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Epidemiological and Epizootological Monitoring and Spatiotemporal Dynamics of Plague in Natural Foci of Kazakhstan

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Abstract

Plague remains a significant natural focal zoonotic infection, maintaining epidemiological relevance in the Republic of Kazakhstan. This study provides a comprehensive assessment of epizootological dynamics in natural plague foci during 2020–2025, integrating historical epidemiological data, phenotypic and molecular characterization of *Yersinia pestis*, and GIS-based spatial analysis. The study utilized long-term surveillance data (1920–2025), epidemiological records of human cases (1926–2003), analysis of 1,526 strains, and whole-genome sequencing of 75 isolates. Epizootological monitoring demonstrated high coverage and stable surveillance capacity, alongside a marked increase in molecular diagnostics. By 2025, expansion of epizootically active areas, a threefold increase in isolated strains, and a substantial rise in PCR-positive detections were observed, indicating intensified pathogen circulation. Despite this, *Y. pestis* populations remained highly stable, with 94.9% phenotypically typical and 97.5% genotypically typical strains, and no evidence of antimicrobial resistance. Spatial analysis revealed significant clustering (Moran's I = 1.627; $p < 0.001$), persistent directional spread, and stable high-risk zones in the North Aral, Betpakdala, Moyynkum, and Kyzylkum foci. No human cases have been recorded since 2003, reflecting effective surveillance. These findings support the integration of spatial modeling and molecular surveillance into risk-oriented plague control strategies.

Keywords: plague; *Yersinia pestis*; natural foci; epizootology; rodents; fleas; resistance factors

1. Introduction

Plague remains one of the most significant zoonotic infections in both historical and epidemiological contexts, caused by *Yersinia pestis* [1,2]. Despite advances in surveillance systems and the development of molecular genetic diagnostic methods, plague persists in natural foci across various regions of the world, including Central Asia, Africa, and certain areas of the Americas [3,4]. At present, the global epidemiological situation of plague remains tense [5,6], with no clear trend toward decline.

Kazakhstan represents one of the largest plague-endemic territories worldwide, where natural foci occupy approximately 1.084 million km², accounting for nearly 40% of the country's total area and more than 50% of the overall area of natural plague foci within the Commonwealth of Independent States [7–9]. These foci encompass diverse ecological and landscape zones, including steppe, desert, semi-desert, and high-mountain regions, creating favorable conditions for the persistence and circulation of *Y. pestis* [10,11]. This also includes transboundary plague foci

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(Kyrgyzstan, Mongolia, China, and Russia), where human outbreaks have repeatedly occurred in the past [12–14]. The last human cases of plague in Kazakhstan were recorded in 2003 [7]; however, the annual registration of epizootics among wild animals continues to pose a biological threat [10,15,16].

In recent years, particular attention has been given to studies by national researchers who have made substantial contributions to the investigation of natural foci, mapping of distribution ranges, assessment of epidemiological risks, and genetic characterization of *Y. pestis* isolates [17–20].

Recent advances in spatial epidemiology, molecular biology, and ecological modeling have significantly expanded the capacity to study plague dynamics. In particular, the application of geographic information systems (GIS), species distribution models, and climate-based projections enables the identification of high-risk areas and the prediction of changes in the distribution of hosts and vectors [21–23]. Under conditions of global climate change, anthropogenic impact, and migration processes, shifts in rodent distribution and expansion of suitable ecological niches are likely, potentially increasing the risk of plague transmission in previously unaffected or low-endemic regions [24,25].

The aim of this study is to assess the current epizootological situation in the natural plague foci of Kazakhstan during 2020–2025, to analyze temporal trends in pathogen circulation based on epizootological monitoring and laboratory diagnostics, and to integrate these findings with historical epidemiological data and risk-oriented approaches in order to enhance understanding of plague dynamics and improve the effectiveness of public health decision-making.

2. Materials and Methods

2.1. Epizootological Monitoring and Field Sampling

The study was conducted within the natural plague foci of Kazakhstan (Figure 1), using widely accepted methodologies for epizootological monitoring [26–28] and incorporating modern GPS-based tools and geographic information system (GIS) technologies. The experimental and analytical base of the research was the Central Reference Laboratory of the M. Aikimbayev National Scientific Center for Especially Dangerous Infections under the Ministry of Health of the Republic of Kazakhstan.

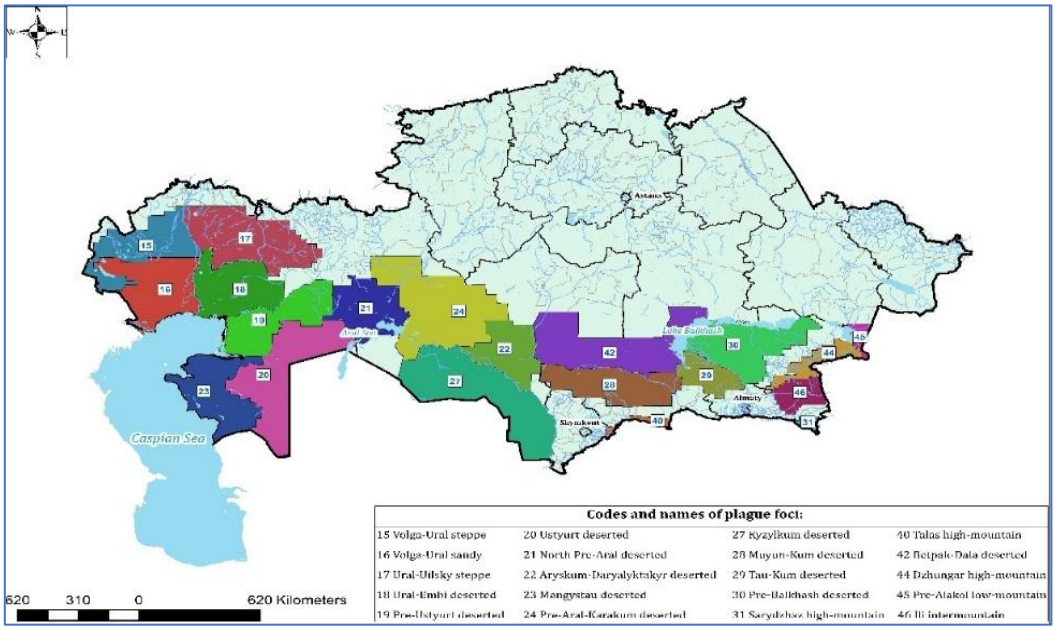

Figure 1. Natural plague foci of the Republic of Kazakhstan.

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Field and laboratory investigations were carried out in accordance with the Resolution of the Chief State Sanitary Physician of the Ministry of Health of the Republic of Kazakhstan "On the implementation of sanitary-anti-epidemic and sanitary-preventive measures in plague-enzootic territories of the Republic of Kazakhstan for 2021–2025" (No. 8, dated February 26, 2021), in compliance with Article 36 of the Code of the Republic of Kazakhstan "On Public Health and the Healthcare System," and taking into account the requirements of the International Health Regulations (2005). The study was conducted to ensure epidemiological safety with respect to plague within the territory of Kazakhstan and was approved by the Bioethics Committee of the Aikimbayev National Scientific Center for Especially Dangerous Infections, part of the QazBioPharm National Holding of the Ministry of Health of the Republic of Kazakhstan.

All animal-related experimental protocols were approved by the Institutional Animal Care and Use Committee of the Aikimbayev National Scientific Center for Especially Dangerous Infections (Application No. 2, dated April 19, 2020).

The study is based on extensive long-term observations derived from epidemiological monitoring of human plague cases (1926–2003) and epizootological surveillance (1920–2025) within the natural plague foci of Kazakhstan. These include 6 major natural foci and 15 autonomous foci, within which more than 100 landscape-epizootological regions have been delineated.

2.2. Study Area and Characteristics of Natural Plague Foci

The study area encompasses the principal natural plague foci of the Republic of Kazakhstan, represented by steppe, desert, and high-mountain ecosystems. The epizootological structure of these foci is characterized by a diversity of primary hosts (rodents and marmots), as well as the circulation of different biovars and phylogenetic lineages of *Yersinia pestis*.

The main characteristics of the natural foci, including focus types, geographic distribution, primary hosts, biochemical properties, and phylogenetic affiliation of the pathogen, are presented in Table 1.

Table 1. Epizootological characteristics of natural plague foci in Kazakhstan: focus types, primary hosts, biovars, and genetic lineages of ***Yersinia pestis*.**

Natural focus (type, code)	Location of enzootic territory / region	Primary host	Subspecies, biovar, phylogenetic lineage	Biochemical characteristics
Steppe foci: Volga–Ural (15), Ural–Uil (17)	West Kazakhstan, Aktobe regions	Little ground squirrel (*Spermophilus pygmaeus*)	Main subspecies, Medievalis biovar, 2.MED1	Do not ferment rhamnose; do not reduce nitrates; ferment arabinose and glycerol. Virulent, epidemiologically significant
Sandy desert foci: Ural–Emba (18), Pre-Ustyurt (19), Ustyurt (20), North Aral (21), Arys-Kum–Daryalyktakyr (22), Mangystau (23), Aral–Karakum (24), Kyzylkum (27), Moyynkum (28), Taukum (29), Balkhash (30), Betpakdala (42), Pre-Alakol low-mountain (45), Ili intermontane (46)	Atyrau, Aktobe, Almaty, East Kazakhstan, Zhetysu, Zhambyl, Karaganda, Kyzylorda, Mangystau, Turkestan, Ulytau regions	Great gerbil (*Rhombomys opimus*)	Main subspecies, Medievalis biovar, 2.MED1	Do not ferment rhamnose; do not reduce nitrates; ferment arabinose and glycerol. Virulent, epidemiologically significant
Sandy desert (Ryn sands): Volga–Ural (16)	West Kazakhstan, Atyrau regions	Midday gerbil (*Meriones meridianus*), tamarisk gerbil (*M. tamariscinus*), red-tailed gerbil (*M. erythrourus*)	Main subspecies, Medievalis biovar, 2.MED1	Do not ferment rhamnose; do not reduce nitrates; ferment arabinose and glycerol. Virulent, epidemiologically significant
High-mountain focus: Saryjaz (31)	Almaty region	Gray marmot (*Marmota baibacina*)	Main subspecies, Antiqua biovar, 0.ANT5	Do not ferment rhamnose; reduce nitrates; ferment arabinose and glycerol. Virulent, epidemiologically significant

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High-mountain focus: Talas (40)	Zhambyl, Turkestan regions	Red marmot (*Marmota caudata*)	Main subspecies, Medievalis biovar, 2.MED1	Do not ferment rhamnose; do not reduce nitrates; ferment arabinose and glycerol. Virulent, epidemiologically significant
	Zhambyl, Turkestan regions	Silver vole (*Alticola argentatus*)	Central Asian subspecies, Talas biovar (Pestoides), 0.PE4t	Ferment rhamnose; do not reduce nitrates; do not ferment arabinose; ferment glycerol. Virulent, epidemiologically significant

2.3. Characterization of Isolated Strains

Phenotypic and molecular-genetic analyses were performed on 75 *Yersinia pestis* strains isolated from natural plague foci in Kazakhstan using standard methodologies [29,30]. All strains were obtained from the depository and live culture collection of the M. Aikimbayev National Scientific Center for Especially Dangerous Infections. All manipulations involving *Y. pestis* strains were conducted in accordance with established biosafety standards and guidelines for handling pathogenic microorganisms.

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, USA). Genotyping of *Y. pestis* strains was carried out using whole-genome sequencing (WGS). DNA library preparation was performed with the Nextera XT DNA Library Preparation Kit (catalog No. FC-131-1024), following the manufacturer's protocol. Sequencing was conducted on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600 cycles; catalog No. MS-102-3003), in accordance with the manufacturer's instructions.

2.4. Quality Control of the Study

Quality control was ensured through the use of reference and control strains maintained in the live culture collection of the M. Aikimbayev National Scientific Center for Especially Dangerous Infections. These included reference strains of *Y. pestis* from different autonomous foci of Kazakhstan, the vaccine strain *Y. pestis* EV, and *Yersinia pseudotuberculosis*.

In addition, reference sequence fragments from four well-characterized strains representing the principal biovars of the plague pathogen were used: Pestoides F strain (Microtus/Antiqua biovar), Nepal516 strain (Antiqua biovar), KIM10 strain (Medievalis biovar), and CO92 strain (Orientalis biovar).

2.5. Epidemic Year Index (EYI)

To quantitatively assess epidemiological risk, the Epidemic Year Index (EYI) was applied, defined as the proportion of years with recorded human plague cases:

$$EYI = \frac{N \text{ epidemic years}}{N \text{ total years}} \text{x} 100\%$$

where:

N epidemic years—number of years with recorded human plague cases; N total years—total number of years under observation.

2.6. Risk Assessment

Risk assessment was performed using a comprehensive approach integrating epizootological, epidemiological, and spatial-analytical indicators. The analysis incorporated the intensity of epizootic activity, frequency of pathogen detection, distribution of host and vector populations, and historical epidemiological data.

A risk-oriented framework was applied to classify natural plague foci according to the probability of human infection, taking into account environmental, biological, and socio-demographic factors. Spatial risk assessment was conducted using GIS-based methods, enabling the identification of high-risk zones and the modeling of potential epizootic spread.

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3. Results

3.1. Epizootological Monitoring

Epizootological monitoring of natural plague foci in the Republic of Kazakhstan during 2020–2025 was characterized by a high level of territorial coverage and a stable surveillance system (Table 2).

The total surveyed area ranged from 834.6 to 896.2 thousand km², showing an increasing trend toward 2025. At the same time, the area of epizootically active territories varied from 3.0 to 8.9 thousand km², with minimum values observed in 2022 and a subsequent increase in 2024–2025, indicating an intensification of epizootic activity.

Table 2. Scope and results of epizootological monitoring and preventive (anti-plague) measures in natural plague foci of Kazakhstan, 2020–2025.

Activity	Unit	2020	2021	2022	2023	2024	2025
Total surveyed area	10^3 km²	834.60	848.49	888.48	845.59	889.48	896.18
Epizootic area	10^3 km²	7.1	5.4	3.0	3.8	5.7	8.9
Rodents examined	10^3 individuals	156.97	150.27	141.70	138.16	131.74	131.73
Ectoparasites examined	10^3 specimens	1270.15	1235.89	1177.60	1456.70	1354.40	1507.08
Other materials examined	10^3 samples	1.782	3.410	2.028	1.654	0.616	0.722
Bacteriological examinations	10^3 samples	186.98	180.15	229.50	218.57	226.76	231.13
Biological assays	10^3 samples	41.226	38.759	33.228	16.584	12.521	12.679
Y. pestis strains isolated	n	31	11	23	35	43	91
Total serological tests	10^3 tests	160.45	156.52	135.32	138.26	155.42	137.38
Positive serological results	n	240	185	51	66	134	212
Total PCR (real-time) tests	10^3 samples	1.20	1.62	9.75	11.96	13.278	53.42
Positive PCR results	n	14	15	22	21	8	71
Protective zones established	n	152	116	196	154	177	180
Total area of protective zones	km²	344.4	354.1	595.0	546.4	394.0	423.2
Settlements surveyed	n	505	507	554	535	521	550
a) for rodent infestation	10^3 m²	5710.1	4510.0	4560.0	4530.0	4510.0	4544.0
b) for flea infestation	10^3 m²	4502.5	3265.0	3268.0	3265.0	3288.0	3272.0
Settlement deratization	10^3 m²	1605.0	1745.0	1755.0	1745.0	1769.0	1761.39
Settlement disinsection	10^3 m²	745.0	805.1	899.7	898.5	899.8	904.49
Humans vaccinated	n	94,032	82,331	60,946	61,802	83,889	64,968
Camels vaccinated	n	87,875	91,547	89,546	90,268	89,864	155,059

The volumes of zoological and parasitological investigations remained substantial: up to 156.9 thousand rodents and more than 1.5 million ectoparasites were examined annually. This was accompanied by a gradual decline in the number of rodents surveyed and a concurrent increase in attention to vectors of infection.

Laboratory diagnostics showed an upward trend in bacteriological testing, reaching 231.1 thousand samples in 2025, alongside a pronounced expansion in the use of molecular methods. In particular, the number of real-time PCR (PCR-RT) assays increased markedly from 1.2 thousand in 2020 to 53.4 thousand in 2025, indicating a substantial shift toward molecular surveillance approaches.

The epizootic activity of the pathogen was confirmed by a steady increase in the number of isolated *Yersinia pestis* strains, from 31 in 2020 to 91 in 2025, as well as by the rise in PCR-positive results (from 14 to 71). The infection prevalence ranged from 0.01% to 0.2%, reaching its peak in 2025.

Spatial analysis revealed pronounced focal heterogeneity (Figure 2). During 2020–2022, epizootic activity was localized, primarily confined to the Ili intermontane, Kyzylkum, and Arys-

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Kum–Daryalyktakyr foci. In contrast, during 2023–2025, an expansion in the geographic distribution of pathogen circulation was observed, involving the North Aral, Balkhash, and Aral–Karakum foci.

By 2025, the North Aral focus became dominant, accounting for more than 75% of all isolated strains, indicating the formation of a major center of epizootic activity.

Serological monitoring revealed pronounced interannual variability: the number of seropositive animals decreased to 51 in 2022 but subsequently increased to 212 in 2025, with a predominance in the North Aral and Moyynkum foci. PCR-based diagnostics likewise demonstrated an expansion in pathogen detection across multiple foci simultaneously, particularly in 2025.

Preventive measures were implemented systematically: up to 196 protective zones were established annually, covering a total area of up to 595 km²; more than 500 settlements were surveyed each year; and the volumes of deratization and disinsection remained consistently high. Vaccination of both humans and animals was conducted annually, with a notable increase in camel vaccination in 2025, corresponding to the rising epizootic intensity.

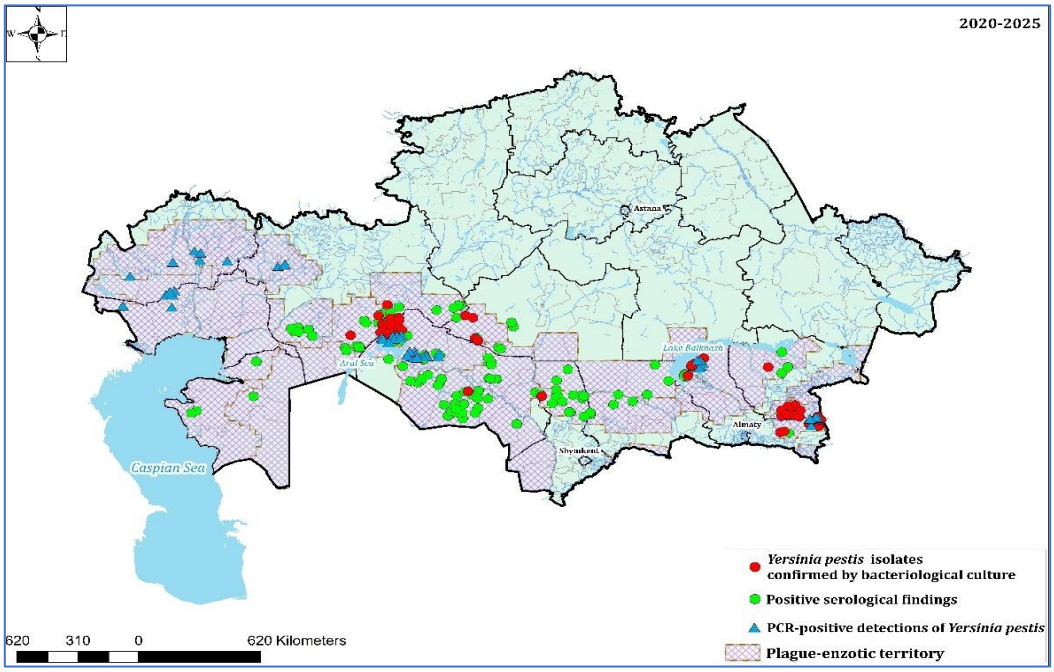

Figure 2. Results of epizootological monitoring: detection of *Yersinia pestis* strains, seropositive animals (F1 fraction), and plague antigen.

Overall, the findings indicate the stable functioning of the epizootological monitoring system, accompanied by a concurrent intensification of epizootic activity in recent years. This is reflected in the expansion of epizootically active areas, an increase in the number of isolated *Yersinia pestis* strains, and improved detection rates associated with the expanded use of molecular diagnostic methods.

The analysis demonstrated coordinated changes in key epizootological indicators: the increase in the area of epizootically active territories was accompanied by a rise in the number of isolated *Y. pestis* strains and an increase in PCR-positive results, indicating a positive association between the spatial spread of the epizootic process and the intensity of pathogen detection.

3.2. Results of Phenotypic and Molecular-Genetic Studies

During the period 2010–2025, a comprehensive analysis of phenotypic and molecular-genetic characteristics was conducted on more than 1,520 *Yersinia pestis* strains isolated from natural plague foci in Kazakhstan, with the aim of identifying atypical variants (Table 3).

The analysis demonstrated that all studied strains conformed to the typical cultural, morphological, biochemical, and enzymatic characteristics of populations circulating within the

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Central Asian desert plague focus. The strains fermented glycerol, glucose, mannitol, maltose, and arabinose, did not ferment rhamnose, were pesticinogenic, and lacked denitrification activity.

Table 3. Results of phenotypic and genotypic characterization of *Yersinia pestis* strains isolated from natural plague foci in Kazakhstan.

Autonomous plague focus	Proportion of strains studied, %	Phenotypic characteristics, %		Genotypic characteristics, %	
		Typical	Atypical	Typical	Atypical
Balkhash	23.4	87.1	12.9	94.4	5.6
Moyynkum	15.6	97.3	2.7	98.3	1.7
Ili intermontane	17.0	97.3	2.7	99.2	0.8
Aral–Karakum	7.6	98.3	1.7	99.1	0.9
Taukum	7.4	97.3	2.7	100	–
North Aral	9.4	98.6	1.4	100	–
Ustyurt	5.2	98.7	1.3	100	–
Kyzylkum	4.4	100	–	100	–
Betpakdala	4.2	100	–	100	–
Arys-Kum-Daryalyktakyr	2.0	100	–	100	–
Pre-Ustyurt	1.3	100	–	100	–
Ural–Emba	0.5	100	–	100	–
Mangystau	0.4	100	–	100	–
Pre-Ustyurt (secondary)	0.3	100	–	100	–
Volga–Ural (sandy)	0.3	100	–	100	–
Ustyurt (secondary)	0.3	100	–	100	–
Mangyshlak	0.2	100	–	100	–
Volga–Ural (steppe)	0.1	100	–	100	–
Ural–Uil	0.1	100	–	100	–
Pre-Alakol	0.1	100	–	100	–
Saryjaz	0.1	100	–	100	–
Talas	0.1	100	–	100	–
Total (%)	**100**	**94.9**	**5.1**	**97.5**	**2.5**

The majority of strains produced the capsular F1 antigen and exhibited high virulence in laboratory animals (LD_{50} for guinea pigs: 1.0×10^6 CFU; for white mice: $1.6–9.0 \times 10^4$ CFU). All strains remained susceptible to antibacterial agents; no antibiotic-resistant or bacteriophage-resistant variants were detected.

At the same time, based on the combined phenotypic characteristics, 94.9% of strains were classified as typical, whereas 5.1% exhibited altered properties. According to genotypic characteristics, 97.5% of strains were typical, while 2.5% lacked the *caf1* gene (plasmid pFra), which encodes the F1 capsular antigen. Atypical strains were predominantly detected in autonomous foci characterized by high epizootic activity and frequent epidemic complications.

Serological testing for the F1 antigen (indirect hemagglutination assay) showed that 98.4% of strains contained the F1 antigen, with concentrations ranging from 1:1,250 to 1:78,125 CFU/mL. The absence of F1 was observed in a limited number of strains, primarily from the Balkhash autonomous focus, as well as in isolated cases from the Ili intermontane, Taukum, and Moyynkum foci.

Strain populations demonstrated a high degree of homogeneity with respect to calcium dependence and pigmentation: the proportion of Ca^{2+}-dependent forms was 98.6 ± 2.4%, and pigment-absorbing strains accounted for 94.3 ± 1.7%. The proportion of Pgm^+ cells was 94.7%, while Pgm^- variants constituted 5.3%. Analysis of pesticin production showed that 99.6% of strains produced pesticin I and remained resistant to it, whereas only 0.4% of strains (from the Balkhash focus) did not produce this factor.

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Analysis of growth factor requirements revealed that the vast majority of strains (n = 1,489) required phenylalanine, methionine, and cysteine. A small number of isolates exhibited dependence on threonine (n = 8) or arginine (n = 29); however, the distribution of these traits did not correlate with the level of epizootic or epidemic activity in the foci.

Comparative analysis showed that in autonomous foci with high epizootic activity and frequent epidemic complications, the frequency of atypical strains was 8.2-fold higher than in foci with low activity.

Overall, *Yersinia pestis* populations in the natural foci of Kazakhstan are characterized by high phenotypic and genotypic stability. The proportion of atypical strains remains low (5.1% phenotypically and 2.5% genotypically), although their distribution is spatially heterogeneous and focal in nature. The absence of the *caf1* gene and variations in phenotypic traits do not substantially affect the overall population structure of the pathogen.

Atypical strains are statistically more frequently observed in foci with elevated epizootic activity, which may reflect increased intensity of pathogen circulation. The observed genetic and phenotypic stability of *Y. pestis* populations, together with the absence of antimicrobial resistance, supports the concept of a highly conserved evolutionary trajectory of the pathogen within natural foci.

3.3. Epidemiological Analysis

A retrospective analysis of long-term data showed that during 1926–2003, more than 560 human plague cases were recorded in the desert foci of Kazakhstan, grouped into 82 epidemic outbreaks. The incidence demonstrated pronounced spatial heterogeneity: the largest proportion of cases occurred in the Kyzylorda (40.53%) and Almaty (32.22%) regions, whereas the contributions of the Mangystau (21.24%), Atyrau (4.42%), and Aktobe (1.59%) regions were substantially lower. This distribution reflects the structure of natural foci and differences in epizootic activity across diverse landscape–ecological zones.

Analysis of temporal dynamics of the epidemic process allowed the identification of three major stages. The pre-antibiotic period (1926–1948) was characterized by high incidence and large outbreaks (1926, 1929, 1945, 1947, 1948), accounting for up to 80.7% of all cases, including episodes of human-to-human transmission. The period of stabilized epidemiological surveillance (1950–1990) was marked by a substantial decline in incidence, associated with the introduction of antibiotic therapy and the establishment of systematic anti-plague measures. In the subsequent period of socio-economic transition (1990–2003), an increase in the proportion of epidemic years was observed, likely linked to reduced effectiveness of epidemiological control and deterioration of sanitary and hygienic conditions. Overall, a statistically significant decrease in incidence following the introduction of antibiotics and structured epidemiological surveillance was identified ($p < 0.05$), followed by fluctuations during the post-Soviet period.

To quantitatively assess long-term epidemiological risk, the Epidemic Year Index (EYI) was applied, defined as the proportion of years with recorded human cases relative to the total observation period. During 1948–2003, the mean EYI was approximately 49%, indicating a high probability of epidemic events within natural foci.

Regional analysis revealed pronounced differentiation in epidemiological risk (Figure 3): Kyzylorda region — 29.0%, Atyrau — 11.0%, Aktobe — 5.45%, Mangystau — 16.36%, and Almaty — 3.6%. In the post-Soviet period (1990–2003), a redistribution of risk was observed: Kyzylorda — 50.0%, Atyrau — 21.42%, Aktobe — 14.28%, Mangystau — 7.14%, and Almaty — 0%.

Horizontal bars represent the proportion of epidemic years across regions. Comparison of the two periods reveals an increase in epidemiological risk in the Kyzylorda, Atyrau, and Aktobe regions after 1990, alongside a concurrent decrease in the Mangystau and Almaty regions. These differences between regions and time periods are statistically significant ($p < 0.05$). The EYI reflects the long-term epidemiological potential of natural foci and can be considered an integral indicator of outbreak risk.

Human cases were strictly associated with periods of increased epizootic activity in natural plague foci. All recorded cases occurred exclusively within zones of active pathogen circulation,

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confirming the secondary (spillover) nature of the epidemic process and its dependence on epizootic dynamics rather than sustained transmission within the human population.

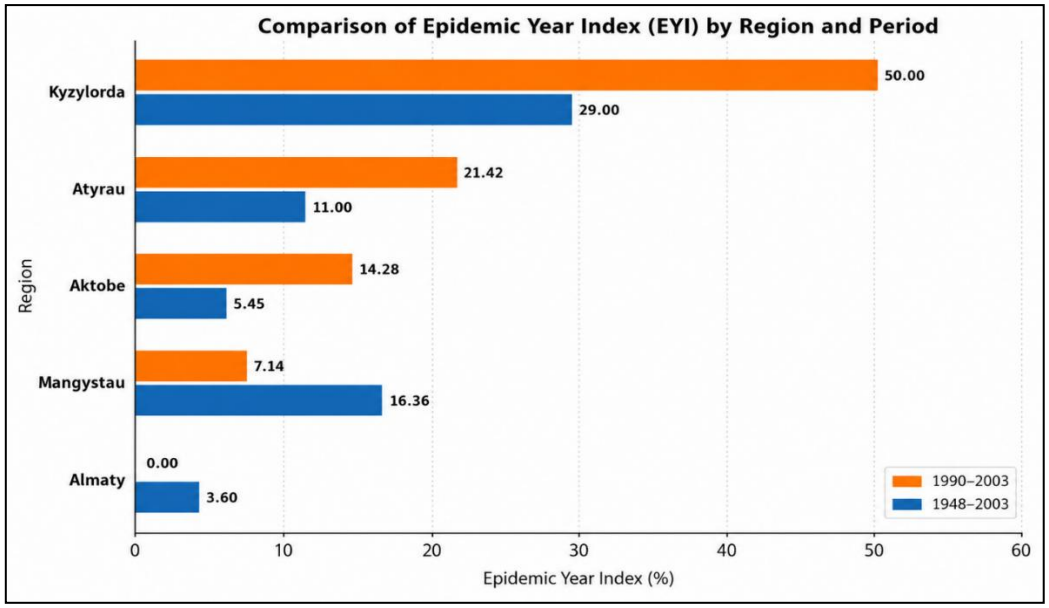

Figure 3. Regional comparison of the Epidemic Year Index (EYI) in Kazakhstan across two periods (1926-2003 and 1990-2003).

The observed patterns have a clear ecological basis and are consistent with the natural focal nature of plague. They are driven by fluctuations in the population dynamics of primary reservoirs (particularly *Rhombomys opimus*), the activity of vector populations (fleas), and the climatic and landscape characteristics of arid ecosystems. Cyclical changes in rodent abundance lead to periodic activation of epizootics, which in turn determines the probability of human infections.

Socio-economic factors also exert an additional influence. The 1990s were characterized by reduced effectiveness of epidemiological surveillance, deterioration of sanitary and hygienic conditions, and increased human contact with natural foci of infection, resulting in a higher proportion of epidemic years and an increased epidemiological risk ($p < 0.05$).

Despite the continued epizootic activity in natural foci, the last human case of plague in the Republic of Kazakhstan was recorded in 2003. This indicates the high effectiveness of the national epidemiological surveillance system and the successful implementation of an integrated One Health approach.

In summary, the epidemic process of plague in Kazakhstan is characterized by pronounced spatiotemporal heterogeneity and is determined by the structure of natural foci. Following the introduction of antibiotic therapy and systematic epidemiological surveillance, a statistically significant reduction in incidence was observed ($p < 0.05$). The Epidemic Year Index (EYI) serves as an informative integral indicator of long-term epidemiological risk and reveals substantial regional differentiation. Human incidence exhibits a secondary (spillover) pattern and is strictly linked to phases of epizootic activity. The dynamics of the epidemic process are governed by a combination of ecological and socio-economic factors.

It is important to emphasize that, despite the persistent epizootic circulation of plague in natural foci among wild animals, no human cases have been recorded in Kazakhstan since 2003. This reflects the effectiveness of state-level organization of surveillance and control measures, as well as the implementation of a comprehensive, integrated system of anti-plague interventions carried out by the national anti-plague service.

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3.4. Assessment of Epidemiological Risk and Epizootic Activity

A retrospective assessment of epizootic and epidemic manifestations of plague in the natural foci of Kazakhstan (total area approximately 1.084 million km²) allowed their differentiation according to levels of epidemiological risk. The classification was based on the frequency of epizootic events and the intensity of human plague cases.

Based on long-term data analysis, all autonomous plague foci were categorized into five risk levels: very high, high, medium, low, and very low. The highest risk level was characteristic of foci with repeated epizootic activity across all epizootic cycles and a high number of human cases, whereas the very low risk level corresponded to areas with sporadic epizootic manifestations and no recorded human infections.

For spatial and quantitative risk assessment, a total of 9327 sectors within natural plague foci were analyzed. Sector classification was performed based on three key parameters: (1) presence of epizootic activity, (2) occurrence of human cases, and (3) population density. Based on the combined evaluation of these parameters, the level of potential epidemic hazard (PEH) was determined.

As illustrated in Figure 4, the conceptual risk matrix demonstrates the relationship between the frequency of epizootic activity and the number of human cases, forming gradients of epidemiological risk.

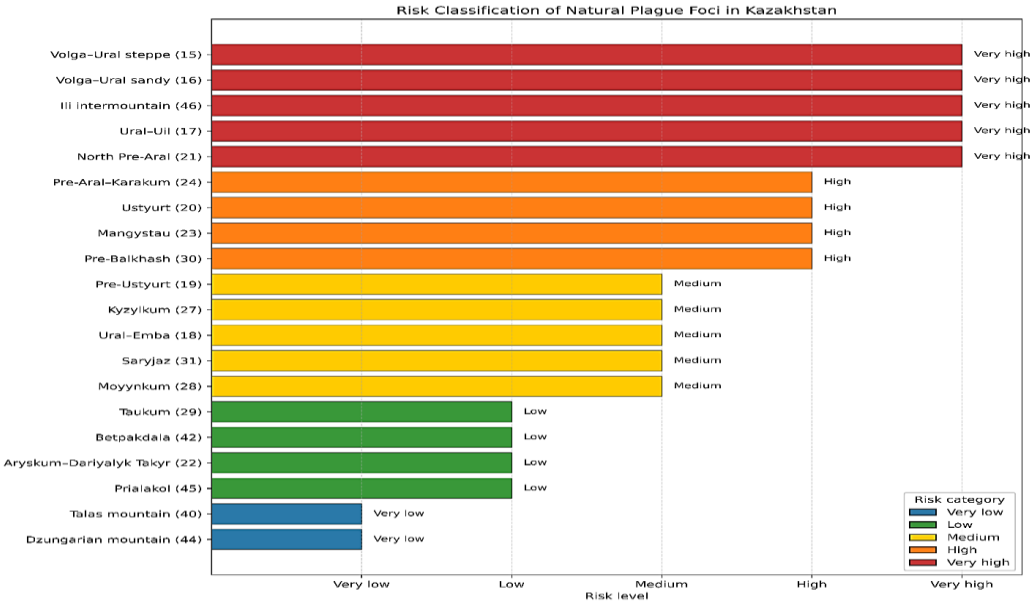

Figure 4. Conceptual risk matrix of plague foci based on epizootic activity and human case occurrence.

The matrix illustrates the relationship between the frequency of epizootic activity and the number of human cases, defining gradients of epidemiological risk.

Note:

(1) *Very high* — epizootics were repeatedly observed in all epizootic cycles, with ≥100 human cases reported;

(2) *High* — epizootics were repeatedly observed across all cycles, with 10–100 human cases;

(3) *Medium* — epizootics were observed in multiple cycles, with 1–10 human cases;

(4) *Low* — epizootics were observed in multiple cycles, with a single human case recorded;

(5) *Very low* — epizootics were recorded within a single cycle, with no human cases.

According to the obtained results, 139 sectors were classified as very high risk, 375 as high risk, 989 as medium risk, 1833 as low risk, and 5991 as very low risk.

The distribution of sectors across risk categories (Figure 5) showed that the majority of territories fall within the very low risk category (64.2%), whereas areas with high and very high risk account for

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only 5.6%. Despite their relatively small proportion, these high-risk zones represent the greatest epidemiological significance as potential sources of outbreak emergence.

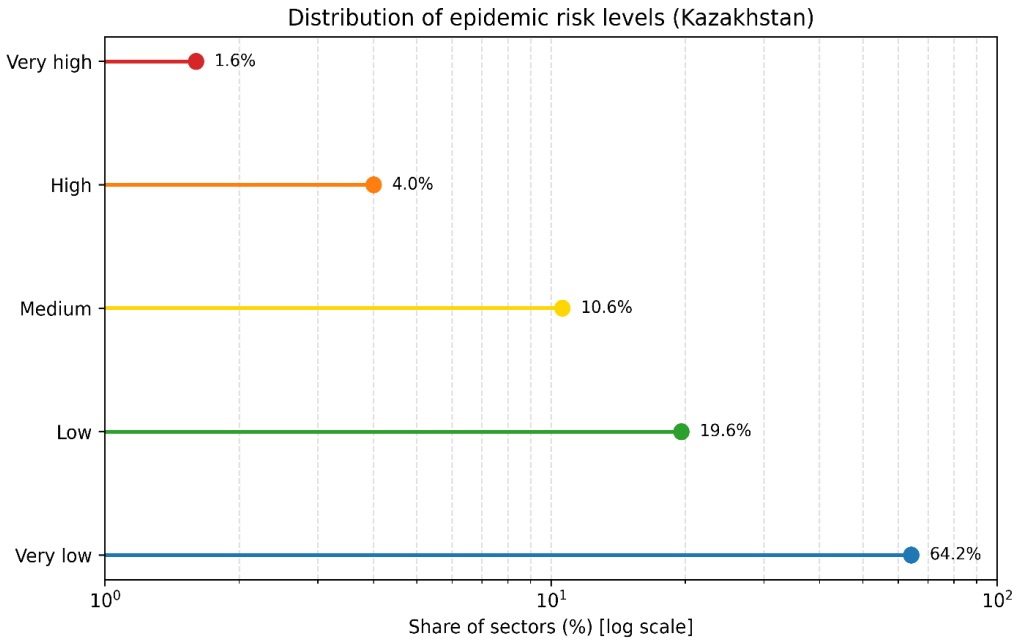

Figure 5. Distribution of sectors by levels of potential epidemic hazard in Kazakhstan (log-scale representation).

A lollipop chart with a logarithmic scale illustrates the proportion of sectors across five risk categories. Color coding reflects increasing epidemiological risk from very low (blue) to very high (red). Due to the highly skewed distribution of sector proportions, a logarithmic scale was applied to improve visualization.

Analysis of spatial distribution revealed that approximately 28% of the territory of natural plague foci is characterized by epizootic activity, while epidemic manifestations are recorded in only 3.7% of the area. This finding confirms that human plague cases represent a localized phenomenon, occurring only under specific combinations of epizootic, environmental, and socio-demographic factors.

Further analysis indicated that foci classified as high and very high risk are characterized by persistent and recurrent epizootic activity, whereas low- and very low-risk foci exhibit sporadic or irregular epizootic patterns. This highlights the pronounced spatial heterogeneity of epidemiological risk across natural plague foci.

Overall, epizootological differentiation and epidemiological zoning of plague foci represent essential tools for planning and optimizing preventive (anti-plague) measures, enabling the identification of priority areas for surveillance and intervention. Epidemiological zoning enables the identification of priority areas for surveillance by integrating epizootic dynamics with demographic and environmental risk factors.

3.5. Spatial Analysis and Forecasting of Epizootic Activity Using GIS Technologies

A comprehensive spatial analysis of the epizootic activity of the plague pathogen (*Yersinia pestis*) in the natural foci of Kazakhstan during 2020–2024 was performed using geoinformation and spatial statistical methods, enabling the identification of patterns of pathogen circulation and the development of a predictive risk model.

Application of the standard distance method allowed for the assessment of spatial dispersion and the central tendency of the distribution of epizootic occurrence points. As shown in Figure 4, the

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majority of cases were concentrated within the area corresponding to one standard deviation from the mean center, indicating the presence of stable cores of epizootic activity. The identification of such zones is of critical importance for defining priority areas for epizootological surveillance.

To assess the directionality of epizootic spread, the directional distribution method was applied. The resulting ellipse (Figure 6) demonstrates a pronounced spatial orientation of epizootics from the southeast to the northwest, indicating the presence of a persistent vector of infection spread. This directional pattern may be driven by a combination of natural factors, including landscape features, migration dynamics of primary reservoirs (particularly *Rhombomys opimus*), as well as the influence of anthropogenic processes.

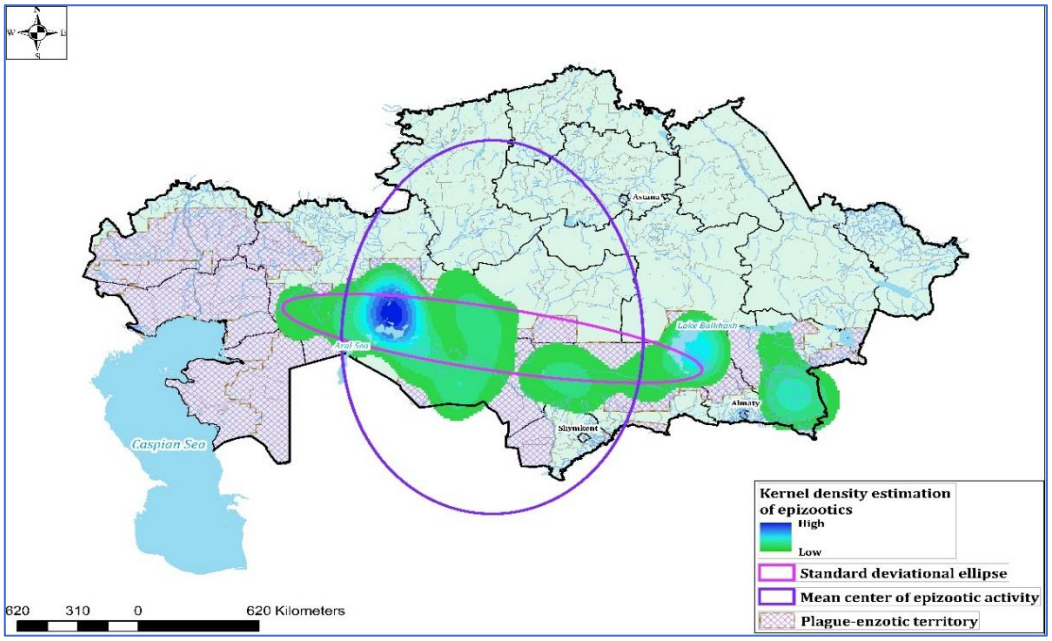

Figure 6. Frequency of epizootic occurrence within one standard deviation from the mean center and the directional distribution ellipse of epizootics (2020–2025), Mercator coordinate system.

Spatial autocorrelation analysis using the global Moran's I index revealed statistically significant clustering of epizootics (Moran's I = 1.627; z = 4.39; $p < 0.001$), confirming the non-random nature of their distribution and the presence of geographically localized zones of increased epizootic activity. The identified spatial structure highlights the need to prioritize surveillance and preventive interventions within these clusters.

To forecast the epizootic situation, the inverse distance weighting (IDW) method was employed, enabling interpolation based on spatial proximity of observations. The resulting predictive map (Figure 7) illustrates a gradient of the probability of epizootic occurrence across the study area.

The modeling results indicated that the highest probability of epizootic activity is associated with the North Aral, Betpakdala, Moyynkum, and Kyzylkum autonomous foci. At the same time, a potential spatial expansion of risk toward adjacent territories was identified, including the Volga-Ural sandy, Pre-Ustyurt, and Ustyurt foci.

Importantly, the high-risk zones identified through interpolation spatially correspond to the directional distribution of epizootics, confirming the internal consistency of the applied analytical approaches. Despite certain limitations related to the uneven distribution of input data, the applied methodology adequately captures the key patterns of pathogen circulation.

Thus, the use of GIS technologies and spatial statistical methods enabled the identification of stable epizootic cores, the determination of the directionality of infection spread, and the development of a predictive model of epizootic risk. The obtained results provide a scientific basis for optimizing epizootological surveillance, improving resource allocation, and enhancing the planning of preventive measures in natural plague foci.

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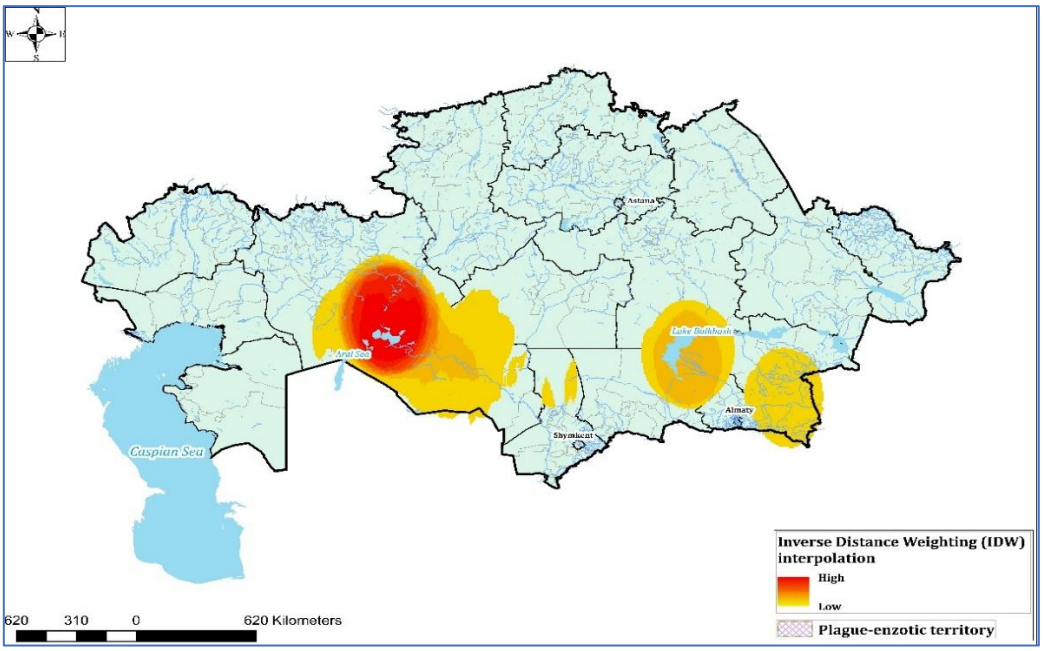

Figure 7. Predicted epizootic distribution based on the inverse distance weighting (IDW) method, Mercator coordinate system.

4. Discussion

The present study integrates long-term epidemiological, epizootological, molecular, and spatial data, providing a comprehensive understanding of plague persistence in one of the largest natural foci globally. The findings highlight a critical epidemiological paradox: increasing epizootic activity without human cases since 2003, reflecting the effectiveness of sustained surveillance systems and targeted interventions. Similar patterns have been reported in other endemic regions where strong surveillance interrupts zoonotic spillover [2,31].

The observed increase in epizootic activity during 2020–2025, including expansion of affected territories and increased pathogen detection, aligns with known ecological cycles of rodent populations and flea vectors in arid ecosystems [32,33]. Notably, the intensification of molecular diagnostics significantly improved detection sensitivity, consistent with global trends in zoonotic disease surveillance [34].

Spatial modeling results indicate that, in the near future, the most probable intensification of epizootic activity will occur in the western sectors of Kazakhstan's desert zone, particularly within the North Aral, Volga–Ural sandy, Pre-Ustyurt, and Ustyurt autonomous foci. In addition, elevated risk is expected in the Betpakdala, Moyynkum, and Arys-Kum–Daryalyktakyr foci. These projections are consistent with the identified spatial clustering and directional spread patterns, suggesting that current epizootic cores may serve as sources for further expansion.

Under conditions of projected climate change, the likelihood of expansion of *Yersinia pestis* circulation zones may increase, potentially elevating the risk of infection in humans and animal hosts, including camels, within enzootic territories. Similar climate-driven shifts in plague distribution have been documented in Central Asia and other endemic regions, where temperature and precipitation variability influence host and vector dynamics [35,36]. These findings highlight the importance of incorporating climate-sensitive surveillance into long-term risk assessment frameworks.

The high phenotypic and genotypic stability of *Y. pestis* populations is consistent with previous genomic studies demonstrating limited evolutionary divergence in natural foci [37,38]. However, the increased frequency of atypical strains in high-activity foci suggests localized ecological pressures and potential microevolutionary processes [39].

Spatial analysis revealed structured clustering and a consistent southeast–northwest directional spread, indicating non-random dynamics of epizootic processes. Comparable spatial patterns have

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been described in Central Asia and China, where landscape connectivity and reservoir migration play a critical role in pathogen distribution [40,41].

The epidemiological analysis confirms the classical natural-focal nature of plague, with human cases strictly dependent on epizootic activity. The Epidemic Year Index (EYI) proved to be a valuable integrative metric for assessing long-term risk, consistent with approaches used in other zoonotic systems [42]. The increase in epidemiological risk during the post-Soviet period further highlights the influence of socio-economic factors on disease dynamics [43].

Overall, these findings support the integration of ecological, molecular, and spatial approaches within a One Health framework. The combination of traditional surveillance, molecular diagnostics, and GIS-based modeling represents a powerful decision-support system for proactive plague control, particularly under conditions of environmental and climatic change.

5. Conclusions

Plague in Kazakhstan remains a persistent natural focal zoonotic infection characterized by stable circulation in wildlife populations and pronounced spatial heterogeneity.

Despite the observed intensification of epizootic activity in recent years, including expansion of affected areas and increased pathogen detection, no human cases have been recorded since 2003. This demonstrates the high effectiveness of the national epizootological and epidemiological surveillance system and the successful implementation of comprehensive anti-plague measures.

The study confirms the high phenotypic and genotypic stability of *Yersinia pestis* populations, with a low proportion of atypical variants and no evidence of antimicrobial resistance, supporting a conserved evolutionary pattern of the pathogen.

Spatial analysis identified stable epizootic cores, significant clustering, and a consistent directional spread of infection, while predictive modeling revealed high-risk zones primarily in western desert regions. These findings indicate the potential for further spatial expansion of epizootic activity, particularly under conditions of climate change.

The Epidemic Year Index (EYI) proved to be an effective integrative indicator of long-term epidemiological risk, highlighting regional heterogeneity and the dependence of human cases on epizootic dynamics.

Overall, the integration of long-term monitoring data, molecular diagnostics, and GIS-based spatial analysis provides a robust scientific framework for risk-oriented surveillance and proactive plague control. Continued development of these approaches within a One Health framework is essential to prevent the re-emergence of human plague in endemic regions.

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