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Isolation and characteristics of keratinophilic fungi from the objects of the external environment

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Abstract

Background and Aim. There is a growing number of fungal skin infections in animals worldwide. The frequent detection of mold fungi in association with dermatomycetes, or as a mono infection, indicates exogenous sources of infection.

The aim of this study is to isolate and characterize keratinophilic fungi from soil and other environmental sources.

Materials and Methods. A total of 198 environmental samples (soil, litter, feed, scrapings from feeders, fences, paddocks, and walls) were collected from Akmola, Almaty, Karaganda, and Pavlodar regions of Kazakhstan. Hair bait technique was used to isolate keratinophilic fungi. Cultural and morphological features were studied on universal dense agar and microscopically at ×10 and ×40 magnifications. Biochemical properties were determined using Hiss media, Christensen medium with 40% urea, and media with milk and gelatin.

Results. Ecologically significant saprophytic keratinophilic micromycetes were identified in the samples: *Trichophyton* spp. – 7%, *Phoma* spp. – 7%, *Marquandomyces* spp. – 7%, *Penicillium* spp. – 13%, *Lecanicillium* spp. – 7%, *Fusarium* spp. – 7%, *Alternaria* spp. – 7%, *Aspergillus* spp. – 6%, *Filobasidium* spp. – 13%, *Mucor* spp. – 26%.

Among isolates, 85% utilized sucrose, 77% maltose, 69% glucose, and 62% mannitol. Urease activity was observed in 70%, and protein-degrading enzyme activity (gelatin and casein) in 50% of isolates. All strains showed varying degrees of keratin assimilation. The most severe hair damage was caused by *Trichophyton* spp., *Penicillium* spp., *Alternaria* spp., and *Aspergillus* spp., which formed dense mycelial sheaths or conidia, leading to thinning and lysis of hair.

Conclusion. The wide distribution of saprophytic keratinophilic fungi in the environment suggests that animal infections are caused not only by classical dermatophytes like *Trichophyton*, but also by opportunistic keratin-degrading molds.

Keywords: dermatophytosis; keratin; keratin hydrolysate; keratinophilic fungi; environmental objects; soil.

Introduction

Currently, there is an active increase in the number of fungal skin infections in animals worldwide. It is believed that this is due to climate change, anthropogenic impacts, and disruptions of the ecosystem – soil, water, and other objects that are natural reservoirs of fungal spores [1, 2]. Dermatophytosis is

the most common fungal infection in animals and is caused by dermatophytes – the filamentous fungi *Micropsorum* spp., *Trichophyton* spp., *Nannizzia* spp., *Arthroderma* spp. and a number of other recently added genera [3, 4]. Dermatophyte-induced diseases are widespread worldwide. The annual number of fungal infections increases not only in wild and farm animals but also in humans and pets [5].

Y.N. Haggag et al. (2017) identified dermatophytes in 150 farm animals, including 50 cattle, 25 buffalo, 50 sheep, and 25 horses. In young animals (up to 2 years old), the infection rate was 38.76% in children and 35.33% in adults. In horses, dermatophytosis was more common in males (44%), whereas in other species, it was more common in females. The high incidence rate is explained by contact transmission, long-term viability of spores in the environment, stress factors (overcrowding, poor sanitation), seasonality (more common in winter), and weak immunity in young animals. In the study, they identified the following dermatophytes: *Trichophyton verrucosum* (47.33%) was the most common, isolated from cattle (48%), buffalo (44%), sheep (64%) and horses (16%); *T. mentagrophytes* (14%) was isolated from cattle (18%), buffalo (32%), sheep (4%) and horses (8%); *T. equinum* (7.34%) was isolated exclusively from horses (44%); and *Microsporum canis* (5.33%) was isolated from cattle (6%) and sheep (10%) [6].

A.P.N. Albano et al. (2012) studied 30 wild cats divided into two groups: 7 cats kept in temporary captivity and 23 caught in the wild. Among them were 11 pumas, 9 Geoffroy's cats, 4 margays, 2 ocelots, 2 tiger cats, 1 jaguarundi, and 1 jaguar. Dermatophytes were found in two animals: a Geoffroy's tiger cat in its ear canal (*T. mentagrophytes*) and a jaguar (infected by an unspecified species of *Trichophyton* spp.) The main cause of infection is probably contact with rodents that wild cats feed on, which confirms the role of these animals as possible asymptomatic carriers capable of spreading the infection among other animals and humans [7]. A study aimed at analyzing the mycobiota of the skin and hair of domestic animals with an emphasis on the role of dermatophytes as a potential source of infections for humans and animals showed that dermatophytes were present in 44.1% of cats and 43.8% of dogs. The most frequently detected species were *M. canis* (17.7% in cats) and *T. mentagrophytes* (23.5% in cats, 25% in dogs). In some cases, dermatophytes were detected in combination with yeasts such as *Candida albicans* and *Rhodotorula mucilaginosa*. Detection of the dermatophytes *M. canis* and *T. mentagrophytes* on the skin and hair of animals indicates a high probability of infection from the external environment, which serves as a natural reservoir for these microorganisms [8]. Fungi play a key role in ecosystems as natural organic matter recyclers that return nutrients to the soil [9]. Soil, air, water, organic materials, and other environmental objects are sources of fungi, including ecologically significant and pathogenic species [10]. Fungi present in various natural environments colonize and decompose animal remains rich in keratin, a protein with high nitrogen and sulfur content. In the soil, keratin from keratinized remains of the skin and its appendages is decomposed by saprophytic microorganism - biodestructors that synthesize the enzyme keratinase, such as *Chrysosporium* spp., *Psuedogymnoascus* spp., *Geomyces* spp., *Pectinotrichum* spp., *Renispora* spp., and a number of others [11, 12]. *Aspergillus, Penicillium, Fusarium, Microsporum, Trichoderma*, and *Chrysosporium* are the most common keratinolytic fungi [13]. The surface and deep soil layers are the largest reservoirs and natural habitats of these keratinophilic fungi, as they contain keratin, most often in the form of mammalian hair (mainly rodents), bird feathers, claws, horns, and a number of other keratinized animal remains [14, 15].

From 40 soil samples collected from urban waste and pastures in Kanpur, 83 fungal species were isolated, among which *Chrysosporium, Microsporum, Trichophyton,* and *Aspergillus* were predominant. The authors found that urban waste and pasture soils are rich sources of keratinophilic fungi, including potential pathogens, highlighting their role in waste biodegradation and associated health risks [16].

Studying the ability of fungi isolated from soil to degrade chicken feathers and human hair, *J. Kumar* et al. (2020) revealed the keratinolytic ability of 11 strains of keratinophilic fungi. The results showed active keratin substrate degradation with the formation of perforating structures and chemical changes, which were particularly noticeable in *Chrysosporium indicum* and *Ch. tropicum* [17].

Isolation and identification of keratinophilic fungi from livestock barn soils in Cayenne, South Khorasan Province, Iran, using the hair bait method and molecular methods from 62 samples revealed 118 fungal isolates belonging to 7 species from 5 genera. The predominant species was *Aphanoascus verrucosus* (59.36%), followed by *Arthroderma quadrifidum, A. terreus, Acremonium* spp*., A. gertleri, Fusarium equiseti,* and *Uncinocarpus reesii* [18].

When analyzing the mycobiota of oil-contaminated soils and the surface air layer in the Binagadi district of Baku, it was found that oil pollution contributes to an increase in the species diversity of micromycetes. The authors identified 34 fungal species from 10 genera in the soil and 25 species from 8 genera in the surface air layer. The genera *Aspergillus, Penicillium, Cladosporium,* and *Fusarium* occupied the main position. For species such as *A. fumigatus* and *A. alternata*, increased rates of development and sporulation were observed [19]. Commonly found in water systems are genera of pathogenic fungi, such as *Aspergillus* spp., *Penicillium* spp., *Candida* spp., *Fusarium* spp., and *Trichoderma* spp. These organisms can contaminate water supplies, posing a serious health threat, especially to immunocompromised animals and humans [20].

In rural Ismailia, Egypt, 15 keratinophilic fungi, including 8 *Chrysosporium* species, were isolated using the hair bait method. *C. zonatum* was mainly isolated from soil collected from fields, animal cages, and the hair of cows and buffaloes [21].

In India, nine fungal species belonging to 6 genera were isolated from the feather samples of 117 birds representing 11 species tested for keratinophilic fungi. Among these, four species were identified as members of the genus *Chrysosporium: Chrys. indicum* (26.4%), *Chrys. tropicum* (11.1%), and *Chrys. Aphanoascus* spp. (2.5%) and *Arthroderma tuberculatum* (3.4%) [22].

The frequent presence of mold fungi in pathological material in association with dermatomyces or the detection of mono infection in mold mycoses of the skin indicates exogenous sources of infection, emphasizing the importance of the ecological aspect in the epidemiology of fungal infections [23]. It is believed that anthropogenic pollution stimulates the functional activity of the soil mycobiota. This contributes to the transformation of saprotrophic fungi into opportunistic forms, increasing the risk of infections, especially in urban environments [5, 19].

When studying pathological material (hair, crusts) from affected skin areas of commercial sables, collected in 2018–2019 in various regions of Russia, 18 taxa of fungi were identified. Keratinophilic dermatophytes (*Arthroderma cuniculi, Chrys. carmichaelii*) and secondary opportunistic pathogens were found in 12% of the samples–including representatives of the genus *Aspergillus* spp. (36%), *Scopulariopsis* spp. (16%), and *Acremonium* spp. (14%) [24].

Analysis of biological material from domestic and wild animals in northern Kazakhstan showed that dermatomyces of the genera *Trichophyton* spp. and *Microsporum* spp. were detected in only 17.1% of cases, whereas opportunistic mold fungi of the genera *Mucor* spp., *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Chaetomium* spp., *Eurotium* spp., *Phoma* spp., *Trichoderma* spp., *Lecanicillium psalliotae*, *Scop. brevicaulis*, and others were present in 50.2%. Yeasts of the genera *Candida* spp., *Rhodotorula* spp., *Exophiala* spp. accounted for 5.1% of cases [25]. Analysis of pathological material samples from calves with clinical signs of dermatophytosis in the Almaty, Turkestan, and Kyzylorda regions of Kazakhstan indicated that the main causative agent of the disease was the fungus *Trichophiton* sp., which was identified using microscopic methods (in 86% of cases), microbiological methods with the isolation of a pure culture (in 79% of cases), and the polymerase chain reaction method, which showed the highest sensitivity (97.9% [26]. In the northern region of Kazakhstan, the most commonly identified etiological agent of dermatophytosis is *Trichophiton* spp.[27]. In Kazakhstan, fungal skin diseases are commonly observed in humans. In eastern Kazakhstan, among the causative agents of dermatomycosis in humans, *Microsporum* spp., *Trichophyton* spp. the frequency of sowing increasing [28]. In 2022, in the Kostanay region, fungal skin diseases affected 51 people. In the first half of 2022 in Kyzylorda, 76 people were infected with dermatophytosis. In Almaty, in 2023, the incidence of fungal skin diseases among people increased by about 1.5 times compared with the previous year. The highest incidence of dermatophytosis was recorded in children agedunder 14 years (85% of all cases) [26].

The data we found during the analysis of the literature emphasize the relevance of soil monitoring for the presence of keratinophilic fungi and the need for prevention to reduce the risk of fungal infections.

The aim of this study was to isolate and identify keratinophilic fungi present in soil and other environmental objects.

Materials and Methods

The material consisted of 113 samples of environmental objects (EO): soil, litter, feed, scrapings from feeders, fences of pens, and walls from different regions of Kazakhstan–Akmola, Almaty, Karaganda, and Pavlodar regions.

To isolate keratinophilic fungi, soil samples were collected from a depth of 5 cm using a metal spatula and placed in wide-necked, sterile glass jars with a capacity of 500 ml. Scrapings were made from walls, fences, posts, and feeders in places where wool- or fat-like accumulations were found. The litter and contents of the feeders were collected using tweezers and packed in zip-lock bags. The feed remains were collected from the bottoms of the feeders, from storage areas at the soil boundary, and in the passages where the animals were fed [29].

The samples were poured into a glass jar and then spread in a 5-mm layer on the bottom of sterile Petri dishes, and hair baits were prepared. For this purpose, hair, previously cut into 3-cm-long pieces and autoclaved at 121 °C for 20 min, was evenly spread on the surface of the samples. Petri dishes wrapped in paper were placed in a dark place at 25 °C for 3-4 weeks, and the soil was periodically moistened as needed [30].

Sabouraud dextrose agar was used for the primary isolation of fungi and for obtaining a pure culture by the direct plate method. Samples of the biomaterial were placed on the surface of the agar medium using sterile tweezers. The Petri dishes were placed in a thermostat and incubated at a temperature of 28 °C. To isolate and identify keratinophilic fungi from EO, pieces of soil, feed, bedding, etc., were transferred to a Petri dish with sterile filter paper soaked in liquid Sabouraud medium, where traps for keratinolytic fungi were laid out in the form of children's or women's hair cut into pieces of 2–3 cm.

To identify the keratinophylic properties of the fungi, we used Sabouraud dextrose agar with the addition of 2% sterilized hair. The medium created favorable conditions for the growth of fungi with keratinolytic activity.

To study saccharolytic properties, Giss nutrient media with glucose, mannitol, lactose, sucrose, and maltose were used. The cultures were sown in test tubes using the prick method and incubated at 28 °C under constant observation for color changes, turbidity, and gas formation. To identify urease activity, we determined the ability of the fungi to decompose urea to ammonia on Christensen medium with the addition of 40% urea. Changes in the medium that occurred during cultivation were visually assessed, and the intensity of the reaction was expressed in crosses (from “+” to “++++”) [31].

The proteolytic activity of the fungi was studied in gelatin and skim milk. Meat-peptone agar with the addition of gelatin was used to assess the ability of fungi to hydrolyze gelatin [32]. Cow’s milk, previously skimmed by centrifugation at 3000 rpm and 2 °C for 60 min, was used to study casein breakdown. Sowing was performed using the prick method in test tubes, after which the tubes were incubated in a thermostat at a temperature of 28 °C for 7 days.

The identification of the resulting cultures was carried out by considering colony growth and morphological characteristics. For microscopy, scotch tape preparations were prepared and examined under a light microscope at a magnification of ×40. The identification of pathogens was carried out using identifiers [33].

Results and Discussion

Analysis of the isolation of fungi and bacteria from samples of EO showed that 57.01% of the samples were positive (Table 1).

Table 1 – Isolation of micromycetes from environmental objects

Sampling location, region	Number of samples	Including						Of which, positive
		soil	cage	feeders	litter	burrow	Feed	
Akmola	107	20	26	22	15	16	8	89
Karaganda	66	13	11	7	15	20	-	12
Pavlodar	15	4	5	1	4	-	0	1
Almaty	10	3	2	3	2	-	-	1
Total:	198	40	44	33	36	36	9	113

As shown in Table 1, the highest frequency of micromycete detection was noted in the Akmola (83.2%) and Pavlodar (73.3%) regions, and lower in the Karaganda (18.2%) and Almaty (10%) regions.

In the Akmola region, 107 samples of EO were examined. Among these, micromycete growth was observed in 89 cases (83.2%). In the Karaganda region, fungal growth was detected in 12 of 66 samples, representing 18.2%. In the Pavlodar region, in 33.3% of cases, no growth of micromycetes was noted. Of the 15 samples, in one case, growth of the micromycete *Mucor plumbeus* was noted, which amounted to 6.7%. At the same time, 10 bacterial strains (66.7%) were identified in the Pavlodar region. In the Almaty region, out of 10 samples, fungal growth was also observed in only one case (*Mucor plumbeus*), corresponding to 10% growth, with no growth in 90% of samples.

Analysis of the spectrum of the identified micromycetes made it possible to establish that the frequency of isolation of *Alternaria* spp. was 38.94%, followed by *Fusarium* spp. – 22.13%, *Mucor* spp. – 14.17%, *Penicillium* spp. – 8.85%. Bacteria were isolated in 8.85% of the cases. Less frequently, fungi of the species *Lecanicillium saksenae* and *Filobasidium magnum* were isolated (each at 1.77%). Other species, including *Aspergillus cristatus, Phoma livicola, Marquandomyces* spp., and *Trichophyton verrucosum*, were detected even less frequently (0.88% in each case) (Figure 1).

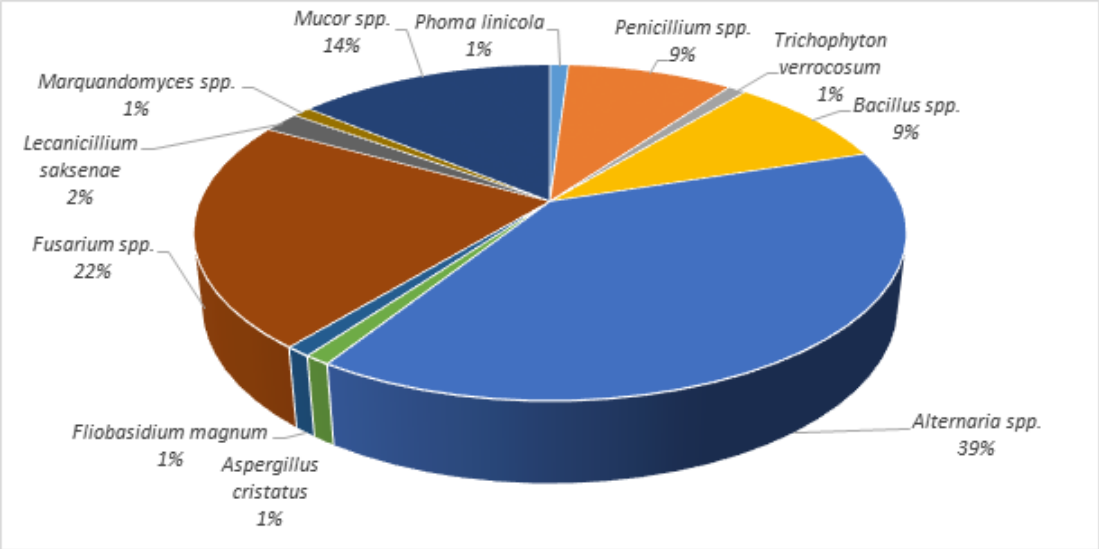

Figure 1 – Spectrum of microorganisms isolated from environmental objects

Cultivation of EO on Sabouraud media with traps for keratinolytic fungi allowed us to isolate colonies of predominantly white fungi (shown by the arrow), which differed in appearance from typical dermatophyte colonies (Figure 2).

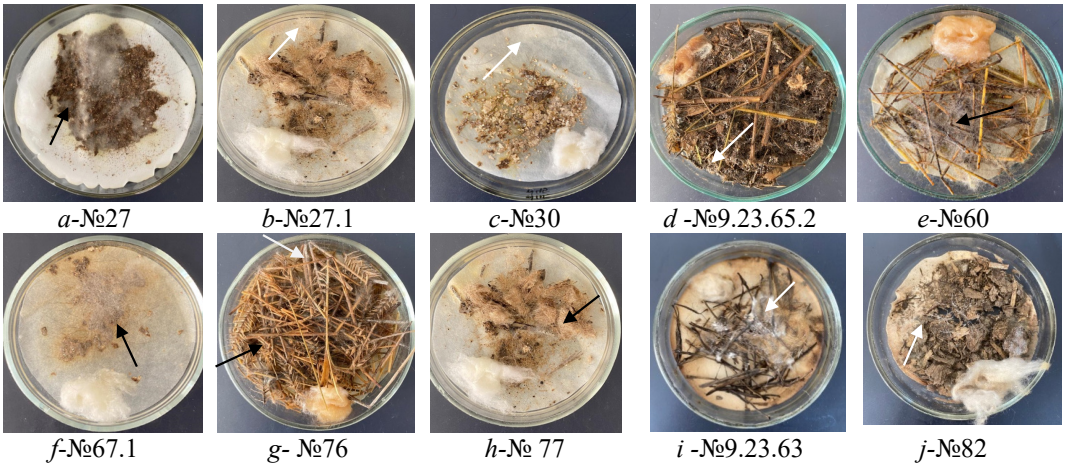

Figure 2 – Isolation of keratinophilic fungal isolates from soil (d, e, f, j), litter (a, b, c), feeders (g, h, i)

Figure 2 shows that in some Petri dishes, colonies of micromycetes developed on the hair. In samples № 27 (litter) and № 67.1 (soil), colony growth occurred along the length of the hair, and the formation of clearly visible white fluffy colonies was observed. In samples № 27.1 (soil) and № 60 (feeder), the formation of white colonies was noted along the entire hair and along the edge of the Petri dish. In sample № 30 (litter), the formation of dense colonies was observed, and in samples № 76, № 77 (scraping from the soil) and in № 9.23.65.2, № 9.23.63, and № 82 - fluffy white colonies were observed. Analysis of all 113 strains of microorganisms isolated from samples of EO from the territory of livestock farms in four regions of Kazakhstan for the presence of keratinophilic properties showed that only 25 strains (22.1%) were positive for keratinase. It should be noted that of the total number of positive samples, keratinophilic bacteria (10 strains) were isolated in 40% of cases, and keratinophilic micromycetes (15 strains) were isolated in 60% of cases, respectively.

In all cases, keratinophilic bacterial strains were isolated from environmental samples collected from the Pavlodar region (Figure 3).

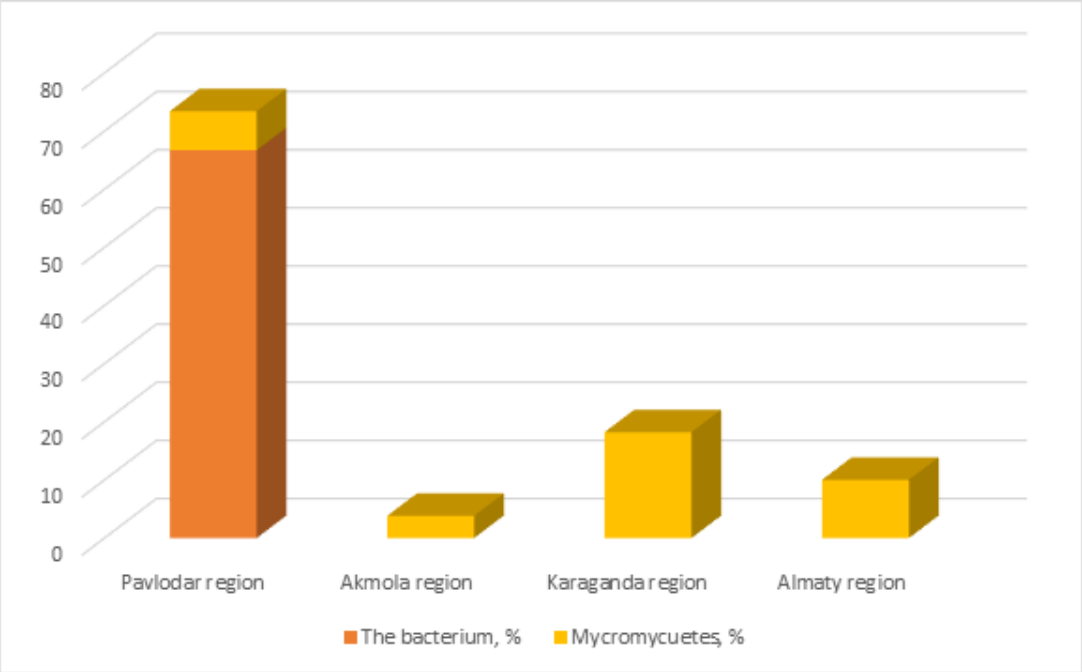

Figure 3 – Results of identifying keratinophilic strains of microorganisms from environmental objects by region of Kazakhstan

As shown in the diagram, by region, the percentage of keratinophilic fungal strains isolated from environmental samples collected in the Akmola, Karaganda, and Almaty regions was 3.8%, 18.2%, and 10.0%, respectively. In the Pavlodar region, bacteria (shown in green) were isolated in 66.7% of samples, which were not analyzed in further studies given the study's goal, and micromycetes were isolated in 6.7% of samples. It should be noted that a larger number of keratinophilic micromycetes were found in the samples of the Karaganda region's organic matter.

The spectrum of the isolated mycelial keratinophilic fungi consisted of representatives of the genera *Trichophyton* spp., *Phoma* spp., *Marquandomyces* spp., *Penicillium* spp., *Lecanicillium* spp., *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Filobasidium* spp., and *Mucor* spp. (Figure 4).

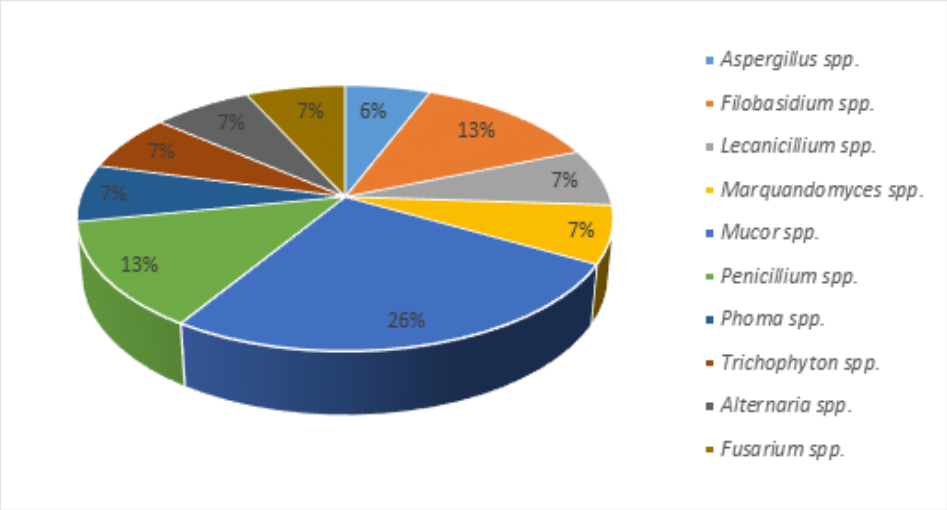

Figure 4 – Spectrum of mycelial keratinophilic fungi isolated from environmental objects

The identification of pure cultures of keratinophilic fungi isolated from the EOS by cultural and morphological properties allowed us to identify *Trichophyton* spp., *Phoma* spp., *Marquandomyces* spp., *Penicilium* spp., *Lecanicillium* spp., *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Filobasidium* spp., *Mucor* spp. The fungal cultures differed in terms of morphological features (Figure 5).

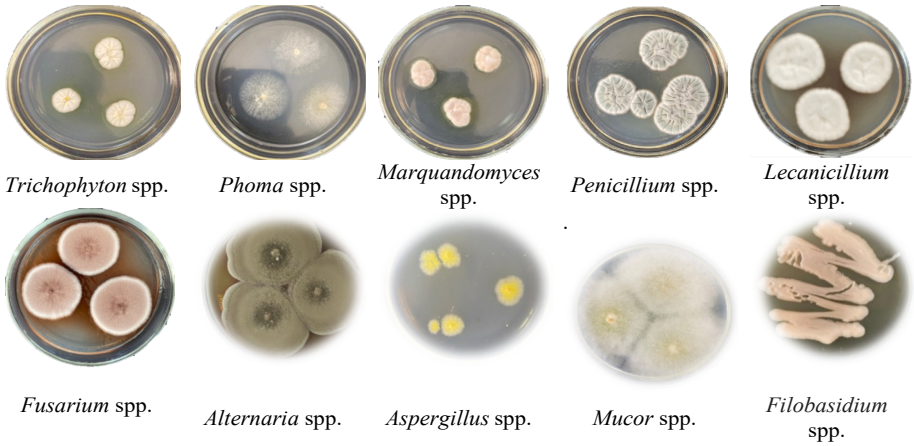

Figure 5 – Keratinophil cultures isolated from environmental objects

Figure 5 clearly shows the morphological features of the strains. The white *Trichophyton* spp. colony had a velvety surface, formed yellow exudate drops, and exhibited moderate growth. The Phoma spp. colonies had white fluffy mycelium on the front side, a light reverse, abundant yellowish exudate, and rapid growth. The *Marquandomyces* spp. isolate colony had a light cream, mealy surface with a translucent edge and dense consistency. The *Penicillium* spp. isolate formed classic, well-known, rounded and dense colonies of rich dark green color that are unevenly distributed over the surface of the substrate. The colony of the *Lecanicillium* spp. isolate had a dense, slightly fluffy texture and a snow-white color. The surface was velvety with clearly defined edges. The *Fusarium* spp. colony was light pink with white edges. It had a convex, folded surface and a crater-shaped center, and its edges were uneven. *Alternaria* spp. colonies were dark green, irregular in shape, with a bumpy profile and a rhizoid center. The edges of the colony were round and irregular, with mycelium in the form of threads. The isolated *Aspergillus* spp. formed colonies of a rounded shape with a bright yellow color, fluffy and velvety in texture, and with wavy, fuzzy edges. The isolated *Mucor* spp. colony was white with a cream center, fluffy, cotton wool-like, with fuzzy, blurred edges. The colony of the *Filobasidium* spp. isolate had a smooth, shiny, and dense texture of cream color. The microscopic identification results of the strains are presented in Figure 6.

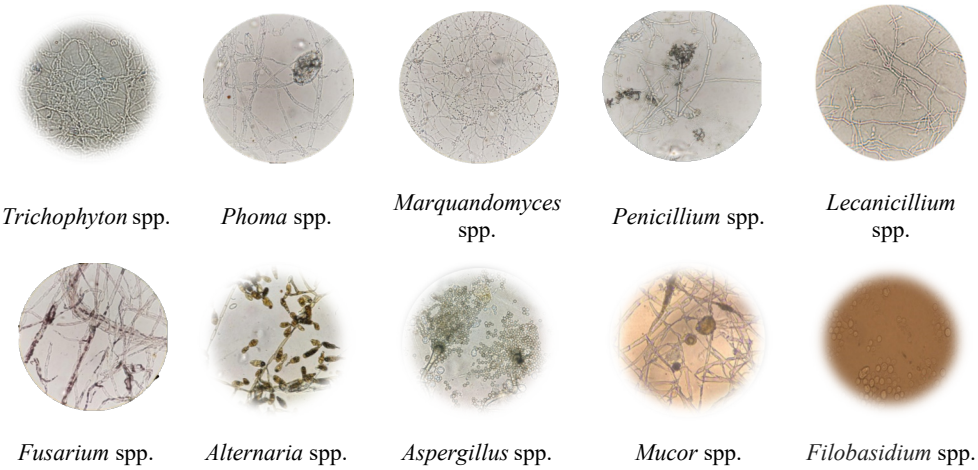

Figure – 6 Results of microscopic identification

As shown in Figure 6, *Trichophyton* spp. have thin septate hyphae, microconidia, and fusiform macroconidia. Upon microscopy, *Phoma* spp. exhibit the formation of pycnids with unicellular ellipsoidal conidia. *Marquandomyces* spp. have separate hyphae. *Penicillium* spp. show tassel-shaped conidiophores and chains of spherical conidia. *Lecanicillium* spp. display branched conidiophores with phialides. *Fusarium* spp. have a septate mycelium of burgundy color. *Alternaria* spp. have septate, light brown mycelium and conidia with three to eight transverse and two longitudinal septa. *Aspergillus* spp. have septate hyphae and conidiophores with a bubble-shaped head covered with phialides and conidia. *Mucor* spp. form wide, aseptate hyphae with large sporangia. *Filobasidium* spp. are yeast-like fungi with thin, septate hyphae and blastospores. The results of the analysis for the presence of saccharolytic activity of some keratinophilic isolates are shown in Figure 7.

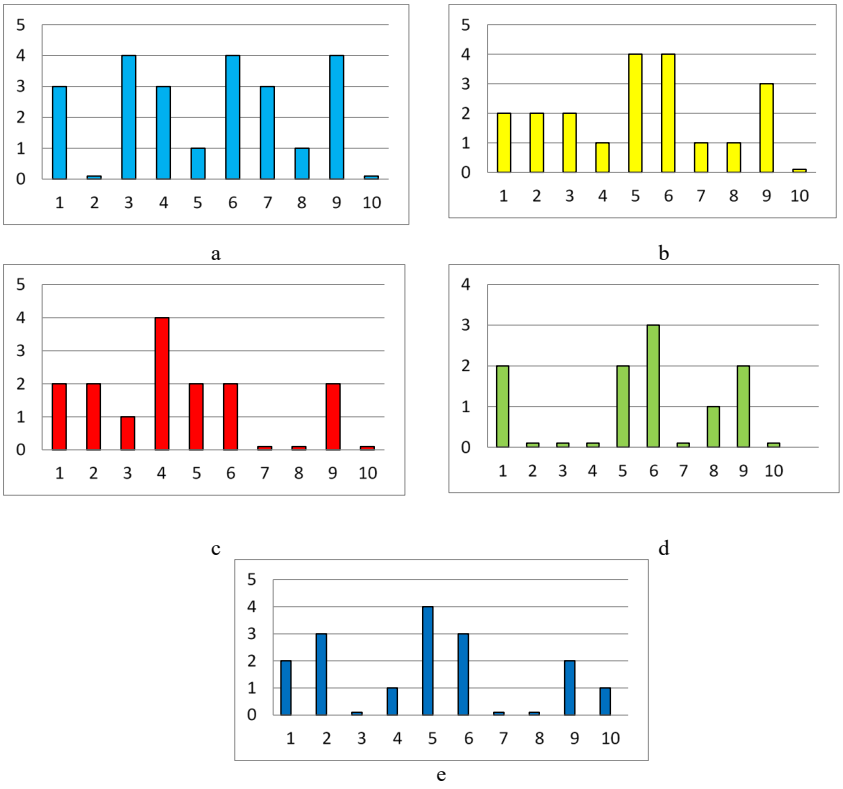

Figure 7 – The results of the saccharolytic activity of isolates:
1- *Trichophyton* spp., 2- Phoma spp., 3-*Marquandomyces* spp., 4-*Mucor* spp., 5-*Penicillium* spp., 6-*Lecanicillium* spp., 7- *Filobasidium* spp., 8- *Aspergillus* spp., 9- *Fusarium* spp., 10- *Alternaria* spp. isolated from environmental objects: a – sucrose, b – maltose, c – glucose, d – lactose, d – mannitol

According to Figure 7, sucrose was metabolized most actively by the isolates (85%), and maltose was well absorbed (77%). Glucose was absorbed in 69% of the isolates, mannitol in 62%, and lactose in 46% of the isolates. Thus, the isolates exhibited the greatest metabolic activity in relation to sucrose and maltose, which is probably due to their adaptation to these substrates in the environment.

The urease and proteolytic activity of the isolates was studied by assessing the cleavage ability of urea, gelatin, and casein on special media. The research results are shown in Figure 8.

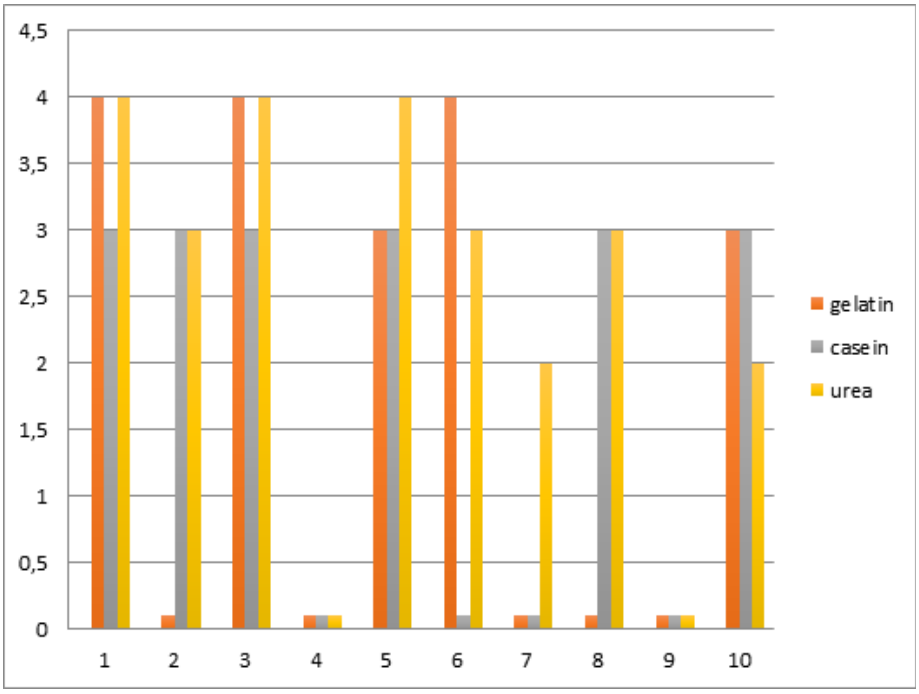

Figure 8 – Results of urease and proteolytic activity of isolates isolated from environmental objects

As shown in Figure 8, the largest number of isolates exhibited urease activity (70%), indicating their high metabolic activity against nitrogen-containing compounds. At the same time, hydrolytic activity against proteins (gelatin and casein) was noted in 50% of the isolates, indicating moderately pronounced proteolytic activity of the studied strains.

Thus, micromycetes exhibit a wide range of enzymatic capabilities, with greater activity toward urea.

The hair on which fungal growth was noted was examined under a microscope from each sample of EO to identify keratinophilic properties. The microscopy results are shown in Figure 9.

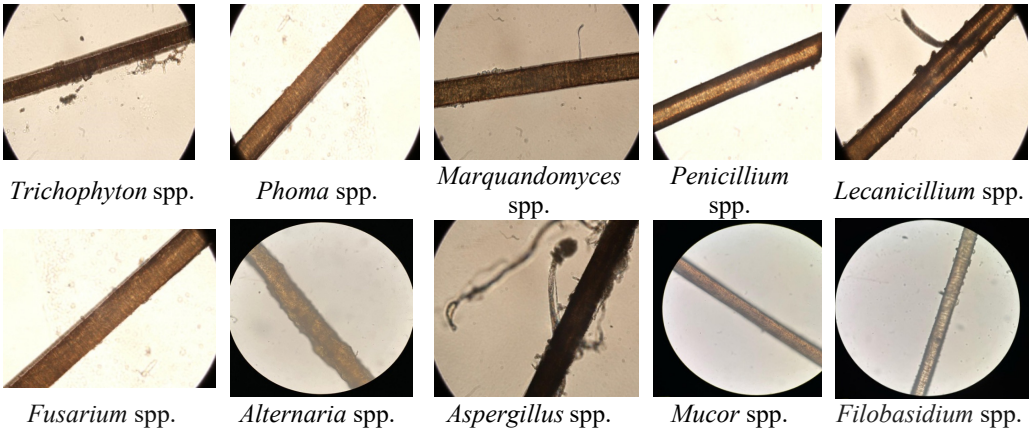

Figure 9 – Microscopy results of hair showing the growth of keratinophilic fungi:
a – *Trichophyton* spp., b – *Phoma* spp., c – *Marquandomyces* spp., d – *Penicillium* spp.,
e – *Lecanicillium* spp., f – *Fusarium* spp., g – *Alternaria* spp., h – *Aspergillus* spp., i – *Mucor* spp.,
j – *Filobasidium* spp.

As shown in Figure 9, *Trichophyton* spp. exhibited mycelium emerging from the hair. A small growth of fungal mycelium was observed in *Phoma* spp. *Marquandomyces* spp. had smooth hair and no visible colonies. *Penicillium* spp. Showed destruction of hair with visible filaments, indicating an intensive decomposition process. *Lecanicillium* spp. had large filaments of fungal mycelium tightly surrounding the hair. *Fusarium* spp. appeared almost clean, but when magnified, individual spores were visible, indicating the presence of keratinophilic properties. The mycelium of *Alternaria* spp. was present around the hair. *Aspergillus* spp. showed good fungal growth around the hair, with a characteristic conidial head. *Mucor* spp. and *Filobasidium* spp. had smooth, even hair.

It is well known that the soil is a reservoir of geophilic keratinophilic fungi, whose biological role is to decompose keratin in the form of keratinized remnants of skin, its derivatives, and other possible sources of animal keratin in the soil, on the surface of the earth, and litter. The keratinophilic fungal species *Chrysosporium* spp., *Psuedogymnoascus* spp., *Geomyces* spp., *Pectinotrichum* spp., and *Renispora* spp. possess biologically significant properties. and a number of others can use predigested keratin residues or by-products of keratin degradation [34].

P. Shivanand, F.H. Yakop (2019) conducted a study on the mycobiota of the soils of forest ecosystems with a focus on geophilic fungi and their pathogenic potential. The studies were conducted on soil samples collected from various natural areas, including pine and mixed forests. Using molecular biological and cultural methods, genetically modified fungal species such as *Aspergillus* and *Penicillium* have been discovered, among which common pathogenic species such as *Aspergillus fumigatus* and *Penicillium marneffei* [31, 35].

As part of the study, we analyzed 117 samples, of which the growth of micromycetes was recorded in 16 samples, representing 13.7% of the total number of samples. In the Akmola region, out of 26 species, only one appeared in one place (*A. cristatus*) for the first time (3.8%). In the Karaganda region, out of 66% of cases, it was noted in 10 cases (15.2%), where species such as *Lecanicillium saksenae, Penicillium chryseogenum, Filobasidium magnum, Mucor plumbeus, Trichophyton verrucosum, Phoma livicola* and *Marquandomyces* spp. were identified. In the Pavlodar region, out of 15 samples, growth was detected in one case (*Mucor plumbeus*), which is 6.7%. A similar situation was noted in the Almaty region, where, out of 10 samples, growth was detected in only one sample (*Mucor plumbeus*), representing a 10% growth rate. Samples in the amount of 101 (86.3%) micromycetes showed no growth, which is probably due to the lack of favorable conditions for micromycetes development. The main similarity between the previous studies and our results lies in the identification of the same dominant genera of fungi (*Aspergillus, Penicillium*).

The study by *Z. Tyszkiewicz* and *M. Krasowska* (2022) was devoted to studying the mycobiota of soils in buffer zones in the agricultural landscape in order to identify the diversity of micromycetes and their impact on carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions. Soil samples were collected from two different zones: under trees near arable land and under grass cover near pastures. Several fungal genera have been identified using cultivation and microscopic analysis methods, including *Penicillium, Pseudogymnoascus* and *Chrysosporium*. *Pseudogymnoascus roseus* showed the highest amount in both soil types. We found that the soil under trees has a higher diversity of fungi as well as higher microbiological activity, which leads to increased emissions of co_2 and NO_2. Both studies used cultural methods of fungal isolation and microscopic analysis and also considered the influence of soil characteristics on the diversity of micromycetes. A similar genus of fungi, such as Penicillium, was also identified in the works, which confirms their widespread distribution. However, unlike our study aimed at identifying keratinophilic fungi with pathogenic potential, the work of *Tyszkiewicz* and *Krasowska* focused on soil-based micromycetes in agricultural buffer zones and their impact on CO_2 and N_2O emissions. In addition, that study did not consider the pathogenic properties of fungi, whereas our analysis focused on the pathogens of opportunistic mycoses [36].

Various authors have reported the detection of fungi of various genera in the affected skin areas of domestic animals. Among them are the genera *Aspergillus, Alternaria, Chaetomium, Phoma, Penicillium, Cladosporium, Candida* and other representatives of mold fungi and yeasts [28, 29]. *Aspergillus, Penicillium, Fusarium, Microsporum, Trichoderma* and *Chrysosporium* are the most common cryptolytic fungi [13].

Our studies confirmed the isolation of representatives of fungal genera such as *Trichophyton* spp., *Phoma* spp., *Marquandomyces* spp., *Penicillium* spp., *Lecanicillium* spp., *Fusarium* spp., *Alternaria* spp., and

Aspergillus spp. from EO such as soil, litter from stalls, scrapings from feeders, and walls of livestock buildings, etc. spp., *Filobasidium* spp*., Mucor* spp. At the same time, a high frequency of isolation of keratinophilic *Aspergillus* and *Penicillium* species has been reported by many [31, 13]. Animals, in turn, are most often infected with fungal infections through contact with objects such as soil, water, and organic materials due to their high content of fungal spores.

Using the Hiss medium allowed us to evaluate the saccharolytic activity aimed at studying the ability of fungi to metabolize various carbohydrates, such as sucrose and maltose, which turned out to be the most digestible (85% and 77% of isolates, respectively), while lactose and mannitol demonstrated lower digestibility.

Proteolytic activity was evaluated to study the ability of fungi to break down proteins; 69% of isolates showed high activity on gelatin, but only 46% on milk, indicating their different enzymatic orientation. It should be noted that, along with keratinophilic properties, these representatives revealed the activity of other enzymes that promote the breakdown of various sugars and animal proteins: sucrose, maltose, mannitol, gelatin.

Urease activity was studied to determine the ability of fungi to decompose urea, which revealed positive results in 67% of the isolates, demonstrating their metabolic flexibility in conditions rich in nitrogen-containing compounds. The presence of high urease activity has been established, which indirectly indicates the potential to live on the skin of living organisms and assimilate nitrogen compounds, particularly urea. The data explain how fungi adapt to various substrates in the external environment, which are their natural reservoirs.

Based on the results obtained, we should agree with the opinion of scientists who stated that the ability of saprophytic fungi to destroy and assimilate keratin found in surface tissues places them in the category of opportunistic pathogens of skin mycoses and determines the pathogenicity of these fungi [10]. The widespread distribution of soil saprophytes with keratinophilic properties in the soil as a reservoir allows us to conclude that animals are infected not only with classical geophilic dermatophytes of the genus Trichophyton (for example, *Trichophyton ajelloi, Trichophyton flavescens, Trichophyton gloriae, Trichophyton terrestre, Trichophyton mentographytes,* variant gypseum, and a number of others), but also with opportunistic ecologically significant keratinophilic fungi.

Conclusion

Samples of EO (soil, litter from stalls, scrapings from feeders and walls of livestock buildings, etc.) were collected and revealed the presence of ecologically significant saprophytic keratinophilic micromycetes, including: *Trichophyton* spp. – 7%, *Phoma* spp. – 7%, *Marquandomyces* spp. – 7%, *Penicilium* spp. – 13%, *Lecanicillium* spp. – 7%, *Fusarium* spp. 7%, *Alternaria* spp. – 7%, *Aspergillus* spp. – 6%, *Filobasidium* spp. – 13%, Mucor spp. – 26%.

Among keratinophilic strains with high enzymatic activity, sucrose (85%), maltose (77%), glucose (69%), and mannitol (62%) were the most actively metabolized. Furthermore, 70% of the isolates exhibited urease activity, indicating high metabolic activity toward nitrogen-containing compounds. The activity of enzymes against proteins (gelatin and casein) was observed in 50% of the isolates.

All strains absorbed keratin to varying degrees. Penicillium spp. demonstrated clear hair destruction with visible filaments, indicating intensive keratin degradation. In contrast, *Trichophyton* spp., *Aspergillus* spp., and *Alternaria* spp. actively formed dense mycelium sheaths around the hair, but without pronounced structural damage, suggesting a strong colonization capacity rather than direct lysis of hair tissue.

Authors' Contributions

YK, TG and PR: Concept development, design and planning of the study, data collection and analysis, critical review of the article and final approval, research, statistical analysis. ZK and GB: Conducted a comprehensive literature search and conducting research. All the authors have read, reviewed and approved the final version of the manuscript.

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UDC 68.41.29 **Research article**

Analysis of the epidemiological and epizootic situation of alveolar echinococcosis in the world

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Abstract

This review article presents literature data on the distribution of alveolar echinococcosis cases across the world over the past 30 years (1993-2023), statistical data from the WOAH for the past 5 years (2020-2024), and the research results reported by domestic scientists.

Alveolar echinococcosis, also known as multilocular echinococcosis, is one of the most dangerous zoonotic parasitic infections. The causative agent, the cestode Echinococcus multilocularis, infects carnivorous animals, small rodents, and humans. Humans, as accidental intermediate hosts, are at high risk for severe complications, including liver and other organ damage. This infection has been recorded in Canada, the USA, Germany, France, Switzerland, Austria, Belgium, the Netherlands, the Czech Republic, Slovakia, Sweden, Denmark, the UK, China, India, Pakistan, Nepal, Bhutan, Iran, Iraq, Mongolia, Lithuania, Latvia, Estonia, Russia, Belarus, the Kyrgyz Republic, and the Republic of Kazakhstan.

The World Health Organization classifies this infection as one of the 17 neglected diseases requiring control and elimination by 2050.

Keywords: alveolar echinococcosis; definitive host; epidemiological situation; epizootiological monitoring; intermediate host; WOAH.

Introduction

Alveolar echinococcosis (AE) is one of the most dangerous zoonoses prevalent in countries with temperate and cold climates. The disease poses a particular threat in regions of Europe, Asia, and North America, where high morbidity rates are detected among wild and domestic animals.

Echinococcus multilocularis has a complex life cycle involving definitive and intermediate hosts. Wild carnivores, such as foxes and raccoon dogs, are the primary carriers, and dogs can become infected by consuming infected rodents. The feces of infected animals contain the parasite eggs, which contaminate the environment and can infect intermediate hosts. Humans serve as intermediate hosts and a biological dead end for *E. multilocularis*.

Human infection occurs through the following three major routes:

- Direct exposure to wild carnivores (such as Arctic foxes and foxes) through contact with their pelts and ingestion of oncospheres present on their fur;
- Consumption of wild plants, berries, or water from sources used by wild carnivores; and
- Exposure to dogs, which become actively infected by feeding on wild rodents. In this case, human infection occurs under the same conditions as in echinococcosis.

High infection risks (within endemic areas) are found among hunters and their families, fur trappers, taxidermists, and rural residents, where dogs play a significant role in daily life and farming. In such cases, children are at particularly high risk.

The most comprehensive analysis of the global distribution of AE cases was conducted by *P.R. Torgerson, K. Keller, M. Magnotta* and *N. Ragland.* In 2010, they published an article using publications from authors worldwide and a stochastic approach to uncertainty modeling. They stated that AE is highly endemic in Sichuan, Gansu, Qinghai, and Ningxia provinces in China, although the actual incidence in the Tibet Autonomous Region may be much higher than the estimated incidence. AE is also found in the USA, Canada, Switzerland, southern Germany, eastern France, and Russia, which is a major endemic zone for AE. Unlike China, Russia lacks publications on mass ultrasound screenings of the population; however, several serological studies confirm the possibility of a high number of cases, especially in Siberia [1].

In China, the majority of human AE cases have been confirmed in Tibet, Xinjiang, Sichuan, Qinghai, and Gansu. In Japan, the endemic zone for AE is Hokkaido Island, where approximately 20 human cases are confirmed annually [2-4]. Studies by *X. Wang, J. Liu* and *Q. Zuo* have demonstrated that voles are probably more important natural intermediate hosts for both *E. multilocularis* and *E. shiquicus* in Shiqu County on the eastern Tibetan Plateau. Therefore, they recommended that future studies on human AE epidemiology should include small mammals as a vital component for research and control purposes [5].

AE foci are generally associated with the habitats of definitive hosts involved in the life cycle of the parasite. In the USA and northern Canada, foci are related to the habitats of the Arctic fox; in Austria, Switzerland, and southern Germany, foci are related to the habitats of the red fox; and in Japan, foci are related to the habitats of other fox species. In Russia, the largest infection foci are found in Chukotka and Kamchatka [6].

A high prevalence of AE has been reported in Kyrgyzstan [7]. According to statistical reports from medical institutions and regional disease prevention centers of the Kyrgyz Republic from 1996 to 2018, the average long-term incidence rate of AE per 100,000 population was 2.2, with a minimum of 0.4 (1996) and a maximum of 3.9 (2015) [8].

An isolated AE case was reported in India in a man from the mountainous regions of Kashmir; however, no animal reservoir has yet been identified [9].

Data regarding AE cases are lacking in Afghanistan. However, the literature reports a case of an Afghan patient treated in the UK [10], suggesting potential future cases, particularly in the north of the country. Between 1948 and 1993, 37 cases of AE were recorded in Iran, or fewer than 1 case per year. One case of AE has been reported in northern Iraq [11].

Data on human AE cases in Belarus are scarce, with reports of only two patients with AE, one from Brest and the other from the Mogilev region. The actual situation concerning AE in Ukraine and Moldova remains unknown due to the lack of published data. AE has been diagnosed in patients from across the Baltic region. In Lithuania, 178 patients were recorded between 1997 and 2014, and the incidence of AE was found to increase from 0.03 in 2004 to 0.57 in 2009 and 0.74 in 2012. To date, only 13 patients with echinococcosis have been registered in Estonia; however, the specific Echinococcus species was not identified [12]. In Latvia, 29 AE cases were reported between 1996 and 2010 [13].

In France, a large-scale study of foxes revealed *E. multilocularis* in 36 of 44 administrative units in the northeastern part of the country. Furthermore, 26 new endemic areas were identified, with prevalence rates of 7% (3/41) and 17% (1/6), respectively [14, 15].

According to the WOAH [16], *E. multilocularis* was detected in domestic and wild animals in regions of America, Asia, and Europe between 2020 and 2024 (Figures 1, 2).

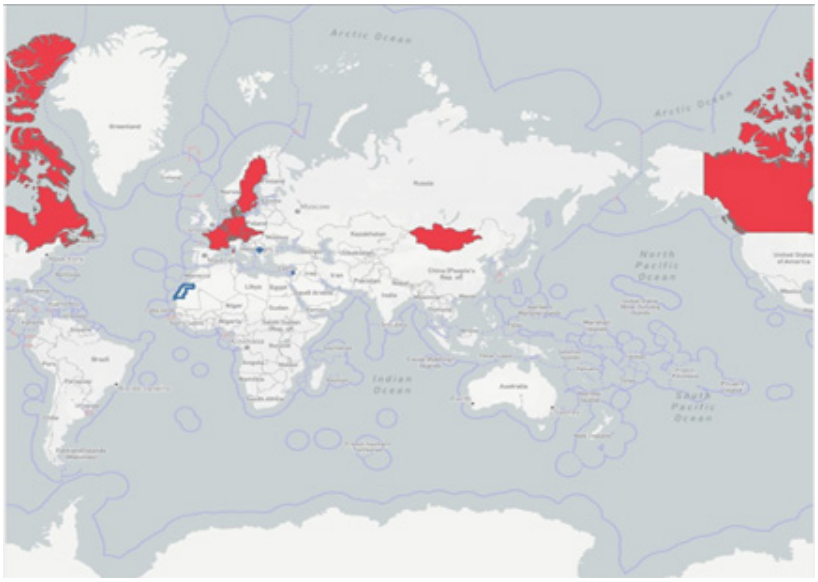

Figure 1 – Map of *Echinococcus multilocularis* occurrence in wild animals according to WOAH data for 2020-2024

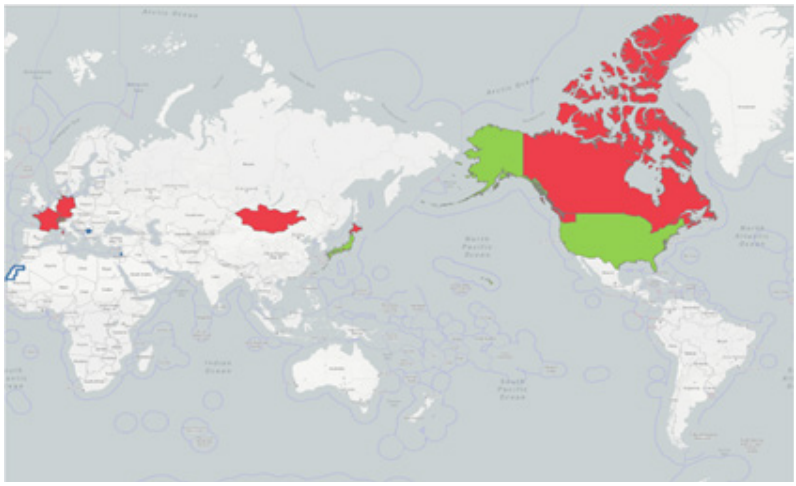

Figure 2 – Map of *Echinococcus multilocularis* occurrence in domestic animals according to WOAH data for 2020-2024

In 2020, AE was recorded among domestic and wild animals in regions of Europe, America, and Asia. Furthermore, *E. multilocularis* was recorded in wild and domestic animals in Canada, Mongolia, France, Germany, and Switzerland; in wild animals in Belgium, the Czech Republic, the Netherlands, and Sweden; and in domestic animals only in Japan. In 2021, E. multilocularis was recorded in wild and domestic animals in Canada, Mongolia, Germany, France, and Switzerland and only in wild animals in Austria, Belgium, the Czech Republic, the Netherlands, Slovakia, and Sweden. In 2022, AE was registered in wild and domestic animals in Canada, Mongolia, Germany, Switzerland, and France and only in wild animals in the Czech Republic, Slovakia, and Sweden. In 2023, AE was recorded among animals only in regions of Europe and Asia. In Sweden and the Czech Republic, it was detected in wild animals, and in Switzerland and Mongolia, it was detected in both wild and domestic animals. In 2024, AE was recorded among animals only in regions of Europe and America. AE was recorded in wild animals in Austria, Belgium, the Czech Republic, Denmark, the Netherlands, Slovakia, and Sweden; only in domestic animals in the USA; and in both wild and domestic animals in Germany, Switzerland, and Canada.

Among neighboring countries sharing land borders with Kazakhstan, AE showed a wide distribution in the Russian Federation and the Kyrgyz Republic [7, 17]. This study investigated *Echinococcus*

granulosus s.l. and *Echinococcus multilocularis* in dog feces and the environment in two Kyrgyz districts. In dog feces, E. granulosus s.l. eggs were found in ~4.2% of samples in Alay and ~3.5% in Kochkor; E. multilocularis eggs in 2.8% and 3.2%, respectively. Environmental contamination was also similar: 8.3 vs. 7.5 eggs/m² for E. granulosus s.l. and 4.4 vs. 5.0 eggs/m² for E. multilocularis. Despite higher human AE incidence in Alay (162 vs. 21 cases per 100,000), no clear association was found between human cases and egg contamination, though contamination increased in autumn after dogs returned from pastures. [18]. In the Russian Federation, *E. multilocularis* was detected in the Taymyr Peninsula in 64.1% of examined Arctic foxes and in one of two red foxes, whereas it was less frequent in dogs and wolves. In Magadan oblast, the parasite was detected in 25.4% of Arctic foxes but not in dogs (>800 examined). Significant infection rates were recorded in Chukotka and Yakutia [19, 20]. In Central Asia, Kyrgyzstan shows the highest burden of alveolar echinococcosis with 140–200 cases annually (AIR 2.62/100,000). Kazakhstan reported 135 cases in 1996–2019 (AIR 0.037/100,000), while Uzbekistan, Iran, Armenia, and Tajikistan had very low AIRs (≤0.1/100,000). In Turkey, 641–918 cases were recorded from 1939–2018, averaging ~20 new cases annually (AIR 0.023/100,000) [21]. In the Kyrgyz Republic, *T.A. Abdyzhaparov* [22] reported the highest percentage of infected animals among gray (4.0%) and red (2.4%) marmots, ground squirrels (3.8%), and forest dormice (2.6%). Moreover, studies by *C.A. Alvarez Rojas, P.A. Kronenberg, S. Aitbaev* et al. [23] in Kyrgyzstan reported that of 43 examined dog fecal samples, *E. multilocularis* was confirmed by PCR in 23 (53.48%) samples, and *E. granulosus* was detected in 20 samples (46.51%). In the Russian Federation, according to data from FBUN «Omsk Research Institute of Natural Focal Infections» of Rospotrebnadzor in Omsk oblast, intermediate hosts (small mammals) with *E. multilocularis* showed an infection rate of 2.2%, whereas definitive hosts (foxes) exhibited infection rates of 30.6%–53.6% [20]. There was 1 case of human disease registered in 2014 (the diagnosis was made posthumously), the disease was characterized by a malignant course for more than 10-15 years, the affected organ was the liver, with characteristic metastases of the mesentery, intestines and peritoneum. Over the past five years, during the mandatory medical examinations of the population, 996 patients with echinococcosis were registered, in the regions of the Arctic zone, 25 people (2.51%) were identified with alveococcosis. [24].

Among the population of the Republic of Kazakhstan, AE was recorded in certain regions but significantly less frequently than cystic echinococcosis. According to data reported by *M.A. Seisembaev, D.S.Toksanbaev, Zh.B. Baimakhanov* [25], over a period of 15 years (1996–2010), 102 patients were diagnosed with hepatic AE and its various complications at the Liver Surgery Department of the National Scientific Center of Surgery named after *A.N. Syzganov,* including 9 (8.8%) patients who had undergone previous ineffective (exploratory) surgeries, 38 (37.3%) men and 64 (62.7%) women, aged 19-69 years, and young and working-age individuals comprising 76.5%.

From 2011 to 2019, 17 cases of human AE were recorded in different regions of the Republic of Kazakhstan. For instance, in 2016, four cases were recorded in Almaty, East Kazakhstan, Karaganda, and Kostanay oblasts, and in 2017, five cases were recorded (one case each in Almaty, West Kazakhstan, and Pavlodar oblasts and two cases in North Kazakhstan oblast). In 2018, two patients with AE were operated in Akmola oblast, three patients with AE were reported in Almaty oblast, and one patient each in Kostanay, Pavlodar, and North Kazakhstan oblasts. In 2019, one resident of Almaty oblast and two residents of North Kazakhstan oblast were operated [26].

In 2020, one AE case was recorded in Almaty and Kostanay oblasts. In 2021, four patients from Almaty oblast and one each from Karaganda, Kostanay, and North Kazakhstan oblasts and Almaty city were operated with an AE diagnosis. In 2022, five patients from Almaty oblast, two patients from East Kazakhstan, one patient from Pavlodar, one patient from Zhetysu oblast, and one patient from North Kazakhstan oblast were operated. In 2023, one patient from Abay oblast, three patients from Almaty oblast, two patients from Zhetysu oblast, and one patient from Pavlodar oblast were operated. In 2024, human AE cases were reported in Almaty, Zhetysu, Zhambyl, North Kazakhstan, Kostanay, Akmola, and Pavlodar oblasts. A total of 10 AE cases were recorded, including 2 in Zhambyl oblast, 3 in Zhetysu oblast, and 1 each in the other abovementioned regions.

Therefore, according to data from the National Center of Public Health of Ministry of Healthcare of RK, 37 patients were operated in Kazakhstan with an AE diagnosis over the past 5 years (2020-2024) (Figure 3).

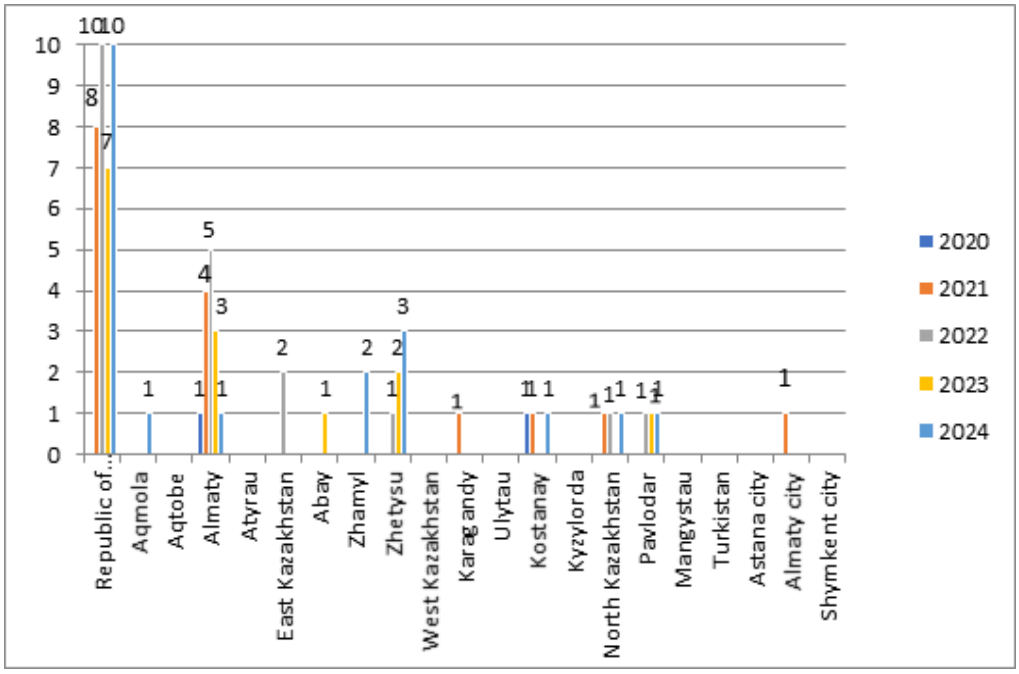

Figure 3 – Absolute incidence rates of alveolar echinococcosis in the population of the Republic of Kazakhstan for 2020-2024

As shown in Figure 3, the number of infected individuals increased in Almaty and Zhetysu oblasts, with the first cases of the disease being recorded in Zhambyl oblast. In North Kazakhstan and Pavlodar oblasts, human AE infections are reported annually.

In the life cycle of the parasite, carnivores (e.g., dogs, foxes, and Arctic foxes) serve as definitive hosts, i.e., carriers and disseminators of mature helminth forms. *R. Uakhit, A. Smagulova* et al. [27] analyzed the sequences of the genes cox1 and nad1 and identified two echinococcosis species, viz., *E. granulosus* in red foxes and wolves and *E. multilocularis* in corsac foxes. Sequencing of mitochondrial genome segments revealed seven pathogen haplotypes in the examined *E. granulosus samples.* Molecular analysis of *E. multilocularis cox1* and *nad1* genes revealed three new haplotypes that exhibited significant variability compared with other investigated Asian haplotypes. Hence, corsac foxes must be included among the definitive hosts that participate in the life cycle of *E. multilocularis* in Kazakhstan. Previous studies had reported the tapeworm form of *E. multilocularis* in foxes and domestic dogs in Kazakhstan [28, 29].

As mentioned earlier, intermediate hosts include small rodents and humans. In Kazakhstan, according to Professor *B.Sh. Shaikenov* [30], *E. multilocularis* larvae were identified in examined highland areas rodent species. The gray and red voles, muskrats, zokors, and great gerbils play the most significant role in forming infection foci. During 2019-2020, *E. multilocularis* larval cysts were detected in the liver and lungs of three common voles in Kostanay oblast among 148 dissected rodents of various species (ground squirrels, great gerbils, wood mice, common voles, harvest mice, field mice, narrow-skulled voles, steppe lemmings, jerboas, and tamarisk gerbils) captured in Mangystau, Kostanay, North Kazakhstan, East Kazakhstan, and West Kazakhstan oblasts [31].

Therefore, based on the literature analysis, the definitive hosts in the life cycle of *E. multilocularis* include dogs, foxes, corsac foxes, and Arctic foxes, and in Kazakhstan, they include dogs, foxes, and corsac foxes. More than 40 species of small mammals have been identified as potential intermediate hosts [32, 33], with 18 species participating in the life cycle of this helminth within our country.

Domestic dogs, foxes, corsac foxes, and Arctic foxes become infected by consuming wild rodents that harbor *E. multilocularis* larvae, and rodents become infected by consuming wild plants, berries, or soil contaminated with helminth eggs. Humans acquire the infection during hunting, carcass dressing, and gathering of wild berries and mushrooms contaminated with E. multilocularis oncospheres.

Conclusion

This review has shown that alveococcosis is a complex parasitic disease, the circulation of which depends on the interaction between definitive hosts (dogs and wild canids) and numerous species of small mammals serving as intermediate hosts. The epidemiological situation in Kazakhstan is determined by the presence of both domestic and wild reservoirs, which maintain the natural foci of *E. multilocularis.* Therefore, according to the epizootiological principle of continuous transmission cycles, it is important to consider the following risk factors:

- Human contact with infected wild carnivores. Foxes are the primary disseminators of helminth eggs in natural biocenoses, whereas dogs (domestic, stray, and feral) play this role in anthropogenic zones. Stray and feral dogs that feed on rodents may play a major role in spreading invasive elements in the environment. When infection rates in domestic dogs increase, the risk of infection spread among the population of the country increases.

- Migration of infected dogs. When dogs are imported into the country from endemic regions where the infection is prevalent, the risk of infection spread increases in anthropogenic zones (rural areas and cities).

- Agricultural lands may become contaminated with E. multilocularis eggs because irrigation water is obtained from canals and open reservoirs inhabited by wild carnivores. This increases the risk of infection for people involved in crop production who do not follow sanitary hygienic guidelines when handling environmental objects (e.g., soil and plants).

The key risk factor for the mass spread of AE is the increasing population of small rodents and foxes, including their appearance in urban and rural areas; however, the most dangerous factor is the inclusion of stray, feral, and domestic dogs in the infection transmission cycle.

Authors contribution

AZh, SB, SK, EK: Conceptualized and designed the study, conducted comprehensive literature searches, analyzed collected data, and prepared the manuscript. AA, ZS: Performed final editing and proofreading of the manuscript. All authors have read, reviewed, and approved the final version of the manuscript.

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UDC 619:616-094 **Research article**

Intestinal Helminth Infections in Small Ruminants: Prevalence in Northern Kazakhstan and a New Treatment Scheme

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Abstract

Background and Aim. Gastrointestinal helminth infections significantly reduce ruminant productivity. This study aimed to determine the prevalence and assess the efficacy of a new treatment regimen against sheep intestinal cestodes and nematodes in Northern Kazakhstan.

Materials and Methods. In 2024, to identify the species and infestation level of parasites, the digestive tracts of 29 sheep, aged 5-9 months, were examined by helminthological dissection, and 200 faecal samples were analysed using the Fülleborn and McMaster methods at two farms in the Tayinsha District. To test the treatment scheme, two groups (control and experimental) were formed, each containing 15 sheep. All animals had mixed infection with strongyles and *Moniezia* spp. Initially, each sheep was treated with Alvet (Nita-Farm, Russia) at the recommended dosage. Additionally, each sheep in the experimental group received 3 g of the phytobiotic Sangrovit Extra (Phytobiotics Futterzusatzstoffe, Germany) daily for 8 weeks. The treatment efficacy was measured on days 30, 60, 90, and 120 after deworming to assess the prevalence and infestation intensity of helminths.

Results. Parasitological research showed that 93.1% of sheep were infected with *Trichostrongylidae* spp., *Moniezia expansa, Trichuris ovis* and *Skrjabinema ovis*. Mixed infestations of 2 to 4 species were found in 68.8% of the sheep. The infection rates of Moniezia spp. and strongyles were 13.3% and 26.7%, respectively, 4 months after deworming in the experimental group. In comparison, these rates were 3.5 and 2.5 times lower, respectively, than in the control group.

Conclusion. Using Sangrovit Extra effectively inhibited small ruminant intestinal helminth infestation.

Keywords: Northern Kazakhstan; sheep; intestinal helminths; prevalence; infestation intensity; treatment regimen; efficacy.

Introduction

The Republic of Kazakhstan is the largest agrarian country in Central Asia. According to the National Bureau of Statistics data in February 2025, the country has a population of 18,528,336 sheep and 1.682.335 goats [1]. Particular attention should be given to sheep farming when addressing the issue of meat supply, as raising young sheep is considerably more manageable than other forms of livestock production.

Higher-quality lamb meat can be achieved by ensuring animal welfare and implementing measures to protect against economically significant infectious and parasitic diseases. Special attention should be paid to parasite infections of sheep, particularly gastrointestinal helminthiases. These diseases result in significant losses due to reduced productivity, deteriorated animal welfare, and increased treatment

and prevention costs. Gastrointestinal helminths, such as species of *Haemonchus, Trichostrongylus, Moniezia, Strongyloides* and *Trichuris* genera, pose a particular threat to husbandry. These parasites are widely distributed among small ruminants worldwide, including countries with nomadic patterns similar to those in Kazakhstan [2]. For example, helminth infections are highly prevalent in sheep populations in Grenada, China, and Central Anatolia, with prevalence from 72% to 100% [3, 4].

Among helminth infections, strongyles infestations cause diseases that lead to severe anaemia in young animals. Anoplocephalata infestations may also act as a primary factor in infectious enterotoxaemia endemic outbreaks in small ruminant herds. The growing resistance of helminths to traditional anthelmintic drugs, such as albendazole and ivermectin, further complicates the issue and necessitates comprehensive treatment approaches. These strategies include the development of targeted programmes aimed at enhancing the resistance of animals to parasitic infections, beginning with the creation of new pharmaceutical treatments [5].

In this context, studying the epidemiology and control of helminth infections in Kazakhstan is strategically important because the seasonal summer pasturing and winter housing system creates specific risks for parasite transmission and potential anthelmintic resistance, which negatively impacts sheep health, welfare and economic productivity.

Such research is critical not only for improving livestock productivity and preserving animal health but also for supporting the sustainable development of the agricultural sector.

The objective of this study was to determine the prevalence of intestinal helminthiases among small ruminants in the Tayinsha District of North Kazakhstan and to evaluate a novel therapeutic model for treating mixed infestations with cestodes and nematodes.

Materials and Methods

The study was conducted in 2024 at the Amanat and Karatomar peasant farms located in the Tayinsha District. Here, the helminthological post-mortem examination of the gastrointestinal tracts from 29 slaughtered indigenous-breed sheep (14 animals from Amanat and 15 from Karatomar farms) aged between 5 and 9 months was carried out in accordance with *K.I. Skryabin* [6] Figure 1).

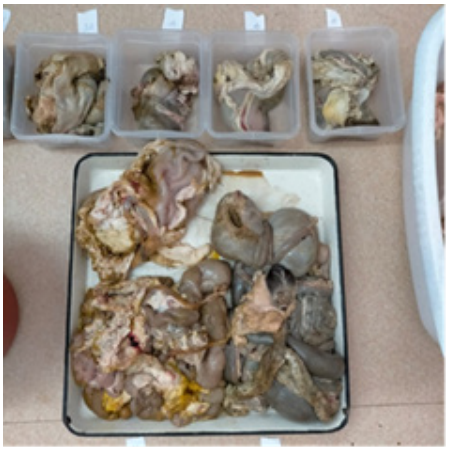

Figure 1 – Helminthological post-mortem examination of the sheep digestive system

To determine the actual infestation level in the sheep population, faecal samples were collected from 94 sheep at Amanat Farm and from 106 sheep at Karatomar Farm. These samples were analysed at the Professor Kadyrov Parasitology Laboratory of Seifullin Kazakh Agrotechnical Research University using the Fuelleborn and McMaster methods.

Next, the newly proposed treatment regimen for mixed infections of sheep caused by nematodes and cestodes was tested under experimental conditions. For this experiment, 30 animals co-infected with strongyles and *Moniezia* spp. were selected based on their identical pre-treatment infection rates and and randomly assigned to two groups of 15 animals.

These animals were subjected to the treatment scheme outlined in Table 1.

Table 1 – Testing trial of new treatment scheme

Group	Days 1 to 9		Day 10		11 days to 8 weeks	
	Feeding scheme	Dosage	Deworming	Dosage	Feeding scheme	Dosage
Control, n=15	-	-	Alvet**	50 mg/kg	-	-
Experimental, n=15	Sangrovit Extra*	3 g/a lamb	Alvet**	50 mg/kg	Sangrovit Extra*	3 g/a lamb

* – administered by mixing with feed, daily, in the morning, using the group method.
**– administered individually *per os* in suspension.

At both farms, the animals received a single treatment with Alvet (Nita-Farm, Russia), administered according to the dosage and methodology recommended by the manufacturer. The sheep from Karatomar Farm (control group) underwent deworming only. In contrast, the sheep from Amanat Farm (experimental group) were fed the phytobiotic Sangrovit Extra (Phytobiotics Futterzusatzstoffe, Germany) for 8 weeks, in conjunction with the deworming treatment (Table 1). During the experimental period, animals from both groups were pastured at the common pasture and received identical concentrated feed.

The treatment scheme efficacy was assessed on the 30th, 60th, 90th, and 120th days, in accordance with the guidance "Efficacy of Anthelmintics: General Requirements" (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medical Products, 2022) [7].

Statistical processing of the obtained results was performed using Excel (Microsoft Corp, Redmond, WA, USA). Results were considered statistically significant when $p < 0.05$.

Results and Discussion

The comprehensive post-mortem helminthological examination data are presented in Tables 2 and 3. The level of gastrointestinal helminth infestation among small ruminants in both farms exhibited a similar pattern.

Table 2 – Intestinal Helminths Identified in Small Ruminants

Species	Amanat Farm, n=14			Karatomar Farm, n=15		
	Number	Prevalence, %	Intensity (M±m)	Number	Prevalence, %	Intensity (M±m)
Trichostrongylidae spp.	12	85.7	450±50	13	86.6	400±100
Trichuris ovis	7	50.0	50±50	11	73.3	100±50
Skrjabinema ovis	1	7.1	50	3	20.0	100.0
Moniezia expansa	6	42.8	4.5±2.5	9	60.0	4.5±1.6

The species composition of the intestinal microbiome included such helminths as *Trichostrongylidae* spp., *Moniezia expansa*, *Trichuris ovis*, and *Skrjabinema ovis* (Table 2, Figures 2-6). Overall, the prevalence of intestinal helminths reached 93.1%. The highest infestation intensity was observed for *Trichostrongylidae* spp. In particular, sheep from Amanat Farm exhibited a mean intensity of 450 ± 50 strongyles, whereas those from Karatomar Farm had a mean intensity of 400 ± 100 strongyles. The infection intensity of other helminths was relatively low.

According to these results, the helminths in the sheep gastrointestinal tracts were detected as either single infestations or as part of mixed infestations involving from two to four species. Most of the examined sheep were found to have mixed helminth infections caused by *Moniezia* spp. and strongyles. The most common mixed infection, comprising *Trichostrongylidae* spp., *M. expans*a, and *T. ovis*, was detected in 24.1% of the animals. A mixed infection consisting solely of *M. expansa* and *Trichostrongylidae* spp. was identified in 17.2% of the examined sheep. *Trichostrongylidae* spp. and *T. ovis*. Single infections were detected in 20.7% of the sheep (Table 3). In total, mixed helminth infections were identified in 68.8% of the animals.

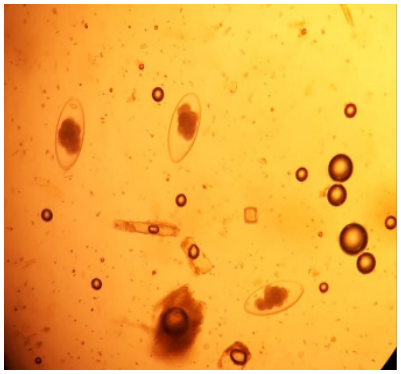

Figure 2 – *Nematodirus* spp. eggs, ×40

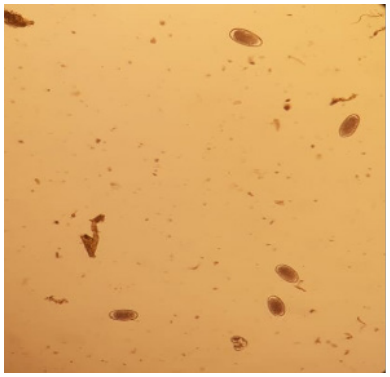

Figure 3 –Trichostrongylidae spp. eggs, ×40

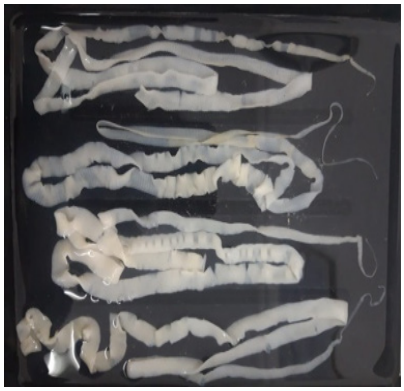

Figure 4 – *Moniezia expansa*

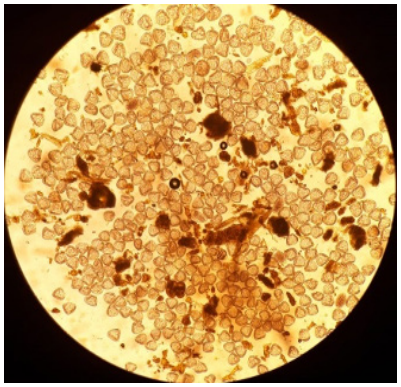

Figure 5 – *Moniezia* eggs ×40

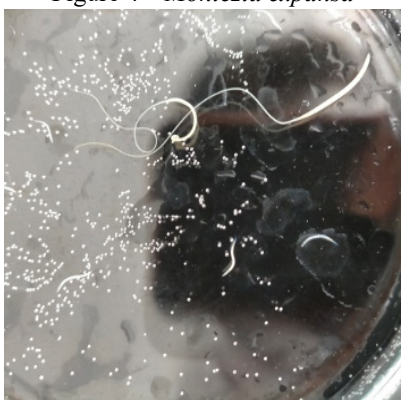

Figure 6 – *Trichuris ovis*

Figure 7 – *Trichuris ovis,* eggs×40

Table 3 – Helminth associations identified in the intestinal biocenosis of sheep (n=29)

Association Members	Number of infected animals	Proportion (%)
Single infestations:		
Trichostrongylidae spp.	4	13.8
T. ovis	2	6.9
Associations with two members:		
Trichostrongylidae spp. + *T. ovis*	4	13.8
Trichostrongylidae spp. + *M. expansa*	5	17.2
Associations with three members:		
Trichostrongylidae spp. + *M. expansa* + *T. ovis*	7	24.1
Trichostrongylidae spp. + *T. ovis* + *S. ovis*	1	3.44
An association with four members:		
Trichostrongylidae spp. + *M. expansa* + *T. ovis* + *S. ovis*	3	10.3

During the faecal sample examination, the eggs of *Moniezia spp.* and strongyles were detected in 88.0% of the sheep. The prevalence of the *Trichostrongylidae* family species was 78%. Among the strongyles, *Nematodirus* spp. showed a prevalence of 42.0%. Sole infestations caused by *Trichostrongylidae* spp. and *T. ovis* were identified in 5.5% and 2.0% of the animals, respectively. The dual infestation involving *Trichostrongylidae* spp. and *T. ovis* was observed in 11.5% of the sheep. Mixed infestations with *Trichostrongylidae* spp. and *Moniezia* spp., as well as with *T. ovis* and *Moniezia* spp., were recorded in 6.5% of the sheep. The most frequent mixed triple infestation, composed of *Trichostrongylidae* spp., *M. expansa*, and *T. ovis*, was found in 26.3% of the sheep. A quadruple mixed infestation caused by *Trichostrongylidae* spp., *Moniezia* spp., *T. ovis*, and *S. ovis* was detected in 3.5% of the animals. Overall, mixed infestations caused by strongyles and Anoplocephalata were highly prevalent, with a prevalence of 42.8%.

The treatment regimens tested reduced and stabilised the parasitic burden in both groups of sheep. As a result of the treatment, the infestation dynamics in the experimental group significantly decreased over time (Table 4).

Table 4 – Efficacy of treatment schemes for mixed infestation of sheep with Moniezia spp. and strongyles

Infection Indicators	Experimental Days			
	30	60	90	120
Control Group, *n = 15*				
Moniezia spp.:				
Prevalence (%)	0	2 (13.3)	4 (26.7)	7 (46.7)
Trichostrongylidae spp.:				
Prevalence (%)	0	3 (20.0)	7 (46.7)	10 (66.7)
Intensity of infestation, number of eggs for 1 g faeces (*M±m*)	0	100±25	300±15	400±25*
Experimental Group, *n = 15*				
Moniezia spp.:				
Prevalence (%)	0	0	1 (6.7)	2 (13.3)
Trichostrongylidae spp.:				
Prevalence (%)	0	1 (6.7)	2 (13.3)	4 (26.7)
Intensity of infestation, number of eggs for 1 g faeces (*M±m*)	0	50	100	150±25*

** - $p < 0.05$*

Four months after deworming, the prevalence of *Moniezia* spp. in the experimental group of sheep was 13.3%, and for *Trichostrongylidae* spp., it reached 26.7%. These figures were 3.5 and 2.5 times lower than those of the control group, respectively. A similar trend was observed in the intensity indicators of *Trichostrongylidae* spp. infestation. Specifically, the quantitative indicator of strongyles egg contamination in the control group of sheep was 2.6 times higher than that of the experimental group (Table 4).

The results of the study indicate a high prevalence of intestinal helminthiases in sheep of the North Kazakhstan region. Helminths of several species were detected in the gastrointestinal tract of 93.1% of slaughtered sheep. These findings are consistent with results reported in other countries. For instance, in Grenada, the prevalence of intestinal parasites among small ruminants ranges between 85% and 95%. Similarly, in the Hinggan region of China, the extent of gastrointestinal helminth infections in sheep has been recorded at 89% [3, 4].

In our study, cestodes belonging to the species *Moniezia expansa* were frequently identified. These findings are consistent with previous research conducted in Central Anatolia (Turkey), where *Moniezia* spp. infections have been reported to be widespread among sheep and have a significant negative impact on livestock productivity [5].

The increasing resistance of helminths to conventional anthelmintic drugs has become a pressing issue in many countries. For example, resistance to albendazole and ivermectin has been frequently

observed [5]. Similarly, in this study, the control group that was treated solely with the conventional drug Alvet exhibited a high reinfection rate (46.7%) within 4 months, indicating the limited efficacy of conventional deworming practices.

In the experimental group, the extended use of the phytobiotic Sangrovit Extra significantly reduced the parasitic load. This phytobiotic is a plant-based feed additive designed to enhance the productivity of livestock. Its active ingredients are alkaloids extracted from the plant *Macleaya cordata*. Sangrovit Extra possesses a broad therapeutic spectrum. It exhibits anti-inflammatory properties, improves appetite and digestion in animals, increases the bioavailability of amino acids, and functions as an anti-stress agent [8].

The results obtained are consistent with international findings that demonstrate the role of phytobiotics in preventing parasitic infections. For example, studies in the Caribbean region have shown that the use of phytobiotics enhances the natural resistance of sheep and significantly improves their resilience against helminths [9]. At the same time, studies conducted in Kazakhstan have also highlighted the effectiveness of an integrated approach that combines anthelmintics with immunomodulators in combating helminth infections in small ruminants [10].

A genetic study of the Anatolian Merino sheep infected with *Moniezia* spp. has indicated that immune tolerance to helminths may be genetically regulated. This highlights the need to enhance research efforts in Kazakhstan focused on selecting and breeding sheep with traits that confer resistance to helminth infections [5].

Overall, the findings of this study confirm the importance of a comprehensive approach to treating gastrointestinal helminth infections, including the use of natural phytobiotics. The proposed treatment method can serve as an effective tool for the integrated management of parasitic diseases and improving the welfare of sheep. This approach has the potential to extend the reinfection interval, reduce veterinary expenses, and enhance the overall economic efficiency of small ruminant farming.

Conclusion

In North Kazakhstan farms, the intestinal helminth fauna of sheep consists of *Trichostrongylidae* spp., *Moniezia expansa, Trichuris ovis* and *Skrjabinema ovis*. The overall infection rate of small ruminants with intestinal helminths reaches 93.1%, with 68.8% of sheep experiencing mixed infections involving 2 to 4 species of nematodes and cestodes. The treatment scheme, which combines deworming with the phytobiotic Sangrovit Extra, reduces the prevalence of *Moniezia* spp. and *Trichostrongylidae* spp. infections by 2.5 and 3.5 times, respectively, within 4 months of treatment. This trial in which the bioactive plant additive was fed to animals infected with gastrointestinal nematodes and cestodes showed that treated animals had reduced nematode and cestode infection levels. Our results show that long feeding of Sangrovit Extra to sheep has beneficial effects on parasite burden and could be recommended to farmers for practice in controlling gastrointestinal parasite infections in Northern Kazakhstan.

Authors' Contributions

AU and AZh: The conceptualisation and design of the study, proofreading and final revision for the manuscript. BY, DS and AT: a literature review, analysis of the research results and drafting the manuscript. All authors have read and approved the final manuscript.

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UDC 619:616.995.132.6 **Research article**

Parasitological aspects of animal introduction and acclimatization

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Abstract

Background and Aim. The introduction and acclimatization of wild ungulates, such as the Bukhara deer (*Cervus elaphus bactrianus*), are widely practiced to restore populations and biodiversity. However, these processes pose parasitological risks, including the loss and acquisition of native and new parasites, respectively. This study evaluated the parasitological outcomes of relocating Bukhara deer to the Ile-Balkhash State Nature Reserve.

Materials and Methods. In total, 45 fecal samples from Bukhara deer were collected during expeditions in 2024-2025. Samples were preserved and examined using the Fulleborn flotation method with an ammonium nitrate solution (density: 1.3). Microscopic analysis was performed to identify protozoan oocysts and helminth eggs based on morphological features, following standard parasitological references.

Results. In the new habitat, the deer retained host-specific protozoa (*Eimeria* spp.) but lost several species-specific helminths, including *Fasciola hepatica, Echinococcus granulosus*, and 13 nematode species. Conversely, they acquired new parasites from the local environment, such as the cestode *Anoplocephala perfoliata* and the nematode *Cylicocyclus insigne*. These shifts in parasite fauna reflect both a "parasitological filter" effect and the potential for local parasites to adapt to introduced hosts.

Conclusion.The introduction and acclimatization of wild ungulates significantly influence host–parasite dynamics. A comprehensive parasitological evaluation is vital for managing biological risks and ensuring the ecological safety of wildlife translocations.

Keywords: acclimatization; animal; helminth; introduction; parasite; protozoan.

Introduction

Humanity has long relied on nature's resources. In prehistoric times, human impact on the environment was minimal, but it gradually increased over time. This growing influence reduced populations of wild animals and birds. Additionally, with the emergence of animal husbandry, natural ecosystems began to change, transforming into pastures for domestic animals. As agriculture development, human pressure on nature intensified.

106 mammalian species and subspecies have been extinct on the planet for 2000 years. Currently, about 600 species and subspecies of vertebrates are on the verge of extinction if they are not taken measures to protect them.

One of these animals listed in the Red Book of Kazakhstan (1991) is the tugai (Bukhar) deer – Cervus elaphus bactrianus Lydekker, 1900 [1, 2].

This deer species inhabits riparian thickets across Central Asia, Kashmir, Afghanistan, Tajikistan, and the Amu Darya region. It is mainly endemic to Central Asia. In Kazakhstan, it lived in the lower and

middle reaches of the Syrdarya River until the mid-20th century. Historically, it may also have inhabited the Almaty region, particularly the groves along the floodplain and mouth of the Ili River. Owing to river flow regulation and extensive development of floodplain groves, Bukhara deer disappeared from Kazakhstan. In 1981, the species was reintroduced to the Karachingil hunting farm on the left bank of the middle Ili River. In the same year, 19 deer were brought from Tajikistan. Within 5 years, the population grew to 60; by the mid-1990s, it reached 200. A March 2001 census recorded 310 individuals. Currently, the hunting farm and adjacent floodplain groves support around 350 Bukhara deer. Reacclimatization efforts are ongoing in the Altynemel National Natural Garden and the groves at the confluence of the Syrdarya and Arys rivers.

The Ile-Balkhash State Natural Reserve was established by Decree No. 381 of the Government of the Republic of Kazakhstan on June 27, 2018. Covering 415.164.2 ha, the reserve is located in the Balkhash district of the Almaty region. It was created with support from the UNDP Project for the Conservation of Biodiversity and Ecosystems. Expanding and creating new specially protected natural areas is a major achievement for Kazakhstan in biodiversity conservation. These efforts align with the country's commitments to the Sustainable Development Goals on protecting and restoring terrestrial ecosystems and addressing climate change.

Kazakhstan has initiated a project to reintroduce the tiger into the Ili Delta and other rivers flowing into Lake Balkhash. The primary goal of the reserve is the reintroduction of tigers in Kazakhstan. To establish a food base for tigers, Bukhara deer were reintroduction here in 2018 and kulan in 2021.

One factor influencing the breeding and reproduction of Bukhara deer is parasites. Therefore, their presence must be studied. Certain parasites markedly affect the reproduction and survival of wild animals. One such parasite is *Eimeria*, which causes pathological changes in the intestines, disrupting their function and impairing the activity of other organs by poisoning the body as a whole. As the body is constantly exposed to these pathogens, infected animals release them into the environment over a prolonged period, leading to their accumulation. This creates a persistent source that poses a continuous threat to susceptible animals.

The importance of *Eimeria* as a pest is considerable. Some species of this genus inhabit the walls or lumen of the intestines in wild and domestic animals, causing large-scale losses among livestock, birds, and other wildlife. All coccidia are true geoprotists, which are especially common in herbivores and birds, as these animals ingest cysts containing spores from food collected on the ground.

Eimeria reproduce asexually in the intestines of infected animals. This leads to a rapid progression of the infection in most cases, increasing the number of destroyed intestinal cells and worsening the condition of the affected animal. Among infected animals, ≥50% may die. Young offspring are especially vulnerable, which is why eimeriosis is often referred to as a "disease of cubs." For this reason, young Bukhara deer calves may also suffer losses. To date, *Eimeria* species specific to Bukhara deer have not been studied.

Helminths are another factor affecting the growth and health of Bukhara deer. Individual helminths are widespread in nature and harm animals in several ways. First, they cause serious damage to the host's organs, including the brain; second, they hinder proper development and reduce productivity. They can also lead to severe illnesses and death. The destructive impact of helminths results from both mechanical damage and toxic effects.

Helminths occupy a prominent place among infectious diseases affecting grazing livestock. Natural biotopes provide favorable conditions for helminth distribution. Wild ungulates, including Bukhara deer, are released into these areas. Given the high number of domestic animals kept in pasture zones, often taxonomically related to wild ungulates and confined to narrow grazing areas, the infestation level in Bukhara deer increases markedly when they interact with domestic livestock.

In Kazakhstan, helminths of Bukhara deer have not yet been studied. The purpose of this study was to identify the current helminth and *Eimeria* fauna of the Kazakh population of Bukhara deer. Helminths of the Bukhara deer were previously studied only in Tajikistan [3]. In total, 14 helminth species were identified there: 2 trematodes, 3 cestodes, and 9 nematodes. The most prevalent were flukes (*F. hepatica* and *D. lanceatum*, – 33-42%, cestoda larvae (*T. hydatigena* and *E. granulosus* – 25%, adult Cestodes (*M. expansa* – 30% and nematodes (*T. skrjabini* – 40%, *G. pulchrum* – 33%, *M. elongatus* – 16,6%)). The most numerous were worms, with an infestation rate reaching 648 individuals, followed by 42 *Gongylonema*, 57 flukes, and 18 adult cestodes.

In the 21st century, researchers from the Institute of Zoology, led by academician O. Berkinbay and Professor *K.K. Baitursinov* from the Yasavi International Kazakh-Turkish University, studied Bukhara deer parasites. Baitursinov's group focused on the Syrdarya River floodplains and Berkinbay Island within the Ile-Balkhash State Nature Reserve. Their research identified three *Eimeria* species in Bukhara deer in Kazakhstan, namely *Eimeria sholpanae, Eimeria kulashae,* and *Eimeria aruzhanae*, as well as 20 helminth species. These included 2 trematodes, 3 cestodes, and 16 nematodes: F*asciola hepatica, Dicrocoelium lanceatum, Echinococcus granulosus* (larval stage), *Moniezia expansa, Anoplocephala perfoliata, Parabronema skrjabini, Onchocerca skrjabini, Setaria cervi, S. digitata, S. labiato-papillosa, Oesophagostomum columbianum, O. radiatum, O. venulosum, Cooperia* sp. (females only), *Haemonchus contortus, Nematodirus spathiger, Nematodirus* sp. (females only), *Dictyocaulus eckerti, D. filaria, Cylicocyslus insigne* and *Trichocephalus skrjabini* (Table 1) [4, 5].

Materials and Methods

The studied material consisted of 45 fecal samples collected from Bukhara deer during expeditions in 2024 and 2025 within the Ile-Balkhash State Nature Reserve.

Lifetime parasitological studies of sheep were carried out using the *O. Berkinbay* et al., 2024 [6] method. Fecal samples (3 g each) were taken from Bukhara deer. The samples were placed in plastic containers and preserved with a 2.5% potassium bicarbonate solution for later analysis in the Nystitut laboratory. The feces were thoroughly mixed in a porcelain dish with 15–20 mL of ammonium nitrate solution (density: 1.3) and left to stand for 45 min. The upper film was then removed using a wire loop, placed on a microscope slide, treated with drops of distilled water, covered with a cover glass, and examined under a microscope.

The species identity of *Eimeria* was determined based on oocyst morphology, including shape, size, color, shell thickness and structure, and the presence of features such as micropyles, polar caps, residual bodies, and refractive bodies. Characteristics of sporocysts (shape, size, and presence of residual and styd bodies), sporozoites (shape, size, and presence of refractive bodies), and the time required for oocyst sporulation were also assessed.

At the same time, the data of *J.P. Dubey* were also taken into account [7].

Helminth eggs were identified based on shape, size, color, and shell structure and thickness; presence of polar caps, miracidia, or yolk-filled eggs; presence of tubercles, spines, or filaments in trematodes; pear-shaped apparatus with an oncosphere in cestodes; and bipolar plugs, crushing balls, or central larvae in nematodes.

Results and Disscusion

The fecal analysis of Bukhara deer (Cervus elaphus bactrianus) conducted across two regions-Turkestan and the Ile-Balkhash State Nature Reserve – revealed the presence of 25 parasite taxa, including protozoa, trematodes, cestodes, and nematodes (Table 1). A total of 22 species were detected in the Turkestan Region and 9 species in the Ile-Balkhash reserve.

Host-specific coccidia of the genus *Eimeria – E. sholpanae, E. kulashae* and *E. aruzhanae*-were identified in samples from both locations, confirming their persistence in the deer population across habitats.

In the Turkestan Region, a broader spectrum of parasites was recorded, including:

Trematodes: *Fasciola hepatica, Dicrocoelium lanceatum*

Larval cestodes: *Echinococcus granulosus*

Adult cestodes: *Moniezia expansa*

Nematodes: Setaria spp., *Oesophagostomum* spp., *Haemonchus contortus*, *Nematodirus* spp., among others

In contrast, the Ile-Balkhash site showed reduced parasite diversity, where only 9 taxa were found. Notably, *Anoplocephala perfoliata* (cestode) and *Cylicocyclus insigne* (nematode) were identified exclusively at this location, indicating potential new parasite acquisition in the reintroduced population.

The overall data indicate a difference in parasite prevalence and composition between the native and reintroduction areas.

Table 1 – Types of parasites found in Bukhara deer

Sequence number	Parasites	Turkestan region	Ile-Balkhash State Nature Reserve
1	*Eimeria sholpanae Berkinbay, Baytursino*v et *Elyubaeva*, 2012	+	+
2	*Eimeria kulashae Berkinbay, Baytursinov* et *Elyubaeva*, 2012	+	+
3	*Eimeria aruzhanae Berkinbay, Baytursinov* et *Elyubaeva*, 2012	+	+
4	*Fasciola hepatica Linnaeus*, 1758	+	-
5	*Dicrocoelium lanceatum Stiles* et *Hassall*, 1896	+	+
6	*Echinococcus granulosus* (*Batsch*, 1786), larvae	+	-
7	*Moniezia expansa* (*Rudolphi*, 1810) *Blanchard*, 1891	+	+
8	*Anoplocephala perfoliata Goeze*, 1782	-	+
9	*Parabronema skrjabini Rassowska*, 1924	+	-
10	*Onchocerca skrjabini Ruchljadew*, 1961	+	-
11	*Setaria cervi* (*Rudolphi*, 1819)	+	-
12	*S. digitata* (*Linstow*, 1906)	+	-
13	*S. labiato-papillosa* (*Alessandrini*, 1838)	+	-
14	*Oesophagostomum columbianum* (*Curtice*, 1890) *Stossich*,1899	+	-
15	*O. radiatum* (*Rudolphi*, 1803) *Railliet*, 1898	+	-
16	*O.venulosum* (*Rudolphi*, 1809) *Railliet* et *Henry*, 1913	+	-
17	*Cooperia* sp. (only the females)	+	-
18	*Haemonchus contortus* (*Rud*., 1803) *Cobb*., 1898	+	+
19	*Nematodirus spathiger* (*Railliet*, 1896) *Railliet et Henry*, 1909	+	+
20	*Nematodirus* sp. (only the females)	+	-
21	*Dictyocaulus eckerti Skrjabin*, 1931	+	-
22	*Dictyocaulus filaria* (*Rudolphi*,1809), *Railliet et Henry*, 1907	+	-
23	*Trichocephalus skrjabini* (*Baskakov*, 1924)	+	-
24	*Cylicocyslus insigne* (*Boulenger*, 1917)	-	+
Total		22	9

The observed disparity in parasite diversity between the native Turkestan Region and the Ile-Balkhash State Nature Reserve aligns with well-documented ecological effects of translocation on host–parasite dynamics. Upon relocation, host populations often lose parasites for which required intermediate hosts or environmental conditions are absent – a process referred to as “parasite release” [8].

Specifically, the absence of Fasciola hepatica and Echinococcus granulosus in the Ile-Balkhash deer likely stems from the scarcity of necessary intermediate hosts – sails and canids – dsrupting their life cycles, as described in wildlife parasite reviews [9].

Conversely, detection of Anoplocephala perfoliata and Cylicocyclus insigne only in Ile-Balkhash indicates possible “parasite acquisition”. Translocated hosts may acquire new parasites endemic to the introduction site, especially when interacting with sympatric species or environmental reservoirs [10, 11, 12].

Host-specific protozoa such as *Eimeria* spp. persisted across both sites, which is consistent with their direct fecal–oral transmission and host fidelity, as described in ruminant infection patterns [13]

Translocation may also alter parasite community structure in unintended ways: captivity or pre-release anthelmintic treatments can eliminate host-specific parasites, potentially increasing susceptibility to endemic parasites post-release due to lack of acquired immunity [14, 15].

From a public health viewpoint, losing zoonotic helminths like *F. hepatica* and *E. granulosus* after relocation may reduce spillover risk to livestock and humans, supporting One Health outcomes. Nonetheless, the risk from newly acquired parasites remains, underscoring the need for continuous monitoring [16, 17].

In summary, the study supports the need to integrate parasitological monitoring into wildlife management and reintroduction protocols. Understanding host–parasite–environment interactions is crucial to ensure the sustainability of conservation efforts under the principles of the One Health approach.

Conclusion

Parasitological aspects of animal introduction and acclimatization are critical factors that markedly influence the success or failure of these biological and ecological efforts. The introduction of animal species into new environments can result in the unintentional transfer of parasites and pathogens, posing risks not only to introduced populations but also to native fauna, potentially disturbing established ecosystems. Parasites may impair health, reduce reproductive success, and lower survival rates in acclimatized animals, thereby compromising conservation and economic objectives. Effectively addressing these challenges requires an integrated, multidisciplinary approach. This includes comprehensive epizootiological monitoring to assess parasite diversity and prevalence, risk assessments to forecast potential outbreaks, and targeted preventive and control measures. Such actions may involve quarantine protocols, veterinary treatment, habitat management, and ongoing postintroduction monitoring.

Sustainable management of biological resources depends on cooperation among parasitologists, ecologists, veterinarians, and wildlife managers. Only coordinated efforts can reduce parasitic risks, conserve biodiversity, and support the long-term viability of animal introduction and acclimatization programs. In summary, understanding and managing parasitological factors is essential for the success of animal introductions and the maintenance of ecosystem health. Future research should focus on developing innovative diagnostic tools, improving integrated pest management strategies, and fostering adaptive management frameworks that respond to emerging parasitological threats under changing environmental conditions.

Authors' Contributions

OB and LZh: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. LZh, BO, NJ and MS: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript".

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UDC 636.068;59.009; 591.135 **Research article**

The main helminths and protozoa of the digestive tract of domestic and wild ungulates in northern and central Kazakhstan

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Abstract

Background and Aim. Parasitic infections in ungulates represent a major challenge to animal health, biodiversity conservation, and agriculture. This study aimed to investigate the diversity, prevalence, and intensity of gastrointestinal helminths and protozoa in domestic and wild ungulates in northern and central Kazakhstan, with a focus on species overlap and ecological implications.

Materials and Methods. Between January 2023 and July 2024, fecal samples (n = 559) from wild and domestic ungulates were collected from five regions. Standard flotation and centrifugation techniques (Fulleborn's method) were used for parasitological analysis. Parasites were identified morphologically, and their prevalence was statistically assessed using chi-square tests.

Results. A broad spectrum of parasites was found, including Strongyle-type eggs, *Eimeria, Trichuris, Nematodirus, Capillaria,* and *Dicrocoelium lanceolatum*. Horses, sheep, and cattle exhibited the highest prevalence and mean intensity of disease, suggesting increased risk due to anthropogenic factors. Wild ungulates had lower infection rates, although cross-species infections were documented.

Conclusion. This study highlights significant interspecies variation in gastrointestinal parasitism, with domestic animals serving as major reservoirs. Monitoring and targeted control are essential at the wildlife–livestock interface.

Keywords: gastrointestinal parasites; wild ungulates; domestic ungulates; helminths; protozoa; Kazakhstan.

Introduction

Ungulates represent a generalized group of mammals with significant economic, hunting, esthetic, and scientific value. Contemporary challenges in fields of veterinary medicine, ecology, epidemiology and biology can be addressed by studying the diversity of helminths and parasitic protozoa in these animals [1].

Parasitic infections of the gastrointestinal tract remains a complex problem for conservation, animal health and zoonotic risk management in ungulates, including domestic species as cattle, sheep, goats, and pigs, and wild ruminants like deer, moose. Main factors contributing to the morbidity and mortality rate among these animal populations are helminths and protozoan parasites. They affect productivity, ecological stability, reproduction and public health.

Substantial variations in the parasitic fauna have been documented by studies conducted in Europe and Asia. While investigating wild ungulates in northeastern Portugal, *Figueiredo* et al. (2020) found that 78.6% of them – particularly red deer, roe deer, and wild boar – were infected with at least one

type of parasite, including zoonotic taxa such as Balantidium coli. The presence of *Strongylidae, Trichostrongylidae, Metastrongylus,* and protozoa like *Eimeria* and *Cystoisospora* underscores the diversity and ecological adaptability of endoparasites in these populations [2]. Similarly, *Swisłocka-Cutter* et al. (2024) used molecular diagnostics to detect high infection rates of *Ostertagia* spp. and other gastrointestinal nematodes in Polish deer [3].

In Russia, numerous studies have reported complex parasitic profiles among captive and free-ranging ungulates. *Tishkov* et al. (2018) documented the presence of gastrointestinal strongyles, Trichuris, and protozoa in maral populations, whereas studies from the Kazan Zoo (*Timerbaeva* et al., 2018) and Moscow zoological parks indicated persistent infections despite routine deworming. These studies confirmed widespread infestations of strongylid and Eimeria spp. among zoo-housed ungulates, highlighting the challenges of parasite control in semi-closed systems. In the Sumorokov Reserve, Russian researchers *Postevoy* and *Andreyanov* (2020) identified up to 17 helminth species in moose, including *Ostertagia, Trichuris, Moniezia,* and *Echinococcus granulosus* [4, 5, 6].

Several studies conducted across various geographic regions and ungulate species have detailed the diversity of helminth and protozoan infections. *Loginova* et al. (2024) examined 233 reindeer (Rangifer tarandus) housed in 50 Russian zoos and identified numerous parasites, including *Fasciola hepatica, Paramphistomum* spp., *Moniezia* spp., *Trichuris* spp., and *Dictyocaulus* spp., with an infection rate of 45%. The study revealed the unique risk posed by captivity, where deer coexist with species that are not normally encountered in the wild [7, 8].

Ecotones in which domestic and wild ungulates intersect further complicate parasite transmission. *Abdybekova* et al. (2020) reported co-infections of *Moniezia, Avitellina, Nematodirus, Marshallagia,* and *Skrjabinema* among sheep and saiga in Kazakhstan, suggesting the potential for parasite spillover. *Barone* et al. (2020) and *Remesar* et al. (2025) emphasized the need for genetic and ecological monitoring, noting the detection of parasite DNA associated with livestock in wild herbivores within conservation landscapes [9, 10].

Environmental contamination with eggs and larvae, particularly in the soils of enclosures and pastures, is also well documented. *Albery* et al. (2018) and *Polaz* (2022) highlighted seasonal fluctuations in larval abundance, peaking in spring and autumn, emphasizing the influence of climatic conditions on parasite life cycles. Given the expanding range of wild ungulates, anthropogenic landscape changes, and ongoing climate shifts, sustained parasitological surveillance is vital for the preservation of biodiversity and agricultural stability. *Panayotova-Pencheva* (2024) reviewed helminth control strategies for captive herbivores in zoos and reserves, focusing on the families *Bovidae, Cervidae*, and *Giraffidae* [11, 12, 13].

Notably, many studies have reported increased anthelmintic resistance among nematode populations. *Gałązka* et al. (2023) and *D'Amico* et al. (2025) documented reduced efficacy of fenbendazole, albendazole, and ivermectin, necessitating molecular surveillance and alternative control strategies. This calls for integrated management approaches that combine targeted therapy, pasture rotation, and biological control. Common challenges include reinfection, drug resistance, and difficulty in administering medications. Ivermectin and fenbendazole have shown variable efficacy, and copper oxide wire particles have also demonstrated potential against nematodes [14, 15].

Additional studies from France by *Verheyden* et al. (2020) established a positive correlation between roe deer helminth burden and livestock density, indicating the considerable potential for interspecies transmission in shared landscapes. *Jones* (2021) presented a geographic perspective on trichuriasis in deer (*Cervidae*), documenting the low but widespread prevalence of Trichuris spp. across the Neotropical and temperate zones. He emphasized the use of molecular tools over morphological methods for accurate species identification, particularly at the wildlife–livestock interface. *Remesar* et al. (2025) demonstrated the value of noninvasive eDNA-based diagnostics in endangered ungulates, confirming the presence of gastrointestinal nematodes in the saola (*Pseudoryx nghetinhensis*) [16, 17, 18].

Global trends confirm the ecological breadth of these parasites. For example, in Nepal, *Achhami* (2016) linked gastrointestinal helminths in blue sheep and Himalayan tahr to shared grazing zones with domestic yaks and goats. Genetic analysis is increasingly revealing host–parasite coevolution, highlighting the necessity of molecular diagnostics in surveillance programs [19].

Thus, the aim of this publication is to comprehensively analyze and summarize the diversity of gastrointestinal helminths and protozoa infecting domestic and wild ungulates, identify overlapping parasite species among host types, and highlight the ecological and veterinary significance of these interactions in the context of parasite control.

Materials and Methods

Sample Collection

Fecal samples from wild ruminants were collected between January 2023 and July 2024, either directly from the animals' rectums or from the ground. Collections were carried out in northern and central Kazakhstan, where the average temperature was 26.5 ℃ (ranging from −21 ℃ to 32 ℃), and included the following five regions: Akmola, Karaganda, Kostanay, North Kazakhstan, and Pavlodar (Fig. 1). Samples were collected by local natural resource agencies, hunters, state veterinarians, and biologists. All sample collectors were provided with protocols and materials for sampling and shipping. Individual samples (10–20 g feces/animal) (n = 559) were obtained from wild and domestic ruminants of varying ages and species, including dappled deer (*Cervus nippon*, n = 23), Kazakhstan mountain sheep (*Ovis ammon collium*, n = 42), wapiti (*Cervus elaphus*, n = 90), moose (*Alces alces*, n = 53), Asian roe deer (*Capreolus pygargus*, n = 37), argali (*Ovis ammon*, n = 42), saiga antelope (*Saiga tatarica*, n = 126), red deer (*Cervus elaphus*, n = 3), European fallow deer (*Dama dama*, n = 3), bison (*Bison bison,* n = 3), wild yak (*Bos mutus*, n = 2), zubr (*Bison bonasus,* n = 1), mouflon (*Ovis gmelini*, n = 1). In addition, 133 samples were collected from domestic cattle (*Bos taurus*, n = 60), domestic sheep (*Ovis aries,* n = 32), and horses (*Equus ferus caballus*, n = 41). Fecal samples were stored in biological sample collection containers, which were numbered and shipped in cardboard boxes at ambient temperature (~18–22 ℃) to the laboratory for further analysis.

Sample Preparation

Fulleborn's method consisted of the following: 3 g of excrement was pre-soaked in a saturated salt solution and centrifuged at 3000 rpm for 15 min. The surface of the film was examined using a parasitological loop with a diameter of 5 mm. Three loops were taken from each sample, and the average number of helminth eggs in each sample was calculated. The morphology of the eggs and larvae obtained from the feces was studied by light microscopy (LM) using an Axio Scope.A1 optical microscope (Zeiss, Germany) equipped with objectives with ×5 (for navigation on the slide), ×10, ×20, ×40, and ×100 magnification (the latter with oil immersion) [20].

Statistical Analysis

The frequency of gastrointestinal parasite isolates detected in fecal samples was statistically compared using the chi-square test, with a 95% confidence interval. The p-value was calculated, and statistical significance was established at $p < 0.05$. (https://www.statskingdom.com).

Results and Discussion

From January 2023 to July 2024, fecal samples were collected from wild and domestic ruminants in the northern and central regions of Kazakhstan. The materials were collected directly from the rectums of the animals and from the soil surface in places where the individuals had recently been present. The study area covers five administrative regions: Akmola, Karaganda, Kostanay, North Kazakhstan, and Pavlodar (Figure 1).

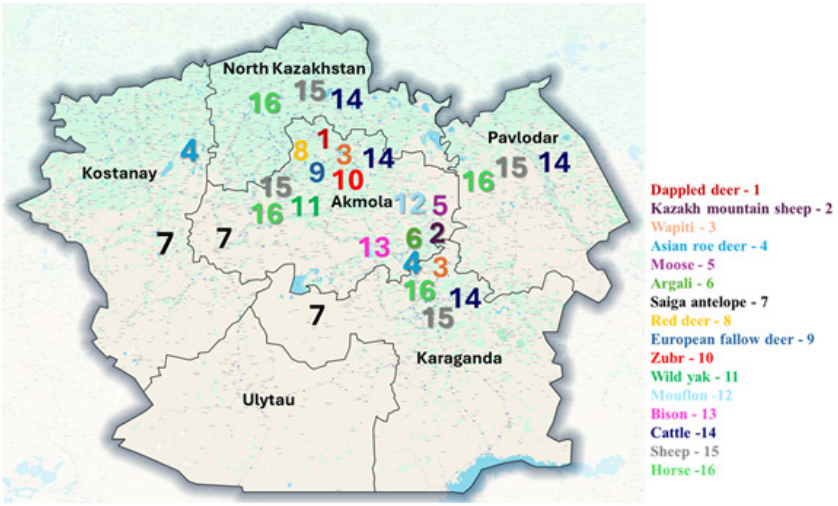

Figure 1 – Map of the regions of northern and central Kazakhstan showing the marked locations of samples collected from domestic and wild ungulates

Figure 1 presents the geolocation data for fecal samples collected from both wild and domestic ungulates across various regions. A noteworthy observation was that many of the samples were from the Akmola region, as this region is notable for its diverse ungulate population. Additionally, a substantial number of samples were collected from the Karaganda region, underscoring its ecological significance. Samples were also obtained from the Kostanay region, where they primarily came from Asian roe deer and saiga antelope, indicating their presence and habitat in this area. On the contrary, the Pavlodar and North Kazakhstan regions obtained feces samples only from domestic farm animals, which shows the difference in ungulate species representation between these regions.

Table 1 presents comparative information on the prevalence and intensity of helminth infections found in domestic and wild ungulates. The data included the number of infected animals, infection prevalence (with 95% confidence intervals), the range of helminth intensity, and the mean intensity with standard deviation.

Table 1 – Prevalence and intensity of helminths in domestic and wild ungulates

№	Host	N infected/N examined	% prevalence (95% CI)	Range of the intensity	Mean (SD) intensity	Helminth species
1	Dappled deer (*Dama dama*)	23/9	39.1 (19.7–61.4)	3-14	8.2 (3.6)	*Strongyle-type egg*
2	Kazakh mountain sheep (*Ovis ammon collium*)	42/13	31.0 (17.6–47.1)	2-7	4.1 (1.9)	*Strongyle-type egg*
3	Wapiti (*Cervus elaphus*)	90/17	18.9 (11.4–28.5)	4-9	6.2 (1.8)	*Strongyle-type egg Eimeria sp. Trichuris sp. Capillaria sp.*
4	Asian roe deer (*Capreolus pygargus*)	37/5	13.5 (4.5–28.7)	3-8	5.0 (1.8)	*Strongyle-type egg Eimeria sp. Trichuris sp.*
5	Moose (*Alces alces*)	53/5	9.4 (3.1–20.6)	2-6	4.0 (1.6)	*Strongyle-type egg Eimeria sp.*

Continuation of Table 1

6	Argali (*Ovis ammon*)	42/4	9.5 (2.6–22.6)	1-8	4.5 (2.6)	*Strongyle-type egg Eimeria sp. Naematodirus sp Dicrocoelium lanceolatum*
7	Saiga antelope (*Saiga tatarica*)	126/20	15.9 (10.0–23.4)	6-26	14.1 (5.8)	*Strongyle-type egg Eimeria sp. Trichuris sp. Naematodirus sp.*
8	Red deer (*Cervus elaphus*)	3	-	-	-	-
9	European fallow deer (*Dama dama*)	3/2	66.6 (9.4–99.1)	2-3	2.5 (0.7)	*Eimeria sp.*
10	Zubr (*Bison bonasus*)	1	-	-	-	-
11	Wild yak (*Bos mutus*)	2/1	-	3	3.0 (0.0)	*Eimeria sp.*
12	Mouflon (*Ovis gmelina*)	1/1	-	3	3.0 (0.0)	*Eimeria sp.*
13	Bison (*Bison bison*)	3	-	-	-	-
14	Cattle (*Bos taurus*)	60/20	33.3 (21.7–46.7)	4-26	13.2 (6.2)	*Strongyle-type egg Naematodirus sp. Eimeria sp.*
15	Sheep (*Ovis aries*)	32/15	46.9 (29.1–65.2)	6-24	12.7 (5.7)	*Naematodirus sp. Eimeria sp. Trichuris sp.*
16	Horse (*Equus ferus caballus*)	41/20	48.8 (32.9–64.9)	5-58	18.6 (11.7)	*Strongyle-type egg Eimeria sp. Trichuris sp.*

95% CI: 95% confidence interval; SD: standard deviation

Among all species examined, European fallow deer (66.6%), horses (48.8%), and sheep (46.9%) showed the highest infection prevalence, indicating that these animals are particularly susceptible to helminth infections. Horses also recorded the highest mean intensity of infection (18.6 ± 11.7 helminths), followed by saiga antelope (14.1 ± 5.8) and cattle (13.2 ± 6.2), reflecting a substantial parasite burden in these hosts. In contrast, species such as moose, argali, and Asian roe deer exhibited relatively low prevalence rates (below 15%) and moderate intensities (approximately 4–5 helminths per infected individual).

Some species, including red deer, zubr, and bison, had incomplete data – either due to a very small sample size or unreported intensity metrics – limiting conclusions about their infection status. Despite this, the data highlight clear interspecies variation in helminth load, with domestic animals tending to have both higher prevalence and intensity than most wild ungulates.

Nematode, trematode, protozoan eggs. The parasites were preliminarily identified based on their morphology and morphometric data (Fig. 2).

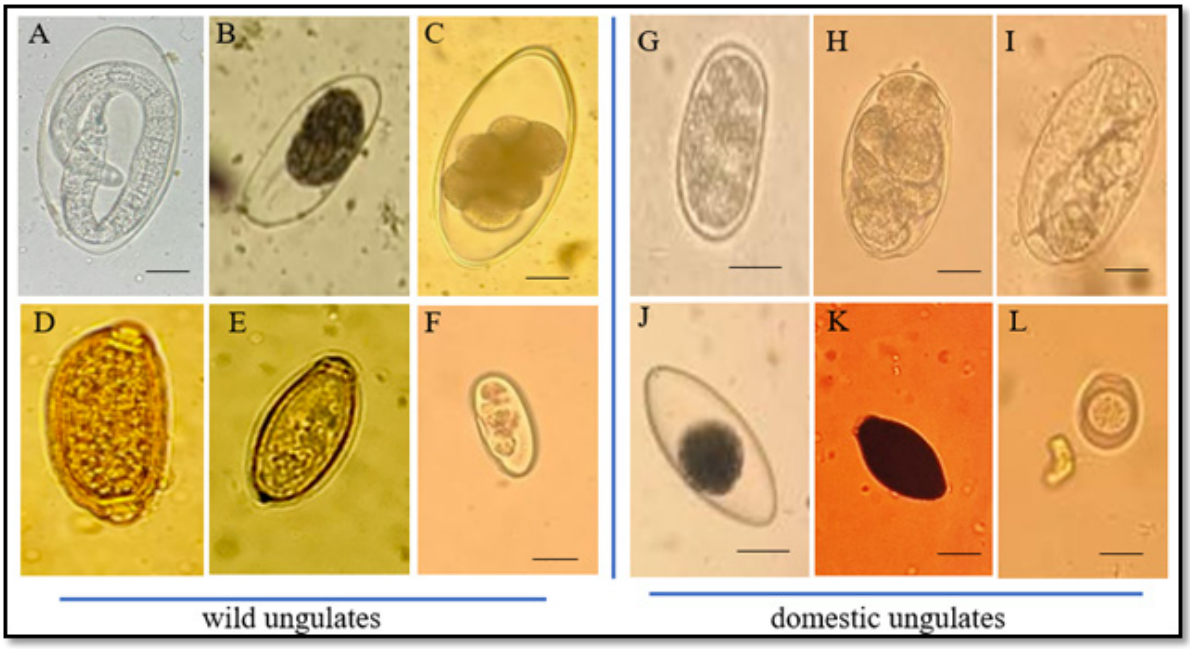

Figure 1 – Diagnostic stages of helminths isolated from wild feces
(A-B. Strongyle-type eggs; C. *Naematodirus* sp.; D. *Capillaria* sp.; E. *Dicrocoelium lanceolatum*;
F. Eimeria sp.) and domestic ungulates (G, H, I. Strongyle-type eggs; J. *Naematodirus* sp.;
K. *Trichuris* sp. L. *Eimeria* sp.)

Bright field microscopy, ×40 objective lens magnification. Scale bar equals 50 μm.

All samples exhibited low parasite intensity. We found 1–5 trematode eggs per 1 g of feces, 1–26 nematode eggs per 1 g of feces, and 1–8 protozoan coccidia. Only one sample from argali (*Ovis ammon*) contained nematodes, trematodes, and protozoa – up to 8 specimens per 1 g. The rest are interpreted as medium intensity or its absence.

Based on the results of the study of fecal samples from wild ungulates (n = 426) and domestic ungulates (n = 133) collected from five regions of northern and central Kazakhstan, these results are consistent with earlier studies showing a higher prevalence of parasites among domesticated ungulates [21, 22]. The ecological plasticity of parasites such as Eimeria and Strongyle-type nematodes was reflected in the results from Portugal, Poland, and Russia (*Figueiredo* et al., 2020; *Swisłocka-Cutter* et al., 2024; *Tishkov* et al., 2018).

In the conducted study, the prevalence and intensity of helminthiasis varied significantly among the examined ungulates. Domestic animals (horses, sheep and cattle) demonstrated higher prevalence and mean intensity values compared to wild ungulates, indicating an increased susceptibility or vulnerability due to housing and environmental conditions. Notably, horses had the highest mean intensity (18.6±11.7), highlighting their potential role as major helminth reservoirs. Whereas, wild species such as elk, argali and Asian roe deer demonstrated lower prevalence and intensity of infection, which may indicate differences in habitat, diet and transmission dynamics of the parasite in the population. These interspecific differences among ungulates highlight the need to develop individual parasite control strategies, especially among domestic animals with a high degree of infection.

Conclusion

This study revealed substantial variation in the prevalence and intensity of gastrointestinal parasites among wild and domestic ungulates in Kazakhstan. The domestic species displayed significantly higher infection burdens, emphasizing their role as key reservoirs. Evidence of parasite overlap between wild and domestic species highlights the importance of controlling cross-transmission, especially in areas where ecotones and shared grazing sites exist. Continued monitoring, species-specific treatment regimes, and coordinated wildlife–livestock health strategies are essential for sustainable parasite control.

Authors' Contributions

Conceptualization, VK; methodology, VK and RU; validation, AS, RU, AN, and LL; formal analysis, VK, RU, and AS; investigation, VK; resources, VK; data curation, AS; writing–original draft preparation, AS, RU, VK; writing–review and editing, VK and RU; visualization, AS, LL and A.N; project administration, VK; funding acquisition, VK. All authors have read and agreed to the publication of the final version of the manuscript.

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UDC 616:619+598.2(574) (045) **Research article**

Spread of highly pathogenic avian influenza in Kazakhstan: investigating the role of bird migration and threats to poultry farming

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Abstract

Background and Aim. Highly pathogenic avian influenza (HPAI) remains a serious threat to industrial poultry farming and human health. The virus is highly variable and can be transmitted through the migratory flows of wild birds, which contribute to its widespread distribution. This study aimed to analyze the epidemiology of HPAI in Kazakhstan and assess the role of migratory birds therein.

Materials and Methods. The study assessed data on outbreaks of HPAI in Kazakhstan for the period 2005 to 2024. Information on migratory bird species in Kazakhstan territories and their migration routes was obtained by the "Institute of Zoology". The research applied classical methods of epidemiological analysis, statistical modeling of epizootics, and data visualization using ArcGIS Pro software.

Results. Over the past 20 years (2005 to 2024), outbreaks of HPAI have been periodically recorded in Kazakhstan, with cases of infection in both domestic and wild birds. The pathogens mainly comprised viruses of the H5N1, H5N8, and other H5 strains. Wild migratory birds played the main role in the emergence and spread of HPAI. The territory of Kazakhstan contained the convergence point of two of their most important migration routes: the Central Asian-Indian and West Asian-African routes. A total of 489 bird species has been registered in Kazakhstan, most of which are seasonal migrants flying through the country; accordingly, they have a significant impact on the epizootic process of HPAI.

Conclusion. Analysis of epizootiological data showed that the dates and locations of HPAI outbreaks directly correlated with the stages and routes of the seasonal migration of migratory birds. Predictive modeling of virus spread showed that the northern and western regions of the country, where most of the country's poultry farms are located, were at the highest risk.

Keywords: Bird migration; epidemiological monitoring; forecasting; highly pathogenic avian influenza; Kazakhstan.

Introduction

Highly pathogenic avian influenza (HPAI) is a highly contagious viral infection that can affect all types of birds. The most susceptible domestic species are turkeys and chickens. Wild birds can serve as carriers of the infection; however, due to their natural resistance, they do not get sick, as a rule, and cover significant distances during migration [1].

HPAI, caused by the type A influenza virus (*Orthomyxoviridae*), poses a serious threat to industrial poultry farming and human health. The virus's high variability and ability to recombine and overcome the interspecies barrier create a constant risk of new epizootics and pandemics [2].

Bird migration has a significant impact on the epizootic process of avian influenza. In Kazakhstan, 489 species of birds have been registered, of which 396 nest in the republic's territory and the rest are seasonal migrants flying through the country during spring and autumn migrations [3].

Since migratory birds are the main reservoir of HPAI, outbreaks of influenza in poultry farms usually occur during their seasonal migrations in spring and autumn. Since 2020, outbreaks of HPAI A (H5N1) have been registered in Kazakhstan and Central Asian countries, confirming the active circulation of the virus in the region. In September 2020, mass cases of infection among poultry occurred in the North Kazakhstan, Akmola, and Pavlodar regions, causing significant mortality and the introduction of quarantine measures. In June 2021, the virus was detected in wild waterfowl in the North Kazakhstan region, indicating its circulation among natural reservoirs of infection [4, 5].

However, the high concentration of birds in a limited area makes poultry farms vulnerable to the introduction of the virus. The main routes of penetration into industrial farms include contact with infected wild birds; viral transfer through contaminated feed, water, and equipment; and mechanical transmission through transport and personnel's shoes and clothing [6]. HPAI outbreaks lead to huge economic losses due to the forced slaughter of infected birds, the introduction of quarantine measures, disinfection, and the temporary cessation of production. For example, in countries affected by HPAI epidemics, losses to the poultry industry are estimated to amount to millions of dollars, including the costs of disease control, compensation to farmers, and the decrease in export potential [7].

In addition, the infection of poultry with HPAI viruses creates a risk of transmission to humans [8]. Historically, HPAI outbreaks have had serious epidemiological consequences; for example, the 1997 H5N1 outbreak in Hong Kong and the 2013 H7N9 outbreak in China resulted in severe infections in humans. Therefore, monitoring bird migration flows, studying the epizootiology of the virus, and developing effective prevention methods are paramount to reduce the risks of infection introduction into poultry farms [9, 10].

Thus, this study aimed to analyze the epidemiology of HPAI in Kazakhstan and assess the role of migratory birds therein.

Materials and Methods

The study used data on avian influenza outbreaks in Kazakhstan for the period 2005–2024, registered in the World Animal Health Information System (WAHIS) of the World Organization for Animal Health, official data from the country's state veterinary service, and our own field studies conducted in 2023 and 2024.

The main research methods included the following:

- collection of epizootiological data on HPAI outbreaks;
- analysis of bird migration routes;
- use of GIS technologies for mapping risk zones; and
- statistical modeling of epizootics using ArcGIS Pro software.

Data on the species of birds migrating through the territory of Kazakhstan and their migration routes were obtained by the "Institute of Zoology." In this institution, through ringing and subsequent monitoring, a large dataset on the timing of seasonal migration, migration routes, and nesting sites was collected. Based on the obtained materials, databases were created, indicating the geographic coordinates of all the parameters of interest, which were subsequently used with the ArcGIS Pro program for data visualization and analysis.

Results and Discussion

Over the past 20 years (from 2005 to 2024), outbreaks of HPAI have been periodically recorded in Kazakhstan. Moreover, outbreaks were recorded both among poultry kept in organized poultry farms and private households and among representatives of wild fauna. Statistics showed the occurrence of HPAI outbreaks in the country approximately every 3 to 4 years. Outbreaks were mainly caused by the H5N1, H5N8, and other H5 influenza subtypes. Given the well-developed poultry sector and the frequency of outbreaks, HPAI is gradually acquiring the status of an endemic infection in Kazakhstan. Data on registered cases of HPAI in Kazakhstan from 2005 to 2024 are presented in Table 1.

Table 1 – Data on outbreaks of highly pathogenic avian influenza in Kazakhstan (2005-2024)

№	Types of birds	Region of outbreak	Genotype/Serotype/ Subtype
1	Black-headed gull (*Chroicocephalus ridibundus*)	Pavlodar	H5 (N not typed)
2	Greylag goose (*Anser anser*)	North Kazakhstan	H5 (N not typed)
3	Greylag goose (*Anser anser*)	North Kazakhstan	H5 (N not typed)
4	Mallard (*Anas platyrhynchos*)	North Kazakhstan	H5 (N not typed)
5	Black raven (*Corvus corone*)	North Kazakhstan	H5 (N not typed)
6	Whistling teal (*Anas crecca*)	North Kazakhstan	H5 (N not typed)
7	Teal (*Anas crecca*)	North Kazakhstan	H5N8
8	Lesser white-fronted goose (*Anser erythropus*)	North Kazakhstan	H5N1
9	Greater white-fronted goose (*Anser albifrons*)	North Kazakhstan	H5N1
10	Mallard (*Anas platyrhynchos*)	Aqmola	H5N1
11	Greylag goose (*Anser anser*)	Qostanay	H5N8
12	Greylag goose (*Anser anser*)	Almaty	H5N1
13	Poultry (*Gallus gallus*)	North Kazakhstan	H5N1
14	Poultry (*Gallus gallus*)	Aqtobe	H5N1
15	Domestic goose (*Anser anser domesticus*)	Atyrau	H5N1
16	Dalmatian pelican (*Pelecanus crispus*)	Mangghystau	H5N1

As shown in Table 1, during the analyzed period, a total of 16 outbreaks of infection were registered in the WAHIS system for the territory of Kazakhstan.

The sample included outbreaks among farm and wild birds, as well as among other representatives of wild fauna. In most outbreaks, the causative agent of the disease belonged to the H5 subtype; however, in several cases, the neuraminidase (N) of the HPAI virus was not typed. Unfortunately, this factor limits the possibilities for epidemiological analysis and accurate identification of the routes of spread of the virus. In some cases, subtypes H5N1 and H5N8 were identified, which confirms the circulation of classical forms of the virus, posing a threat to both birds and humans.

Subsequently, based on epidemiological data, we produced a cartographic visualization of HPAI outbreaks registered in the country for the period 2020 to 2024 (Figure 1).

Figure 1 – Visualization of outbreaks of highly pathogenic avian influenza in Kazakhstan in 2020-2024

Most outbreaks of HPAI were registered in the country's northern regions, especially North Kazakhstan. Outbreaks were also noted in the western (Atyrau, Mangistau) and southeastern (Almaty) regions. It is worth mentioning that when HPAI was registered in the country, the pathogen was not only found in domestic and wild birds but also isolated from some wild animals. At the same time, in most cases, the source of infection remained unknown, even though it is known from the disease epidemiology that wild migratory birds are the main reservoirs and carriers.

Birds become infected with HPAI through direct contact with the source of infection, as well as through environmental objects (most often water) contaminated with the saliva, nasal secretions, and feces of infection carriers. As the experience of Asian countries shows, the contamination of water bodies with the pathogen is caused not only by the presence of infected birds but also by feeding farmed fish with bird droppings.

The Republic of Kazakhstan, with its vast territory and diverse landscapes, is not only a nesting place for birds but also a region where two of their most important migration routes converge: the Central Asian-Indian and the West Asian-African routes. Millions of birds flying along these routes use the territory of Kazakhstan for molting and stopovers. Migration is influenced by wetlands, which are especially abundant in the northern part of the country.

There are 489 species of birds registered in Kazakhstan, of which 396 nest in its territory, with the rest being seasonal migrants flying through the country during spring and autumn migrations. At the same time, bird migration has a significant impact on the epizootic process of avian influenza.

The main carriers of avian influenza of epidemiological significance for Kazakhstan are as follows: swans (*Cygnus* spp.): mute swan (*Cygnus olor*), whooper swan (*Cygnus cygnus*), Bewick's swan (*Cygnus columbianus*); geese (*Anser* spp.): greylag goose (*Anser anser*), white-fronted goose (*Anser albifrons*), lesser white-fronted goose (*Anser erythropus*); ducks (*Anas spp.*): mallard (*Anas platyrhynchos*), teal (*Anas crecca*), northern pintail (*Anas acuta*); order Charadriiformes: migratory waterbirds, gulls (*Larus* spp.): herring gull (*Larus argentatus*), black-headed gull (C*hroicocephalus ridibundus*); waders (*Scolopacidae, Charadriidae*): black-tailed godwit (*Limosa limosa*), Eurasian curlew (*Numenius arquata*); order Pelecaniformes, pelicans (*Pelecanus spp*.): Dalmatian pelican (*Pelecanus crispus*), great white pelican (*Pelecanus onocrotalus*); cormorants (*Phalacrocorax* spp.): great cormorant (*Phalacrocorax carbo*).

Currently, most scientists hold a point of view that can be called "synthetic": the flight of birds covers a wide front, but within it are areas with increased concentrations: migration routes (flyway) that form migration corridors when merged. The main corridors of migratory birds through the territory of the Republic of Kazakhstan are as follows (Figure 2):

- routes from African and South European wintering grounds in the Mangistau, Atyrau, and West Kazakhstan regions;

- routes from Pakistani and Indian wintering grounds in the South Kazakhstan, Kyzylorda, Kostanay, and North Kazakhstan regions; and

- routes from South Asian wintering grounds in the Almaty, East Kazakhstan, and Pavlodar regions.

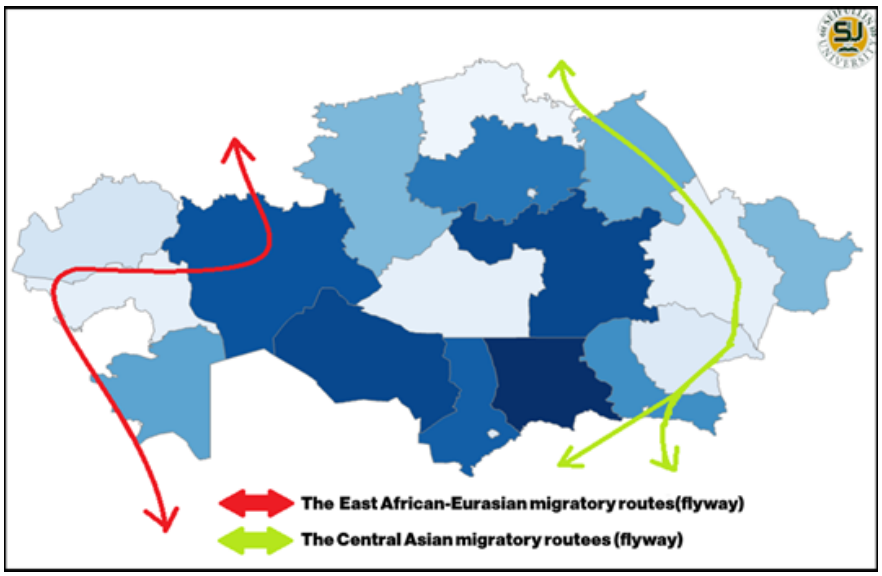

Figure 2 – Main migration routes of birds

The available epidemiological data on the locations and dates of the primary outbreaks of HPAI in Kazakhstan demonstrated a direct correlation with the stages and routes of seasonal migration of migratory birds through the country. At the same time, predictive modeling of the emergence and spread of the virus showed that the northern and western regions were at the highest risk. Importantly, most of the country's poultry farms are located in areas with a high risk of HPAI outbreaks. Currently, 65 poultry farms are operating in the country for meat and egg production, most of which are located in the northern, northwestern, and western regions of the country (Figure 3).

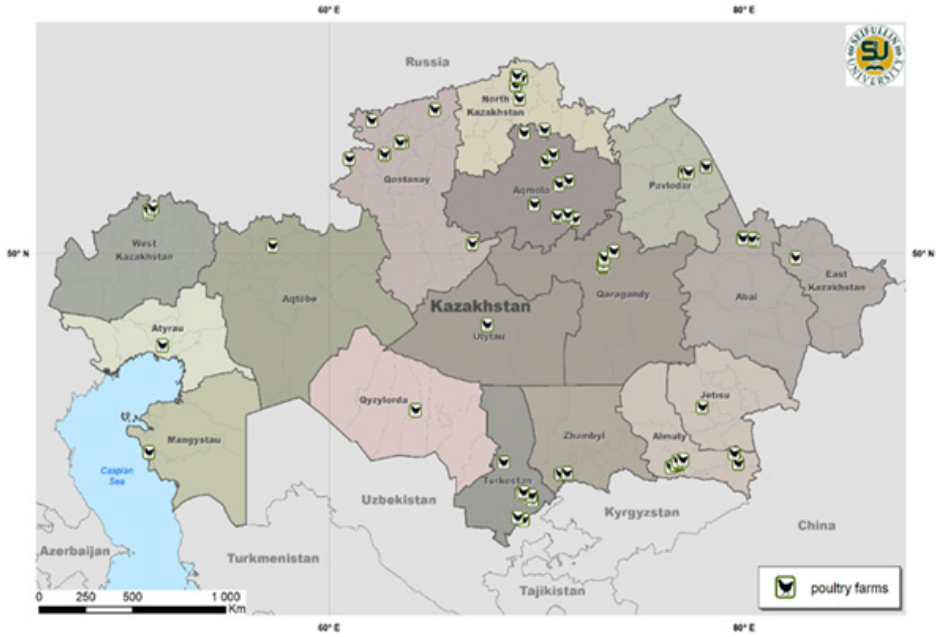

Figure 3 – Visualization of the location of industrial poultry farms in Kazakhstan

In Kazakhstan, industrial poultry farms contain more than 80% of the total poultry population. That is, outbreaks of HPAI at such enterprises always lead to an exacerbation of the epizootic situation and huge economic losses. Therefore, the obtained data show the importance of continuous epidemiological surveillance of HPAI in Kazakhstan. Enhanced biological safety measures, monitoring of migratory birds, and the development of diagnostic centers for the early detection of infection are necessary.

HPAI poses a threat to many countries due to the impact of the disease on wild bird popula-tions, direct and indirect losses to the poultry industry, and potential impact on public health [11, 12]. Seasonal migrations of wild birds play an important role in the intercontinental spread of the disease, and transmission occurs at the interface between wild and domestic birds, despite efforts by governments in both developed and developing countries, including biosecurity and other preventive measures [13, 14].

Kazakhstan, located in the center of Central Asia, is under constant threat of avian influenza, as evidenced by outbreaks in recent years in the northern, eastern, and southern regions [15, 16].

Considering the epizootic situation of this disease in Kazakhstan in previous years, as well as the global situation this year, the probability of an epizootic outbreak remains high. The main threat of spread stems from wild migratory birds, especially ducks, geese, and swans [17, 18].

Given the geographical location of the republic, Kazakhstan's reservoirs are the most important reserves in Asia, housing aquatic and near-water bird species. In the republic, 489 species of birds have been registered during the nesting, molting, seasonal migration, and wintering periods. Every year, the number of nesting bird species reaches 10 million, 2–3 million birds fly in for molting, and about 50 million migratory birds stop at the reservoirs during spring and autumn migrations [19].

Several seasonal bird migration routes pass through the territory of Kazakhstan. The Central Asian-Indian and West Asian bird migration routes intersect with the Black Sea-Mediterranean and East African-West Asian channels in the west of the republic [20].

The present study of the epizootic situation in Kazakhstan identified migratory corridors, nesting sites, and water bodies as vulnerable points for the monitoring and control of HPAI. Outbreaks of the disease are statistically associated with a high density of water resources, which necessitates in-tensive surveillance in these regions. Waterfowl are extremely important carriers of the avian influen-za virus, as they can transmit it over long distances. Interaction between different bird species in their usual resting and breeding areas can facilitate the transmission of the avian influenza virus between species [21].

The patterns identified in Kazakhstan are consistent with data obtained in other regions along the main Eurasian migratory flyways. For example, studies in China, Mongolia, Russia, and Kazakhstan have also demonstrated a close association between H5N1 outbreaks and the presence of wetlands that serve as resting and feeding sites for migratory waterfowl, and have proposed various hypotheses to explain the movement of H5N1 from China to Mongolia, Russia, and Kazakhstan [22]. These findings support the view that aquatic ecosystems serve as critically important reservoirs for the persistence and spread of avian influenza viruses. Researchers reported two significant patterns of H5N1 spread from Asia. A comprehensive analysis of bird migration routes and wild bird behavior suggested that the introduction of H5N1 into Mongolia occurred as a result of wild bird movements from China several months before the outbreaks. These events were repeatedly linked to the seasonal migrations of swans, geese, and ducks, with H5N1 and H5N8 subtypes predominating [23]. This is consistent with data from Kazakhstan, where the northern and western regions with dense wetlands and a high level of poultry farming are the most vulnerable. This indicates that the role of wild birds as long-distance carriers is not unique to Kazakhstan but is part of the global epidemiological picture. Nevertheless, the central geographical position of Kazakhstan and the convergence of numerous fly-ways appear to intensify these risks, creating overlapping migratory corridors that increase opportuni-ties for virus exchange. This highlights Kazakhstan as a particularly important "epidemiological bridge" between Asia, Europe, and Africa. Taken together, these comparisons show that although the specific outbreak patterns observed in Kazakhstan are determined by local landscapes and the distri-bution of domestic poultry, they are also consistent with broader global mechanisms of highly patho-genic avian influenza spread. Thus, the conclusions drawn from this study contribute not only to na-tional veterinary surveillance but also to the general understanding of avian influenza epidemiology across continents. A comprehensive understanding of the spread of HPAI requires an integrated ap-proach that includes monitoring bird migration routes, strengthening biosecurity measures at poultry farms, and informing the public about potential threats and ways to prevent them. Cooperation among international and regional organizations in sharing information and developing prevention strategies is also important. Effective risk management of HPAI requires the integration of data from multiple sources and sectors to develop a holistic strategy to protect bird health and prevent human epidemics.

Conclusion

Analysis of epizootological data shows that the dates and locations of HPAI outbreaks directly correlate with the stages and routes of seasonal migration of migratory birds. Predictive modeling of the spread of the virus showed that the northern and western regions of the country, where most of the country's poultry farms are located, are at the highest risk.

Authors' Contributions

SA, YM, AM and SR: developed the concept and design of the study. SR conducted a comprehensive literature search, analyzed the collected data, and drafted the manuscript. SA and YM, AM: Performed final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript.

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UDC 577.2:579.637.0 **Research article**

Microbiological and molecular genetic characteristics of *Staphylococcus aureus* isolated from raw horse meat

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Abstract

Background and Aim. Staphylococcal food poisoning is caused by the consumption of food contaminated with *Staphylococcus aureus* and represents a global public health concern. Food products of animal origin can serve as reservoirs for multi-drug resistant strains of *S. aureus*. This study aimed to characterize the microbiological and molecular genetic properties of *S. aureus* isolated from raw horse meat.

Materials and Methods. Species identification of *S. aureus* was performed using 16S rRNA gene analysis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The sensitivity of the isolate to antibiotics was investigated using the disc diffusion method. Genetic determinants of resistance were identified using whole-genome sequencing. High-throughput sequencing was performed on an Illumina MiSeq platform. DNA quantity was assessed spectrophotometrically, and quality was evaluated by electrophoresis on a 1% agarose gel. Genome assemblies based on short reads were obtained using SPAdes v. 4.0.0. Assembly quality assessment, organism verification, and initial annotation were performed using FastQC v.0.11.9. Antibiotic resistance genes were identified using the CARD and PATRIC databases.

Results. The *S. aureus* 76_KZ strain was isolated from a raw horse meat sample. Morphologically, it is a Gram-positive coccus arranged in irregular clusters resembling grape bunches. The identified antibiotic resistance profile of *S. aureus* 76_KZ characterizes this strain as a multidrug-resistant isolate with sensitivity to a limited spectrum of antibacterial drugs. This study presents the results of whole-genome sequencing of *S. aureus* isolated from raw horse meat in the Republic of Kazakhstan. The sequencing yielded a genome coverage of 173×. The sequenced genome of the *S. aureus* 76_KZ isolate, consisting of 2.616.354 bp, has been deposited in the GenBank genetic database under accession number JBNBZR000000000.1.

Conclusion. The whole-genome data obtained for *S. aureus* 76_KZ enable the assessment of the isolate's resistance to antimicrobial drugs and facilitate the identification of genetic features relevant to epidemiological typing.

Keywords: food products; resistance; *Staphylococcus aureus*; whole genome sequencing.

Introduction

Food contamination by pathogenic microorganisms remains a key issue in food safety and public health [1]. Foodborne infections affect both men and women of various age groups living in both rural and urban areas alike, and can occur sporadically or in the form of epidemics. Every year, foodborne infections affect approximately 20% of the population in industrialized countries [2]. To date, over 250 different foodborne diseases have been identified worldwide, two-thirds of which are caused by bacteria.

Staphylococcus aureus is the third most common foodborne pathogen globally [3]. Staphylococcal infections cause significant morbidity and mortality in both developing and developed countries.

Various foods, such as meat and meat products, milk and dairy products, poultry, eggs, fish, vegetable salads, and cream-filled pastries, have been implicated in staphylococcal food poisoning. Unhygienic food handling is an important source of staphylococcal contamination [2]. The main sources of staphylococcal contamination in food products are humans and animals with purulent inflammatory processes (such as abscesses, furuncles, or purulent wounds) who carry these microorganisms. Staphylococci can be transferred from humans to food products via airborne droplets, direct contact, or during processing activities such as equipment handling, slaughtering, and carcass cutting.

S. aureus is a gram-positive, non-motile, non-spore-forming, facultatively anaerobic, commensal, and opportunistic pathogen that can cause a wide range of infections, from mild skin infections to life-threatening conditions, such as bacteremia, endocarditis, necrotizing pneumonia, toxic shock syndrome, and food poisoning [4]. Food poisoning results from ingesting preformed staphylococcal enterotoxins. Five serologically distinct enterotoxins (A, B, C, D, and E) have been identified, with enterotoxin A being the most common cause of food poisoning outbreaks. An estimated 30% to 80% of the global population carries *S. aureus*, and 50% of these carrier's harbor variants associated with food poisoning. *S. aureus* can grow over a wide range of temperatures (7 to 48.5 °C; optimum 30 to 37 °C), pH (4.2 to 9.3; optimum 7 to 7.5), and sodium chloride concentrations up to 15% NaCl. It is resistant to desiccation and can survive in potentially dry and stressful conditions, such as on the human nose and skin, and on surfaces such as clothing. These characteristics facilitate the growth of organisms in many food products [2].

Contamination of food products with antibiotic-resistant bacteria poses a serious threat to public health because antibiotic resistance determinants can be transmitted to other bacteria of clinical importance. Monitoring the spread of antibiotic-resistant bacteria and their resistance genes is a key factor in efforts to prevent antibiotic resistance. It has been established that *S. aureus* strains have developed resistance mechanisms to virtually all antimicrobial drugs used in treatment. The most important factor is resistance to drugs most often used in the treatment of gram-positive infections, such as beta-lactams, glycopeptides, and oxazolidinones [5].

S. aureus is able to develop antibiotic resistance through various mechanisms, including efflux pumps, biofilm formation, and enzymatic modification of antibiotics [6, 7, 8]. Whole-genome sequencing is increasingly used to analyze the genetic profiles of drug-resistant strains and the mechanisms of resistance gene transfer.

Identifying antibiotic-resistant foodborne *S. aureus* strains and assessing their pathogenic potential are urgent issues in food safety research. Currently, there are results on the study of phenotypic and genotypic resistance to resistance to antibacterial medications (ABMs) in S. aureus strains isolated from the milk of cattle in Northern Kazakhstan. The genotypic study of strains targeted genes related to resistance to β-lactam antibiotics (blaZ, 193 bp), macrolides (ermC 142 bp), and tetracyclines (tetK, 167 bp) [9]. In the study by *R. Rychshanova* et al., the results of the research shown were that *S. aureus* isolates obtained from cows' milk samples at the stage of subclinical mastitis were resistant to many antibiotics of the tetracyclines and *β*-lactam groups which are commonly used to treat mastitis [10]. There are also studies investigating the complete genomes of clinical *S. aureus* isolates obtained from hospitals in Kazakhstan [11, 12].

In this regard, the study aimed to perform microbiological and molecular genetic characterization of an S. aureus isolate obtained from raw horse meat. The novelty of this study lies in the implementation of whole-genome sequencing of non- clinical *S. aureus* isolates, providing information on the clustering of resistance genes, as well as pathogenicity, adhesion, and invasion factors.

Materials and Methods

Research object

A culture of *S. aureus*, isolated from a raw horse meat sample, was obtained from the Republic of Kazakhstan in 2025. Isolation, identification, and determination of the sensitivity of the bacterial isolates to antimicrobial drugs were performed at the National Scientific Laboratory of Biotechnology for Collective Use of the National Centre for Biotechnology LLP (Astana, Kazakhstan).

Species identification and storage of the microorganism

Species identification of the bacterium was performed based on analysis of the nucleotide sequence of the 16S rRNA gene, as well as by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using MALDI Biotyper 3 software. 1 (Bruker Daltonics, Germany). The criterion for reliable species identification by MALDI TOF MS was a score ≥ 2.0. The bacterial isolate was stored at -80 °C in Luria-Bertani broth supplemented with 50% glycerol.

Determination of sensitivity to antimicrobial drugs

The antimicrobial susceptibility profiles of the *S. aureus* food isolate were determined using the disc diffusion method on Mueller-Hinton agar for the following antibiotics: moxifloxacin (5 μg), ciprofloxacin (5 μg), rifampicin (5 μg), clindamycin (2 μg), erythromycin (15 μg), tobramycin (10 μg), amikacin (30 μg), and tetracycline (30 μg). Antimicrobial susceptibility was determined based on the growth inhibition zone breakpoints established by EUCAST (version 15.0 dated 01.01.2025). Quality control of sensitivity testing was performed on *S. aureus* ATCC29213 cultures.

Double disk approximation test (D-test)

The isolates that were resistant to erythromycin were tested for inducible clindamycin resistance by double disk approximation test (D-test) as per EUCAST 15.0 guidelines. In this test, a 0.5 McFarland's standard suspension of *S. aureus* was prepared and plated onto MHA plate (Mueller Hinton agar). An erythromycin disk (15 μg) and clindamycin (2 μg) were placed 15 mm apart edge-to-edge on the MHA plate. Plates were analyzed after 18 h of incubation at 37 °C [13].

DNA extraction and sequencing

Genetic determinants of resistance were identified in a multidrug-resistant *S. aureus* isolate from raw horse meat by whole-genome sequencing. DNA was extracted using cetyltrimethylammonium bromide/ NaCl. Bacteria grown on solid nutrient medium were collected with a bacterial loop and transferred to a clean test tube with 1.5 μL of TE buffer, and a uniform suspension was prepared. The mixture was centrifuged at 10.000 rpm for 3 min. The supernatant was then removed. DNA was extracted using the Kate Wilson method [14]. A centrifuge (Eppendorf 5415 D, Germany) was used at a centrifugation force of 12,000 × g.

Total DNA was quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific) at a wavelength of 260 nm. RNase A (Thermo Fisher Scientific) was used to remove RNA impurities. For a more accurate determination of DNA concentration, a Qubit 2.0 fluorometer (Invitrogen/Life Technologies, Carlsbad, USA) was used. Qualitative characterization of total DNA was performed by electrophoresis on a 1% agarose gel (Applichem, Darmstadt, Germany).

The library was obtained using PS DNA Library Prep Kit with UD Indexes (Thermo Fisher Scientific) per the manufacturer's instructions. The genome sequencing of *S. aureus*, strain 76_KZ was performed using Illumina Miseq platform and Miseq kit v3 (Illumina, Cambridge, UK) which allows to obtain 300 bp long paired-end reads.

Bioinformatic analysis

Genome assemblies based on short reads were obtained using SPAdes v. 4.0.0 [15].

Assembly quality assessment, organism verification, and initial annotation were performed using FastQC v.0.11.9 software [15]. Antibiotic resistance genes were identified using the Comprehensive Antibiotic Resistance Database (CARD) and PATRIC (http://patricbrc.org) databases.

Statistical processing of results

Statistical processing of the research results was performed using standard descriptive statistical methods in Microsoft Office Excel 2010. The statistical significance of differences in the proportion of resistant cultures was assessed using Student's t-test at a significance level of $\alpha < 0.05$.

Results and Discussion

Isolation and primary identification of Staphylococcus spp. isolated from fresh horse meat

For the isolation and primary identification of Staphylococcus spp. from the samples studied, we used the selective chromogenic medium, Chromatic S. aureus agar (Liofilchem, Teramo, Italy) (Figure 1). Figure 1 shows that *S. aureus* colonies were round with a smooth surface and a pinkish-purple color.

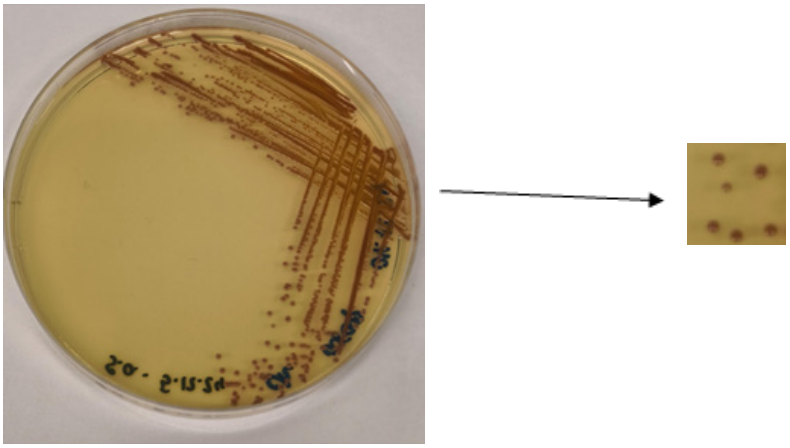

Figure 1 – Growth pattern of the studied isolate on Chromatic S. aureus agar (Liofilchem, Teramo, Italy)

The isolate was identified using MALDI-TOF MS by comparing the spectra of the constant proteins of the microorganisms with the MALDI Biotyper database (Bruker Daltonics GmbH, Bremen, Germany). The isolated strain was identified as *S. aureus* 76_KZ.

Next, the morphological and biochemical properties of the horse meat isolate were studied, namely, Gram staining and hemolytic activity of the isolated culture. The studied isolate, *S. aureus* 76_KZ, is a gram-positive coccus (Figure 2).

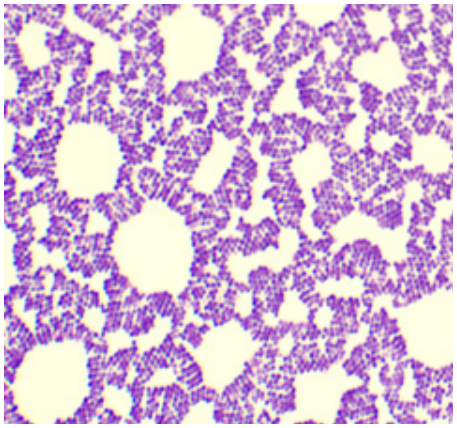

Figure 2 – Gram staining of *S. aureus* isolate 76_KZ

Figure 3 illustrates the growth of *Staphylococcus aureus* on the Columbia blood agar.

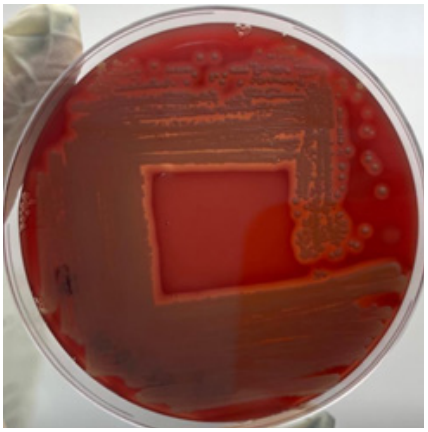

Figure 3 – Growth of *S. aureus* 76_KZ on a solid nutrient medium (Columbia blood agar, Liofilchem, Teramo, Italy)

As shown in Figure 3, the growth of the isolate on blood agar was marked by the formation of round colonies with a convex surface and smooth edges; a distinctive feature of the growth was the demonstration of β-hemolysis, which manifested itself in the formation of a transparent zone of lysis around the colonies.

When cultured on Bayer-Parker medium, it forms shiny grey-black colonies surrounded by a zone of medium clarification. This is because Staphylococcus aureus reduces tellurium from potassium tellurite, staining the colonies black, and also has lecithinase activity, which causes the surrounding medium to lighten due to the breakdown of egg yolk (Figure 4).

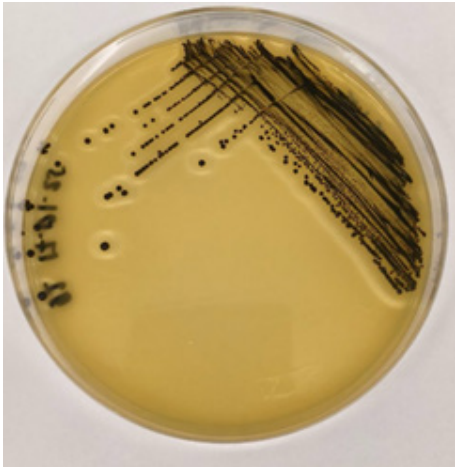

Figure 4 – Growth of *S. aureus* 76_KZ on Bayer-Parker medium

Study of antibiotic resistance

The antibiotic resistance profile of the S. aureus 76_KZ isolate was assessed using the disc diffusion method in accordance with the current EUCAST 15.0 (2025) interpretation criteria (Table 1).

Table 1 – Antibiotic susceptibility of *S. aureus* strains 76_KZ and ATCC 29213 determined by the disk diffusion method according to EUCAST standard v.15.0 (2025)

№	Antibiotic	Name of microorganism				Clinical breakpoints (EUCAST 2025-01-01)
		S. aureus 76_KZ		*S. aureus* ATCC 29213		
		Inhibition zone diameter (mm)	Interpretation (S/R)*	Inhibition zone diameter (mm)	Interpretation (S/R)*	
1	Tobramycin	18.3±0,6	S	18.0 ± 2.8	S	≥18: S; <18: R
2	Moxifloxacin	19.3±1,2	R	18.5 ± 3.5	R	≥25: S; <25: R
3	Rifampicin	22.7±2,1	R	21.5 ± 5.0	R	≥26: S; <26: R
4	Ciprofloxacin	16.0±0,0	R	15.0 ± 1.4	R	≥50: S; <17: R
5	Erythromycin	20.3±0,6	R	19.0 ± 1.4	R	≥21: S; <21: R
6	Clindamycin	19.7±0,6	R	17.0 ± 4.2	R	≥22: S; <22: R
7	Amikacin	17.7±1,2	S	15.5 ± 6.4	S	≥15: S; <15: R
8	Tetracycline	21.7±1,5	S	19.0 ± 1.4	R	≥22: S; <22: R
Note: *Interpretation (S – susceptible, R – resistant)						

The average inhibition zone diameter of the *S. aureus* 76_KZ strain ranged from 16.0 ± 0.0 mm (ciprofloxacin) to 22.7 ± 2.1 mm (rifampicin). Similarly, the average inhibition zone diameters of the S. aureus ATCC 29213 strain ranged from 15.0 ± 1.4 mm (ciprofloxacin) to 21.5 ± 5.0 mm (rifampicin).

Unlike the control strain S. aureus ATCC 29213, the clinical strain *S. aureus* 76_KZ showed susceptibility to tetracycline, whereas the control strain was resistant to it. For the other antibiotics

tested, the clinical strain *S. aureus* 76_KZ demonstrated similar susceptibility and resistance patterns as the control strain S. aureus ATCC 29213.

The experimental data demonstrated a heterogeneous pattern of sensitivity of the studied strain to antimicrobial drugs from various pharmacological groups. Analysis of the growth inhibition zones revealed resistance to several key antibacterial agents. Thus, the *S. aureus* 76_KZ strain showed resistance to fluoroquinolones, moxifloxacin, and ciprofloxacin, indicating the overexpression of NorC efflux systems, including resistance to moxifloxacin. This is consistent with the data of Que Chi Truong-Bolduc [16], who found that overexpression of *norC* contributes to the development of a quinolone resistance phenotype in the *mgrA* mutant. In our opinion, the overexpression of *norA* efflux systems may also be associated with ciprofloxacin resistance, which is consistent with the data of the authors [17]. In addition, pronounced resistance to rifampicin was observed, indicating potential modifications in the β-subunit of bacterial RNA polymerase encoded by the *rpoB* gene [18].

Of particular note is the presence of constitutive MLSB in this strain, a phenotype associated with simultaneous resistance to two classes of antibiotics: macrolides and lincosamides (to erythromycin (15 μg) and clindamycin (2 μg)) [19].

In this study, we investigated the inducible resistance of *S. aureus* 76_KZ to clindamycin (phenomenon D) (Figure 4). Some Streptococcus strains exhibit inducible resistance to clindamycin in the presence of erythromycin. This is because erythromycin activates the resistance mechanisms [20].

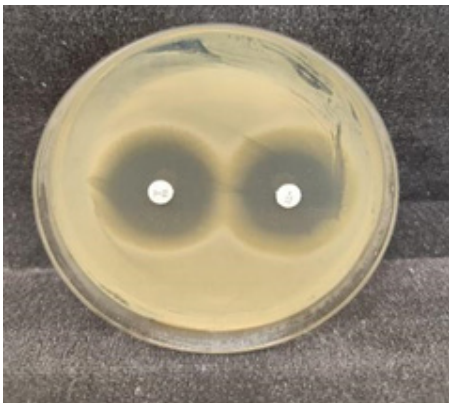

Figure 5 – Detection of inducible resistance of staphylococci to clindamycin
(D phenomenon, E: erythromycin 15 μg, CD: clindamycin 2 μg)

Figure 5 shows the results of a test to detect induced resistance to clindamycin (D-test) in the *S. aureus* 76_KZ strain. As can be seen in Figure 5, there is no characteristic D-shaped deformation of the inhibition zone, which indicates the absence of the D phenomenon and the presence of a constitutive resistance phenotype (cMLS phenotype) of resistance, which may be due to ribosomal mutations of the 23S rRNA gene, manifested by resistance to both erythromycin and clindamycin [21].

As shown in Figure 5 the test result was negative; that is, clindamycin was not inhibited by the antagonist erythromycin. Nevertheless, the S. *aureus* 76_KZ isolate retained its sensitivity to aminoglycosides, tobramycin, and amikacin, which may indicate the absence of aminoglycoside-modifying enzymes or mutations in the binding site in the 30S subunit of the ribosome. Moreover, sensitivity to tetracycline was preserved, indicating the absence of active resistance mechanisms such as tetracycline efflux proteins (Figure 6 and Figure 7).

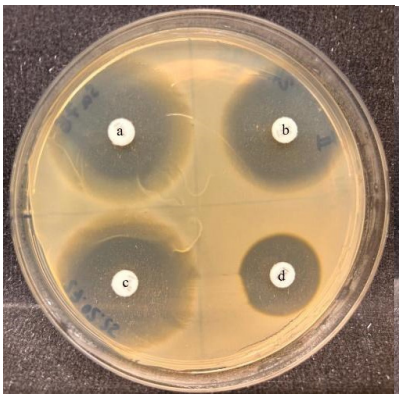

Figure 6 – Antibiotic resistance of *S. aureus* 76_KZ to erythromycin (a), tetracycline (b), clindamycin (c) and amikacin (d)

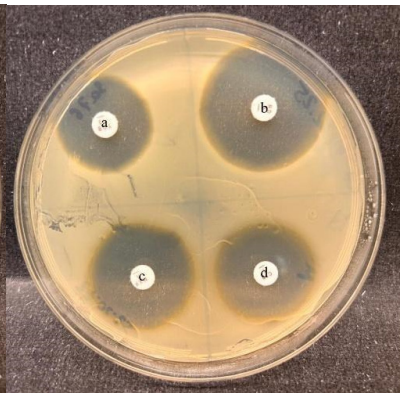

Figure 7 – Antibiotic resistance of *S. aureus* 76_KZ to tobramycin (a), rifampicin (b), ciprofloxacin (c) and moxifloxacin (d)

Therefore, the identified antibiotic resistance profile of *S. aureus* 76_KZ characterized this strain as a multidrug-resistant isolate with sensitivity to a limited range of antibacterial drugs.

Analysis of the whole genome of S. aureus 76_KZ

Whole-genome sequencing data were obtained using a MiSeq platform (Illumina, USA). The whole genome of the *S. aureus* 76_KZ isolate was 2.616.354 bp in length. The genome was sequenced with 173× coverage, an average read length of 150.24 bp, and a G+C content of 32.76%. A total of 899.768 reads were obtained. Read quality was checked using FastQC v.0.11.9. Sixteen contigs were assembled using SPAdes version 4.0.0. Final assemblies were annotated using the NCBI Prokaryotic Genome Annotation Pipeline v.6.10. Default parameters were used for all software packages. Annotation of the *S. aureus* 76_KZ genome revealed 2.577 genes, 2.488 of which encode proteins. In addition, 27 tRNAs and two complete rRNAs were detected.

Next, we performed a phylogenetic analysis of the whole genome of the isolate using the complete genomes of representative strains from the *Staphylococcaceae* family available in the NCBI database (Figure 8).

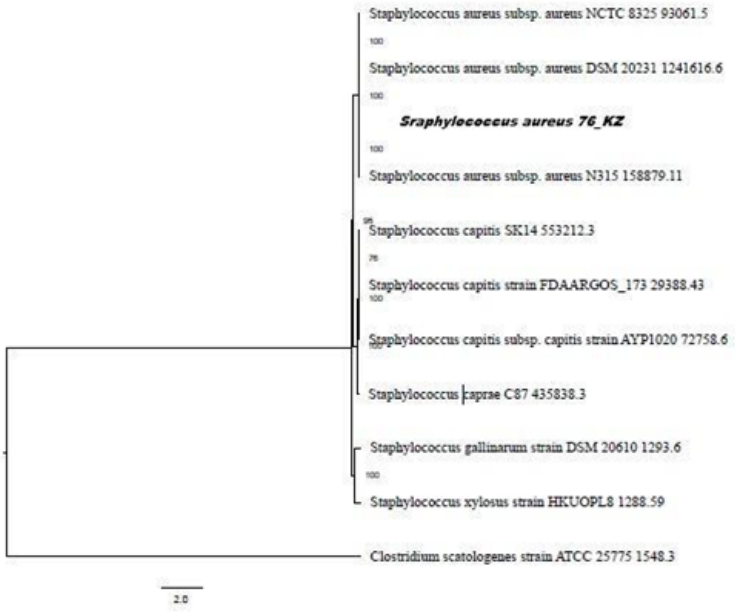

Figure 8 – Phylogenetic tree of representative *Staphylococcus spp*. genomes taken from the NCBI database using CSI Phylogeny v1.4

Figure 8 shows that the Kazakh isolate *S. aureus* 76_KZ forms a single branch with strains belonging to the species *S. aureus* and is located between the strain *Staphylococcus aureus* subsp. aureus DSM 20231 1241616.6, which was isolated from a human in 1953. *S. aureus* N315 is methicillin-resistant (MRSA) and was first isolated in 1982.

Antibiotic resistance genes

Antibiotic resistance genes were identified using the PathoSystems Resource Integration Centre (PATRIC) (https://www.patricbrc.org) (Table 2).

Table 2 – Antibiotic resistance genes

Resistance mechanisms	Genes
Antibiotic inactivation enzyme	FosB
Antibiotic resistance gene cluster, cassette, or operon	TcaA, TcaB, TcaB2, TcaR
Antibiotic target in susceptible species	Alr, Ddl, EF-G, EF-Tu, folA, Dfr, folP, gyrA, gyrB, inhA, fabI, Iso-tRNA, kasA, MurA, rho,rpoB, rpoC, S10p, S12p
Antibiotic target in susceptible species	BceA, BceB, NorA, Tet (38)
Gene conferring resistance via absence	gidB
Protein altering cell wall charge conferring antibiotic resistance	GdpD, MprF, PgsA
Regulator modulating expression of antibiotic resistance genes	BceR, BceS, LiaF, LiaR, Lia

Table 2 demonstrates that the norA gene has been identified, which encodes a multi-component efflux pump in *S. aureus* and provides resistance to fluoroquinolones and other structurally unrelated antibiotics, such as acriflavine [21]. It should be noted that the isolate under study was phenotypically resistant to fluoroquinolones, such as moxifloxacin. The gyrA gene, which is resistant to ciprofloxacin, was also identified during the analysis of the inhibition zones with this antibiotic. Interestingly, despite the isolate's resistance to both clindamycin and erythromycin, the genes responsible for the resistance to these drugs were not detected. Coutinho et al. also described six *S. aureus* isolates that were resistant to both erythromycin and clindamycin but did not carry any resistance genes [22, 23]. In addition, the *S. aureus* 76_KZ isolate contains the rpoB gene, which causes resistance to the antibiotic rifampicin, and is phenotypically evident. Thus, the phenotypic profile data were correlated with the identified genetic determinants of resistance, except for clindamycin and erythromycin resistance.

Virulence genes

Virulence genes are specific DNA segments that encode proteins or molecules contributing to the pathogenicity of microorganisms. These genes play key roles in the ability of pathogens to cause disease in their hosts. The identification of S. aureus virulence genes is important for assessing disease development (Table 3).

Table 3 – Virulence genes of *S. aureus*

Name	Virulence factor	Identity	Position in the contig	Protein function	NCBI number
S.aureus_ exoenzyme	aur	100.0	27108..28637	aureolysin	BA000018.3
S.aureus_ exoenzyme	splA	100.0	548336..549043	serine protease splA	BA000018.3
S.aureus_ exoenzyme	splB	100.0	547489..548211	serine protease splB	BA000018.3
S.aureus_toxin	hlgA	100.0	37327..38256	gamma-hemolysin chain II precursor	BA000018.3
S.aureus_toxin	hlgB	100.0	39772..40748	gamma-hemolysin component B precursor	BA000018.3
S.aureus_toxin	hlgC	100.0	38823..39770	gamma-hemolysin component C	BA000018.3

Continuation of Table 3

S.aureus_toxin	lukD	100.0	553174..554157	leukocidin D component	BA000018.3
S.aureus_toxin	lukE	100.0	554159..555094	leukocidin E component	BA000018.3
S.aureus_toxin	lukE	100.0	554159..555094	leukocidin E component	CP001781.1
S.aureus_toxin	seg	99.87	560236..561012	enterotoxin G	CP001844.2
S.aureus_toxin	sei	100.0	563014..563742	enterotoxin I	BA000018.3
S.aureus_toxin	sei	100.0	563014..563742	enterotoxin I	CP011147.1
S.aureus_toxin	sem	100.0	563777..564496	enterotoxin M	BA000018.3
S.aureus_toxin	sen	100.0	561295..562071	enterotoxin N	BA000018.3
S.aureus_toxin	seo	100.0	564777..565559	enterotoxin O	BA000018.3
S.aureus_toxin	seu	99.61	562089..562860	enterotoxin U	HE681097.1
S.aureus_toxin	tst	100.0	94247..94951	toxic shock syndrome toxin-1	AP009324.1

The table 3 shows that the isolate under investigation contains several exotoxins, which are divided into three groups based on their known functions: cytotoxins, superantigens (SAgs), and cytotoxic enzymes. Cytotoxins affect the membranes of host cells, leading to the lysis of target cells and inflammation. Superantigens mediate cytokine production and induce T- and B-cell proliferation. The secreted cytotoxic enzymes damage mammalian cells. Collectively, these exotoxins modulate the host immune system and are crucial for infections caused by *S. aureus* [24]. *S. aureus* secretes a metalloproteinase known as aureolysin, encoded by the aur gene. Aureolysin stimulates T and B lymphocytes via polyclonal activators and suppresses the production of lymphocyte immunoglobulins [25]. The toxin γ-hemolysin is encoded by the hlgA, hlgB, and hlgC genes. γ-hemolysin mimics leukocidins, forming pores in the membranes of host cells, promoting bacterial survival and evading immunity [26]. The toxin known as Panton-Valentine leukocidin (PVL) belongs to the family of synergistic chemotactic toxins, which also includes γ-hemolysin and other leukocidins such as LukE-LukD. Leukocidins destroy leukocytes and inhibit phagocytosis [27]. Serine protease-like proteins (Spls), encoded by the *splA* to *splF* genes, were discovered nearly three decades ago; however, their pathophysiological basis and biological functions during infection remain largely unknown [28]. Enterotoxin-like (SEl) toxin, encoded by the genes *seg, sei, sem, sen, seo,* and *seu*, is the most significant virulence factor involved in food poisoning, toxic shock syndrome, and staphylococcal infectious diseases in humans. These toxins belong to a broad family of pyrogenic superantigens that stimulate nonspecific T-cell proliferation [29]. Thus, identifying S. aureus virulence genes is important for the evaluation of isolated strains and the subsequent development of the disease.

Phylogenetic analysis

A comparative genome analysis was performed using the complete genome of the studied isolate, *S. aureus* 76_KZ. The comparison included reference genomes of S. aureus strains circulating in Russia, Japan, South Korea, and China. An isolate circulating in Kazakhstan was also included in the comparison (Table 4, Figure 9).

Table 4 – Presents the characteristics of the strains included in the phylogenetic analysis (country, year, and source of isolation)

№	Accession	Name strain	Country	Year	Source of isolation
1	AP017922.1	JP080	Japan	2005	human
2	NZ_BRBM00000000.1	JARB-OU1260	Japan	2018	human
3	NZ_JANVJN000000000.1	GD9M30A	China	2019	milk
4	NZ_CP113018.1	Taliyah	Taiwan	2019	environmental
5	NZ_WNKR00000000.1	1709	Russian Federation	2018	milk
6	NZ_JALJBR000000000.1	Crie-F374	Russian Federation	2019	Minced pork cutlet
7	NZ_JAJNOL010000000	SA201503,	China	2015	anal swab (human)
8	CP134071.1	4233	Kazakhstan	2022	water
9	CP030138.1	M48	China	2012	pig
10	CP121204.1	SA0907	China	2022	eccrine
11	NZ_PQWU00000000.1	0257-2201-2015	Russia	2015	Skin lesions (human)
12	CP080562.1	HL21008	South Korea	2017	Human blood
13	CP080560.1	HL17064	South Korea	2015	Human blood
14	JBNBZR000000000.1	76_KZ	Kazakhstan	2025	raw horse meat
15	CP082815	SCAID WND1-2021	Kazakhstan	2021	Swab from Wound (human)
16	CP082813	SCAID OTT1-2021	Kazakhstan	2021	Swab from ear (human)

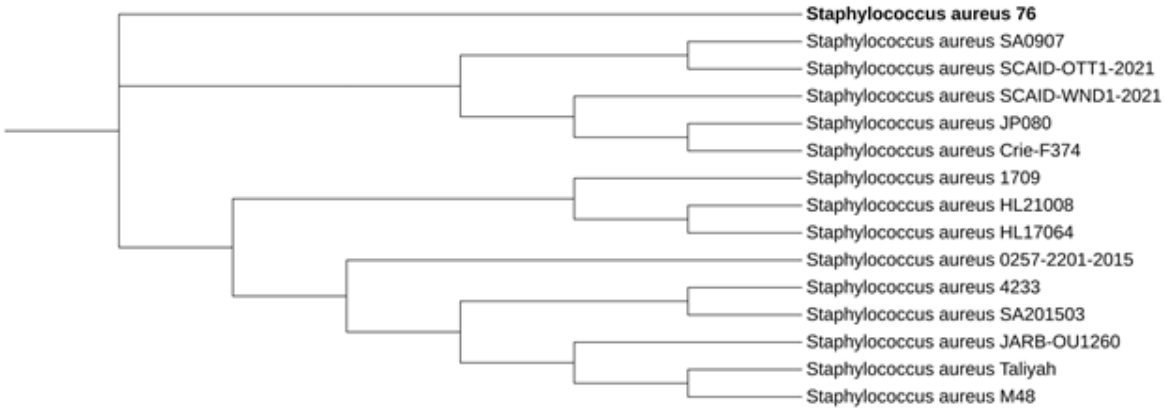

Figure 9 – Phylogenetic analysis of the genomes of the studied isolate *S. aureus* 76_KZ and complete genomes circulating in different countries using CSI Phylogeny v1.4

Figure 9 demonstrates that *S. aureus* 76_KZ is significantly distant from the complete *S. aureus* genomes included in the phylogenetic tree. The closest relatives are strain SA0907, isolated in China, and strain SCAID OTT1-2021, isolated in Kazakhstan.

Conclusion

Whole-genome sequencing of *S. aureus* isolates was performed to provide information on the clustering of resistance genes, as well as factors of pathogenicity, adhesion, and invasion, representing the novelty of this study. The identified antibiotic resistance profile of *S. aureus* 76_KZ characterizes this strain as a multidrug-resistant isolate with sensitivity to a limited spectrum of antibacterial drugs. Phenotypic resistance data were consistent with the identified genetic determinants of resistance. Notably, *S. aureus* 76_KZ isolate was resistant to erythromycin and clindamycin, but did not carry

known resistance genes associated with these antibiotics. This discrepancy requires further study to elucidate the underlying mechanism of resistance. Genes associated with pathogenicity that characterize the isolate under study were identified as pathogenic determinants.

The results obtained are important for the epidemiological monitoring of the spread of resistant clones of *S. aureus*. Findings related to the *S. aureus* 76_KZ isolate may be used to analyze the genetic characteristics of foodborne pathogenic strains circulating in Kazakhstan.

Authors' Contributions

SKh: Design and control of microbiological experiments, writing a publication on the microbiological part. EZh: Conceptualized and designed the research, processing, and interpretation of genetic data, writing the rest of the publication. DZh, AT, and AB: Conducted experimental work: microbiological experiments, preparation of DNA libraries, and DNA sequencing. All authors have read, reviewed, and approved the final manuscript.

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Based on the results of whole-genome analysis, the S. aureus 76_KZ genome sequence was deposited in GenBank with the accession number JBNBZR000000000.1. Raw data were obtained from BioProject No. PRJNA1245609 was submitted to the NCBI Sequence Read Archive under accession number SRX28236276. This research is funded by the Ministry of Agriculture of the Republic of Kazakhstan (BR22885795) We would like to express our gratitude to Professor A.K. Bulashev for his advisory assistance.

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Spatiotemporal Clustering of Animal Rabies in Kazakhstan: Insights from ArcGIS Pro-Based Analysis

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Abstract

Background and Aim. Rabies is a zoonotic viral disease that continues to pose a major public health threat worldwide, particularly in regions with limited access to vaccination and disease monitoring. Kazakhstan remains endemic for animal rabies, with variable patterns across geography and species. The objective of this study is to identify spatial and temporal clusters of rabies outbreaks among animals in Kazakhstan from 2013 to May 2025, using GIS-based methods to inform regional control and prevention strategies.

Materials and Methods. This study applied three geospatial tools within ArcGIS ProHot Spot Analysis using Getis-Ord Gi*, Kernel Density Estimation (KDE), and Space-Time Kernel Density to a national dataset of confirmed rabies cases among animals. The database included geographic coordinates, species type, dates of confirmation, and number of infected animals. KDE and Getis-Ord Gi* were used to detect spatial clusters and density gradients, while Space-Time Kernel Density enabled tracking of outbreak patterns over time.

Results. Hot Spot Analysis identified high-risk zones in northeastern (North Kazakhstan, Pavlodar oblasts) and western regions (Aktobe oblast), potentially linked to wild animal migration and insufficient vaccination coverage. KDE revealed additional high-density areas in southern and eastern regions. Space-time analysis showed persistent clusters in Zhambyl, Kostanay oblasts, and western Kazakhstan, while central regions exhibited low density likely due to geographic and demographic isolation.

Conclusion. Spatial and spatiotemporal analyses demonstrated that rabies outbreaks in Kazakhstan are not randomly distributed but form distinct species- and region-specific clusters. These insights support the need for differentiated veterinary approaches. Timely and geographically targeted vaccination programs particularly in identified hot spots are critical to reducing the incidence of rabies.

Keywords: animal rabies; ArcGIS Pro; epidemiology; Kazakhstan; spatial analysis; vaccination.

Introduction

Rabies is a fatal zoonotic disease caused by lyssaviruses that affects the central nervous system of mammals, including humans. Once clinical symptoms emerge, the disease is almost invariably lethal. Despite the long-standing availability of effective vaccines and post-exposure prophylaxis, rabies continues to cause tens of thousands of human deaths globally each year, with a disproportionate impact on rural communities in low- and middle-income countries [1, 2].

In Kazakhstan, rabies remains a significant public and veterinary health concern, with confirmed outbreaks recorded annually in both domestic and wild animal populations. Between 2013 and 2023,

hundreds of cases were reported across the country, with notable fluctuations in intensity by season, region, and species involved [3]. The persistence of rabies in Kazakhstan is driven by several interrelated factors, including low vaccination coverage among stray and rural domestic animals, the ecological dynamics of wildlife reservoirs such as foxes and wolves, and limited surveillance infrastructure in sparsely populated areas [4, 5].

Recent studies have also highlighted the role of transboundary dynamics, particularly in northern and western regions bordering Russia, which may facilitate the reintroduction and circulation of rabies virus strains across administrative boundaries [6]. These challenges were tragically underscored in May 2025, when a fatal human case of rabies was reported in Lisakovsk, Kostanay Region. The case involved a woman who was bitten by a domestic cat and did not receive timely post-exposure prophylaxis, pointing to ongoing systemic weaknesses in rabies awareness and response mechanisms [7].

To improve disease control and allocate limited resources effectively, spatial epidemiology offers a valuable approach. Geographic Information Systems (GIS) enable the visualization, modeling, and interpretation of complex epidemiological data. Recent advances in GIS platforms, such as ArcGIS Pro, allow for high-resolution analysis of disease patterns by incorporating multiple data layers, including case locations, host species distribution, demographic and environmental factors [8, 9].

The present study applies a suite of spatial and spatiotemporal analytical tools within ArcGIS Pro specifically Getis-Ord Gi* Hot Spot Analysis, Kernel Density Estimation, and Space-Time Kernel Density to identify high-risk zones of animal rabies in Kazakhstan. By integrating geographic, temporal, and biological dimensions, the research aims to support the development of regionally adapted and evidence-based rabies control measures.

Beyond its direct public health implications, rabies also exerts a considerable socio-economic burden, particularly in agrarian economies such as Kazakhstan. Livestock losses due to rabies outbreaks reduce household income in rural areas and create barriers to food security, while costs associated with post-exposure prophylaxis place additional strain on healthcare systems. Moreover, the persistence of rabies undermines international trade in animal products, as importing countries impose strict sanitary regulations. These factors underscore the urgent need for evidence-based spatial risk assessments that can guide targeted interventions, optimize vaccination strategies, and enhance cross-sectoral collaboration under the One Health framework [10, 11].

Materials and Methods

This study utilized confirmed rabies case data collected from veterinary surveillance reports in Kazakhstan between 2013 and May 2025 [3, 4]. Each record included spatial coordinates, date of confirmation, species involved, and number of infected animals [3, 6]. All spatial analyses were performed using ArcGIS Pro (Esri, Redlands, CA), which provided integrated tools for geostatistical evaluation [8, 9].

To identify spatial clusters of rabies cases, Hot Spot Analysis was performed using the Getis-Ord Gi* statistic, a standard method for detecting areas of significantly high or low values in spatial data [3, 8]. This method identifies spatial clusters with elevated (hot spots) or diminished (cold spots) rabies incidence based on Z-scores and P-values [9]. A high positive Z-score and low P-value indicate statistically significant clustering of high values (hot spots), while a high negative Z-score and low P-value suggest clustering of low values (cold spots) [6, 9]. Interpretation of the outputs included Z-score-based classification: areas with values above 1.96 ($p<0.05$) were classified as statistically significant [3, 9]. The analysis was applied to point data from the 2013–2025 rabies case database using the number of infected animals as the population field [3, 8].

Kernel Density Estimation (KDE) was used to assess the spatial intensity of outbreaks by generating a continuous raster surface where each cell value reflected the density of rabies cases in its vicinity [3, 9]. The KDE parameters included: input points (rabies cases), a defined search radius based on average inter-case distances, and a population field representing infected animal counts [4, 9]. Outputs were visualized as heatmaps where areas of higher density were indicated by more intense coloration [3, 9].

For spatiotemporal analysis, the Space-Time Kernel Density method was applied [3, 9]. This technique expands traditional KDE by integrating the temporal dimension, allowing for detection of outbreak clusters that persist or evolve over time [3, 9]. Inputs included the same point dataset, with daily

time intervals and distance measured in meters [3, 6]. The outputs were rendered in three-dimensional space-time cubes, where purple dots represented individual rabies cases across time, and darker areas within the color gradient reflected higher densities of outbreaks [3, 9]. Dashed boundary lines indicated administrative divisions of Kazakhstan [3, 4].

All analyses adhered to established methodological standards and were designed to be fully reproducible using documented workflows in ArcGIS Pro [8, 9]. Data processing and visual interpretation were performed consistently using heatmap and cluster detection outputs to facilitate accurate and actionable conclusions [3, 9].

Results and Discussion

Between 2013 and May 2025, a total of 1,010 confirmed rabies outbreaks were reported across Kazakhstan, involving 1,243 infected animals. These outbreaks affected more than ten animal species, including companion, agricultural, and wild animals. Approximately 58% of outbreaks occurred in rural and urban settlements, with repeated cases documented in major cities such as Semey (35 cases), Taraz (29), and Shymkent (28).

The highest cumulative outbreak numbers were recorded in the northeastern (n = 236) and western (n = 219) regions, followed by the southern (n = 196) and northern (n = 130) zones (Figure 1). Annual data revealed consistent case reporting throughout the entire period, with seasonal peaks observed in spring and late autumn, and a noticeable contribution from agricultural and companion animal populations.

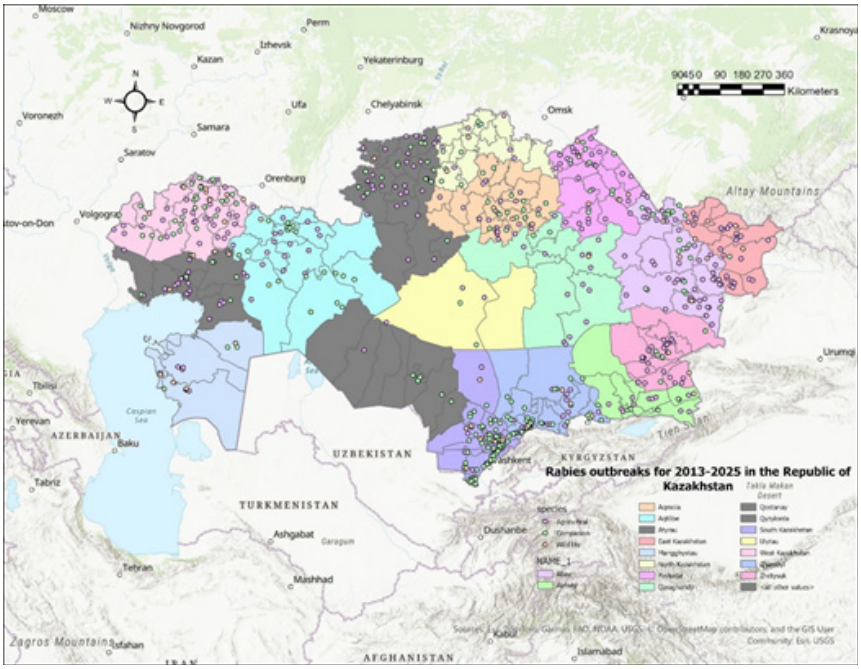

Figure 1 – Spatial Distribution of Rabies Foci in the Republic of Kazakhstan, 2013-2025 (may)

This long-term pattern reflects a persistent endemic status of rabies in Kazakhstan, with clear regional clustering and repeated emergence in certain high-risk areas. These findings emphasize the importance of spatially focused control measures, especially in endemic zones with recurrent activity.

The Hot Spot Analysis (Getis-Ord Gi) revealed statistically significant spatial clustering of rabies outbreaks in Kazakhstan from 2013 to 2024 (Figure 2). Hot spots areas with significantly elevated rabies incidence were predominantly located in northeastern Kazakhstan (North Kazakhstan and Pavlodar oblast) and western Kazakhstan (Aktobe oblast). These clusters are likely associated with active wildlife migration or insufficient effectiveness of vaccination programs. In particular, western regions demonstrated high animal density and low vaccination coverage only 26% of total vaccinations targeted livestock and 8.8% domestic animals. Moreover, no oral vaccination of wild carnivores is carried out in western Kazakhstan, as bait distribution polygons for wildlife immunization are absent in this region, which likely contributes to sustained transmission among wildlife reservoirs and cross-species spillover.

In contrast, cold spots zones with significantly low incidence were detected in the southern regions of the country, specifically in Almaty and Zhambyl oblasts. According to the methodological framework of Hot Spot Analysis (Getis-Ord Gi*), such cold spots represent statistically significant clustering of low values, which may indicate suppression or control of disease transmission in those areas. In the context of rabies, this suggests a likely impact of effective prevention efforts. As supported by previous cluster and outlier analyses, these areas may reflect the positive outcomes of consistent domestic animal vaccination campaigns and elevated levels of oral immunization of wildlife conducted during 2015-2017 and 2022-2023. The spatial concentration of low-incidence values in these regions may thus reflect a measurable containment of the epizootic process through targeted immunoprophylaxis.

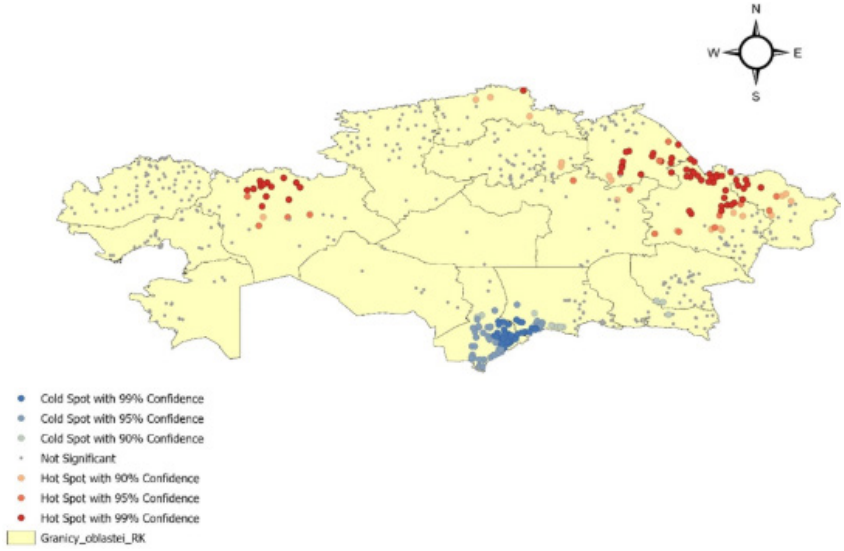

Figure 2 – Hot Spot Analysis of Rabies Cases in Kazakhstan

Kernel Density Estimation illustrated the spatial distribution of rabies cases across Kazakhstan, revealing high-density outbreak zones in Western (West Kazakhstan and Aktobe), Northern and Eastern (North Kazakhstan, Pavlodar, East Kazakhstan), and Southern (Zhambyl and Almaty) oblasts. Central Kazakhstan (Karaganda oblasts) showed relatively low case density, potentially due to sparse human and livestock populations or improved control and vaccination measures in certain southern districts (Figure 3).

Figure 3 – Kernel Density Estimation of Rabies Case Distribution

Spatiotemporal analysis using Space-Time Kernel Density demonstrated dynamic clustering patterns over time. Medium-density clusters (represented as shaded purple regions) were prominent in West Kazakhstan, North Kazakhstan (Kostanay oblast), and most notably in the South (Zhambyl oblast). Central Kazakhstan remained less affected, possibly due to lower population densities, limited livestock activity, and geographic isolation (Figure 4).

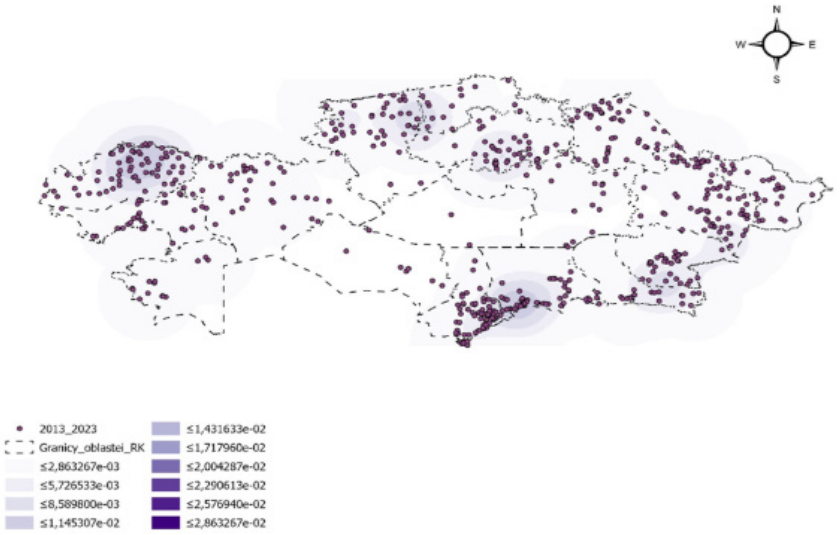

Figure 4 – Space-Time Kernel Density Map of Rabies Outbreaks 2013-2024

Conclusion

The applied spatial and spatiotemporal analysis methods (Getis-Ord Gi* Hot Spot Analysis, Kernel Density, and Space-Time Kernel Density) successfully identified distinct clusters and high-risk territories for rabies outbreaks in Kazakhstan, which correspond with species-specific distributions of infected animals [8, 9, 12]. Companion animals formed clusters predominantly in southern Kazakhstan, agricultural livestock were concentrated in eastern and western regions, and wild animals were most prevalent in western, southern, and northeastern parts of the country [3, 4]. Rabies emergence appears to be strongly influenced by environmental conditions that affect wildlife reservoirs, a factor that should be integrated into the planning of veterinary interventions [5, 6, 13].

These findings support the development of differential control and surveillance strategies and provide baseline analytical and visual tools essential for evaluating the efficacy of rabies prevention programs [1, 2, 14]. Specific clusters identified in eastern and western regions (livestock), southern regions (domestic animals), and northeastern and western territories (wildlife) indicate the need for tailored preventive approaches. For instance, the domestic animal cluster in southern Kazakhstan represents a restrained epizootic scenario influenced by veterinary measures, as supported by Anselin Local Moran's I and cold spot detection [8, 12]. However, despite the planned 70% vaccination coverage, challenges persist due to large populations of pets and the unaccounted stray animal population [14, 15, 16]. This suggests the need for stronger state veterinary oversight and measures to control both owned and stray animal populations.

Vaccination in southern regions should align with seasonal declines in rabies cases ideally between July and September. In eastern Kazakhstan, where low-infection livestock clusters are surrounded by high-risk zones, early and simultaneous vaccination efforts should be scheduled between October and January, coinciding with the housing season for livestock [3, 14]. In western Kazakhstan, the resumption of oral vaccination is advised due to climatic changes affecting wildlife populations and migration patterns [4, 6, 13]. Rising average annual temperatures (+0.38 °C) driven by warm air masses from the Caspian Sea may increase wildlife activity and interspecies contact during winter–spring months [6, 13]. Wildlife migration from bordering regions of the Russian Federation and milder winters, combined with anomalously warm spring and autumn periods, have contributed to sustained rabies activity in North Kazakhstan and Pavlodar regions [3, 5, 12]. These areas confirmed as high-incidence zones by hot and

cold spot analyses require enhanced preventive efforts through adjusted timing and expanded coverage from September to November.

Authors' Contributions

AK: Conducted laboratory research and wrote the first draft of the manuscript. AK, MY: Developed the aims, objectives, and methodology of the work; AA: prepared the article per the publication requirements. SA: Performed statistical analysis and reviewed the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

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Estimation of the prevalence of feline leukaemia virus in Astana, Kazakhstan

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Abstract

Background and Aim. Feline leukemia virus (FeLV) is one of the most important retroviral pathogens in domestic cats, causing immunosuppression, anemia, lymphoma, and leukemia. FeLV exists in two forms: exogenous (exFeLV), which is infectious and horizontally transmitted, and endogenous (enFeLV), which is inherited as integrated proviral sequences. Differentiating between these forms is essential for accurate epidemiological assessment and diagnostics, since enFeLV sequences can interfere with molecular assays and lead to false-positive results. This study aimed to evaluate the prevalence of endogenous FeLV sequences in domestic cats in Astana and to determine whether exogenous FeLV was actively circulating in this population.

Materials and Methods. A total of 203 whole-blood samples from domestic cats were collected during routine veterinary examinations, and genomic DNA was extracted using a modified Kanai method. Two independent real-time PCR systems were employed: primers targeting the conserved *env* region to detect enFeLV, and primers specific to the U3 region of the long terminal repeat (LTR) to identify exFeLV. Amplifications were performed on the CFX96 Touch platform, and samples with quantification cycle (*Cq*) values <40 were interpreted as positive.

Results. Of the 203 analyzed samples, 197 (97%) were positive for enFeLV sequences, confirming the widespread presence of endogenous retroviral elements in the genome of cats, while no amplification was detected with U3-specific primers, indicating the absence of active exFeLV infection. The *Cq* values for enFeLV-positive samples ranged from 11.24 to 37.04, reflecting variability in proviral copy number among individuals. These findings demonstrate that enFeLV is nearly ubiquitous among domestic cats in Astana, while no evidence of exFeLV circulation was detected.

Conclusion. The results underscore the importance of using *U3-LTR* specific assays to reliably differentiate endogenous from exogenous forms of FeLV and to avoid false-positive diagnoses. Further studies should focus on monitoring potential recombination events between enFeLV and exFeLV, evaluating the expression of endogenous loci, and assessing their role in disease pathogenesis.

Keywords: Feline leukemia virus; cats; PCR; Exogenous forms (exFeLV); endogenous forms (enFeLV); sequencing.

Introduction

Feline leukemia retrovirus (FeLV) is one of the most significant infectious agents in domestic cats, causing immunosuppression, anemia, lymphoma, and leukemia [1-3]. FeLV is divided into exogenous and endogenous forms. Exogenous forms (exFeLV) are clinically relevant exogenous forms and are

transmitted horizontally, whereas endogenous forms (enFeLV) are viral sequences integrated into the genome of cats during evolution and inherited [1, 4-6].

Endogenous retroviruses, including enFeLV, are widespread in domestic cat populations and may vary in copy number and preservation levels [7-9]. Their importance lies not only in the evolutionary aspect, but also in the actual pathobiology: they can have both protective and pathogenic effects on the body. For example, enFeLV expression can compete with an exogenous virus for cellular receptors, reducing the effectiveness of infection [10]. On the other hand, there is a risk of recombination between enFeLV and exFeLV, leading to the emergence of new pathogenic subtypes such as FeLV-B and FeLV-D [4, 11-13]. These recombinant variants have a modified tropicity spectrum and are often associated with more severe clinical manifestations.

Epidemiological data indicate that most domestic cats contain between 8 and 12 copies of enFeLV in their genome, although the exact number may vary between populations and breeds [7]. It is noteworthy that animals with a high copy of enFeLV may have a lower severity of clinical manifestations when infected with exFeLV, presumably due to the formation of immune tolerance to viral proteins [5]. These observations confirm the complex nature of the interaction of endogenous and exogenous forms of the virus.

From the point of view of molecular diagnostics, the presence of enFeLV is a significant problem. When using non-specific primers, especially for gag and env sites, both exogenous and endogenous sequences are amplified, which can lead to false positive results [14]. This significantly complicates the interpretation of the results of PCR analysis and can lead to errors when screening clinically healthy animals, especially in the absence of obvious clinical signs.

To minimize the risk of diagnostic errors, primer systems are used that target unique areas that are missing from enFeLV. One of these regions is the U3 LTR region, which is specific to exFeLV [4, 14]. The use of such markers makes it possible to reliably distinguish between endogenous and exogenous forms of the virus. This approach is critically important both for practical veterinary medicine (screening in kennels, during the sale and movement of animals), and for scientific research aimed at studying the prevalence and evolution of retroviruses in cats.

In the present study, a PCR analysis of 203 DNA samples of domestic cats was performed in order to assess the prevalence of endogenous FeLV sequences and exclude active exogenous infection. To improve the diagnostic accuracy, two primer systems were used, developed on the basis of modern publications [4, 14]. Additionally, data on the epidemiological situation in the region were taken into account, which made it possible to assess not only the infection rate, but also the potential risks of recombinant forms of the virus in the population.

Materials and Methods

Collecting samples

The samples for the study were collected in veterinary clinics in Astana. Whole blood taken from domestic cats during routine veterinary examinations was used as biological material. Sampling was carried out in vacuum tubes (vacutainers) containing the anticoagulant EDTA-K_2, a reagent widely used in diagnostic practice, as it effectively prevents blood clotting by chelating calcium ions, which allows samples to be kept in a stable state until the DNA isolation stage.

The use of EDTA-K2 also ensures minimal damage to blood cells and the preservation of nucleic acids, which is critically important when conducting molecular research, including PCR analysis. After sampling, the samples were transported to the laboratory under controlled conditions of temperature and storage time, which excluded degradation of the genetic material.

DNA isolation

DNA isolation was carried out according to the method described by *Kanai* et al. [15], with minor modifications that make it possible to efficiently extract genomic DNA even from clotted blood left after standard biochemical analyses. For isolation, 200 µl of a blood sample (including frozen and partially coagulated samples) was used, which were transferred to sterile 1.5 ml micro-samples containing 250 µl of a lysing buffer of the following composition: 720 mcg/ml of proteinase K, 150 mM NaCl, 50 mM EDTA and 2% SDS. The tubes were incubated at 60 °C for 3 hours with periodic stirring on a vortex to improve cell lysis and protein degradation. After incubation, 300 µl of 6 M NaCl and 600 µl of a mixture

of chloroform and isoamyl alcohol (24:1) were added to the lysate. The contents of the tubes were stirred on a vortex and centrifuged at 5000 rpm (≈1667 g) for 5 min. After phase separation, the aqueous phase containing nucleic acids was transferred to a new tube with 800 µl of isopropanol for DNA deposition. The samples were centrifuged at the same parameters, after which the resulting precipitate was washed with 70% ethanol, dried at room temperature, and eluted in 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The resulting genomic DNA preparations were used to set up PCR reactions.

PCR testing

Two independent sets of primers and temperature conditions were used for amplification, borrowed from the following publications:

1. The protocol is based on the methodology from the article by *Cavalcante* et al. [4]. A primer system specific to the LTR U3 region was used to detect exogenous FeLV.

Type	Name	Sequence
Forward primer	FeLV-U3-exo-f	5'-AACAGCAGAAGTTTCAAGGCC-3'
Reverse primer	FeLV-U3-exo-r	5'-TTATAGCAGAAAGCGCGCG-3'
Probe	FeLV-U3-probe	5'-FAM-CCAGCAGTCTCCAGGCTCCCCA-TAMRA-3'

Amplification was performed in a volume of 25 µl using HS qPCR (Biolabmix, Russia). The temperature regime consisted of 95 °C - 5 min of preliminary denaturation, followed by 40 cycles including: 95 °C - 15 sec, 60 °C - 1 min (annealing/elongation)

2. A protocol based on *Powers* et al. [14]. To determine the presence of endogenous copies of FeLV, a system of primers aimed at a conserved env site was used:

Type	Name	Sequence
Forward primer	enFeLV-F	5′-GTCTTATCCTAAGTCCACCGTTTA-3′
Reverse primer	enFeLV-R	5′-CTAGGCTCATCTCTAGGGTCTATC-3′
Probe	enFeLV-probe	FAM-5′-CCTGGCCCTAAGATGGGAATGGAAA- BHQ1-3′

The reaction was also carried out in 25 µl volume using the previously mentioned real-time PCR kit. The amplification conditions included: 95 °C - 5 min, 40 cycles, 95 °C - 5 sec, 60 °C - 15 sec.

Each system was started independently. The reactions were performed on the CFX96 Touch platform (BioRad, USA), which allows multiplex analysis of up to 5 targets simultaneously in 96 samples. The fluorescence threshold was determined automatically, and Cq values <40 were interpreted as positive.

Results and Discussion

Of the 203 analyzed DNA samples from domestic cats, 197 (97%) showed a positive result of amplification using primers aimed at the conservative site of *env*. The data obtained confirm that endogenous FeLV sequences are almost universally distributed in the studied population. Such a high percentage of positive samples is consistent with the literature data, according to which most domestic cats contain from 8 to 12 copies of enFeLV in their genome. [7]. Thus, our results once again confirm that the endogenous retroviral FeLV sequences are a stable component of the *Felis catus* genome and are preserved in the vast majority of individuals, regardless of their clinical status.

At the same time, none of the studied samples demonstrated amplification using a system of primers specific to the LTR U3 region of exogenous FeLV. This indicates the absence of active circulation of replication-competent exogenous forms of the virus among the examined animals within this sample. Negative results for exFeLV may reflect a relatively low level of infection of the population with exogenous forms in the conditions of Astana city or the effectiveness of measures to prevent the spread of the virus among domestic cats. It is important to note that in shelters or large nurseries where animal crowding is higher, the detection rate of exFeLV is usually significantly higher [4, 5], this highlights the value of our data specifically for assessing the epidemiological situation in the urban population.

The *Cq* (cycle quantification) values equivalent to Ct (cycle threshold) recorded for positive samples ranged from 11.24 to 37.04, with an average value of 18.85. Lower *Cq* values reflect the high representation of endogenous FeLV copies in the genome of individual animals, which may be due to the

variability in the number of copies between individuals and breeds. On the contrary, values approaching cycle 37 indicate the presence of samples with a relatively low number of copies, which confirms the interindividual differences in the integration of enFeLV. Taken together, such data demonstrate significant genetic heterogeneity in the level of endogenous retroviral load, which may be important for the formation of different animal susceptibility to exogenous FeLV and for the variability of clinical manifestations of infection.

Thus, the study showed that in the studied sample of cats from Astana, endogenous copies of FeLV were detected in the vast majority of animals, while exogenous forms of the virus were not detected.

These results have not only local epidemiological significance, but also confirm the data of other authors on the widespread occurrence of enFeLV and the complexity of differential diagnosis between endogenous and exogenous forms. Considering that it is recombination between endogenous and exogenous sequences that can lead to the formation of new pathogenic subtypes (for example, FeLV-B and FeLV-D), further monitoring of the cat population using specific molecular markers is an important direction for veterinary practice and scientific research (Figure 1).

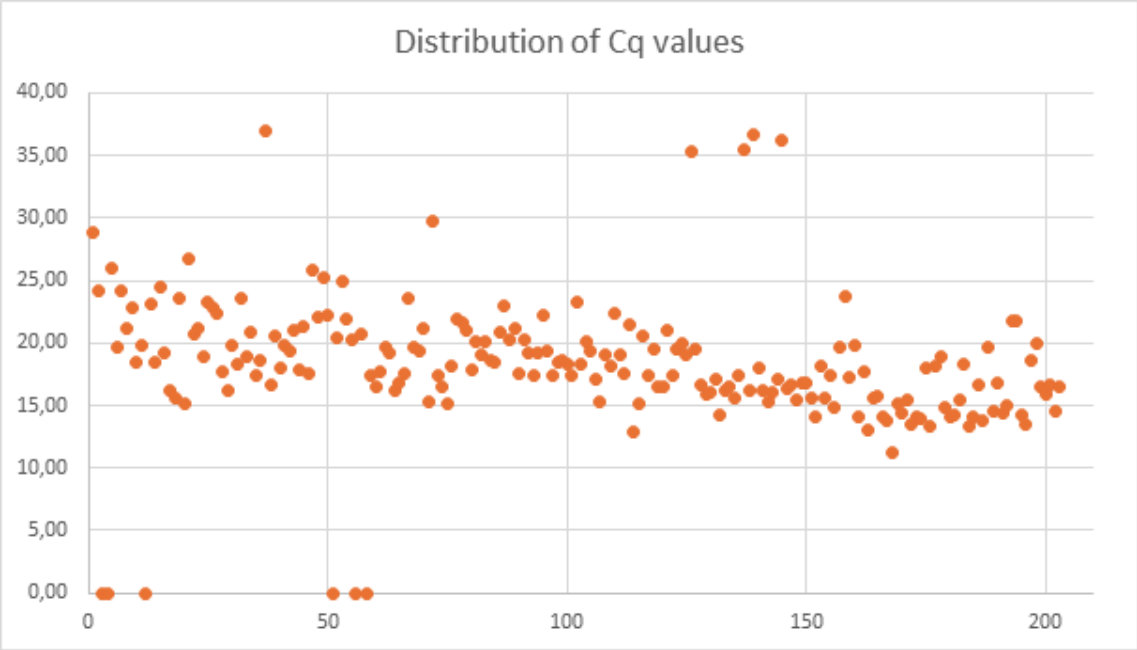

Figure 1 – Distribution of *Cq* values obtained by DNA amplification using primers to the enFeLV site. The X - axis shows the number of samples. Y- axis - displays the value of *Cq*

The results of this study confirmed the widespread occurrence of endogenous FeLV retrovirus among cats, with complete absence of signs of exogenous infection based on amplification of the U3 region. This is completely consistent with previous observations, according to which enFeLV is present in the genome of almost all domestic cats and varies in the number of copies depending on individual and population characteristics [7, 16]. The data obtained indicate that the detected amplification signals when using common primers to env reflect the presence of an embedded provirus, rather than an active exogenous infection. This result highlights the need to use highly specific diagnostic systems focused on unique areas missing from enFeLV, such as the LTR U3 region, which is especially critical when examining animals in populations with low morbidity [14, 17].

In addition to their purely diagnostic significance, endogenous FeLV retroviruses are also of interest in a broader biological context. They serve as a model for studying viral evolution, as well as interactions between the virus and the host. In particular, the ability of enFeLV to recombine with exogenous forms of the virus has been confirmed in a number of molecular studies [4, 9, 11, 18]. Such recombination events can lead to the formation of new pathogenic subtypes, including FeLV-B and FeLV-D, with an altered spectrum of cellular tropicity and a more severe clinical course. In addition, the transcriptional

activity of individual endogenous loci can influence the expression of host genes, the functioning of the immune system, and even the formation of individual resistance or susceptibility to infection [5, 10, 19].

The practical significance of enFeLV is also evident in the context of prevention. According to current European veterinary guidelines, FeLV vaccination should be combined with mandatory testing of animals for exogenous forms using U3-specific systems [20]. This minimizes the risk of false positive results due to endogenous copies and increases the reliability of epidemiological control. This approach is especially important in shelters and nurseries, where crowded conditions increase the likelihood of retrovirus activation due to stressful factors [21-23].

Thus, the results of this study not only confirm the almost universal presence of enFeLV in the genome of domestic cats, but also emphasize the urgency of the problem of differential diagnosis of endogenous and exogenous forms of the virus. The absence of amplification by U3 primers, combined with the high frequency of positive reactions to the conserved env site, demonstrates the importance of choosing the right molecular targets for PCR assays. In a broader sense, the findings complement the existing evidence base on enFeLV and highlight the need for further research aimed at: 1. evaluation of the expression of endogenous sequences; 2. study of their recombination potential; 3. Epidemiological monitoring of the circulation of exogenous FeLV in various cat populations. These areas may be of critical importance not only for veterinary practice and infection prevention, but also for understanding the role of endogenous retroviruses in the mammalian genome.

Conclusion

Analysis of 203 DNA samples from domestic cats showed the almost ubiquitous presence of endogenous FeLV sequences in the complete absence of signs of exogenous infection. This result emphasizes that endogenous forms of retrovirus are preserved in the animal genome as an inherited element and reflect the features of the evolutionary interaction of the virus and the host. The absence of amplification of the U3 region indicates that there is no active circulation of exogenous forms of the virus in the studied population.

The data obtained confirm the key role of differential diagnosis in detecting FeLV. The use of PCR with primers aimed at conservative sites (for example, env), without taking into account the endogenous origin of the sequences, can lead to false positive results and incorrect epidemiological conclusions. In conditions of low incidence of exogenous forms, this is especially critical, since a diagnostic error can affect the tactics of treatment, vaccination, and animal movement control.

The use of primers specific to the unique regions of the exogenous virus (in particular, the LTR U3 region) is a prerequisite for reliable detection of active infection. This approach is already reflected in international guidelines for testing and vaccination of cats and should be considered as a standard for the molecular diagnosis of FeLV.

Future research in this area should focus not only on improving diagnostic systems, but also on a deeper study of the role of enFeLV in the pathogenesis of diseases. Of particular interest are the issues of expression of endogenous loci, their potential involvement in recombination with exogenous forms, and their effect on the animal's immune response. Such data is necessary both to develop strategies for the prevention and control of infections in cats, and to understand the general patterns of interaction between retroviruses and the mammalian genome.

Authors' Contributions

GY, BA: supervision, conceptualisation, writing - original draft preparation, writing - review and editing; AK, GM, and IA: methodology, validation and formal analysis. All authors have read and agreed to the final version of the manuscript.

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Using indirect hemagglutination assay for the diagnosis of cattle brucellosis

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