, 6], and commercial DNA extraction kits [7]. The CTAB method, based on the cationic detergent cetyltrimethylammonium bromide, effectively removes polysaccharide and protein contaminants, which is especially important when working with tissues rich in secondary metabolites. However, the CTAB method is significantly more time-consuming compared to commercial kits, and each step of the protocol is subject to modification, creating reproducibility issues in the absence of a detailed description of the extraction conditions [8].

Commercial kits, such as the PureLink Genomic DNA Kit (Thermo Fisher Scientific), offer standardized, reproducible procedures that can ensure more consistent DNA quality and reduce the impact of human error [9]. However, a direct comparison of the effectiveness of the CTAB method and a commercial kit for a specific type of biomaterial (sheep ear notches) remains insufficiently studied.

This task is particularly relevant in the Republic of Kazakhstan, where indigenous sheep breeds, particularly the Kazakh fat-tailed coarse-wool, remain insufficiently studied at the genomic level, and WGS data for them is virtually nonexistent [10]. Building a reliable genetic base for such populations requires dependable and reproducible methods for obtaining DNA suitable for WGS.

The aim of this study was to evaluate the effectiveness of the CTAB method and the commercial PureLink Genomic DNA Kit for extracting genomic DNA from ear notches of Kazakh fat-tailed sheep based on the quantitative and qualitative characteristics of the obtained DNA and to verify its suitability for WGS.

Materials and Methods

Ethical approval

Institutional Review Board Statement: The study was approved by the Bioethics Commission of PHPEI “West Kazakhstan Innovation and Technology University”, Kazakhstan (approval date: 02 September 2024, protocol number 15/09).

Sampling

Ear tissue samples were collected from 40 sheep, including 20 Kazakh coarse-wool fat-tailed (KCWFT) and 20 Kazakh semi-coarse-wool fat-tailed (KSCWFT) sheep, at the Bepen farm in the Beskaragay District, East Kazakhstan Region, Republic of Kazakhstan. One ear tissue sample was collected from each animal using sterile instruments and placed individually in a sterile, labeled tube. Thus, a total of 40 biological samples were obtained. For the paired comparison of the two DNA extraction methods, two portions were taken from the same ear tissue sample of each animal; no additional tissue samples were collected. For each extraction method, approximately 0.025 g of tissue was used. Consequently, DNA obtained by the two methods represented paired measurements from the same individual animal. Both DNA extraction procedures were performed on the same day, thereby eliminating differences in storage duration between the two methods. The samples were stored at −20 °C until DNA extraction.

Stage 1: DNA Extraction Using CTAB

In the first stage of the study, genomic DNA was extracted from ear tissue samples using a CTAB-based method. Approximately 25 mg (0.025 g) of each tissue sample was placed in a sterile 1.5-mL microcentrifuge tube, followed by the addition of 200 µL of PBS. The tissue was mechanically minced into small pieces using sterile scissors to facilitate subsequent lysis. After homogenization, 500 µl of CTAB buffer was added to the samples. (CTAB: 20.00 g/L (2% w/v), EDTA-Na2 x 2H2O: 7.44 g/L (20 mM), Tris: 12.11 g/L (100 mM), Sodium chloride: 81.82 g/L (1.4 M)) и 4 µl of proteinase K (20 mg/ml). Incubation was carried out in a thermo-shaker at 56 °C for 4 hours at 1000 rpm.

After lysis, 500 µl of a 24:1 mixture of chloroform and isoamyl alcohol were added to the samples, thoroughly mixed with a vortex, and centrifuged at 11,400 x g for 5 minutes. The upper aqueous phase

was carefully transferred to a clean tube, avoiding the interfacial protein film. To precipitate the DNA, sodium acetate (1/10 the supernatant volume) and 350 µl of isopropanol were added. The mixture was then incubated for 10 minutes at 23 °C temperature and centrifuged at 12,100 x g for 5 minutes.

The resulting DNA precipitate was washed with 70% ethanol, centrifuged again, and any residual liquid was removed. The precipitate was dried at 23 °C temperature for 10–15 minutes and then dissolved in 50 µl of deionized water. The resulting DNA samples were stored at +4 °C or –20 °C until further use. The concentration and purity of the isolated DNA were assessed using a NanoDrop 2000 spectrophotometer (ThermoScientific).

Step 2: DNA Extraction with a Commercial PureLink Kit

Genomic DNA extraction from ear notch samples was also performed using the PureLink Genomic DNA Mini Kit according to the manufacturer's recommendations with minor modifications.

For DNA extraction, 0.025 g of ear notch tissue was minced into small fragments using sterile scissors and placed in 1.5 ml tubes containing 180 µl of Digestion Buffer and 20 µl of Proteinase K. The samples were incubated in a thermo-shaker at 56 °C for 4 hours at 1000 rpm until complete tissue lysis.

After incubation, the samples were centrifuged at 11,400 x g for 3 minutes to pellet insoluble particles. The supernatant was transferred to a new tube, and 20 µl of RNase was added. The mixture was briefly vortexed and incubated at 23 °C temperature for 2 minutes.

Then, 200 µl of lysis buffer was added to the samples, mixed thoroughly, and incubated at 70 °C for 10 minutes without shaking. Then, 200 µl of 96–100% ethanol was added and vortexed. The resulting mixture was transferred to filtration columns and centrifuged at 6,200 x g for 2 minutes. If necessary, additional centrifugation was performed to ensure complete passage of the liquid through the membrane. During the wash step, 500 µl of Wash Buffer 1 was added to the column and centrifuged at 6,200 x g for 2 minutes. Then, 500 µl of Wash Buffer 2 was added and centrifuged at 11,400 x g for 1 minute. After washing, the filtration column was transferred to a clean 1.5 ml tube.

To elute the DNA, 70 µl of Elution Buffer, preheated to 70 °C, was applied to the column membrane, incubated for 2 minutes, and centrifuged at 11,400 x g for 1 minute. The resulting DNA samples were stored at +4 °C or –20 °C until further use. Quantitative and qualitative assessment was performed using three methods: NanoDrop 2000 spectrophotometry (concentration, A260/280 and A260/230); Qubit 4 fluorimetry with the Qubit dsDNA BR Assay complex; agarose gel electrophoresis (1% agarose gel, 100 V voltage, TAE buffer) to assess DNA integrity.

Preparation for Whole Genome Sequencing

The isolated DNA was sent to Novogene (London, UK) for library construction and whole genome sequencing on the Illumina platform. The laboratory tested the DNA for library preparation, including assessing its concentration, integrity, and the absence of inhibitors. Based on the analysis results, all samples were deemed suitable for sequencing, and FASTQ files were generated.

Statistical analysis

Statistical analysis was performed using Microsoft Excel. Descriptive statistics were calculated for DNA concentration and purity parameters (A260/280 and A260/230 ratios) and are presented as mean ± standard deviation (SD), together with the minimum and maximum values. Differences between the KCWFT and KSCWFT breeds were assessed using an independent-samples Student's t-test. A p-value < 0.05 was considered statistically significant.

Results

Isolation of CTAB with buffer

In the first stage of the study, DNA was isolated from sheep ear notches (n=40) using the CTAB method. Analysis on a NanoDrop2000 spectrophotometer revealed that the average KCWFT breed DNA concentration was 293.5 ± 47.8 ng/µL (range 221.8–421.8 ng/µL). The A260/280 ratio ranged from 1.70 to 1.86 (mean 1.78 ± 0.04), indicating the presence of protein impurities in some samples. The A260/230 ratio was significantly lower–1.52 ± 0.13 (range 1.28–1.84), indicating a significant content of organic compounds and residual amounts of CTAB buffer. DNA concentrations for the KCWFT are shown in Table 1.

Table 1. Qualitative parameters of the KCWFT breed DNA isolated by the CTAB method

Sample	Concentration, ng/µL	A260/280	A260/230
1	285.6	1.79	1.52
2	308.1	1.82	1.58
3	262.3	1.75	1.41
4	341.5	1.83	1.65
5	294.2	1.80	1.54
6	415.3	1.84	1.72
7	253.8	1.74	1.38
8	301.9	1.81	1.56
9	328.6	1.82	1.61
10	244.1	1.73	1.35
11	278.5	1.78	1.48
12	365.4	1.84	1.68
13	309.7	1.81	1.59
14	232.5	1.72	1.32
15	268.8	1.77	1.45
16	339.2	1.83	1.64
17	221.8	1,70	1.28
18	288.4	1.79	1.51
19	303.5	1.80	1.57
20	255.9	1.76	1.43
Mean (n=20)	294.4 ± 48.0	1.77 ± 0.05	1.53 ± 0.14

When analyzing the breeds separately, no statistically significant differences were found ($p > 0.05$). For the KCWFT breed (n=20), the indicators were: concentration 294.4 ± 48.0 ng/µl, A260/280 1.77 ± 0.05, A260/230 1.53 ± 0.14.

For the KSCWFT breed (n=20): concentration 292.6 ± 48.1 ng/µl, A260/280 1.79 ± 0.04, A260/230 1.52 ± 0.12. (Table 2).

Table 2. Qualitative parameters of DNA of the KSCWFT breed, isolated by the CTAB method

Sample	Concentration, ng/µL	A260/280	A260/230
21	281.3	1.78	1.50
22	312.4	1.81	1.60
23	258.7	1.74	1.39
24	345.2	1.82	1.66
25	290.5	1.79	1.53
26	408.6	1.85	1.73
27	249.3	1.73	1.36
28	298.4	1.80	1.55
29	332.1	1.81	1.62
30	240.6	1.72	1.34
31	275.2	1.77	1.46
32	358.9	1.83	1.67
33	305.3	1.80	1.58

Continuation of Table 2

34	228.9	1.71	1.31
35	264.2	1.76	1.44
36	335.7	1.82	1.63
37	225.4	1.70	1.29
38	284.9	1.78	1.52
39	300.1	1.79	1.56
40	251.3	1.75	1.42
Mean ± SD	292.6 ± 48.1	1.79 ± 0.04	1.52 ± 0.12

Agarose gel electrophoresis (1% agarose, TAE buffer, 100 V, 30 min) of DNA isolated from ear notches of Kazakh fat-tailed sheep using the CTAB method demonstrated significant variability in quality between individual samples. All wells of the gel contained visible DNA bands, but their morphology varied. Samples #4 and #11 and #12 were characterized by relatively compact bands of high-molecular-weight DNA, while samples #2, #10, and #11 demonstrated pronounced smearing in the range of low-molecular-weight fragments, indicating fragmentation of genomic DNA during the extraction process (Figure 1). Sample #9 was additionally distinguished by a broad, smeared band, indicating heterogeneity of the fractional composition.

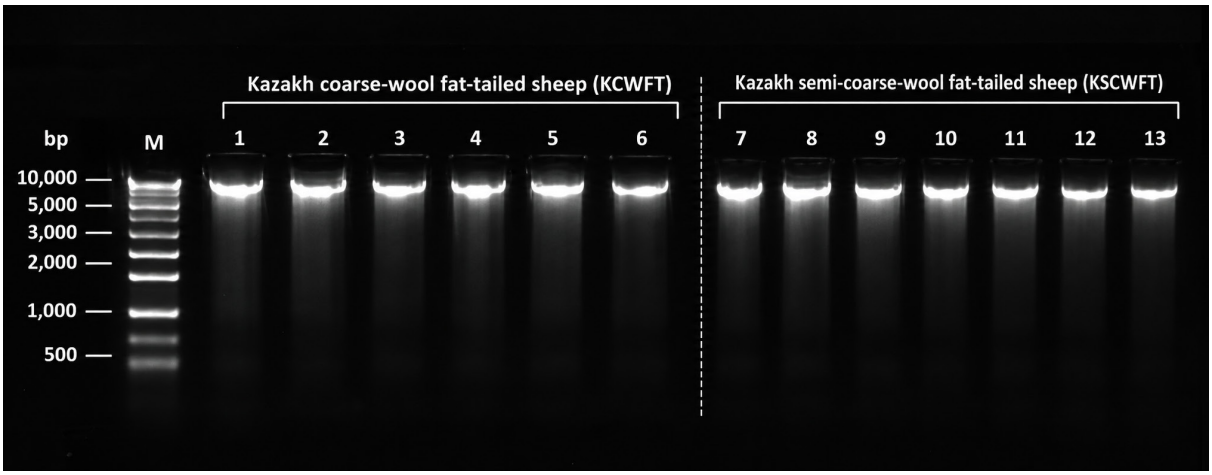

Figure 1. Agarose gel electrophoresis of extracted genomic DNA samples of two sheep breeds. High-molecular-weight DNA bands are visible near the wells, with varying degrees of DNA smearing among the samples

Based on these results, it was decided that a commercial kit was needed to obtain DNA with higher and more stable purity

DNA Extraction with the PureLink Commercial Kit

In the second stage, the commercial PureLink Genomic DNA Mini Kit was used to extract DNA from the ear notches of all 40 sheep. The isolated DNA concentration ranged from 131.5 to 585.4 ng/µL and averaged 350.2 ± 96.9 ng/µL. The A260/280 ratio was 1.86 ± 0.01, indicating high DNA purity without significant protein or phenol impurities. The A260/230 ratio was 2.13 ± 0.08, indicating minimal organic impurities. Data for the KCWFT breed are presented in Table 3.

Table 3. Qualitative parameters of KCWFT breed DNA isolated using the PureLink Genomic DNA Kit

Sample	Concentration, ng/µL	A260/280	A260/230
1	386.4	1.88	2.16
2	425.4	1.88	2.21
3	444.0	1.88	2.23

Continuation of Table 3

4	444.6	1.87	2.21
5	365.8	1.88	2.20
6	585.4	1.85	2.21
7	342.3	1.87	2.17
8	281.4	1.87	2.16
9	389.8	1.87	2.11
10	273.2	1.86	2.08
11	266.9	1.85	2.18
12	371.1	1.86	2.15
13	335.4	1.86	2.09
14	242.2	1.86	2.05
15	263.7	1.86	2.01
16	357.8	1.86	1.98
17	245.8	1.86	2..03
18	131.5	1.88	2.20
19	338.8	1.87	2.02
20	325.0	1.86	2.18
Mean ± SD	340.8 ± 96.5	1.87 ± 0.01	2.13 ± 0.08

No statistically significant differences were revealed in the separate analysis of breeds. For the KCWFT breed (n=20), the indicators were: concentration 340.8 ± 96.5 ng/µl, A260/280 1.87 ± 0.01, A260/230 2.13 ± 0.08 (Table 3). For the KSCWFT breed (n = 20): concentration 359.6 ± 97.6 ng/µl, A260/280 1.86 ± 0.01, A260/230 2.13 ± 0.09 (Table 4).

Table 4. Qualitative parameters of DNA of the KSCWFT breed isolated by the PureLink Genomic DNA Kit

Sample	Concentration, ng/µL	A260/280	A260/230
1	498.0	1.86	2.23
2	351.3	1.87	2.17
3	476.1	1.87	2.21
4	404.8	1.86	2.20
5	309.5	1.86	2.10
6	159.0	1.87	2.15
7	292.6	1.85	1.90
8	446.1	1.85	2.08
9	361.5	1.87	2.22
10	466.6	1.85	2.13
11	396.9	1.85	2.14
12	348.4	1.85	2.14
13	300.1	1.86	2.22
14	414.9	1.85	2.19
15	428.7	1.86	1.98
16	390.5	1.85	2.16
17	329.2	1.86	2.13

Continuation of Table 4

18	189.7	1.87	2.21
19	503.8	1.85	2.04
20	325.0	1.86	2.18
Mean ± SD	359.6 ± 97.6	1.86 ± 0.01	2.13 ± 0.09

Agarose gel electrophoresis revealed the presence of a high-molecular-weight DNA fraction in all samples obtained using the PureLink kit, indicating that the molecular integrity of the DNA was preserved during the extraction process (Figure 2).

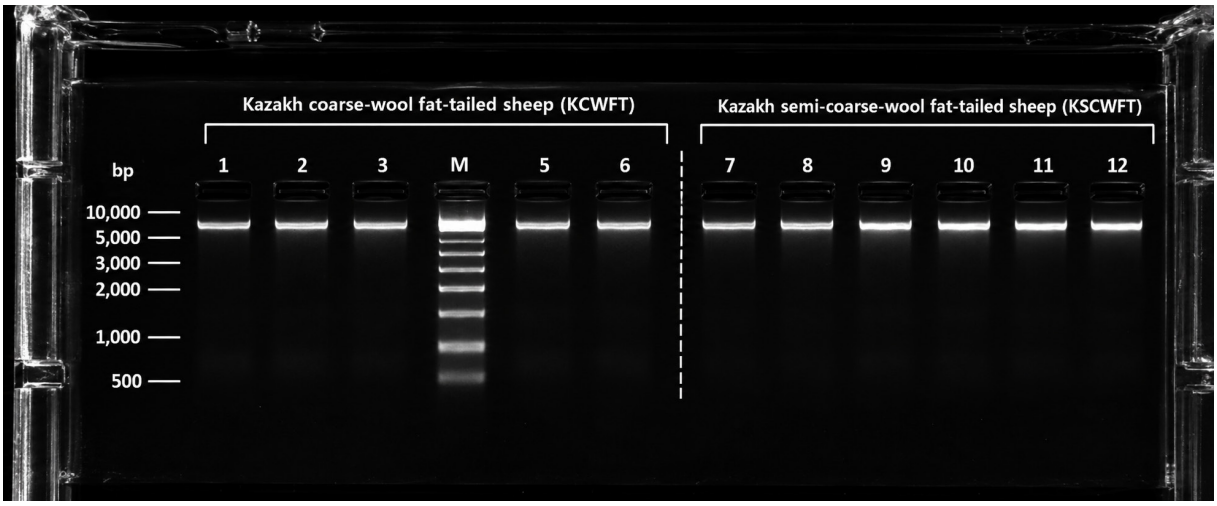

Figure 2. Agarose gel electrophoresis of extracted genomic DNA samples of two sheep breed. High-molecular-weight DNA bands are visible near the wells, with varying degrees of DNA smearing among the samples

All DNA samples isolated using the PureLink kit were sent to a commercial laboratory for library construction. Based on quality control performed at the lab, all 40 samples were deemed suitable for library preparation and sequencing. The resulting FASTQ files demonstrate the functional suitability of the isolated DNA for WGS.

Discussion

Comparative DNA Yield Analysis

The results of this study demonstrate that both the CTAB method and the commercial PureLink Genomic DNA Kit provide sufficient yields of genomic DNA from sheep ear notches for subsequent WGS. The average DNA concentration, determined spectrophotometrically, was 293.5 ng/µL for samples isolated by the CTAB method and 350.2 ng/µL for samples obtained with the PureLink kit. Both values significantly exceed Illumina's minimum library construction requirements, which are 100–500 ng of total DNA, or ~10 ng/µL concentration at a typical eluate volume [https://knowledge.illumina.com/library-preparation/dna-library-prep/library-preparation-dna-library-prep-faq-list/000001262].

Literature data show that DNA yields are generally higher using the CTAB method than commercial kits. For example, a study on DNA extraction from grape leaves showed that the CTAB method yields higher DNA concentrations compared to commercial kits from Qiagen, VWR, and MP [11]. Similar results were obtained when working with highly processed animal by-products, where the CTAB method demonstrated the greatest efficiency in DNA recovery [8]. In our study, the difference in concentration between the methods was only ~19% in favor of PureLink, which is smaller than typical differences described in the literature. This may be explained by the specific nature of ear tissue and the high density of connective tissue, which equally impedes lysis in both protocols.

DNA Purity Assessment: A260/280 Ratio

The A260/280 absorbance ratio is a key indicator of the presence of protein contaminants in DNA samples. For high-quality genomic DNA, this ratio should be in the range of 1.8–2.0. In our study, the

CTAB method yielded average A260/280 values of 1.77 ± 0.05 (KCWFT) and 1.79 ± 0.04 (KSCWFT), which is at the lower end of the acceptable range and, in some samples, was outside it (the minimum value was 1.63). This indicates the presence of residual protein contaminants in DNA preparations isolated using the CTAB method. In contrast, the PureLink Genomic DNA Kit provided significantly higher and more consistent A260/280 values: 1.87 ± 0.01 (KCWFT) and 1.86 ± 0.01 (KSCWFT), fully meeting Illumina requirements.

Coefficient of variation (CV) analysis showed that the spread of A260/280 values using the CTAB method was 2-fold higher (CV ~3.4%) than using PureLink (CV ~1.6%). This confirms the data of *Schenk* et al., 2023 [8], who noted that "numerous modifications to the CTAB protocol create a critical problem, as studies become neither transparent nor reproducible." The high variability of the CTAB protocol is due to the fact that each step from mechanical tissue disruption to sediment conditioning is subject to individual variations. At the same time, commercial kits such as PureLink offer standardized, reproducible procedures, which reduces the influence of human factors and ensures interlaboratory comparability of results.

A260/230 Ratio Analysis

The most significant differences between the two methods were revealed in the A260/230 ratio, which reflects the presence of organic impurities. For DNA suitable for high-throughput sequencing, this ratio should exceed 2.0, while values below 1.5 indicate high concentrations of contaminants that can negatively impact enzymatic reactions. In our study, the CTAB method yielded average A260/230 values of 1.53 ± 0.14 (KCWFT) and 1.52 ± 0.12 (KSCWFT), which are significantly below the recommended threshold. However, in a number of samples (Nos. 3, 7, 10, 11, 14, and 15), this ratio fell below 1.5, indicating significant organic impurities. These data are consistent with publications reporting that classical CTAB protocols are prone to leaving polysaccharide contaminants in DNA preparations.

In contrast, the PureLink Genomic DNA Kit provided high and CTABle A260/230 values: 2.13 ± 0.08 (KCWFT) and 2.13 ± 0.09 (KSCWFT), fully meeting the requirements of Illumina (2.0–2.2) and Novogene (OD260/230 without specific limitations, but with the requirement of no degradation) (Novogene Sample Requirements). A key advantage of the PureLink kit is its use of a silica gel column purification, which effectively removes polysaccharides, oligosaccharides, and other polar compounds remaining after CTAB extraction.

DNA Integrity Analysis by Agarose Gel Electrophoresis

Agarose gel electrophoresis is the method of choice for assessing genomic DNA integrity prior to high-throughput sequencing.

DNA isolated by CTAB was characterized by significant fragmentation. Minor blotches were observed in the gel, along with a major band of high-molecular-weight DNA. This may indicate degradation of the genomic DNA during the extraction process.

Unlike CTAB, the PureLink Genomic DNA Kit recovered high-molecular-weight DNA in all 40 samples (100%). Clear, compact bands were visualized on the gels, with no signs of blotches or low-molecular-weight fragments. This demonstrates that PureLink effectively inactivates endogenous nucleases early in the extraction process, while column purification minimizes mechanical stress on DNA molecules. In a study comparing DNA extraction methods from grape leaves, the CTAB method demonstrated a predominance of high-molecular-weight DNA with minimal smear, while commercial kits using mechanical homogenization (bead beating) showed significant fragmentation. However, in our case, the CTAB method yielded the opposite result of fragmentation, which is likely due to the specific nature of the animal tissue (dense connective tissue).

All 40 samples (20 KCWFT and 20 KSCWFT) isolated with the Purelink kit were sent to the Novogene commercial laboratory for library creation and sequencing. Based on Novogene's internal QC, all samples isolated with the PureLink kit were deemed suitable for sequencing, and FASTQ files were generated.

The data obtained in this study have important practical implications for the development of genomic studies of indigenous sheep breeds in the Republic of Kazakhstan. Validation of the method for obtaining high-quality genomic DNA from ear notches opens the possibility of large-scale WGS studies of Kazakh fat-tailed breeds, which will enable: (1) the construction of reference genomes for the main indigenous breeds; (2) the identification of genetic variants associated with economically valuable

traits (meat productivity, wool, adaptation to climatic conditions); (3) conduction of a comparative analysis of the genomic structure of Kazakh breeds with global sheep populations; (4) the development of molecular markers for breeding programs. Thus, this study provides a methodological foundation for the formation of a reliable genetic database of indigenous sheep breeds in Kazakhstan, which is of strategic importance for the conservation of genetic resources and the development of sheep farming in the region.

Conclusion

The study demonstrated that the commercial PureLink Genomic DNA Kit provides more CTABle and reproducible DNA quality parameters from sheep ear notches compared to the classical CTAB method. The concentration, purity, and integrity of DNA obtained using the PureLink kit met the requirements for WGS.

Authors' contributions

ShO, and AKh: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript. ZKh, BK, DS: conducted final editing and proofreading of the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

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Conflict of Interest

The authors declare that they have no known financial interests or personal relationships that could have influenced the results presented in this work.

References

1 Li, X., Yang, J., Shen, M. et al. (2020). Whole-genome resequencing of wild and domestic sheep identifies genes associated with morphological and agronomic traits. *Nat Commun.*, 11, 2815. DOI: 10.1038/s41467-020-16485-1.

2 Jacinto, J., Letko, A., Gentile, A., Otter, A., Floyd, T., Collins, R., Richey, M., Carty, H., Scholes, S., Jones, A., Fuller, H., Häfliger, I.M., Strugnell, B., Studer, E., Benazzi, C., Bolcato, M., Starič, J., Diana, A., Weber, J., Freick, M., Drögemüller, C. (2025). Exploring skeletal disorders in cattle and sheep: a WGS-based framework for diagnosis and classification. *Genet. Sel. Evol.,* 57(1), 51. DOI: 10.1186/s12711-025-01002-z.

3 Kruasuwan, W., Sawatwong, P., Jenjaroenpun, P., et al. (2024). Comparative evaluation of commercial DNA isolation approaches for nanopore-only bacterial genome assembly and plasmid recovery. *Sci Rep.,* 14, 27672. DOI: 10.1038/s41598-024-78066-2.

4 Devonshire, A.S., Morata, J., Jubin, C. et al. (2025). Interlaboratory evaluation of high molecular weight DNA extraction methods for long-read sequencing and structural variant analysis. *BMC Genomics*, 26, 698. DOI: 10.1186/s12864-025-11792-7.

5 Doyle, J.J., Doyle, J.L. (1987). A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochem. Bull.*, 19, 11–15.

6 Winnepenninckx, B., Backeljau, T., De Wachter, R. (1993). Extraction of high molecular weight DNA from molluscs. *Trends Genet.,* 9(12), 407. DOI: 10.1016/0168-9525(93)90102-n.

7 Uda-Shimoda, C.F., Colli, C.M., Pavanelli, M.F., Falavigna-Guilherme, A.L., Gomes, M.L. (2014). Simplified protocol for DNA extraction and amplification of 2 molecular markers to detect and type Giardia duodenalis. *Diag. Microbiol. Infect. Dis.*, 78(1), 53–58. DOI: 10.1016/j.diagmicrobio.2013.09.008.

8 Schenk, J.J., Becklund, L.E., Carey, S.J., Fabre, P.P. (2023). What is the "modified" CTAB protocol Characterizing modifications to the CTAB DNA extraction protocol. *Appl. Plant Sci.,* 11(3), e11517. DOI: 10.1002/aps3.11517.

9 Dossybayev, K., Amandykova, M., Ualiyeva, D., Kapassuly, T., Kozhakhmet, A., Ciani, E., Bekmanov, B., Amzeyev, R. (2025). Genome-Wide SNP Analysis Reveals the Unique Genetic Diversity Represented by Fat-Tailed Coarse-Wooled Sheep Breeds of Kazakhstan. *Biology,* 14(11), 1478. DOI: 10.3390/biology14111478.

10 Buljević, N., Preiner, D., Šikuten, I., Tomaz, I. (2025). Comparison of Different DNA Isolation Methods from Grapevine (Vitis vinifera) Leaves. *Separations,* 12(11), 316. DOI: 10.3390/separations12110316.

11 Filipa-Silva, A., Castro, R., Rebelo, M., Mota, M.J., Almeida, A., Valente, L.M.P., Gomes, S. (2024). Enhancing the authenticity of animal by-products: harmonization of DNA extraction methods from novel ingredients. *Front. Chem.,* 12, 1350433. DOI: 10.3389/fchem.2024.135043.

Research article

Identification of a *Salmonella* Abortusequi isolate based on morphological and serological characteristics and assessment of its antimicrobial resistance profile

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Abstract

Background and Aim. Equine salmonellosis-associated abortion remains an important infectious disease of horses, with significant implications for reproductive health and horse breeding. *Salmonella enterica* subsp. *enterica* serovar Abortusequi is recognized as one of the etiological agents associated with abortion in mares and may persist within affected horse populations. This study is aimed to characterize and identify an *S.* Abortusequi isolate recovered from pathological material obtained from an aborted equine fetus in the Aiyrtau District of the North Kazakhstan Region through comprehensive assessment of its morphological, cultural, biochemical, and serological characteristics, and to determine its antimicrobial susceptibility profile.

Materials and Methods. A pure bacterial culture was obtained through sequential subculturing followed by the selection of colonies exhibiting characteristic morphological features. Identification of the isolate was performed using an integrated approach based on morphological, cultural, biochemical, and antigenic characteristics. Cellular morphology and staining properties were examined by light microscopy, while cultural characteristics were evaluated based on growth patterns and colony morphology on selective and differential diagnostic media. Biochemical activity was assessed using standard diagnostic tests. The antigenic profile of the isolate was determined by slide agglutination with polyvalent and monoreceptor diagnostic antisera specific for *Salmonella* O- and H-antigens. Species-level identification was additionally confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Antimicrobial susceptibility was assessed against 12 antimicrobial agents using the disk diffusion method, with results evaluated based on the diameters of bacterial growth inhibition zones.

Results. The recovered isolate exhibited morphological, cultural, and biochemical characteristics consistent with the genus Salmonella, including Gram-negative, motile, rod-shaped cells, facultative anaerobic growth, characteristic colony morphology on selective and differential media, and hydrogen sulfide (H2S) production. Biochemical testing demonstrated glucose fermentation with acid and gas production, absence of lactose and sucrose fermentation, negative indole and urease reactions, and positive citrate utilization. Serological analysis revealed specific agglutination with O:4 (O-IV) and O:12 (O-XII) diagnostic antisera, assigning the isolate to *Salmonella* serogroup B; the antigenic formula was determined as 4,12:-: n,x, consistent with *Salmonella enterica* subsp. *enterica* serovar Abortusequi. Identification was additionally supported by MALDI-TOF MS analysis. Antimicrobial susceptibility

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testing showed inhibition zone diameters ranging from 26 to 41 mm, with the largest zones observed for imipenem (41 mm), ofloxacin (40 mm), and ceftriaxone (38 mm), followed by amoxicillin/clavulanic acid and norfloxacin (35 mm each), cefoxitin (34 mm), co-trimoxazole and tobramycin (32mm each), ampicillin (31 mm), gentamicin (30 mm), azithromycin (27 mm), and tetracycline (26 mm).

Conclusion. Comprehensive assessment of the morphological, cultural, biochemical, serological, and mass spectrometric characteristics enabled identification of the isolate recovered from pathological material of an aborted equine fetus as *S.* Abortusequi. Disk diffusion testing demonstrated growth inhibition zone diameters ranging from 26 to 41 mm across the panel of 12 antimicrobial agents tested. These findings provide a phenotypic characterization of the *S.* Abortusequi isolate and describe its antimicrobial susceptibility profile, contributing to the laboratory characterization of this pathogen associated with equine abortion.

Keywords: antimicrobial resistance; equine salmonellosis-induced abortion; epizootic strain; *Salmonella* Abortusequi; serological identification.

Introduction

Identifying the biological properties represents a critical step in the laboratory diagnosis of equine salmonellosis-induced abortion and serves as the basis for taxonomic verification of isolates [1, 2]. Contemporary studies emphasize the need to comprehensively assess both the phenotypic and molecular-genetic characteristics of strains [3, 4, 5].

Morphologically, *S.* Abortusequi is a Gram-negative, motile rod, facultative anaerobe that does not form spores. These characteristics have been confirmed both through classical microbiological identification and in the characterization of strains isolated in Kazakhstan and China [1, 2, 3].

Its cultural properties include forming smooth colonies on solid nutrient media and producing hydrogen sulfide (H_2S) on differential diagnostic media, which are utilized in primary laboratory diagnostics [2, 3]. Its biochemical profile is characterized by glucose fermentation with acid and gas production, absence of lactose and sucrose fermentation, negative indole and urease reactions, and the ability to utilize citrate [1, 4]. These characteristics retain diagnostic significance for differentiation from other *Salmonella* serovars.

Antigenic structure plays a decisive role in identification. Serotyping is based on the detection of somatic (O) and flagellar (H) antigens. *S*. Abortusequi is characterized by the antigenic formula O: 4,12: -: e,n,x, corresponding to serological group B [1, 3, 5]. Genomic studies have confirmed the stability of this antigenic formula and its conformity with the international nomenclature of serovars [3, 5].

Modern molecular methods, including whole-genome sequencing, have significantly enhanced identification capabilities by enabling precise determination of strains' phylogenetic affiliation and the detection of virulence genes and antimicrobial resistance markers [3, 5, 6]. Genomic analyses of Kazakhstani isolates indicate phylogenetic relatedness among strains while maintaining typical phenotypic characteristics [3].

Serological diagnostic methods, particularly the development of domestic enzyme-linked immunosorbent assays and the use of recombinant antigens, aim to enhance the sensitivity and specificity of confirming a pathogen's antigenic structure [4, 7, 8, 9, 10]. Their application is especially important for epizootic monitoring and for detecting latent carriers [11, 12, 13, 14].

The scientific novelty of this study is represented by the comprehensive characterization of an epizootic isolate of *S.* Abortusequi recovered from pathological material of an aborted equine fetus in the North Kazakhstan Region. The isolate was characterized using an integrated approach combining cultural, morphological, biochemical, serological, and mass spectrometric methods, together with phenotypic assessment of its antimicrobial susceptibility. The findings provide additional data on the phenotypic and serological characteristics of *S*. Abortusequi associated with equine abortion and contribute to improving its comprehensive laboratory identification. The established antimicrobial susceptibility profile provides a basis for further comparative studies of independent *S.* Abortusequi isolates and for assessing the variability of antimicrobial susceptibility within this serovar.

Materials and Methods

Ethical approval

This study was approved by the local Bioethics Committee of the Institute of Animal Science and Veterinary Medicine at S. Seifullin Kazakh Agrotechnical Research University (protocol number: 3, approval date: January 12, 2026) and conducted in accordance with bioethical principles.

Isolate Preparation

The study material consisted of a single aborted equine fetus at 5-6 months of gestation, obtained in February 2026 from a horse-breeding farm in the Aiyrtau District of the North Kazakhstan Region. The fetus was transported to the laboratory in accordance with established veterinary and sanitary requirements for the packaging and transportation of pathological material.

The packaging and transportation of pathological material were carried out in accordance with the requirements of the Rules for Sampling of Transported Objects and Biological Material, approved by Order No. 7-1/393 of the Minister of Agriculture of the Republic of Kazakhstan dated April 30, 2015, as well as applicable guidelines for the collection of biological material for the laboratory diagnosis of infectious diseases in farm animals. Necropsy of the aborted fetus and collection of internal organ samples for bacteriological examination were performed under laboratory conditions at S. Seifullin Kazakh Agrotechnical Research University in accordance with aseptic procedures and precautions aimed at preventing secondary contamination of the collected material.

For bacteriological examination, samples of the liver, spleen, kidneys, and regional lymph nodes were aseptically collected using sterile instruments. From each tissue sample, 1.0 g was homogenized in 9 mL of sterile 0.85% sodium chloride solution to obtain a 1:10 suspension. The resulting tissue suspensions were subsequently used for bacterial isolation and identification.

Primary isolation was performed by inoculating the prepared tissue suspensions onto differential and selective media, including Endo agar and *Salmonella*-Shigella (SS) agar. The inoculated plates were incubated aerobically at 37 °C for 18–24 h. Following incubation, bacterial growth and colony morphology were evaluated, and colonies exhibiting cultural characteristics consistent with the genus *Salmonella* were selected for further purification. A pure culture was obtained by sequential subculturing using the Drygalski plating method and was subsequently used for identification and antimicrobial susceptibility testing.

Isolate Characterization

The morphological characteristics of the isolated culture were examined by light microscopy of Gram-stained smears. Cell shape, size, arrangement, and Gram reaction were evaluated. Cultural characteristics were assessed based on bacterial growth and colony morphology on Endo agar and Salmonella-Shigella (SS) agar following incubation at 37 °C for 18–24 h. Biochemical characterization included assessment of glucose, lactose, and sucrose fermentation, hydrogen sulfide (H2S) and indole production, urease activity, and citrate utilization. The combined morphological, cultural, and biochemical characteristics were used for preliminary identification of the isolate as a member of the genus *Salmonella*.

Serological identification of the pure culture was carried out by slide agglutination using *Salmonella* diagnostic antisera (SSI Diagnostica A/S, Hillerød, Denmark). Initial serogroup determination was performed using the *Salmonella* Sero-Quick Group Kit, containing diagnostic antisera for the major *Salmonella* serogroups. Assignment of the isolate to serogroup B was based on a positive reaction with the corresponding group-specific antiserum detecting the somatic O:4 antigen. Further characterization of the somatic antigenic profile was performed using Salmonella Factor O:12 antiserum, while the flagellar antigenic profile was assessed using Salmonella Phase H,n,x antiserum (SSI Diagnostica A/S, Hillerød, Denmark). Slide agglutination tests were conducted according to the manufacturer's instructions, with the formation of visible specific agglutination interpreted as a positive reaction. The resulting profile of somatic O- and flagellar H-antigens was interpreted according to the White-Kauffmann scheme for *Salmonella* serovar determination.

Species-level verification was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) at the National Center for Biotechnology (Astana, Republic of Kazakhstan). Spectra were recorded using a Microflex LT MS and Biotyper software (Bruker Daltonics, Bremen, Germany); identification was considered reliable when the score was ≥2.0.

Antimicrobial susceptibility of the isolated culture was assessed using the disk diffusion method. The isolate was tested against 12 antimicrobial agents: co-trimoxazole (sulfamethoxazole/trimethoprim), 25 µg (23,75/1,25 µg); amoxicillin/clavulanic acid, 30 µg (20/10 µg); ampicillin, 10 µg; cefoxitin, 30 µg; ceftriaxone, 30 µg; gentamicin, 10 µg; tobramycin, 10 µg; ofloxacin, 5 µg; imipenem, 10 µg; norfloxacin, 10 µg; azithromycin, 15 µg; and tetracycline, 30 µg.

Following incubation, the diameters of the growth inhibition zones around the antimicrobial discs were measured in millimeters. The obtained values were used to characterize the phenotypic antimicrobial susceptibility profile of the isolate. Results were interpreted according to the applicable EUCAST criteria. When no validated clinical breakpoint was available for a specific organism-

antimicrobial combination, the measured inhibition zone diameter was reported without categorical interpretation.

The study was limited to the characterization of a single bacterial isolate recovered from a single case of equine abortion. Samples collected from different organs of the same fetus were used exclusively for pathogen isolation and identification and were not treated as independent biological or epidemiological observations.

Results

The sampling and subsequent bacteriological analysis of pathological material obtained from an aborted equine fetus (5-6 months of gestation) from the Aiyrtau District, North Kazakhstan Region, resulted in the isolation of a microorganism taxonomically assigned to the genus *Salmonella* (Figure 1).

Figure 1. The aborted equine fetus (Aiyrtau District, North Kazakhstan Region)

During postmortem examination of the aborted equine fetus, samples of the liver, spleen, kidneys, thyroid lymph nodes, and mesenteric lymph nodes were collected for bacteriological examination. Analysis of the collected pathological material resulted in the isolation of a bacterial culture, which was subsequently subjected to phenotypic, serological, and mass spectrometric identification (Figure 2).

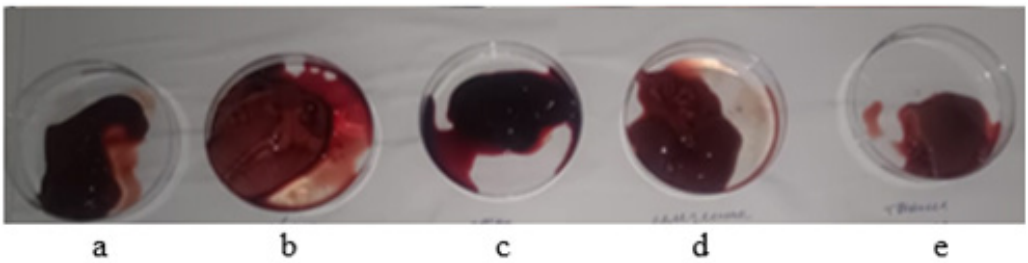

Figure 2. Pathological material collected from the internal organs and regional lymph nodes of an aborted equine fetus for bacteriological examination

Note: a-kidney; b-spleen; c-liver; d- thyroid lymph node; e- mesenteric lymph node.

Following sample homogenization and initial cultivation on selective and differential diagnostic media (Endo and *Salmonella-Shigella* agar) at 37 °C for 18–24 hours, colonies exhibiting morphology typical of the Salmonella genus were observed. On *Salmonella-Shigella* agar, the colonies appeared colorless with a characteristic black center, indicating H_2S production. Subsequent subculturing yielded a pure culture that maintained stable morphological characteristics.

Microscopic examination of Gram-stained smears revealed Gram-negative rods 3-5 µm long and 0.5 µm wide, occurring singly or in pairs (Figure 3). The observed morphology was consistent with bacteria belonging to the genus *Salmonella*.

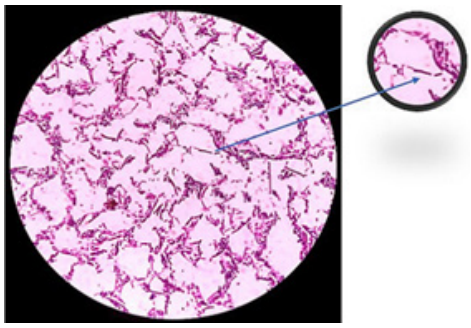

Figure 3. Morphological characteristics of the *Salmonella* Abortus equi isolate revealed by Gram staining and light microscopy

In meat-peptone broth, the culture exhibited uniform turbidity and moderate sedimentation. The isolate was characterized biochemically by glucose fermentation with acid and gas production, absence of lactose and sucrose fermentation, negative indole and urease reactions, the ability to utilize citrate, and H2S production. This combination of biochemical characteristics corresponds to the diagnostic criteria for the genus *Salmonella*.

The phenotypic antimicrobial susceptibility profile of the *S.* Abortusequi isolate was evaluated against 12 antimicrobial agents from different pharmacological groups using the disk diffusion method. The growth inhibition zone diameters varied depending on the antimicrobial agent tested and ranged from 26 to 41 mm. The results of antimicrobial susceptibility testing are presented in Table 1.

Table 1. Antimicrobial susceptibility profile of the epizootic *S*. Abortus equi isolate determined by the disk-diffusion method

№	Antimicrobial agents and their concentrations	S ≥	R<	ATU	Inhibition zone diameter, мм	Result
1	Co-trimoxazole (sulfamethoxazole trimethoprim) – 25 µg (23.75/1.25 µg)	15	15	-	32	S
2	Amoxiclav (amoxicillin/clavulanic acid) – 30 µg (20/10 µg)	19	19	19–20	35	S
3	Ampicillin – 10 µg	14	14	-	31	S
4	Cefoxitin – 30 µg (screen only)	19	19	-	34	S
5	Ceftriaxone – 30 µg	27	24	-	38	S
6	Gentamicin – 10 µg	17	17	-	30	S
7	Tobramycin – 10 µg	16	16	-	32	S
8	Ofloxacin (5 µg)	24	22	-	40	S
9	Imipenem (10 µg)	22	19	-	41	S
10	Norfloxacin (10 µg)	24	24	-	35	S
11	Azithromycin (15 µg)	12	12	-	27	S
12	Tetracycline (30 µg)	30	30	-	26	S

Abbreviations: ATU = area of technical uncertainty, R = resistant, S = susceptible

Analysis of the antimicrobial susceptibility results showed that the largest growth inhibition zone was observed for imipenem (41 mm), followed by ofloxacin (40 mm) and ceftriaxone (38 mm). Inhibition zone diameters of 35 mm were recorded for both amoxicillin/clavulanic acid and norfloxacin, followed by cefoxitin (34 mm), co-trimoxazole and tobramycin (32 mm each), ampicillin (31 mm), and gentamicin (30 mm). Smaller inhibition zones were observed for azithromycin (27 mm) and tetracycline (26 mm), with tetracycline showing the smallest zone among the 12 antimicrobial agents tested.

Comparison of the measured inhibition zone diameters with the EUCAST interpretive criteria presented in Table 1 showed that the results for co-trimoxazole, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftriaxone, gentamicin, tobramycin, ofloxacin, imipenem, norfloxacin, and azithromycin met the corresponding susceptibility criteria. For amoxicillin/clavulanic acid, the observed inhibition zone diameter of 35 mm was also outside the Area of Technical Uncertainty (ATU; 19-20 mm) specified by EUCAST.

For tetracycline, the inhibition zone diameter was 26 mm, which was below the susceptibility breakpoint (S ≥ 30 mm) applied in this study; therefore, this result did not meet the specified susceptibility criterion. Overall, the S. Abortusequi isolate met the applied susceptibility criteria for most antimicrobial agents included in the tested panel, whereas tetracycline produced the smallest growth inhibition zone among the antimicrobial agents examined.

The obtained data indicate that the studied *S*. Abortusequi isolate exhibited in vitro susceptibility to most of the antimicrobial agents tested, based on the interpretive criteria applied in this study. These findings characterize the phenotypic antimicrobial susceptibility profile of the isolate and provide baseline information on its response to antimicrobial agents. However, given that the present study was limited to a single isolate, further investigation involving a larger collection of *S*. Abortusequi isolates from different geographical regions and epidemiological settings is required to assess the distribution and potential emergence of antimicrobial resistance in equine salmonellosis.

Species identity was confirmed by MALDI-TOF MS. The obtained mass spectra, analyzed with the Bruker Biotyper software, yielded a score ≥2.0, providing reliable species-level identification as *S*. Abortusequi (Figure 4).

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
A1 (+++) (A)	A1	Salmonella sp	2.353	Salmonella sp	2.259
B1 (++) (A)	B1	Salmonella sp	2.151	Salmonella sp	2.086
C1 (+) (B)	C1	Salmonella sp	1.742	not reliable identification	1.626
D1 (-) (C)	D1	not reliable identification	1.684	not reliable identification	1.653
E1 (++) (A)	E1	Salmonella sp	2.162	Salmonella sp	2.158
F1 (++) (A)	F1	Salmonella sp	2.009	Salmonella sp	1.974
G1 (-) (C)	G1	not reliable identification	1.502	not reliable identification	1.353
(++) (A)	H1	Salmonella sp	2.215	Salmonella sp	2.152
A2 (++) (A)	A2	Salmonella sp	2.161	Salmonella sp	2.089
B2 (++) (A)	B2	Salmonella sp	2.189	Salmonella sp	2.146

Figure 4. Mixed-sample analysis demonstrating the detection of protein profiles characteristic of members of the genus *Salmonella*. The labeling of the analyzed samples corresponds to the following anatomical sites: A1 = kidneys; B1 = spleen; C1 = liver; E1, F1 = thyroid (cervical) lymph nodes; H1, A2, B2 = mesenteric lymph nodes

During the pathological examination of the aborted equine fetus (5-6 months of gestation), pronounced morphological changes were observed in the internal organs, indicative of a generalized infection process. The kidneys (A1) exhibited signs of parenchymal dystrophy, moderate cortical hyperemia, and vascular congestion, reflecting the toxico-septic impact of the infectious agent. The spleen (B1) was enlarged, with pronounced blood engorgement and a tense capsule, characteristic

of reactive lymphoid hyperplasia in septicemia. The liver (C1) showed congestive hyperemia, focal granular hepatocyte dystrophy, and a dull cut surface, consistent with intrauterine bacterial infection. The thyroid lymph nodes (E1 and F1) were enlarged and firm, with pronounced vascular injection, reflecting immune system activation in response to antigenic stimulation. The mesenteric lymph nodes (H1, A2, and B2) exhibited signs of serous inflammation, vascular injection, and tissue edema, indicating systemic dissemination of an infectious agent. This combination of pathological changes corresponds to intrauterine septicemia, confirming the development of a generalized infection-inflammatory process typical of equine salmonellosis-induced abortion caused by members of the genus *Salmonella*.

Serological characterization of the isolate was performed by slide agglutination using diagnostic O- and H-antisera. The isolate was assigned to serogroup B based on a positive agglutination reaction with antiserum specific for the somatic O:4 antigen. Further serotyping demonstrated a positive reaction with O:12 antiserum, confirming the presence of the corresponding somatic antigen. Analysis of the flagellar antigenic profile revealed specific agglutination with H-antiserum e,n,x. The results of serological characterization are presented in Table 2.

Table 2. Serotyping results of the *S.* Abortus equi isolate according to the Kaufmann-White scheme

№	Diagnostic sera used	Detected antigens	Agglutination result	Interpretation
1	Salmonella Sero-Quick Group Kit, Group B	O:4	++++	Belonging to serogroup B
2	Salmonella Factor O:12 serum	O:12	+++	Positive reaction; presence of somatic antigen O:12 confirmed
3	Salmonella Phase H:e,n,x serum	H:e,n,x	+++	Positive reaction; flagellar antigens e,n,x detected
4	Antigenic formula	O:4,12:-:e,n,x	-	Consistent with S. Abortus equi

The combined results of serological typing established the antigenic profile of the isolate as O:4,12:-:e,n,x. The detection of somatic antigens O:4 and O:12 in combination with flagellar antigens e,n,x was consistent with the antigenic characteristics of *S*. Abortusequi according to the White-Kauffmann scheme. These findings were consistent with the cultural, morphological, and biochemical characteristics of the isolate, while additional identification by MALDI-TOF MS confirmed its taxonomic assignment to *S*. Abortusequi.

Thus, serological typing constituted an important component of the comprehensive identification of the isolate and enabled characterization of its somatic and flagellar antigenic profile. In combination with the morphological, cultural, biochemical, and mass spectrometric findings, the serological results provided consistent evidence supporting the identification of the isolate as *S*. Abortusequi.

Discussion

The obtained results demonstrate the diagnostic value of an integrated approach combining cultural-morphological, biochemical, serological, and mass spectrometric methods for the identification of *S.* Abortusequi. These findings are consistent with previous studies demonstrating the association of *S.* Abortusequi with reproductive disorders, particularly abortion, in horses and other equids [8, 15, 16]. Notably, an epidemiological study conducted in China between 2017 and 2022 reported the isolation of 243 *S*. Abortusequi strains from 377 examined samples, all of which were characterized by the antigenic formula 4,12:-:e,n,x [17]. The consistency of this antigenic profile among a large collection of isolates highlights the diagnostic relevance of serotyping for the identification and characterization of *S.* Abortusequi, particularly when used in combination with complementary phenotypic and instrumental identification methods.

Current research emphasizes the importance of combining conventional bacteriological methods with molecular approaches for the reliable detection and characterization of *S*. Abortusequi. *Wang* et al.

[8] developed a specific real-time PCR assay for the detection of *S.* Abortusequi and demonstrated high analytical sensitivity and specificity, supporting its potential application for the examination of clinical samples. Whole-genome sequencing provides additional opportunities for detailed characterization of isolates, including assessment of their phylogenetic relationships, virulence-associated factors, and genetic determinants of antimicrobial resistance [8, 10]. Whole-genome analysis of three *S.* Abortusequi strains isolated from cases of equine abortion in Kazakhstan revealed a high degree of genomic similarity, 12 *Salmonella* pathogenicity islands, three prophages, and the antimicrobial resistance-associated gene aac (6')-Iaa [10]. These findings demonstrate the complementary value of molecular methods for the confirmation and further characterization of *S*. Abortusequi isolates initially recovered by conventional bacteriological methods.

The antimicrobial susceptibility testing performed in the present study provides a phenotypic characterization of the S. Abortusequi isolate examined. Growth inhibition zone diameters ranged from 26 to 41 mm, with the isolate meeting the applied susceptibility criteria for most of the antimicrobial agents tested. However, published studies indicate variability in antimicrobial susceptibility among *S.* Abortusequi isolates. *Manikandan* et al. [5] reported resistance to 8 of 26 antimicrobial agents in an isolate from India and observed only 32% concordance between phenotypic resistance and the presence of corresponding genetic resistance determinants. This discrepancy demonstrates that the detection of antimicrobial resistance genes does not necessarily predict phenotypic resistance and highlights the importance of integrating phenotypic susceptibility testing with molecular characterization of resistance determinants when evaluating antimicrobial resistance in *S*. Abortusequi.

Thus, the present findings provide phenotypic and serological characterization of a single *S.* Abortusequi isolate recovered from a case of equine abortion and contribute additional data on the characteristics of this pathogen. In light of recent studies, further investigations should include larger collections of epidemiologically independent *S*. Abortusequi isolates from different geographical regions and combine standardized antimicrobial susceptibility testing with molecular genetic approaches. Such studies will provide a more comprehensive understanding of the phenotypic and genomic diversity of *S*. Abortusequi and facilitate the assessment of antimicrobial resistance patterns among circulating isolates.

This study provided a comprehensive characterization of a single *S*. Abortusequi isolate recovered from pathological material obtained from an aborted equine fetus. The findings established its morphological, cultural, biochemical, and serological characteristics and defined its phenotypic antimicrobial susceptibility profile. Given that the study was limited to a single isolate, further investigations should include a larger collection of epidemiologically independent *S*. Abortusequi isolates from different farms and geographical regions. Comparative phenotypic and molecular genetic analyses of such isolates would provide a more comprehensive understanding of the diversity, epidemiological characteristics, and antimicrobial resistance patterns of this pathogen.

Conclusion

This study provided a comprehensive characterization of an epizootic isolate of *S*. Abortusequi recovered from pathological material obtained from an aborted equine fetus. The combined assessment of morphological, cultural, biochemical, serological, and mass spectrometric characteristics enabled reliable identification of the isolate, while antimicrobial susceptibility testing defined its phenotypic susceptibility profile *in vitro*. The findings contribute additional data on the phenotypic and antigenic characteristics of *S*. Abortusequi and demonstrate the value of an integrated laboratory approach for its identification. However, the antimicrobial susceptibility results obtained in this study are specific to the examined isolate and should not be extrapolated to *S*. Abortusequi populations as a whole or interpreted as evidence of the clinical efficacy of the antimicrobial agents tested. Further studies involving epidemiologically independent isolates from different farms and geographical regions, together with standardized phenotypic susceptibility testing and molecular genetic analyses, are required to better characterize the diversity of *S*. Abortusequi and the distribution and genetic basis of antimicrobial resistance.

Author Contributions

The authors jointly developed the concept and study design; TB, ZhB: collected pathological material and performed bacteriological, morphological, cultural, biochemical, and serological analyses. ZhB: conducted; MS: verification of the isolate using MALDI-TOF MS, determined its antimicrobial susceptibility profile according to EUCAST standards. ZhB, GE, AK, IA, MB: analyzed and interpreted the data, prepared the illustrative material and the manuscript. SA and ZhB: approved the final version of the manuscript for publication.

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Conflict of Interest

The authors declare that they have no known financial interests or personal relationships that could have influenced the results presented in this work.

References

1 Mai, Z., Fu, .H, Miao, R. et al. (2024). Serological investigation and isolation of Salmonella abortus equi in horses in Xinjiang. *BMC Vet Res.,* 20(1), 103. DOI:10.1186/s12917-024-03955-7.

2 Bakishev, T.G., Bakisheva, Zh.S., Yessembekova, G.N. et al. (2025). Epizootic situation and comprehensive diagnosis of equine salmonellosis-associated abortion in the Republic of Kazakhstan. *Herald Sci. Seifullin Kazakh Agrotech. Univ.: Vet. Science*, 2(010), 45–52.

3 Bakishev, T., Amirgazin, A., Kuibagarov, M., Shevtsov, A., Bakisheva, Z., Yessembekova, G., Kairzhanova, A., Kadyrov, A., Guo, K., Wang, X., Abdrakhmanov, S., Borovikov, S. (2025). Genome-wide characterization and comparative phylogenomics of three Salmonella Abortusequi strains isolated from equine abortions in Kazakhstan. *Vet World*, 18(6), 1571–1580. DOI:10.14202/vetworld.2025.1571-1580.

4 Borovikov, S.N., Syzdykova, A.S. (2022). Development of enzyme immunoassay for serological diagnosis of equine salmonella abortion. *Herald Sci. Seifullin Kazakh Agrotech. Univ.: Vet. Science,* 3(114), 12–20. DOI: 10.51452/kazatu.2022.3(114).1115.

5 Manikandan, R., Rajagunalan, S., Malmarugan, S., Gupta, C. (2024). First report on whole genome sequencing and comparative genomics of Salmonella enterica serovar Abortusequi isolated from donkey in India. *Sci. Rep*., 14, 23455.

6 Zhao, J., Wang, J., Zhang, C., Xu., Sh., Ren, H., Zou, L., Ma, J., Liu, W. (2024). Characterization of a Salmonella abortus equi phage 4FS1 and its depolymerase. *Front. Vet. Sci.*, 11. DOI: 10.3389/fvets.2024.1496684.

7 Suleimanova, N.S., Borovikov, S.N. (2024). Study of recombinant antigens of Salmonella Abortusequi. SRED, 6.

8 Guo, K., Zhang, Y., Wang, X., Li, Y., Chen, H., Liu, Q., Zhao, L., Wang, J. (2023). Development and application of a competitive ELISA for the detection of antibodies against Salmonella Abortusequi in equids. *J Clin Microbial.*, 61(11), 1–9. DOI: 10.1128/jcm.00273-23.

9 Leon, A., Pillon, C., Tebourski, I., Bruyas, J.-F., Lupo, C. (2023). Overview of the causes of abortion in horses, their follow-up and management. *Reprod Domest Anim.,* 58(2), 93–101. DOI: 10.1111/RDA.14406.

10 Guo, K., Wang, J.H., Zhang, Z.N., Yang, Y., Zhang, W.G., Chu, X.Y., Wang, Y.X., Liu, D.Q., Guo, W., Hu, Z., Wang, X.J. (2024). Aetiological and serological surveillance of equine abortus salmonellosis in equids in China from 2017 to 2022. *Equine Vet. J.,* 56(S60), 16. DOI:10.1111/evj.14222.

11 Ruby , R.E., Janes, J.G. (2023). Infectious Causes of Equine Placentitis and Abortion. *Vet. Clin. N. Am. Equine Pract.,* 39(1), 73–88. DOI: 10.1016/j.cveq.2022.11.001.

12 Mayhew, K.K., Clarke, L.E., Howerth, W. (2021). Salmonella enterica subsp. arizonae-associated abortion in a mare. *Equine Vet. Educ.*, 33(12), e449–e452. DOI: 10.1111/eve.13437.

13 Wilson, C.S., Carrick, J., Shearer, P., Heller, J., Brookes, V.J. (2025). A retrospective case-control study of pregnancy failure in Thoroughbred horses in Australia. *Prev. Vet. Med.,* 237, 106424. DOI: 10.1016/j.prevetmed.2025.106424.

14 Akter R., El-Hage, C.M., Sansom, F.M., Carrick, J., Devlin, J.M., Legione, A.R. (2021). Metagenomic investigation of potential abortigenic pathogens in foetal tissues from Australian horses. *BMC Genomics,* 22, 713. DOI: 10.1186/s12864-021-08010-5.

Research article

Comparative acaricidal activity of aqueous and ethanolic extracts of medicinal plants against the mite *Psoroptes cuniculi*

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Abstract

Background and Aim. *Psoroptes cuniculi* is one of the most widespread causative agents of psoroptic mange in rabbits, causing significant economic losses in rabbit breeding. Given the increasing need for environmentally friendly alternatives to synthetic acaricides, the investigation of acaricidal properties of medicinal plants represents a promising research direction. The aim of the present study was to conduct a comparative evaluation of the acaricidal activity of aqueous and ethanolic extracts of medicinal plants against *Psoroptes cuniculi* mites under laboratory conditions.

Material and Methods. The study objects included extracts of *Mentha piperita, Origanum vulgare* L., *Thymus vulgaris, Ferula foetida, Cinnamomum cassia* (L.) D. Don, *Curcuma phaeocaulis* Valeton, and *Eugenia caryophyllata* Thunb. at concentrations of 200, 500, and 1000 µl/ml. A 1% dimethyl sulfoxide (DMSO) solution was used as a negative control, while permethrin at concentrations of 1, 2.5, 5, 10, and 20 µl/ml served as a positive control. Acaricidal activity was assessed by mite mortality rates. Statistical analysis was performed using the Kruskal-Wallis test.

Results. It was established that the extraction method significantly influenced the efficacy of the preparations. Overall, ethanolic extracts induced significantly higher mite mortality compared to aqueous extracts (30.33% and 19.70%, respectively; $P < 0.01$). The most pronounced acaricidal activity was demonstrated by the ethanolic extract of *Eugenia caryophyllata* Thunb., which achieved 100% mite mortality at a concentration of 1000 µL/mL, whereas the aqueous extract caused only 28% mortality. High efficacy was also shown by ethanolic extracts of *Cinnamomum cassia* (69%) and *Curcuma phaeocaulis* (48%) at the maximum concentration. Extracts of *Mentha piperita, Origanum vulgare, Thymus vulgaris*, and *Ferula foetida* were characterized by considerably lower acaricidal activity.

Conclusion. The obtained results indicate the high potential of ethanolic extracts of *Eugenia caryophyllata, Cinnamomum cassia,* and *Curcuma phaeocaulis* as sources of natural acaricidal compounds for the development of alternative control agents against Psoroptes cuniculi. Further in vivo studies are required to confirm their practical efficacy, focusing on therapeutic effectiveness and safety of application.

Keywords: acaricidal activity; aqueous extracts; ethanolic extracts; medicinal plants; natural acaricides; *Psoroptes cuniculi*.

Introduction

Tick and mite infestations represent one of the most pressing challenges in veterinary parasitology and livestock production worldwide. Among the causative agents of mange in rabbits, particular significance is attributed to mites of the genus *Psoroptes* – the etiological agents of psoroptic mange

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(ear mange), which cause severe lesions of the external auditory canal and adjacent skin areas. *Psoroptes cuniculi* (Delafond, 1859) is a non-burrowing mite that does not penetrate the deep layers of the skin but instead localizes on the epidermal surface within the auricular region, where it feeds on lymph, exudate, and desquamated epidermal cells. The life cycle of the parasite lasts approximately 21 days, and mites are capable of surviving off the host for up to 21 days, which accounts for the high contagiousness of the disease [1]. Rabbit psoroptic mange is characterized by the formation of dark-brown crusts in the external ear canal, pruritus, head shaking, and, in severe infestations, the spread of the pathological process to the face, neck, and limbs. The disease leads to a significant reduction in rabbit productivity, deterioration of pelt quality, development of secondary bacterial complications, and, in some cases, animal mortality, all of which result in substantial economic losses for rabbit breeding enterprises.

Currently, the control of *Psoroptes* mites in veterinary practice relies predominantly on the use of synthetic acaricides, such as ivermectin, fipronil, pyrethroids (deltamethrin, cypermethrin), amitraz, and their combinations [2]. However, prolonged and uncontrolled use of these agents has led to the development of resistance in mite populations, significantly reducing the therapeutic efficacy of synthetic compounds [2, 3]. Moreover, synthetic acaricides possess a number of substantial drawbacks, including high toxicity to target and non-target organisms, accumulation of residues in livestock products (meat, pelts), adverse environmental impact, and detrimental effects on beneficial entomofauna [2, 3, 4].

For instance, studies conducted in East and West African countries have revealed moderate to high levels of resistance to chlorfenvinphos and amitraz in populations of *Rhipicephalus microplus, Amblyomma variegatum*, and *Rhipicephalus appendiculatus*; notably, *R. microplus* demonstrated high resistance to alpha-cypermethrin and cypermethrin, while populations of *A. variegatum* (West Africa) and *R. appendiculatus* (Uganda) exhibited moderate resistance to ivermectin [3]. These findings underscore the urgent need for alternative, environmentally safe methods for tick and mite control. Given the limited resources of chemical acaricides and the growing problem of resistance, plant-derived products – including phytopreparations, essential oils, and plant extracts – are attracting increasing research interest [4, 5]. Plants synthesize a wide array of secondary metabolites (monoterpenes, sesquiterpenes, phenylpropanoids, flavonoids, alkaloids, tannins) that possess insecticidal, repellent, and acaricidal activities. Botanical acaricides are characterized by multi-component mechanisms of action, which substantially reduces the risk of resistance development in parasites; they are less toxic to mammals and non-target organisms, degrade rapidly in the environment, and leave no residues in livestock products [4, 5, 6]. Review studies confirm that natural products, including plant extracts and essential oils, represent promising agents for the control of ticks, mange mites, and other ectoparasites of animals [7].

Despite the fact that aqueous and ethanolic extracts of medicinal plants are simpler to produce and have lower production costs, their acaricidal activity has been significantly less investigated [8, 9]. Aqueous extracts predominantly contain polar compounds - phenolic acids, flavonoids, glycosides, tannins, and saponins – whereas ethanolic extracts enable the recovery of a broader spectrum of compounds, including less polar alkaloids, terpenoids, coumarins, and phenylpropanoids.

In this context, the aim of our study was to conduct a comparative evaluation of the acaricidal activity of aqueous and ethanolic extracts of medicinal plants against the mite *P. cuniculi.*

Materials and Methods

Collection and identification of plant materials

Plant materials from seven species were used. The inner bark of *Cinnamomum cassia* (L.) D. Don was purchased from Jiangsu Yabang Chinese Medicine Co., Ltd. (Jiangsu Province, China) and ground before extraction. Roots of *Ferula foetida* L. were collected in the vicinity of Shymkent in late September 2025. The aerial parts of *Thymus vulgaris* L. were collected in the Almaty Region in July 2025. The aerial parts of *Origanum vulgare* L. were collected in the Trans-Ili Alatau, Almaty Region, in 2025. Leaves of *Mentha piperita* L. were collected in the vicinity of Shymkent in early July 2025. Rhizomes of *Curcuma phaeocaulis* Valeton and dried unopened flower buds of *Eugenia caryophyllata* Thunb. were purchased from Jiangsu Yabang Chinese Medicine Co., Ltd. (Jiangsu Province, China).

The botanical identity of the field-collected specimens was confirmed at the Research Center for Medicinal Plants of Al-Farabi Kazakh National University. Taxonomic identification was based on morphological characteristics using appropriate botanical identification keys. Following collection, plant materials were sorted, washed when necessary, air-dried to remove surface moisture, and dried in a drying oven at 40–50 °C until completely dry. The materials were cooled to room temperature, ground to a homogeneous powder using a laboratory mill, and stored in sealed moisture-resistant containers at 20–25 °C, protected from direct sunlight, until extraction.

Preparation of plant extracts by ultrasonic extraction. Thirty grams of dried, ground plant material was mixed with 240 mL of extractant (1:8, w/v) in a 500-mL conical flask. Extraction was performed using a KQ5200B ultrasonic bath at 40 kHz and room temperature (20–25 °C) for 30 min and was repeated three times. Between extraction cycles, the mixtures were maintained at room temperature and mixed to ensure uniform contact between the plant material and extractant.

For ethanolic extraction, 96% ethanol was used as the extractant; distilled water was used for aqueous extraction. After extraction, the mixtures were filtered through paper filters. Ethanolic filtrates were concentrated using an EYELA N-1300 rotary evaporator at 45 °C until the solvent was removed and a concentrated extract was obtained. Aqueous filtrates were concentrated under the same temperature conditions and, when necessary, subjected to gentle additional drying. The extracts were stored in sealed containers at 4–8 °C until testing.

Preparation of working extract solutions. Concentrated extracts were weighed and used to prepare 10 mg/mL stock solutions. Water-soluble extracts were dissolved in distilled water. When an extract was insufficiently soluble in water, DMSO was used as a co-solvent. For example, 40.65 mg of extract was dissolved in a total volume of 4.065 mL, including 0.4065 mL DMSO, to obtain a 10 mg/mL stock solution containing 10% (v/v) DMSO.

The 1000 µg/mL working solution was prepared by mixing 0.1 mL of the 10 mg/mL stock solution with 0.9 mL distilled water. The 500 µg/mL solution was prepared by mixing 0.1 mL of the 1000 µg/mL solution with 0.1 mL water, and the 200 µg/mL solution was prepared by mixing 0.1 mL of the 1000 µg/mL solution with 0.4 mL water. The final DMSO concentrations in these solutions did not exceed 1% (v/v).

Mite collection

P. cuniculi mites were collected from auricular lesions of six naturally infested New Zealand White rabbits. Three rabbits had lesions predominantly involving the external surface of the ear, whereas three had lesions involving the internal surface of the pinna. The collected material was transferred to sterile glass Petri dishes and incubated at 35 °C for 30 min. Active adult mites were selected under a stereomicroscope using a fine entomological needle. After sample collection, the rabbits were treated with ivermectin. The procedures were conducted in accordance with the regulations of the Gansu Provincial Experimental Animal Centre and the applicable national requirements in China.

In vitro acaricidal assay

Filter paper was placed in 60-mm Petri dishes and treated with 30 µL of the corresponding working solution. Twenty active adult mites were transferred to each dish. Each treatment was tested in five independent replicates, corresponding to 100 mites per treatment condition. Permethrin, a synthetic pyrethroid acaricide manufactured in China, was used as the positive control at 1, 2.5, 5, 10, and 20 µL/mL. A 1% (v/v) aqueous DMSO solution without plant extract, representing the maximum vehicle concentration used in the assay, served as the negative control.

The dishes were sealed and incubated at 25 °C and 75% relative humidity. Mortality was assessed 24 hours after exposure. Mites were considered dead when no movement was observed, including after mechanical stimulation. The experiment included 42 plant-extract treatment conditions (seven plants × two extraction methods × three concentrations), five permethrin conditions, and one negative-control condition. Overall, 4800 mites were examined: 4200 in the plant-extract groups, 500 in the permethrin groups, and 100 in the DMSO control group.

Statistical analysis

Analyses were performed using R version 4.4.2. Mortality was calculated separately for each Petri dish and expressed as mean percentage ± standard deviation (SD) across five independent replicates. Overall mortality associated with aqueous and ethanolic extraction was compared using the paired

Wilcoxon signed-rank test, with plant species and concentration treated as matched conditions. The effect of concentration was evaluated using the Friedman test, with plant species–extraction method combinations treated as blocks. Differences among plant species were evaluated using the Kruskal–Wallis test. Where pairwise comparisons were performed, p-values were adjusted using the Holm method. Statistical significance was defined as $p < 0.05$. Because mortality in the negative control was 7%, the table reports observed, uncorrected mortality values [10, 11, 12].

Results

Mortality varied substantially among plant species, extraction methods, and concentrations (Figure 1). Across all plant–concentration combinations, ethanolic extracts produced a mean mortality of 27.43%, compared with 13.67% for aqueous extracts. The overall difference between extraction methods was statistically significant (paired Wilcoxon signed-rank test, $p = 0.0033$).

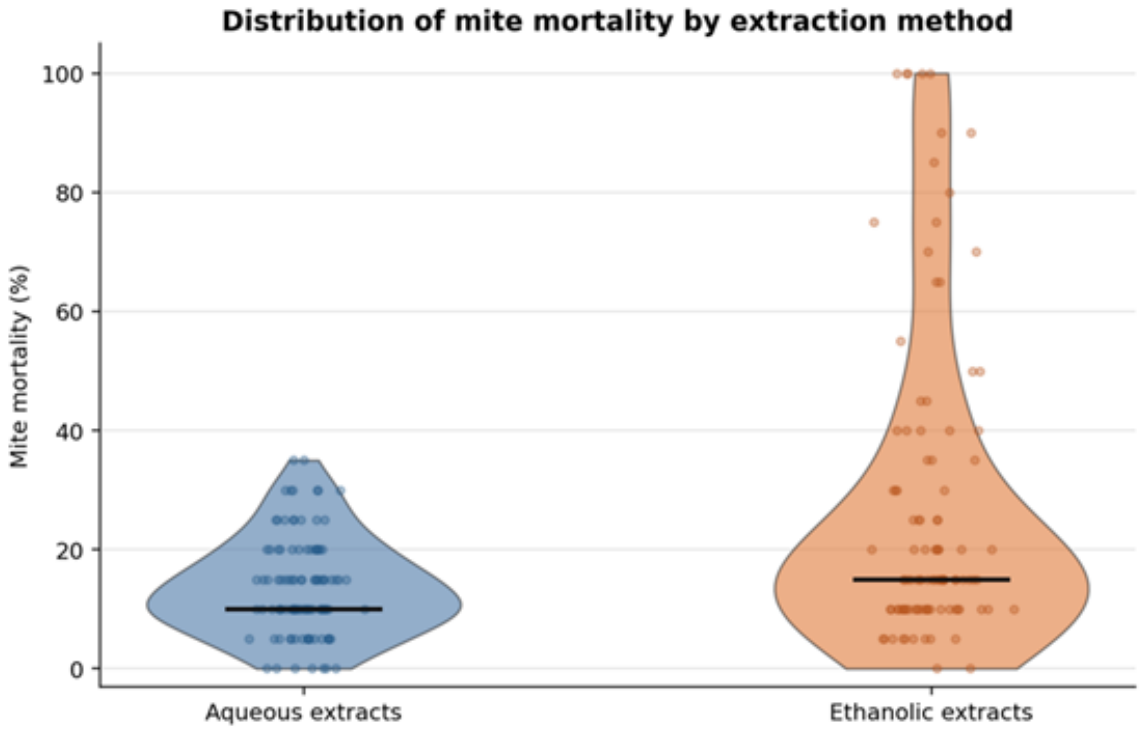

Figure 1. Distribution of *P. cuniculi* mortality following exposure to aqueous and ethanolic plant extracts. Points represent replicate-level mortality values across the seven plants and three concentrations; horizontal lines indicate medians

Concentration had a significant overall effect on mortality (Friedman $\chi2 = 19.44$, $p < 0.001$), and acaricidal activity differed significantly among plant species (Kruskal–Wallis $H = 22.76$, $p < 0.001$). Concentration-dependent responses for each plant and extraction method are shown in Figure 2.

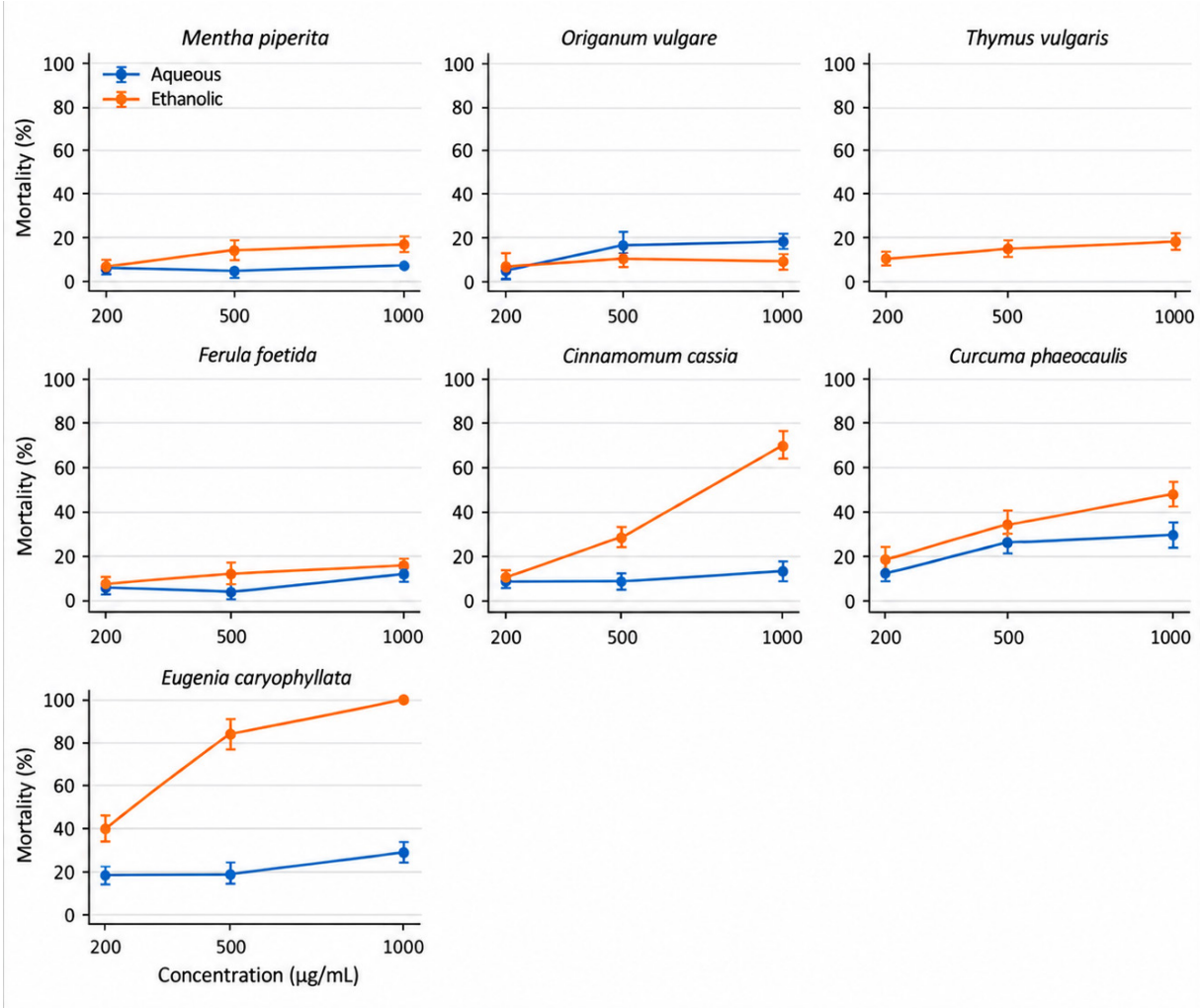

Figure 2. Concentration-dependent acaricidal activity of aqueous and ethanolic plant extracts against *P. cuniculi.* Values are means ± SD from five independent replicates, with 20 mites per replicate

The highest mortality was produced by the ethanolic extract of *Eugenia caryophyllata* at 1000 µg/mL (100.00 ± 0.00%), whereas the corresponding aqueous extract produced 28.00 ± 4.47% mortality. At the same concentration, the ethanolic extract of *Cinnamomum cassia* produced 69.00 ± 4.18% mortality, compared with 12.00 ± 4.47% for its aqueous extract. *Curcuma phaeocaulis* showed a consistent concentration-related increase, reaching 48.00 ± 5.70% mortality with the ethanolic extract and 29.00 ± 5.48% with the aqueous extract at 1000 µg/mL.

Table 1. Mortality of *P. cuniculi* following exposure to plant extracts and control treatments

Treatment	Concentration	Aqueous extract, mortality (%) ± SD	Ethanolic extract, mortality (%) ± SD
Control treatments			
1% DMSO	1% (v/v)	7.00 ± 4.47	
Permethrin	1 µL/mL	25.00 ± 3.54	
Permethrin	2.5 µL/mL	42.00 ± 5.70	
Permethrin	5 µL/mL	52.00 ± 8.37	
Permethrin	10 µL/mL	53.00 ± 25.88	
Permethrin	20 µL/mL	64.00 ± 10.84	
Plant extracts			

Continuation of Table 1

Mentha piperita	200 µg/mL	7.00 ± 2.74	7.00 ± 2.74
Mentha piperita	500 µg/mL	5.00 ± 3.54	14.00 ± 4.18
Mentha piperita	1000 µg/mL	7.00 ± 6.71	17.00 ± 2.74
Origanum vulgare	200 µg/mL	5.00 ± 3.54	6.00 ± 6.52
Origanum vulgare	500 µg/mL	16.00 ± 6.52	11.00 ± 4.18
Origanum vulgare	1000 µg/mL	18.00 ± 2.74	9.00 ± 4.18
Thymus vulgaris	200 µg/mL	11.00 ± 2.24	11.00 ± 2.24
Thymus vulgaris	500 µg/mL	15.00 ± 3.54	15.00 ± 3.54
Thymus vulgaris	1000 µg/mL	18.00 ± 2.74	18.00 ± 2.74
Ferula foetida	200 µg/mL	6.00 ± 4.18	8.00 ± 2.74
Ferula foetida	500 µg/mL	4.00 ± 4.18	12.00 ± 4.47
Ferula foetida	1000 µg/mL	12.00 ± 2.74	16.00 ± 2.24
Cinnamomum cassia	200 µg/mL	9.00 ± 2.24	11.00 ± 2.24
Cinnamomum cassia	500 µg/mL	8.00 ± 2.74	28.00 ± 2.74
Cinnamomum cassia	1000 µg/mL	12.00 ± 4.47	69.00 ± 4.18
Curcuma phaeocaulis	200 µg/mL	13.00 ± 2.74	19.00 ± 5.48
Curcuma phaeocaulis	500 µg/mL	26.00 ± 4.18	34.00 ± 6.52
Curcuma phaeocaulis	1000 µg/mL	29.00 ± 5.48	48.00 ± 5.70
Eugenia caryophyllata	200 µg/mL	19.00 ± 4.18	39.00 ± 4.18
Eugenia caryophyllata	500 µg/mL	19.00 ± 4.18	84.00 ± 6.52
Eugenia caryophyllata	1000 µg/mL	28.00 ± 4.47	100.00 ± 0.00

Values are presented as mean mortality (%) ± standard deviation (SD) from five independent replicates, with 20 mites per replicate. Observed, uncorrected mortality values are shown. Plant-extract concentrations are expressed in µg/mL; permethrin concentrations are expressed in µL/mL

Mortality remained comparatively low for *Mentha piperita, Thymus vulgaris, Ferula foetida,* and *Origanum vulgare*. At 1000 µg/mL, ethanolic-extract mortality was 17.00 ± 2.74% for *M. piperita*, 18.00 ± 2.74% for *T. vulgaris*, 16.00 ± 2.24% for *F. foetida*, and 9.00 ± 4.18% for *O. vulgare*. For *T. vulgaris,* aqueous and ethanolic extracts produced identical mean mortality at all three concentrations. *O. vulgare* showed a different pattern, with higher mortality for the aqueous extract at 500 and 1000 µg/mL.

The negative control produced 7.00 ± 4.47% mortality. Permethrin mortality increased from 25.00 ± 3.54% at 1 µL/mL to 64.00 ± 10.84% at 20 µL/mL. Because plant extracts were expressed as mass concentrations and permethrin as volume concentrations, dose-matched inferential comparisons between these agents were not performed.

Discussion

The results demonstrate that in vitro acaricidal activity against *P. cuniculi* depended on plant species, extraction method, and concentration. Overall, ethanolic extracts produced significantly higher mortality than aqueous extracts (27.43% vs. 13.67%; $p = 0.0033$), although the magnitude and direction of the extraction-method difference varied among plants.

Ethanol can extract a broader range of moderately polar and less polar constituents than water, whereas aqueous extracts are enriched in highly polar compounds. These differences in phytochemical composition may explain the higher overall activity of the ethanolic preparations. However, the present assay did not quantify individual constituents, and the contribution of specific compounds cannot be established from the mortality data alone [13].

The strongest response was observed for the ethanolic extract of *Eugenia caryophyllata*, which produced complete mortality at 1000 µg/mL. Ethanolic extracts of *Cinnamomum cassia* and *Curcuma*

phaeocaulis also showed marked concentration-related activity. By contrast, *Mentha piperita, Origanum vulgare, Thymus vulgaris*, and *Ferula foetida* showed low or modest activity under the present conditions.

Eugenia caryophyllata flower buds are rich in eugenol, and clove essential oil has previously shown dose- and time-dependent activity against *P. cuniculi* [14, 15]. These findings support the biological plausibility of the high activity observed here. Nevertheless, an essential oil, a crude ethanolic extract, and purified eugenol are chemically distinct preparations; direct quantitative comparison among them should therefore be made cautiously.

Cinnamomum cassia bark contains cinnamaldehyde-rich volatile fractions, and both extracts and essential oils of *C. cassia* have shown acaricidal activity against *Haemaphysalis longicornis* [16, 17, 18]. The present results extend this evidence to a crude ethanolic extract tested against *P. cuniculi,* but differences in target species, extraction procedures, chemical composition, and exposure conditions preclude direct comparison of potency.

The Curcuma species examined in the present study was *Curcuma phaeocaulis,* whereas much of the published acaricidal literature concerns *Curcuma longa* [19–21]. These are distinct species and should not be considered interchangeable. Evidence for *C. longa i*s therefore cited only to indicate the broader acaricidal potential of Curcuma-derived constituents. Species-specific phytochemical characterization of *C. phaeocaulis* is required before its active components and mechanisms can be defined.

Conclusion

Under the conditions of this in vitro assay, the ethanolic extracts of *Eugenia caryophyllata, Cinnamomum cassia*, and *Curcuma phaeocaulis* showed the greatest acaricidal potential against *P. cuniculi.* The ethanolic extract of *E. caryophyllata* produced complete mortality at 1000 µg/mL. These findings identify promising candidates for further investigation but do not establish therapeutic efficacy or safety in animals. Future studies should characterize the active constituents, assess repeatability and toxicological safety, and evaluate efficacy in controlled in vivo trials.

Authors' Contributions

BO: conceived and designed the study, defined the aims and objectives, provided scientific supervision, coordinated the research process, and approved the final version of the manuscript. GI, AD: performed the literature review and analysis, developed the research methodology, participated in the preparation of plant material and extraction procedures, drafted the initial version of the manuscript, prepared the "Materials and Methods" and "Results" sections, and prepared the manuscript for submission.

ED: performed the statistical analysis of the data, analysed and interpreted the results, and prepared the tables and figures. DKh: critically revised the manuscript, contributed to the discussion of the results, verified the scientific content, performed linguistic and scientific editing, and approved the final version of the article. XSh: conducted the in vitro acaricidal activity experiments, prepared the biological material, and recorded the experimental data. BV: collected the medicinal plant raw material, performed extraction and fractionation of the plant extracts, and supervised the laboratory procedures.

All authors made a substantial contribution to the study, participated in the discussion of the results, read and approved the final version of the manuscript, and agreed to be accountable for the accuracy and integrity of the data presented.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1 University of Missouri, College of Veterinary Medicine. (2026). *Psoroptes cuniculi.* Diseases of Research Animals (DORA). https://cvm.missouri.edu/diseases-of-research-animals-dora/rabbits/psoroptes-cuniculi/

2 Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J., Iqbal, Z. (2014). Acaricide resistance in cattle ticks and approaches to its management. *Vet. Parasitol.*, 203(1–2), 6–20. DOI: 10.1016/j.vetpar.2014.03.006.

3 Evans, A., Madder, M., Fourie, J., Halos, L., Kumsa, B., Kimbita, E., Byaruhanga, J., Mwiine, F.N., Muhanguzi, D., Adehan, S.B. (2024). Acaricide resistance status of livestock ticks from East and West Africa and in vivo efficacy of acaricides to control them. *Int. J. Parasitol. Drugs Drug Resist.*, 25: 100541, 1–11. DOI: 10.1016/j.ijpddr.2024.100541.

4 Pavela, R., Benelli, G. (2016). Essential oils as ecofriendly biopesticides? *Trends Plant. Sci.,* 21: 12, 1000–1007. DOI: 10.1016/j.tplants.2016.10.005.

5 Isman, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture. *Annu. Rev. Entomol.*, 51, 45–66. DOI: 10.1146/annurev.ento.51.110104.151146.

6 Tavares, W.R., Barreto, M.C.L., Seca, A.M.L. (2021). Aqueous and ethanolic plant extracts as bio-insecticides. *Plants*, 10(5), 920. DOI: 10.3390/plants10050920.

7 Liao, F., Han, C., Deng, Q., Zhou, Z., Bao, T., Zhong, M., Tao, G., Li, R., Han, B., Qiao, Y., Hu, Y. (2023). Natural products as mite control agents in animals: a review. *Molecules*, 28: 19, 6818. DOI: 10.3390/molecules28196818.

8 Chermenskaya, T.D., Stepanycheva, E.A., Shchenikova, A.V., Chakaeva, A.S. (2010). Insectoacaricidal activity of plant extracts. *Ind. Crops Prod.*, 32(2), 157–163. DOI: 10.1016/j.indcrop.2010.04.009.

9 Ntalli, N.G., Ozalexandridou, E.X., Kasiotis, K.M., Samara, M., Golfinopoulos, S.K. (2020). Nematicidal activity and phytochemistry of Greek Lamiaceae species. *Agronomy*, 10(8), 1119. DOI: 10.3390/agronomy10081119.

10 Angon, P.B., Mondal, S., Jahan, I., Datto, M., Antu, U.B., Ayshi, F.J., Islam, Md.S. (2016). Integrated pest management (IPM) in agriculture and itsrole in maintaining ecological balance and biodiversity. *Adv. Agric.*, 2023, 1–19. DOI: 10.1155/2023/5546373.

11 Aphalo, P.J. (2017). OpenIntro Statistics. 3ed by D.M. Diez, C.D. Barr, M. Cetinkaya-Rundel. *UV4Plants Bulletin*, 2016(2), 51–53. DOI:10.19232/uv4pb.2016.2.90.

12 Dutbayev, Y., Bolatbekova, A., Kharipzhanova, A., Sabitova, A., Bakhytuly, K., Tsygankov, V., Bastaubayeva, S., Tsygankov, A., Kumarbayeva, M., Keishilov, Z., Kokhmetova, A. (2025). Genetic and Environmental Interactions in Spring Wheat Resistance to Bipolaris Sorokiniana Root Rot in Kazakhstan. *OnLine J. Biol. Sci.*, 25(4), 1050–1063. DOI:10.3844/ojbsci.2025.1050.1063.

13 Chatepa, L.E.C., Mwamatope, B., Chikowe, I., Masamba, K.G. (2024). Effects of solvent extraction on the phytoconstituents and in vitro antioxidant activity properties of leaf extracts of the two selected medicinal plants from Malawi. *BMC Complement. Med. Ther.,* 24, 317. DOI: 10.1186/s12906-024-04619-7.

14 Fichi, G., Flamini, G., Giovanelli, F., Otranto, D., Perrucci, S. (2007). Efficacy of an essential oil of Eugenia caryophyllata against Psoroptes cuniculi. *Exp. Parasitol.,* 115(2), 168–172. DOI: 10.1016/j.exppara.2006.07.005.

15 Shahina, Z., Dahms, T.E.S. (2024). A comparative review of eugenol and citral anticandidal mechanisms: partners in crimes against fungi. *Molecules,* 29(23), 5536. DOI: 10.3390/molecules29235536.

16 Shang, X.F., et al. (2019). The acaricidal activity and mechanism of eugenol on Psoroptes cuniculi. *Vet. Parasitol.,* 266, 56–62. DOI: 10.1016/j.vetpar.2018.12.014.

17 Nwanade, C.F. et al. (2021). Acaricidal activity of Cinnamomum cassia (Chinese cinnamon) against the tick Haemaphysalis longicornis is mediated by (E)-cinnamaldehyde. *Parasit. Vectors,* 14, 330. DOI: 10.1186/s13071-021-04823-1.

18 Bai, L., Gao, Z., Xu, X., Lv, W., Wang, Y., Dong, K., Yu, Z., Yang, X. (2024). The acaricidal activity and enzymatic targets of the essential oils of Cinnamomum cassia and Cinnamomum camphora and their major components against Haemaphysalis longicornis (Acari: Ixodidae). *Ind. Crops Prod.*, 209, 117967. DOI: 10.1016/j.indcrop.2023.117967.

19 Damodharan, S. et al. (2024). Curcuma longa essential oil as an acaricidal agent for the control of cattle ectoparasitic tick - Rhipicephalus (Boophilus) annulatus. *Sci. Rep.,* 14, 28138. DOI: 10.1038/s41598-024-78716-5.

20 Abdel-Shafy, S., Allam, N.A., Metwally, A.A., El-Kady, G.A., Haridy, F.M., El-Sherif, H.A. et al. (2020). Efficacy and safety of ethanolic Curcuma longa extract as a treatment for sand tampan ticks in a rabbit model. *Vet World*, 13(4), 812–820. DOI: 10.14202/vetworld.2020.812-820.

21 Chouhan, K., Gautam, V., Jain, S., Mishra, P., Chourasia, A., Meshram, A. (2024). Evaluation of Acaricidal Efficacy of Curcuma Longa, Curcumin and Nanocurcumin Against Rhipicephalus (B.) microplus. *Acta Sci Vet Sci.*, 6(11), 36–44. DOI: 10.31080/ASVS.2024.06.0941.

Research article

Comparative assessment of the biological effectiveness of a domestic medium for bovine oocyte aspiration

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Abstract

Background and Aim. *In vitro* embryo production is an important reproductive biotechnology for accelerating genetic improvement in cattle. The efficiency of this technology largely depends on the quality and developmental competence of cumulus–oocyte complexes (COCs) recovered during ovum pick-up (OPU). Aspiration media play an important role in maintaining oocyte viability during collection and subsequent handling. This study aimed to evaluate the biological efficacy of a newly developed domestic aspiration medium for bovine oocyte recovery and to compare its performance with that of a commercial aspiration medium.

Materials and Methods. The study was conducted using six physiologically healthy Galloway donor cows aged 1–4 years. Oocyte aspiration was performed twice in each donor at a 14-day interval. The recovered COCs were assigned to experimental and control groups according to the aspiration medium used. A commercial aspiration medium was used in the control group, whereas a newly developed domestic medium based on modified Dulbecco's medium was used in the experimental group. COC quality was evaluated immediately after aspiration by morphological assessment and after 24 h of *in vitro* maturation using fluorescence and polarization microscopy. Nuclear maturation, cumulus morphology, reactive oxygen species (ROS) levels, mitochondrial activity, and apoptotic changes were assessed. Differences were considered statistically significant at $p < 0.05$.

Results. A total of 125 COCs were recovered using the commercial medium and 121 using the experimental medium, with mean yields of 20.8 ± 3.6 and 20.5 ± 3.2 COCs per OPU procedure, respectively. Morphologically normal COCs accounted for 81.6% in the control group and 80.1% in the experimental group, with no statistically significant differences between the groups. After in vitro maturation, the proportion of oocytes reaching metaphase II and the presence of the meiotic spindle were comparable between groups. No significant differences were detected in cumulus morphology, ROS levels, mitochondrial activity, or apoptotic changes. The proportion of oocytes with high mitochondrial activity was 76.4% in the control group and 74.2% in the experimental group.

Conclusion. The newly developed domestic aspiration medium demonstrated biological efficacy comparable to that of the commercial medium and maintained the morphological quality, nuclear maturation, mitochondrial activity, and overall viability of bovine oocytes. These findings indicate that the medium is promising for further evaluation in OPU and *in vitro* embryo production. However, validation in a larger number of donor animals, including assessment of fertilization, cleavage, blastocyst development, embryo transfer, and pregnancy outcomes, is required before conclusions can be drawn regarding its broader practical application.

Keywords: cattle; cumulus-oocyte complexes; embryo transfer; *in vitro* embryo production; oocyte aspiration; oocyte aspiration medium.

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Introduction

In the Russian Federation, cattle breeding is a key sector of the animal husbandry industry as a whole. The effectiveness of its further development depends largely on the implementation of modern biotechnological procedures, one of which is the production of *in vitro* embryos. This technology makes it possible to significantly accelerate genetic progress in herds, make fuller use of the genetic potential of outstanding animals, and produce more offspring from them.

The first step in *in vitro* embryo production technology is ovum pick-up (OPU), during which cumulus-oocyte complexes (COCs) are obtained from the cow's ovaries using minimally invasive methods, which are then used to produce embryos [1]. This step is critical, as it largely determines the potential of the oocyte for subsequent development, fertilization, and culture of the resulting zygote [2]. During OPU, the oocyte is exposed to various stress factors, including fluctuations in environmental pH, mechanical stress, oxidative stress, and temperature deviations from the physiological norm [3]. To maintain its ability to further develop, the use of a special fluid, which is an aspiration medium, is necessary [4].

Currently, imported commercial oocyte aspiration media are widely used in the Russian Federation; however, they are expensive, and their availability depends on foreign supplies. An alternative is the use of laboratory-prepared experimental media of varying compositions, the preparation of which requires specialized equipment and resources. Domestic commercial aspiration media, in turn, are virtually nonexistent.

To date, studies have been conducted that provide sufficient information on the impact of aspiration medium components such as HEPES buffer [5, 6], various antioxidants, and various salts for maintaining osmotic pressure [7] on COC quality. However, given the lack of domestic commercial media, a comprehensive evaluation of the effectiveness of a domestic aspiration medium relative to its commercial counterpart is a promising direction.

Consequently, it was decided to conduct an experimental study of a newly developed domestic medium for oocyte aspiration. Its overall effectiveness was compared with that of a commercial medium used for this stage of *in vitro* embryo production. The obtained results fill the existing gap in data on domestic alternatives to imported aspiration media.

The objective of the study was to evaluate the biological effectiveness of a new domestic aspiration medium for use in in vitro bovine embryo production technology based on the data obtained during the experimental study.

Materials and Methods

The study was conducted from March to April 2026. The ovum pick-up (OPU) procedures were performed at the Smolensk Galloway Breeding Center, LLC, and the laboratory stage was conducted in the embryology laboratory of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy. The study subjects were cumulus-oocyte complexes (COCs) obtained from physiologically healthy Galloway cows aged 1 to 4 years with no estrous cycle disorders.

Oocyte aspiration was performed twice in each donor at a 14-day interval [8], once using the commercial medium and once using the experimental medium. The order of media application was balanced: in three animals the experimental medium was used for the first aspiration, and in the other three the commercial medium. In the control group, OPU was performed using the commercial aspiration medium OPU Medium (cat. No. 51001, IVF Bioscience, UK). According to the manufacturer, it is a serum-free medium enriched with vitamins and amino acids and supplemented with heparin and gentamicin. The quantitative composition of the medium is not disclosed by the manufacturer. OPU media maintain a pH of 7.2 – 7.4 and an osmolality close to that of the internal environment of the body, which is 290 - 310 mOsm/kg. In the experimental group, a new domestic medium developed for bovine oocyte aspiration was used.

The domestic medium was prepared on the basis of modified Dulbecco's phosphate-buffered saline (mDPBS), in which the NaCl concentration was reduced to 114 mM to compensate for the osmotic contribution of the buffer components. It contained a buffer system consisting of HEPES and TES; sodium heparin to prevent blood clotting; polyvinyl alcohol, used instead of bovine serum albumin [7] to prevent adhesion of oocytes to the walls of the needle and tubes; gentamicin to prevent bacterial

contamination; and L-cysteine and L-carnitine as antioxidants. The quantitative composition of the medium is given in Table 1. The pH of the medium was adjusted to 7.4 with 1 M NaOH; the osmolality of the medium was 290 mOsm/kg.

Table 1. Composition of the experimental oocyte aspiration medium

Component	Concentration	Amount per 1 L of medium
NaCl	114 mM	6.66 g
KCl	2.68 mM	0.20 g
Na2HPO4(anhydrous)	8.1 mM	1.15 g
KH2PO4	1.47 mM	0.20 g
HEPES	25 mM	5.96 g
TES	10 mM	2.29 g
Polyvinyl alcohol	1.5 mg/mL	1.5 g
Sodium heparin	7.5 IU/mL	7.500 IU
L-cysteine	0.2 mM	24.2 mg
L-carnitine	0.5 mM	80.6 mg
Gentamicin	75 µg/mL	75 mg
NaOH (1 M solution)	to pH 7.4	0.65 g

After OPU, the resulting aspirate, according to standard protocols [9, 10], was subjected to primary filtration, placed in a transport medium, and transported in a thermal container at a temperature of 37, 38 °C for no more than 3 hours [11, 12]. The cumulus-oocyte complexes were then washed in a special medium, after which they underwent an initial morphological assessment under a stereomicroscope at ×40 and ×80 magnification [13]. Morphologically normal COCs were identified as oocytes with uniform, dark-granulated cytoplasm without visible vacuoles or with minor cytoplasmic heterogeneity and with 2-4 layers of cumulus cells. These were immediately transferred to *in vitro* oocyte maturation medium in 100-µl droplets covered with a layer of sterile mineral oil [14].

After 24 hours, the COCs were re-evaluated for quality. Nuclear maturity of oocytes was assessed by fluorescence microscopy by performing complete denudation using gentle pipetting, fixation, and DAPI staining, after which the metaphase stage II was determined [14]. The presence of the meiotic spindle was also assessed using polarized light microscopy [15]. The degree of expansion, compactness, and the presence of degenerative signs of the cumulus were separately recorded. In addition, the level of reactive oxygen species was determined using the fluorescent probe DCFH-DA, and mitochondrial activity was assessed using the fluorescent dye MitoTracker [16]. The level of apoptosis was also determined using the TUNEL assay [17].

Statistical data processing was performed using Statistica 10.0 and Microsoft Excel 2019. Quantitative data with a normal distribution were presented as mean ± standard deviation (SD). Normality was assessed using the Shapiro-Wilk test. COC yield per procedure was compared using a linear mixed model with medium and aspiration order as fixed factors and animal as a random effect. Indicators assessed at the level of individual oocytes were compared using pooled group data with Pearson's χ^2 test and Fisher's exact test. Fluorescence intensity was compared using Student's t-test for independent samples. Differences between groups were considered statistically significant at a probability level of $p < 0.05$.

Results

During oocyte aspiration using a commercial medium, 125 COCs were obtained, while 121 COCs were obtained in the experimental group using a new domestic aspiration medium. The average yield of complexes per OPU procedure was 20.8 ± 3.6 in the control group and 20.2 ± 3.2 in the experimental group. Differences in the number of oocytes retrieved were not statistically significant ($p = 0.28$), nor was the effect of aspiration order ($p = 0.82$). A preliminary assessment of the COCs was conducted, and the results are presented in Table 2.

Table 2. Distribution of COCs by morphological grade in the control and experimental groups

Control group	Number of COCs	Proportion of COCs, %	Experimental group	Number of COCs	Proportion of COCs, %
Total	125	100	Total	121	100
1 grade	64	51.2	1 grade	59	48.8
2 grade	38	30.4	2 grade	38	31.4
3 grade	14	11.2	3 grade	15	12.4
4 grade	9	7.2	4 grade	9	7.4
Morphologically normal COCs	102	81.6	Morphologically normal COCs	97	80.2

The total proportion of grade 1 and 2 complexes in the experimental group was 80.2%, which is 1.4% lower than in the control group. Statistical analysis revealed no significant differences between the groups in the distribution of COCs by grade ($\chi^2 = 0.17$; $p = 0.98$). The obtained data are consistent with published data, according to which, when using OPU, the proportion of morphologically intact cumulus-oocyte complexes ranges from 70 to 85% of the total number of retrieved oocytes [18, 19].

During a secondary assessment of cumulus-oocyte complexes, the data presented in Figure 1 were obtained. The results of the secondary assessment of cumulus–oocyte complexes are presented in Figure 1.

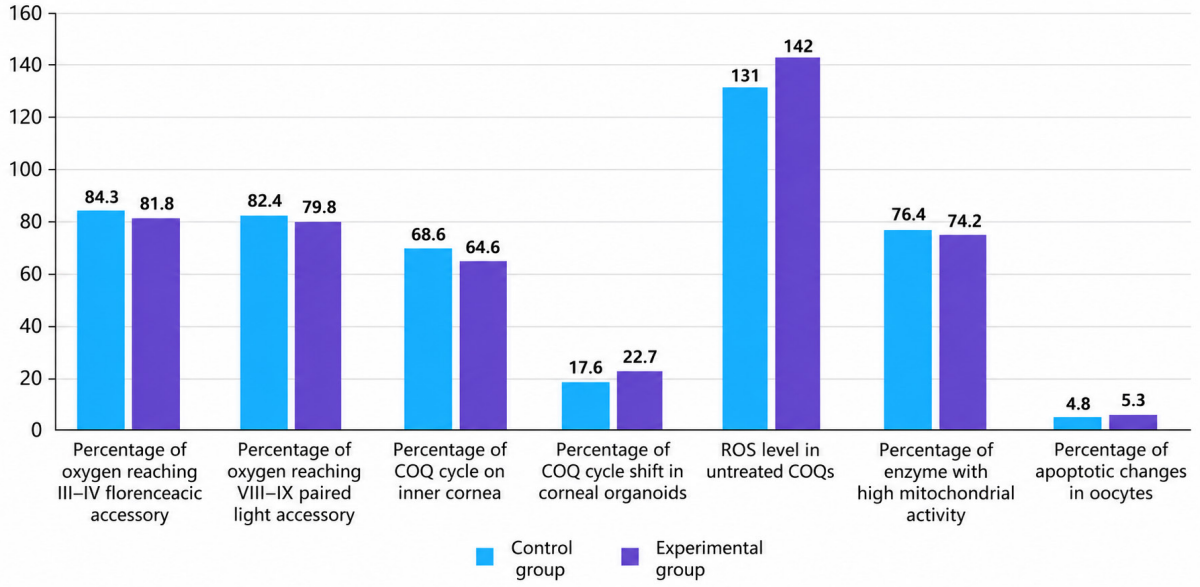

Figure 1. Comparison of the experimental and control groups by indicators of nuclear and cytoplasmic oocyte maturation

Following IVM, matured oocytes were examined using polarized light microscopy. The meiotic spindle was visualized in approximately 80% of oocytes in both the control and experimental groups. Fluorescence microscopy then showed that the proportion of oocytes reaching metaphase II was 2.5 percentage points higher in the control group ($p > 0.05$); the results of fluorescence and polarized light microscopy were comparable. Both media provided an acceptable level of nuclear maturation typical of standard IVM protocols, which is consistent with studies on *in vitro* oocyte maturation [20, 21].

The secondary evaluation of COCs also included assessment of morphological changes in the cumulus. Overall cumulus morphology after IVM remained within the physiological norm in both groups; no statistically significant differences were found between the two groups [22]. Additionally, ROS levels were measured, revealing that the average fluorescence intensity was slightly higher in the experimental group. The difference compared to the control group averaged 10.5 arbitrary units, which is statistically insignificant. These results are consistent with published data on the permissibility

of moderate fluctuations in oxidative stress levels without significantly affecting oocyte maturation [23].

Mitochondrial activity was also assessed, but no statistically significant differences were found: the proportion of oocytes with intense mitochondrial fluorescence was 76.4% in the control group and 74.2% in the experimental group. The absence of differences in these data indicates the maintenance of the effectiveness of the oocyte mitochondrial system, confirming their cytoplasmic maturation.

Finally, apoptotic changes were assessed and occurred at a low frequency. This is also consistent with published data showing that apoptosis levels remain minimal under optimal oocyte retrieval conditions [24, 25].

Limitations of this study include the small number of donor animals, which limited the total number of cumulus-oocyte complexes studied, limiting the statistical power of the study. Furthermore, only an early assessment of oocyte quality indicators was conducted; however, monitoring the progress of *in vitro* embryo production up to their transfer to recipients could have yielded more reliable results.

The obtained results demonstrated that the domestic medium exhibits comparable performance to a commercial one. The obtained results are within the range of values for standard commercial oocyte aspiration media [26, 27]. These data may indicate the need for further research on the domestic medium. The absence of statistically significant differences between groups suggests that the experimental medium is successfully performing its functions. The observed tendency towards a slight decrease in individual indicators requires further verification of the data, for which it is worth considering the possibility of repeating a similar experiment on a larger sample with different donors, as well as longer-term monitoring of the obtained oocytes [28].

Discussion

The comparable biological responses observed with the experimental and commercial aspiration media indicate that the composition of the newly developed domestic medium provides an appropriate microenvironment for bovine oocytes during OPU and subsequent handling. The buffer system based on HEPES and TES is intended to maintain stable physicochemical conditions, while polyvinyl alcohol protects oocytes from undesirable adhesion to aspiration equipment. The inclusion of L-cysteine and L-carnitine as antioxidant components may contribute to maintaining cellular homeostasis during oocyte recovery and maturation. Importantly, the experimental medium did not produce detectable adverse effects on nuclear or cytoplasmic maturation, oxidative status, mitochondrial function, or apoptotic processes. These observations suggest that replacement of the commercial aspiration medium with the developed formulation did not compromise the principal biological characteristics associated with oocyte quality and developmental competence. From a practical perspective, the development of a domestically produced aspiration medium may reduce dependence on imported commercial products and improve the accessibility of OPU-based reproductive technologies. Nevertheless, the relatively small number of donor animals and the assessment of oocytes only during the early stages of in vitro embryo production should be considered when interpreting the findings. Further studies involving a larger and more diverse donor population, fertilization, embryo culture, blastocyst development, and subsequent embryo transfer are required to comprehensively evaluate the reproductive efficiency of the developed medium.

Conclusions

The experiment assessed the biological efficacy of a new domestic aspiration medium for use in *in vitro* bovine embryo production technology, confirming its suitability for further testing.

The number of oocytes recovered, their quality, and maturation parameters obtained using the new domestic medium were comparable to those obtained using a commercial medium.

No statistically significant differences were found between the two groups, indicating the comparability of the domestic medium with its commercial counterpart.

These results indicate that the tested medium is promising for further evaluation in OPU procedures. Validation in a larger and more diverse donor population is required, together with assessment of fertilization, cleavage, blastocyst development, embryo transfer, and pregnancy outcomes. Further studies should also evaluate whether optimization of the medium composition is warranted.

Authors' Contributions

Conceptualization: AA, YK, DS; methodology: AA, YK, SF and DS; investigation: AA, YK and DS; data curation: AA and YK; statistical analysis: YK and DS; writing original draft preparation: AA and YK; writing review and editing: SF and DS; supervision: SF. All authors have read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare that they have no competing interests.

References

1 Kasyanov, R.O., Pleshkov, V.A., Smolovskaya, O.V. et al. (2025). Razrabotka komponenta pitatel'noi sredy iz biologicheskih zhidkostei reproduktivnoi sistemy korov dlya povysheniya effektivnosti proizvodstva embrionov krupnogo rogatogo skota in vitro [Development of a culture medium component derived from bovine reproductive tract fluids to enhance the efficiency of in vitro cattle embryo production]. *Bulletin of KrasGAU*, 6, 160–181. [*In Russian*].

2 Agarwal, A., Gupta, S., Sharma, R. (2005). Oxidative stress and its implications in female infertility: a clinician's perspective. *Reproductive BioMedicine Online*, 11(5), 641–650. DOI: 10.1016/S1472-6483(10)61174-1.

3 Aguila, L. et al. (2020). Oocyte selection for in vitro embryo production in bovine species: noninvasive approaches for new challenges of oocyte competence. *Animals*, 10(12), 2196. DOI: 10.3390/ani10122196.

4 Bahrami, M., Morris, D., Monaghan, L. et al. (2022). Culture conditions for in vitro maturation of oocytes: a review. Animal Reproduction Science, 246, 107141. DOI: 10.1016/j.repbre.2022.04.001.

5 Bohlooli, S. et al. (2015). HEPES buffer in ovary-transportation medium influences developmental competence of cattle oocytes. *S. Afr. Anim Sci.*, 45(3), 299–308. DOI: 10.4314/sajas.v45i5.11.

6 Bols, P.E., Vandenheede, J.M., Van Soom, A., de Kruif, A. (1995). Transvaginal ovum pick-up (OPU) in the cow: a new disposable needle guidance system. *Theriogenology*, 43(3), 677–687. DOI: 10.1016/0093-691x(94)00073-4.

7 Choi, B.H. *et al.* (2019). Effect of serum and serum-free media on the developmental competence of OPU-derived bovine IVP embryo. *J Anim Reprod Biotechnol.,* 34(4), 305–312. DOI: 10.12750/JARB.34.4.305.

8 Combelles, C.M.H., Greenfeld, C.M., Racowsky, C. (2009). Could oxidative stress influence the in-vitro maturation of oocytes? *Reproductive BioMedicine Online,* 19(1), 55–67. DOI: 10.1016/S1472-6483(10)61174-1.

9 Ferré, L.B., Baruselli, P.S., Bó, G.A. et al. (2020). Recent advances in bovine in vitro embryo production: reproductive biotechnology history and methods. *Animal*, 14(Suppl 1), 1–15. DOI: 10.1017/S1751731119002775.

10 Ferré, L.B., Baruselli, P.S., Bó, G.A. et al. (2023). Transvaginal ultrasound-guided oocyte retrieval in cattle: state-of-the-art and its impact on the *in vitro* fertilization embryo production outcome. *Reprod Dom Anim*., 58(3), 363–378. DOI: 10.1111/rda.14303.

11 Fry, R.C., Niall, E.M., Simpson, T.L., Squires, T.J., Reynolds, J. (1997). The collection of oocytes from bovine ovaries. *Theriogenology,* 47(5), 977–987. DOI: 10.1016/s0093-691x(97)00054-x.

12 Gómez-López, N. et al. (2024). Chemical gasification: An alternative approach to in vitro maturation of bovine oocytes. *Reprod Dom Anim.*, 59. DOI: 10.1111/rda.14701.

13 Guérin, P., El Mouatassim, S., Ménézo, Y. (2001). Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Hum Reprod Update*, 7(2), 175–189. DOI: 10.1093/humupd/7.2.175.

14 Hansen, P.J. (2023). Laboratory guide for production of bovine embryos in vitro (ver. 06.28.2023). Gainesville: University of Florida, *Animal Sciences Department*, 78. https://animal.ifas.ufl.edu/hansen/lab-protocols/

15 Hara, S., Shida, M., Abe, K., Shirasuna, K., Iwata, H. (2025). Effect of bovine oocyte transportation system on embryonic quality. *J Reprod Dev.*, 71(6), 301–309. DOI: 10.1262/jrd.2025-031.

16 Jin, J.-I., Ghanem, N., Kim, S.-S. et al. (2016). Interaction of donor age, parity and repeated recovery of cumulus–oocyte complexes by ovum pick-up on in vitro embryo production and viability after transfer. *Livestock Science*, 188, 43–47. DOI: 10.1016/j.livsci.2016.04.002.

17 Krisher, R.L. (2024). Bovine embryo production *in vitro*: evolution of culture media and commercial perspectives. *Animal Reproduction*, 21(3), e20240018. DOI: 10.1590/1984-3143-AR2024-0051.

18 Lizarraga, C. et al. (2024). Scoping review of primary studies evaluating bovine in vitro oocyte maturation and embryo development. *Veterinaria México OA,* 11. DOI: 10.22201/fmvz.24486760e.2024.1315.

19 Mogessie, B. et al. (2018). Assembly and positioning of the oocyte meiotic spindle. *Annual Review of Cell and Developmental Biology*, 34, 381–403. DOI: 10.1146/annurev-cellbio-100616-060553.

20 Naspinska, R., Kulus, M., Kranc, W. *et al.* (2023). Current advances in bovine in vitro maturation and embryo production using different antioxidants: a review. *Animals*, 13(18), 2894. DOI: 10.3390/jdb11030036.

21 Palasz, A.T. et al. (2008). The effect of different zwitterionic buffers and PBS used for out-of-incubator procedures during standard in vitro embryo production on bovine oocytes and embryos. *Theriogenology*, 70, 1461–1470. DOI: 10.1016/j.theriogenology.2008.06.092.

22 Pascottini, O.B., Van Soom, A. et al. (2018). Holding immature bovine oocytes in a commercial embryo holding medium: high developmental competence for up to 10 h at room temperature. *Theriogenology*, 106, 139–146. DOI: 10.1016/j.theriogenology.2017.10.040.

23 Pytel, A.T. et al. (2024). Factors defining developmental competence of bovine oocytes collected for in vitro embryo production. *Biol Reprod.,* 111(1), 1–15. DOI: 10.1093/biolre/ioae065.

24 Salek, F. et al. (2025). Factors Affecting the Success of Ovum Pick-Up, In Vitro Production and Cryopreservation of Embryos in Cattle. *Animals*, 15(3), 344. DOI: 10.3390/ani15030344.

25 Singh, P. et al. (2026). Effect of CO_2-free HEPES-buffered media and semen preparation methods on developmental competence of OPU-derived Sahiwal cattle oocytes. *SSRN Electronic Journal,* 61.

26 Sprícigo, J.F.W. et al. (2015). Effects of different maturation systems on bovine oocyte quality, plasma membrane phospholipid composition and resistance to vitrification and warming. *PLoS ONE*, 10(6), e0130164. DOI: 10.1371/journal.pone.0130164.

27 Suttirojpattana, T. et al. (2017). Transporting bovine oocytes in a medium supplemented with different macromolecules and antioxidants: effects on nuclear and cytoplasmic maturation and embryonic development in vitro. *Animal Science Journal,* 88, 160–166. DOI: 10.1111/asj.12623.

28 Viana, J.H.M. (2021). Statistics of embryo production and transfer in domestic farm animals. *Embryo Technology Newsletter,* 40(4), 22–40.

29 Ward, F., Lonergan, P., Enright, B.P., Boland, M.P. (2000). Factors affecting recovery and quality of oocytes for bovine embryo production in vitro using ovum pick-up technology. *Theriogenolog*y, 54(3), 433–446. DOI: 10.1016/S0093-691X(00)00360-5.

Publisher Correction

Publisher Correction to: "Parasite fauna of wild ungulates in Kazakhstan: current state and analysis"

Kidiraliyev, E.Zh., Zhaksylykova, A.A., Aryssova, E.A., Abdybekova, A.M.

Central Asian Journal of Veterinary Science. **2026; 2(014), 25–34.**
Original article DOI: https://doi.org/10.51452/cajvs.2026.2(014).2211

Publisher Correction

Following publication of the article *"Parasite fauna of wild ungulates in Kazakhstan: current state and analysis"*, the Editorial Office identified a production error that occurred during the typesetting process.

The **Materials and Methods** section contained in the final manuscript accepted for publication was inadvertently omitted. Instead, text belonging to the subsequent **Results** section was placed under the heading *Materials and Methods*.

The correct *Materials and Methods* section reads as follows:

Materials and Methods

The literature review was conducted through a systematic search of the Scopus, Web of Science, and RSCI databases, as well as peer-reviewed journals indexed by the Committee for Quality Assurance in Science and Higher Education of the Republic of Kazakhstan and other relevant official sources. To ensure comprehensive coverage of the regional literature, specialized monographs and doctoral dissertation abstracts were also examined. The search strategy employed targeted keywords in Kazakh, Russian, and English, including *wild ungulates, parasite diversity, host–parasite relationships, epidemiology,* and *Kazakhstan*. The review primarily covered publications published between 1989 and 2026, while earlier landmark studies were included where necessary to provide a taxonomic and historical baseline. An initial search yielded approximately 120 publications, of which 50 peer-reviewed studies met the predefined selection criteria and were included in the final review.

Study selection was based on explicit inclusion and exclusion criteria. Eligible publications focused on parasite biodiversity, host–parasite relationships, and epidemiology in Kazakhstan and neighboring regions. Studies lacking reliable species-level identification, employing insufficiently documented parasitological methods, or falling outside the geographical and ecological scope of the review were excluded. The selected literature was subsequently organized according to parasite taxa, host species, and geographic distribution. The available data were synthesized using a qualitative comparative approach, incorporating infection parameters (prevalence and infection intensity) together with morphological evidence to identify regional epizootiological patterns and highlight existing knowledge gaps.

The online version of the article has been corrected by restoring the Materials and Methods section as presented in the final manuscript accepted for publication and by relocating the inadvertently duplicated text to its appropriate position within the article.

This correction does not affect the results, interpretation, conclusions, or scientific findings of the article. The Editorial Office apologizes to the authors and readers for this production error.

The original article has been corrected.

Central Asian Journal of Veterinary Science

GUIDELINES FOR AUTHORS

GENERAL PROVISIONS

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c) final approval of the version to be published.

Individuals who contributed to the work but do not meet the criteria for authorship, as well as organizations that provided financial support for the research, should be acknowledged in the **Acknowledgements** section at the end of the article.

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• **Discussion:** Provide a concise interpretation of the most significant findings, comparing the obtained results with those of other studies, and identifying patterns and/or discrepancies.

• **Conclusion:** Summarize the main conclusions and outline prospects for further research on the studied topic/problem.

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Examples:

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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MANUSCRIPT STRUCTURE

Research article

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

Sergey N. Borovikov[1], Kanatbek N. Mukantayev[2], Aitbay K. Bulashev[1], Kanat A. Tursunov[2] Alfiya S. Syzdykova[1], Ainagul B. Begenova[1]

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Abstract (max. 300 words)

Background and Aim.....
Materials and Methods.....
Results.....
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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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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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