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EPIDEMIOLOGY AND MICROBIOLOGY OF THE PLAGUE (*YERSINIA PESTIS*) PATHOGEN IN THE REPUBLIC OF UZBEKISTAN

Monograph

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The monograph is dedicated to the characteristics of natural plague (Yersinia pestis) foci in Uzbekistan and the microbiology of the plague pathogen. It presents scientific research conducted on the role of climatic changes in natural plague foci and on the microbiological aspects of the pathogen, along with recommendations for their application.

This monograph is intended for microbiologists, epidemiologists, parasitologists, students of medical universities, bachelor's and master's students, laboratory specialists, and others.

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I dedicate this to the cherished memory of my father and mother.

INTRODUCTION

“The person who studies the plague pathogen — this is not just a profession, in fact, it is a way of life," — says Doctor of Medical Sciences, Professor, Academician of the Russian Academy of Medical and Technical Sciences, A.S. Ne'matov.”

Describing the characteristics of natural foci of quarantine and especially dangerous infectious diseases — particularly the laboratory diagnosis of plague (*Yersinia pestis*) — is one of the essential areas of focus for anti-plague service personnel. It is especially important for those combating plague to be well-informed about modern laboratory diagnostic methods.

In many countries around the world, the epidemiological situation regarding plague remains quite severe. According to data from the World Health Organization,more than 51 million people die each year globally, including over 20 million due to infectious diseases »[1] [127;]. In this regard, identifying the dynamics and structure of natural foci of quarantine and highly dangerous diseases — including plague — forecasting incidence rates, applying modern early diagnostic methods, preventing disease emergence, ensuring effective treatment, and improving existing laboratory methods and preventive measures are among the top priorities for specialists in this field.

A number of scientific studies are being carried out around the world to investigate the epidemiological characteristics of plague, ensure its early diagnosis, and enhance the effectiveness of preventing its introduction from external sources. In this context, special attention is given to identifying the molecular-genetic and microbiological properties of the pathogen, determining the main factors contributing to the spread of plague, identifying sources and transmission routes to humans, substantiating the causes of infection, forecasting plague incidence rates, harmonizing specific and non-specific preventive measures across transboundary plague foci, creating a unified global epidemiological information center on the current state of natural plague foci, and developing and implementing a platform to improve control at border crossing points. Formulating strategic directions for early

[1]Global Burden of Disease 2020.World Health Organization Geneva.http://www.who.int/publications

detection, prevention, and improvement of epidemiological surveillance of the disease holds particular importance.

In our country, special attention is being given to research aimed at improving medical services, aligning them with international standards, and enhancing the use of modern technologies in the prevention, diagnosis, and treatment of infectious diseases. In this regard, the following objectives have been set: "…to increase the effectiveness, quality, and accessibility of medical care provided to the population in our country; to establish a system of medical standardization; to introduce high-tech methods of diagnosis and treatment; to promote a healthy lifestyle; and to prevent diseases."[2]. Successfully fulfilling these tasks will make it possible to reduce disability and mortality rates caused by diseases by applying modern technologies in the diagnosis and treatment of highly dangerous infectious diseases and elevating the quality of medical services to a new level.

The Decrees of the President of the Republic of Uzbekistan — No. PD-6110 dated November 12, 2020, "On introducing completely new mechanisms into the activities of primary healthcare institutions and further increasing the effectiveness of ongoing healthcare reforms"; No. PD-60 dated January 29, 2022, "On the Development Strategy of New Uzbekistan for 2022–2026"; Resolution No. PR-4891 dated November 12, 2020, "On additional measures to ensure public health by further increasing the effectiveness of preventive healthcare activities"; and Resolution No. PR-215 dated April 25, 2022, "On additional measures to bring primary healthcare services closer to the population and improve the effectiveness of medical services", as well as other relevant regulatory legal documents, serve as a foundation for the implementation of the tasks outlined therein. This monograph contributes, to a certain extent, to achieving these goals.

Scientific research is being actively conducted on improving the diagnosis, treatment methods, and prevention of plague, as well as on optimizing strategic directions to prevent the spread of the pathogen from infection sources. Leading international scientific centers and higher educational institutions around the world are carrying out in-depth studies focused on the application of molecular-genetic methods.

Globally, a number of scientific studies are being conducted to improve the prevention of highly dangerous plague, including in the

[2]Decree No. 5590 of the President of the Republic of Uzbekistan dated December 7, 2018, "On comprehensive measures for the radical improvement of the healthcare system."

following priority areas: early diagnosis of plague-infected patients, enhancing the effectiveness of treatment methods and preventive measures, addressing the insufficient understanding of infection dynamics in carrier organisms and ectoparasites, and recognizing the high contagiousness of plague, the rapid progression of its severe clinical forms, and its high fatality rate without effective emergency medical assistance — all of which suggest that the potential for its spread is underestimated. Thus, it is essential to further develop the foundations for rapid laboratory diagnostics and to enhance integrated preventive measures for plague among the population.

CHAPTER I. CHARACTERISTICS OF THE NATURAL FOCI OF KYZYLKUM AND USTYURT IN THE REPUBLIC OF UZBEKISTAN AND THE PROPERTIES OF PLAGUE PATHOGENS

Scientists from many countries around the world are paying great attention to the study of the problem of natural foci of plague pathogens (Motin V., 2002; Hinnebusch B.J., 2016; Sun Y.C., 2016). Nevertheless, the epidemiological and epizootological situation of this infection, as well as the need for rapid laboratory diagnosis, remains urgent (Zenkevich Ye.S., 2016; Meka-Mechenko T.V. et al., 2016; Popov N.V., 2017; Verzhusky D.B., 2018; Magnanou Ye, Fons R, 2004; Anisimov A.P., 2005; Plano G.V., 2013). In Uzbekistan, many scientific articles have been dedicated to the fight against plague (Kenjebaev A.Ya., 2002; Rivkus Yu.Z., 2004; Khakimov M.M., 2005; Miragzamov A.M., 2006; Ne’matov A.S., 2008; 2022; Khusanov O.A., 2022).

The research findings of various authors (Kutirev V.V., 2016; Balakhanov S.V., 2017; Matrosov A.N., 2020; Popov N.V., 2022) are mainly devoted to the specific epidemiological features of plague in certain regions, important aspects of epidemic monitoring, the system of preventive measures, and the nature of epizootics. Comparative characterization of plague epizootics in the natural foci of the Kyzylkum and Ustyurt regions, as well as their laboratory diagnosis, is currently of great relevance. However, modern-level studies of the plague pathogen’s microbiological, genetic, and virulence traits — its diagnostic, epidemiological, and preventive significance, and the variability mechanisms affecting these properties — remain insufficiently explored.

In recent years, progress in epidemiological and microbiological studies of plague has expanded our understanding of the variability of *Yersinia pestis*, allowing for deeper study of this infection in the active natural foci of Kyzylkum and Ustyurt. In this regard, in-depth analysis of our long-term observations and collected materials through the natural-scientific lens of epizootology, epidemiology, microbiology, and prevention using molecular-genetic diagnostic methods (PCR) is necessary. This also demands the development of a scientifically based and improved system of integrated preventive measures.

The plague control system in Uzbekistan was established in 1924 and has remained one of the key directions in safeguarding public health [82; pp. 5–10]. The territory of Uzbekistan spans 448,900 square kilometers. It is a landlocked, arid country in Central Asia with one of the highest population densities in the region and several natural endemic plague zones in desert and mountainous areas [42; pp. 5–8]. Currently, the Kyzylkum and Ustyurt territories — including the Republic of Karakalpakstan, and the regions of Navoi, Bukhara, Khorezm, Kashkadarya, and Tashkent — are considered epidemiologically significant (see Figure 1.1).

Mountainous epizootic plague areas are located in the regions of Tashkent, Andijan, Namangan, Fergana, Kashkadarya, and Surkhandarya. Thus, except for Tashkent City, Sirdarya, Jizzakh, and Samarkand, epizootic zones exist in nearly all regions of Uzbekistan [42; pp. 5–8, 136–290].

Natural reservoirs of plague in Uzbekistan include wild rodents such as large, diurnal, and red-tailed sand rats, thin-fingered and yellow marmots, small moles, hares, foxes, weasels, and ferrets. Among domestic animals, camels and cats serve as plague reservoirs [9; pp. 39–44; 19; pp. 7–17; 82; pp. 5–10].

From the perspectives of epizootology and epidemiology, the natural foci of Kyzylkum and Ustyurt have been comprehensively studied. However, due to the drying of the Aral Sea, new ecological regions have emerged in the arid zone that was once underwater. The majority of the dried seabed — approximately 2.5 million hectares — lies within the territory of Uzbekistan, which has had a significant impact on the flora and fauna of the region [3; pp. 12–14; 14; pp. 18–21].

We present to your attention the data related to the natural foci of Kyzylkum and Ustyurt.

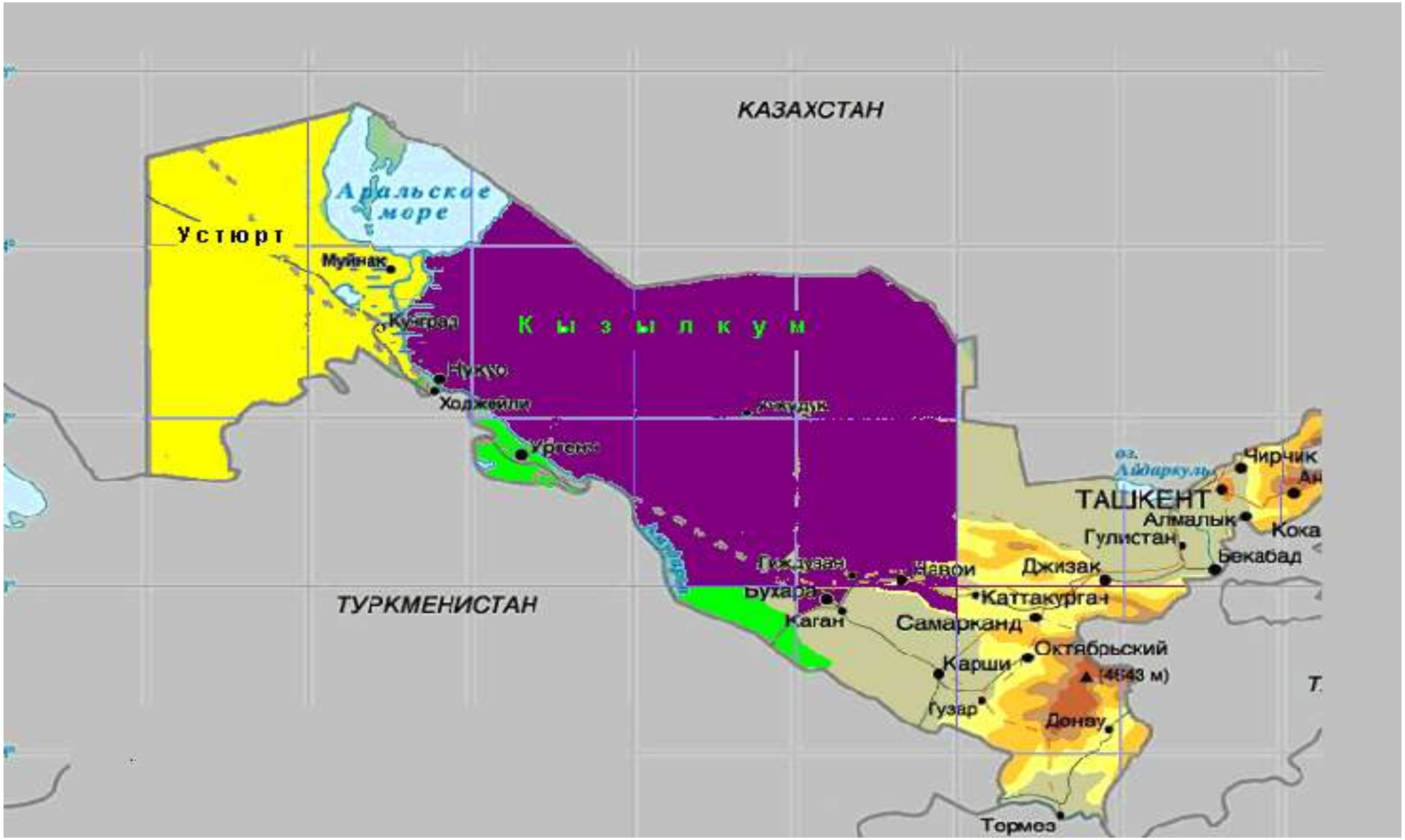


Figure 1.1 – Natural Foci of Plague in the Republic of Uzbekistan

§1.1. The Kyzylkum Natural Focus of Plague

One of the vast deserts located in Central Asia is the Kyzylkum Desert. This plain is devoid of surface water channels. Three major tectonic elements are expressed in Kyzylkum:
a) The uplifts and depressions of the Central Kyzylkum zone extend into its eastern regions.
b) The group of structures in the Gazli region.
c) The Eastern Aral depression, part of which lies within the Aral Sea area. Approximately 2.5 million hectares of the Aral Sea bed have dried up [6; p. 29]. In the central part of Kyzylkum — in the Tomditau, Kuldjuktau, and Bukantau regions — Paleozoic mountains are located. All mountains and slopes within Kyzylkum are separated from one another by sand massifs. About 60% of the total area is covered by ridge-hollow sand relief, formed as a result of the fragmentation and distribution of Pliocene–early Quaternary deposits. The soil cover consists mainly of gray-brown soils [31; pp. 62–66].

Kyzylkum's vegetation includes around 900 plant species, primarily various types of saxaul, several species of wormwood, and mosses. On shifting sands, selin, sand acacia, kandym, and feather grass are found. On the dried seabed, black saxaul and tamarisk grow. All types of shrubs are present in Kyzylkum, mainly white saxaul and juzgun, as well as sand sedge, semi-shrub wormwood, and small shrubs such as boyalich and sengren. Riparian vegetation is found along the shores of the Aral Sea and Aydarkul reservoir bordering the Kyzylkum Desert [16; p. 440].

The climate of Kyzylkum is characteristic of a desert: very low humidity, abundant sunlight and heat, and long dry periods. Summers are hot and dry with little to no precipitation. Due to the harsh climatic conditions, catastrophic events can occur in the lives of rodents, ectoparasites, and other animals, leading to sharp declines in their populations [27; pp. 163–164; 63; p. 215].

According to the biocenotic characteristics of the Kyzylkum natural focus, it is divided into four types: sandy, gravelly, saline deserts, and residual upland biocenoses. The main carrier of plague in Kyzylkum is the great sand gerbil, which is the key edifier of the desert biocenosis. The main role in pathogen transmission is played by fleas of the *Xenopsylla* genus [96; pp. 86–87].

The great sand gerbil, the main carrier of plague in Kyzylkum, is characterized by:

- A high and relatively stable population size,
- Strong burrows,
- High resilience, and
- Moderate susceptibility to plague [90; p. 215].

Fleas of the *Xenopsylla* genus are widely distributed in Kyzylkum and meet all criteria for primary vectors. Moreover, these fleas are also found as secondary and additional carriers. Adults are present year-round, and even in depression years their numbers remain relatively high. *Yersinia pestis* is transmitted by these fleas from March to December.

The distribution of great sand gerbils in various ecological zones of Kyzylkum is uneven. Several types of settlements exist: continuous habitats, diffuse-mosaic habitats, and ravine habitats [92; pp. 96–101]. In general, for the Kyzylkum region, the psammophilic (sand-loving) rodent community plays the most important role in the natural cycle of plague. The formation of plague foci can also be linked to regions of mountainous plains and low mountains [91; pp. 122–126].

The periodicity of epizootics is significantly influenced by a combination of factors, one of which is the relationship between the pathogen and its carrier [7; pp. 59–72; 23; pp. 19–26]. Great sand gerbils have varying levels of infectious susceptibility and predisposition to plague infection [74; pp. 75–78; 82; pp. 5–10]. Kyzylkum gerbils tend to exhibit more intense and prolonged bacteremia, which activates the epizootic process involving carriers and transmitters of the disease in Kyzylkum’s natural focus areas [19; pp. 7–17].

The identified carriers and transmitters of plague in the Kyzylkum natural focus are listed in **Tables 1.1 and 1.2** [63; p. 215].

1.1-Table

List of Carriers Found in the Natural Foci of Plague in the Kyzylkum Region

№	Carriers
1.	Great gerbil – *Rhombomys opimus*
2.	Red-tailed gerbil – *Meriones libycus*
3.	Southern gerbil – *Meriones meridianus*
4.	Crested gerbil – *Meriones tamariscinus*
5.	Yellow ground squirrel – *Spermophilus (Citellus) maximus*
6.	Narrow-fingered ground squirrel – *Spermophilopsis leptodactylus*
7.	Small jerboa – *Allactaga elator*
8.	Lichtenstein's jerboa – *Eremodipus lichtensteini*
9.	Thick-furred jerboa – *Dipus sagitta*
10.	Tolai hare – *Lepus tolai*
11.	Grey hamster – *Cricetulus migratorius*
12.	Comb-fingered jerboa – *Paradipus ctenodactylus*
13.	Common mole vole – *Ellobius tancrei*
14.	House mouse – *Mus musculus*
15.	Weasel – *Mustela nivalis*
16.	Steppe polecat – *Mustela eversmanni*
17.	Marbled polecat – *Mustela (Vormela) peregusna*
18.	Fox – *Vulpes vulpes*
19.	Domestic cat – *Felis lybica*
20.	Dromedary camel – *Camelus dromedarius*

The natural focus of the Kyzylkum consists of 15 landscape-ecological regions (LER) [63; p. 215], with a total area of 38.5 million hectares. The population of great gerbils varies significantly from low to high (1 to 20 animals per hectare). The epizootic index (EI) of the districts — that is, the ratio of the number of years plague epizootics were recorded to the total number of years the area was studied — ranges from 0.1 to 0.33.

1.2-Table

List of ectoparasites (fleas and ticks) found in the natural plague foci of the Kyzylkum			
1.	*Xenopsylla gerbilli caspica*	16.	*Stehoponia vlasovi*
2.	*Xenopsylla hirtipes*	17.	*Stehoponia conspecta*
3.	*Xenopsylla conformis*	18.	*Stehoponia pallidus*
4.	*Xenopsylla magdalinae*	19.	*Synosternus logispinus*
5.	*Ceratophyllus tarsus*	20.	*Parapoxopsyllus teretifrons*
6.	*Ceratophyllus laeviceps*	21.	*Echidnophaga oschanini*
7.	*Ceratophyllus turkmenicus*	22.	*Cterophthalmus dolichus*
8.	*Ceratophyllus aralis*	23.	*Mesopsylla eucta*
9.	*Ceratophyllus fidus*	24.	*Rostropsylla daca*
10.	*Ceratophyllus trispinus*	25.	*Pulex irritans*
11.	*Coptopsylla latmellifer*	26.	*Hyalomma Asiaticum*
12.	*Coptopsylla olgae*	27.	*Ornithodorus tartakovskyi*
13.	*Coptopsylla bairamaliensis*	28.	*Heamophysalis numdiana*
14.	*Rhadiopsylla cedestis*	29.	*Gamasidae*
15.	*Rhadiopsylla social*		

1. In Northern Kyzylkum, plague epizootics are regularly observed, with an epizootic index (EI) of 0.3. The burrows of great gerbils are arranged in striped and island-like patterns. Their population is relatively stable, with long-term average data showing 3–4 individuals per hectare. The flea density (dominated by *X. g. caspica* and *X. hirtipes*) is moderate, exceeding 300 vectors per hectare. The area of the region is approximately 1.7 million hectares. This region includes sandy-clay areas of the ancient delta, intersected by dry canals and abandoned irrigated lands, with fragmented hard dunes on elevated terrain. The plant groups consist of tamarisk, black saxaul, and itsigek shrubs.

2. The Staroreche area of Janadarya is considered the most persistent plague epizootic zone, with an epizootic index (EI) of 0.33. The burrows of the host species have a diffuse and mosaic distribution pattern. The population is stable, with 5 to 10 animals per hectare.
   Flea prevalence is low (with *X. g. caspica* being dominant), ranging from 100 to 300 vectors per hectare. The total area of the district is 1.9 million hectares. This region includes the ancient valley of the Syrdarya river, featuring alternating ridged and fragmented sand formations with meridionally stretched takyr-like plains. The vegetation includes black saxaul, salt-tolerant plants, and biyurgun shrubs.
3. In the Western Aral region, plague epizootics are relatively regularly detected, with an epizootic index (EI) of 0.33. The distribution of great gerbils is diffuse, and their population density is high — ranging from 10 to 20 individuals per hectare. The number of vectors (*X. g. caspica*) is also high, reaching 1,500 fleas per hectare. The area covers approximately 2.2 million hectares. The landscape consists of moderately and softly undulating sand dunes, as well as saline plains along the seashore. The dominant vegetation includes Jitnyakov–saltwort and saxaul–wormwood plant communities.

4. The Oqchadarya region exhibits frequent enzootic activity, with an epizootic index (EI) of 0.27. The primary host species is mainly diffusely distributed. Its population is relatively stable, with 5 to 10 individuals per hectare.
   The dominant flea species is *X. hirtipes*, with a low density — up to 300 fleas per hectare. The total area of the region is approximately 1.8 million hectares. The landscape consists of ancient delta plains and sieve-like static sand dunes.
   The vegetation cover is dominated by white saxaul, sand acacia, cherkez, and ephedra.
5. In the Beltog region, plague epizootics are regularly observed, with an epizootic index (EI) of 0.18. The diffuse distribution of great gerbils predominates. Their population is relatively stable and high, with 6 to 8 individuals per hectare. The flea density is low — approximately 300 vectors per hectare. The total area of the region is about 0.6 million hectares. The landscape consists of soil-covered

areas surrounded by static sandy dunes. The vegetation includes saxaul, sand acacia, and white wormwood.

6. The Nukus Sands represent a periodic pattern of epizootic activity, with an epizootic index (EI) of 0.19. There is a predominance of diffuse distribution of great gerbils, which exist in low numbers — fewer than 5 individuals per hectare. The dominant flea species is *X. hirtipes*, with a low insect density ranging from 100 to 300 fleas per hectare. The area covers approximately 1.2 million hectares. This region consists of sandy plateaus covered with white saxaul, cherkez, and ephedra.
7. The Northwestern Kyzylkum is a low-frequency epizootic zone, with an epizootic index (EI) of 0.17. The primary distribution type of great gerbils is diffuse, with fewer island-type colonies. Their numbers are low, with no more than 4 animals per hectare. The flea prevalence is very low, with approximately 100 fleas per hectare. The total area of the region is 1.1 million hectares. The landscape consists of elevated sandy plains with sparse saxaul, juzgun, and wormwood vegetation.
8. The Western Kyzylkum is a low-frequency epizootic zone, with an epizootic index (EI) of 0.14. The habitats of the main host species are scattered, and their population is unstable. According to long-term data, the species occurs infrequently, with approximately 3 individuals per hectare. The flea population is very low, fluctuating around 100 fleas per hectare.

   The area of the region is approximately 2.5 million hectares. The terrain consists of sandy hills, and the vegetation includes white saxaul, acacia-cherkez-ephemeral plant communities.
9. The Northeastern Kyzylkum is a frequently occurring epizootic zone, with an epizootic index (EI) of 0.24. The burrows of the main host species are diffusely distributed, and occasionally island-like. The long-term average population is about 3.5 individuals per hectare. The dominant flea species (*X. hirtipes*, *X. g. caspica*) have a low density, with approximately 200 fleas per hectare. The area of the region is 3.0 million hectares. The landscape consists of takyr-like plains interspersed with island-shaped sand dunes. Vegetation includes white saxaul, juzgun–acacia–wormwood plant communities.

10. The Eastern Kyzylkum is a region where plague epizootics are not rare, with an epizootic index (EI) of 0.18. The burrows of great gerbils are scattered and island-like, and in approximately one-third of the area, the animals are sparsely populated. Their number is low — fewer than 5 individuals per hectare. The flea population (*X. g. caspica*, *X. hirtipes*) is also low, with about 150 vectors per hectare. The area is approximately 5.1 million hectares. The terrain includes elevated ridge-like sandy wind-blown plains, as well as ancient riverbeds. The vegetation cover is dominated by saxaul–wormwood–ephemeral plant communities, and to a lesser extent, saltwort–juzgun associations.

11. In the Central Kyzylkum, plague epizootics occur at long intervals, with an epizootic index (EI) of 0.24. The burrows of the main host species are diffusely distributed and sharply defined. The population is unstable, with significant fluctuations over the years. The flea density from the Xenopsylla family is low, ranging from 90 to 180 specimens per hectare. The total area of the region is approximately 7.3 million hectares. This is the highest-elevation zone among the plague foci, formed by residual rocky uplifts. The spaces between the ridges are occupied by sandy plains. The vegetation is characterized by desert grasses and white saxaul.

12. In the Central Kyzylkum, plague epizootics occur with long intervals, with an epizootic index (EI) of 0.24. The distribution of the main host's burrows is diffuse and sharply defined. The population size is unstable, showing sharp fluctuations over the years. The abundance of fleas from the Xenopsylla family is low, ranging from 90 to 180 specimens per hectare. The total area of the region is approximately 7.3 million hectares. This is the highest part of the plague focus, formed by residual rocky uplifts. The gaps between isolated ridges are filled with sandy plains. The vegetation is characterized by desert grasses and white saxaul.

13. Southern Kyzylkum is a region with sporadic occurrences of epizootics. That is, the epizootic index is -0.1. The colony type of large sand gerbils is diffuse. Their population is highly unstable and tends toward a deeply extended depressive form. The flea density is low, amounting to 100–200 carriers per hectare. The area is approximately 3.0 million hectares. It is covered by a gypsum-sandy plain associated with saline and saltwort vegetation. The land is being intensively developed under irrigated agriculture conditions.

14. The Lower Zarafshan region – epizootics are very rare here, with an epizootic index of less than 0.1. Large sand gerbils inhabit this area in a scattered and linear pattern and have an unstable population level. The number of fleas is very low, with fewer than 100 insects per hectare. The area is approximately 2.1 million hectares. It is a valley divided by numerous dry canals and riverbeds. The vegetation consists of salt-tolerant black saxaul.

15. The Karshi Steppe – epizootics are very rare, with an epizootic index of less than 0.1. The distribution pattern of large sand gerbils is insular. Their population is low and often experiences deep declines. The number of fleas is very low. The area covers approximately 3.5 million hectares. It is an alluvial-deltaic plain region with elevated plateaus and sandy massifs. Salt-tolerant vegetation and saltwort are characteristic.

16. Sandikli Sands – the plague pathogen is rarely found in these areas, with an epizootic index of less than 0.1. The nests of the main carrier are of a diffuse type. The number of rodents is extremely low and unstable. The flea population is also very low, with fewer than 100 insects per hectare. The area is approximately 1.5 million hectares. It is a rugged, hilly, and solid massif. The most widespread vegetation is the grainworm–saltwort complex.

The climate is continental due to large fluctuations in temperature throughout the year and is uneven across the seasons, mainly in winter and spring [20; pp. 93–103]. These regions are predominantly characterized by vegetation typical of sandy deserts formed on sandy and relatively compact sand deposits. The dominant plant species include white saxaul, certain species of tamarisk, rabbit ears (*Salsola*), singren (*Astragalus*), iloq (*Artemisia*), and others.

White saxaul is a tree-like shrub that can reach a height of up to 5 meters, with roots penetrating more than 15 meters deep. It is a key edifying species of white saxaul pastures and one of the main fodder plants. Its nutritional value: 100 kg of air-dry matter contains 52 feed units and 6.8 kg of protein. White saxaul is also considered an important plant for fixing sand.

Black saxaul is a leafless tree that grows up to 8–10 meters tall and is found on saline and compact takyr soils. It can also take the form of a shrub 1.5–2 meters tall. Its root system reaches areas fed by underground mineralized waters. It grows on takyr soils, valley-like sandy edges, and old riverbeds. Flowering begins at the end of March to early April, and fruiting occurs by mid-September.

Astragalus kosmateyshii (singren) is a shrub up to 1 meter tall that grows on compact stony or sandy soils. Its root system is poorly developed, penetrating up to 12 cm deep. Flowering occurs in the second half of April. Astragalus is considered a good forage plant.

Tree-like Salsola (Boyalych) is a highly branched shrub 20–100 cm in height. It blooms and bears fruit from June to September. It grows in sandy areas and is consumed by camels throughout the year.

Sprawling wormwood (djusan) is a shrub 25–40 cm tall with a well-developed root system, drought-resistant, and begins flowering at the end of March. This type of wormwood is especially valuable as feed for sheep (17% protein content). 100 kg of dry wormwood provides 35 feed units in terms of protein value.

Tamarisk (Juzg'un / Qandim) is a branched shrub up to 2 meters in height with a deep root system extending 10–12 meters, of a universal type. It flowers in April. Its nutritional value: 100 kg of dry matter provides 71.8 feed units. The plant contains 10–13% tannins and typically grows in sandy pastures.

Selin is a short-rooted perennial plant forming a shrub 30–50 cm tall. It is a good forage desert plant consumed by sheep and horses in late spring and early summer. It is harvested as hay for winter use and plays an important role in sand stabilization.

Other plants found in the desert zone include: desert herbs, half-shrub species like qairaq, swollen iloq (a type of wormwood), and sandy iloq. Among poisonous plants, *shoxbosh* is notable – a spring annual ephemeral that grows in clay, sandy soils, gravel, and saline areas. It contains toxic substances that are neutralized by drying, but it is highly poisonous to sheep during the flowering stage. Precautionary measure: before allowing sheep to graze, it is necessary to carefully inspect the pasture area [3; pp. 12–14].

§1.2. Description of the microbiological characteristics of the plague pathogen in the natural focus of the Kyzylkum Desert

In the study of the properties of plague pathogen strains from different epizootiological zones of the Kyzylkum Desert, it was found that regardless of the location where the strains were isolated, they exhibit cultural-morphological and other characteristics typical of the plague pathogen. The strains are sensitive to plague and pseudotuberculosis phages, produce acid from glycerol without gas, and ferment maltose, glucose, galactose, arabinose, xylose, and mannitol. However, they do not ferment lactose, rhamnose, dulcitol, or erythritol and produce Fraction 1 antigen. They show high sensitivity to streptomycin, tetracycline, chloramphenicol, gentamicin, ciprofloxacin, oleandomycin, lincomycin, and penicillin. They grow on basic media containing cysteine, methionine, threonine, and phenylalanine. Growth appears after 3–4 days in medium lacking threonine, but no growth is observed without phenylalanine. After 6–8 days at 28 °C, isolated colonies appear on sodium sulfite or cysteine-containing media [57; pp. 112–121].

Based on our research, alongside cultures highly sensitive to streptomycin, we encountered for the first time streptomycin-resistant strains (though still sensitive to other antibiotics). On Jackson-Burrows nutrient medium, they grow in the form of pigmented colonies. Most cells are calcium-dependent and produce pesticin. The plague pathogen strains are highly virulent. However, there are strains with characteristics not typical of Kyzylkum cultures.

During different phases of the epizootic process in Kyzylkum, the cultures we studied grew in synthetic media containing four amino acids: cysteine, methionine, phenylalanine, and threonine. For some strains, growth on the third day was stimulated by eight amino acids: the four mentioned above, along with glutamic acid, lysine, leucine, and valine. A comparative summary is presented in Table 1.3.

In epizootics, not only large sand gerbils but also secondary small carriers are present and participate. Therefore, the nutritional requirements of plague pathogens in Kyzylkum are not dependent on the phase of the epizootic process. The appearance of strains with varying dependencies on amino acids or other components—uncharacteristic for a specific natural focus—requires further investigation. This may indicate the evolution of the plague microbe and explain the emergence of specific ecotypes.

In the natural focus area of the Kyzylkum Desert, acute epizootics can occur near human settlements, which poses a real epidemiological risk. In addition to landscape, geographical, climatic, ecological, and similar features, plague foci may have a characteristic composition of rodents and ectoparasites, within which pathogens with specific traits circulate, thus forming a distinct epizootic structure of the focus [8; p.186, 21; pp.105–109, 34; pp.51–55, 54; pp.19–22, 143; pp.571–582].

Under current conditions, in order to guide epidemiological surveillance, it is necessary to compare landscape structures when identifying epizootic activity and differentiating between focal territories.

1.3 -table

Comparative indicators of the characteristics of plague strains

№	Strain Characteristics	Kyzylkum	Ustyurt
1	Motility	–	–
2	Sensitivity to phages: - Pokrovsky plague phage - Pseudotuberculosis phage	 + +	 + +
3	Fermentation: - Glycerin - Rhamnose - Arabinose - Urea	 + – + –	 + – + –
4	Denitrification	–	–
5	Fibrinolytic activity	+	+
6	Coagulase activity	+	+
7	Requirement for essential amino acids for growth: - Methionine - Threonine - Phenylalanine - Cysteine - Leucine	 + + – + + (–)	 + + + + + (–)
8	Calcium affinity	+	+
9	Virulence: - White mice - Guinea pigs	 high high	 high high

§1.3. The Ustyurt is the natural Plague-Endemic Zone

Ustyurt is the only elevated plateau in Western Asia, located between Mangyshlak and the Kara-Bogaz-Gol Bay in the west and the Aral Sea in the east. In the north and northwest, it borders the Pre-Caspian lowland. The total area of the lowland is 200,000 square kilometers. The western part of Ustyurt belongs to the Mangyshlak region. The eastern (or Pre-Aral) part lies within the territory of Karakalpakstan, and the southwestern part is located within the Republic of Turkmenistan. The boundary of the lowland is sharply demarcated by high (150–200 m) eroded escarpments that form a surrounding ridge. The total area is 15.8 million hectares [63; p. 215].

In terms of its relief, the Ustyurt natural plague focus belongs to the group of arid-denudational mountain plateaus, characterized by diverse forms of microrelief in the form of dry valleys. It can be divided into 11 landscape-epizootiological zones [6; p. 29].

1. Northern Ustyurt Plain – Epizootic cases are recorded occasionally. The epizootic index (i.e.) is 0.28. The primary carrier inhabits in various forms. The average long-term density is 4 specimens per hectare. According to long-term data, the dominant flea species (*X. skrjabini*) accounts for 453 fleas per hectare. The area is about 1.4 million hectares and consists of sandy-limestone plains with *buyurgun* and *boyalych* vegetation.

2. Mataykum Sands – Local epizootics have been recorded with long inter-epizootic periods. The epizootic index is 0.1. Large sand gerbils live in sandy areas and along slopes, numbering no more than 4 per hectare. Flea numbers are also low—about 300 fleas per hectare. The estimated area is 0.4 million hectares, consisting of ridge-hill massifs sparsely covered with psammophilous plants.

3. Qirqquduq Plain Region – Epizootic cases are very rare. The epizootic index is 0.2. The main carriers' nesting pattern is diffuse and linear. The long-term average is no more than 6 specimens per hectare. Flea density is moderate (384 per hectare). The area is about 0.7 million hectares and consists of an alluvial plain fragmented by channels, chalk deposits, saline spots, and takyr-like depressions. Vegetation consists of wormwood–grass associations.

4. Sam Region – Epizootics are regularly recorded. The epizootic index is 0.76. The burrows of sand gerbils are of a diffuse type. Long-term data show low density: 4 specimens per hectare. The main parasite (*X. skrjabini*) averages 529 fleas per hectare. The area is approximately 0.4 million hectares and consists of ridge-hill sandy terrain. Dominant vegetation includes wormwood–grass and *teresken*.

5. Qoratul Region – Epizootics are observed almost annually. The epizootic index is 0.6. Gerbil burrows are diffuse and broadly linear. Their number does not exceed 4 per hectare. Flea density is high—804 fleas per hectare. The area covers 1.1 million hectares. It is a compact plain with many ravines. The plant association is dominated by a wormwood–saltwort complex, with rare saxaul trees.

6. Plakorno Plain – Epizootics are recorded with significant interruptions. The epizootic index is 0.4. The primary carrier's burrows are mostly diffuse, forming linear bands along railways and gas-oil pipelines. The long-term average density is 3–5 specimens per hectare. Flea density is low (173 per hectare), with *X. skrjabini* in the north and *X. g. caspica* in the south. The area spans about 6.6 million hectares. The clay plateau is covered with a mix of *boyalych*, wormwood–saltwort vegetation, and rare *buyurgun*.

7. Eastern Uvalist Region – No epizootics have been recorded. Gerbil burrows are scattered. According to long-term data, their density does not exceed 5 per hectare. Flea density is very low—fewer than 100 per hectare. The region's area is 0.4 million hectares, consisting of gently sloping grey soil elevations (250 m above sea level). The vegetation is dominated by *boyalych* and *shuvoq-buyurgun*.

8. Central Uvalist Region – Epizootiologically under-researched. Gerbil burrows are diffuse and linear (along ridges). Density is low—3–5 rodents per hectare. Fleas (*X. nyttalli*, *X. g. caspica*) are very few—under 100 per hectare. The area is about 2.2 million hectares and includes a chain of ridges crossing the plateau from west to southeast with many ravines, dry riverbeds, and some ruins on mountain slopes. Vegetation consists of *buyurgun–wormwood–keireuk* complexes, including rare saxaul.

9. Sor Borsa-Kelmes – Plague enzootics occur periodically. The epizootic index is 0.1. The primary carrier's burrows are of the diffuse type, and the population is relatively stable—5–10 rodents per hectare. Flea numbers range from 100 to 3000 per hectare. The area is about 0.2 million hectares, characterized by saline basins with associations of *saline black saxaul*.

10. Ossake-Oudan Lowland – No epizootics detected. Gerbil burrows are rare and diffuse. The density is low—fewer than 5 specimens per hectare. Flea numbers are very low—under 100 per hectare. The area is about 1.2 million hectares, slightly undulating, and sparsely covered with black saxaul, saltwort, and *sarsazan*.

11. Southern Uvalist Region – Poorly studied. Epizootics are very sporadic. The epizootic index is 0.1. Large sand gerbil burrows are of the diffuse type, with densities ranging from 1 to 5 per hectare. Fleas (*X. g. caspica*) are few—100 to 300 per hectare. The area is about 1.2

million hectares and includes gravelly and gypsum lands with ephemeral wormwood–saltwort vegetation.

The desert landscape of the main part of Ustyurt belongs to the clay-wormwood and wormwood–saline desert types, which serve as green pastures in spring and autumn [127; pp. 26–33]. Precipitation is extremely low—100 to 150 mm per year, mostly in summer, and it may not occur at all. The air is extremely dry. In the north, the average temperature of the hottest month is -27°C, while in the south it is +28.5°C. The maximum temperature reaches +45–46°C. Additionally, there are large diurnal temperature fluctuations of up to 26–28°C. Winters are mostly snowless, and the snow cover does not exceed 20 cm in open areas. In January, the average temperature is -8.5°C in the north and -6°C in the south, with maximum recorded values of -33°C and +32°C, respectively.

Ustyurt is characterized by dense gray-brown soils with thick gypsum layers near the surface and a gravel crust on top. Poor drainage and high soil salinity—exacerbated by lack of wind—hinder the development of distinct vegetation cover. The complexity of the vegetation is related to the specific influence of plants on the soil, particularly their ability to draw salts to the surface, affecting desalinization and leaching of the soil. The northern part of the plateau is characterized by saline brown desert complexes—desert soils with takyr-like features and loose sedimentary, highly alkaline soils.

Vegetation varies from north to south. In the north, it includes hawthorn, qairaq (halophytic shrubs), and saltwort; in the south: *buyurgun* (halophytes), hawthorn, wormwood, and other lithophytes. On gravelly soils grow *tas-buyurgun*, thorny, and curly thorned species.

The fauna includes more than 20 species of rodents [38; pp. 38–40, 62; pp. 116–123]. In northern Ustyurt, in addition to sand gerbils, marmots are widespread and frequently encountered. Also found are jerboas, common voles, and double-toothed rodents. Mouse-like rodents are rare. Among predators: wolves, corsacs, foxes, wild cats, pale weasels, weasels, and sables [39; p. 23]. Hunting of animals throughout the region is prohibited because they are potential sources of plague infection for humans.

In Ustyurt, the spread of disease among rodents arises due to the introduction of the microbe from zones where it is permanently present. Since local conditions are not favorable for the prolonged existence of the plague pathogen, epizootics are typically short-lived, and in some areas, outbreaks may not occur for many years. This suggests that some regions of Ustyurt lack the necessary conditions for stable plague foci and serve as zones where the epizootic dies out.

The typical main host in the focus is the **great sand gerbil**, which has no equal in terms of population and distribution among rodents. They primarily inhabit small hilly sands, depressions between sand dunes, bases of ridge sands, saline areas, and moist riverbanks. In the eastern part of Ustyurt, burrows are found mainly in the depressions between coarse sandy hills.

Secondary carriers include **crested** and **red-tailed sand gerbils** and small marmots [35; pp. 387–397, 40; pp. 34–38]. Accidental hosts include yellow marmots, jerboas, tarbagans, house mice, and moles.

In almost all of Ustyurt's territory, fleas of the genus ***Xenopsylla*** are widespread [1; pp. 25–33]. Other flea genera such as *Ceratophyllus*, *Coptopsylla*, and *Stenoponis* are also found in the foci and serve as additional or accidental ectoparasitic vectors [2; pp. 40–44].

We conducted an epizootiological analysis taking into account landscape and geographic differences. Detailed study of these differences allowed us to identify epizootiological heterogeneity across individual parts of Ustyurt. This supports the role of ecological and geographic factors in shaping the uneven distribution of the main carrier. The most stable development of plague in Ustyurt is observed in the **Northern Ustyurt basin**, a region mostly referred to as the *Cam* sands. The ridge plains of the western part also play a role in the spread of epizootics. Saline areas with small sand patches are present. The **densest colonies of great sand gerbils** have been recorded in the **Uali area** and near the **Soy-Utes railway station** [15; pp. 44–51, 47; pp. 20–29].

Our observations indicate that when passenger and freight trains arrive with grain products, the influx of people and food increases, attracting rodents searching for food. As a result, rodent migration increases, leading to population surges year after year. This facilitates the wider spread of infection not only along railways but also in adjacent areas and nearby settlements.

In the mountainous plains of the plateau, gerbil burrows are rare, but additional vectors exist: crested and diurnal sand gerbils and small marmots. However, this list of vectors may not be sufficient to maintain long-lasting epizootics. Due to the lack of necessary conditions for stable infection and microbial reproduction, plague foci in these areas

1.4-table

List of Carriers and Ectoparasites of the Ustyurt Natural Focus

No.	Carriers	No.	Ectoparasites
1	Great gerbil – *Rhombomys* opimus	1	*Xenopsylla skrjabini*
2	Red-tailed gerbil – *Meriones* libycus	2	*Xenopsylla gerbilli caspica*
3	Southern gerbil – *Meriones meridianus*	3	*Ceratophyllus laeviceps*
4	Crested gerbil – *Meriones tamariscinus*	4	*Ceratophyllus trispinus*
5	Yellow ground squirrel – *Citellus maximus*	5	*Ceratophyllus tarsus*
6	Small ground squirrel – *Citellus pygmaeus*	6	*Ceratophyllus tesquorum*
7	Small jerboa – *Allactaga elator*	7	*Coptopsylla lamellifer*
8	Underground hare (Tarbagan) – *Allactagulus pygmaeus*	8	*Rhadiopsulla cedestis*
9	Grey hamster – *Cricetulus migratorius*	9	*Stehoponia conspecta*
10	House mouse – *Mus musculus*	10	*Stehoponia vlasovi*
11	Weasel – *Mustela nivalis*	11	*Prapoxopsullus repandus*
12	Steppe polecat – *Mustela eversmanni*	12	*Echidnophaga oschanini*
13	Corsac fox – *Vulpes corsac*	13	*Cterophthalmus dolichus*
14	Domestic cat – *Felis lybica Forst*	14	*Hyalomma*
15	Camel – *Camelus dromedarius*	15	*Haemophysalis*
16	Fox – *Vulpes vulpes*	16	*Ornithodorus*
17	Fur-bearing animals (marten, sable) – *Mustela peregusna*	17	*Ixodes*

persist only briefly—sometimes just a few days. In certain regions, no epizootics may occur for several years.

We present a list of typical carriers and ectoparasites specific to the Ustyurt Plateau [63; p. 215] (see Table 1.4).

Thus, in **southern Ustyurt**, due to the desiccation of the Aral Sea, new conditions are emerging for the preservation and formation of new epizootics. At the same time, this region is considered a potential zone for eliminating plague infections and conducting comprehensive epizootiological surveys across the landscape. Geographic differences in this area enhance our understanding of the flea fauna and habitats in the study region.

In general, the epizootic process in Ustyurt is characterized by a scattered pattern in spring and intensification as it progresses. The main biological host, the core of the biocenosis, is the great sand gerbil and fleas of the genus Xenopsylla.

§1.4. Description of the Microbiological Characteristics of the Plague Pathogen in the Ustyurt Natural Focus

The strains we studied exhibit cultural and morphological characteristics typical of the plague pathogen. They ferment glycerol, glucose, galactose, arabinose, and hexose without gas production, but do not ferment lactose, rhamnose, sucrose, sorbitol, xylose, erythritol, inositol, or dulcitol [44; pp. 43–48]. The cultures give negative reactions for denitrification and nitrification and produce Fraction 1 antigen. They are highly sensitive to antibiotics such as tetracycline, streptomycin, chloramphenicol, monomycin, and others, and also to plague and pseudotuberculosis phages. Growth is observed in nutrient media containing cysteine, methionine, threonine, and phenylalanine, as well as in Jackson-Burrows nutrient medium. For some strains, growth is also noted with leucine. A distinct calcium dependency is observed. All strains are highly virulent.

A characteristic feature of the strains isolated from the Ustyurt natural focus is the manifestation of **pigment sorption (Psb)** on the bacterial cell membrane, which reflects the bacterium's ability to absorb iron ions and correlates with virulence. Selective phage tests showed that the proportion of Psb-positive cells varied between 0.01–5% depending on how long the cultures were preserved under laboratory conditions [45; pp. 140–144]. All Psb-positive and calcium-dependent strains demonstrated high virulence in laboratory animals and expressed Fraction 1 antigen, as detected by PGAR (titers 1:40–1:320).

All Ustyurt strains are resistant to pesticin 1 and do not grow in media lacking amino acids. However, some strains among the plague pathogen population show dependence on particular amino acids. These differences in amino acid requirements among strains from different natural foci suggest that their genetic characteristics are shaped by the ecological and geographical features of the respective foci [49; pp. 62–67, 136; p. 290].

One of the stable traits of Ustyurt strains is **leucine dependency**, which usually persists even during long-term storage of cultures. To study the effects of leucine, we evaluated the ability of plague bacteria to grow in media with high concentrations of growth factors. Cultures from different areas of the Ustyurt natural focus were tested, allowing us to examine the impact of excessive amino acid concentrations. The effects were expressed in three forms: changes in colony size, number of colonies, and delayed growth. The sensitivity to these amino acids was associated with their role in biosynthesis [138; pp. 38–43, 148; pp. 356–361].

Our findings suggest that both leucine-positive and leucine-negative strains are equally affected by high concentrations of specific amino acids (leucine, valine, isoleucine, methionine, etc.). This indicates that plague bacteria can be classified into a group of microorganisms that exhibit 100% growth inhibition at critical concentrations of DL-leucine.

Thus, all of the above is important not only for differential diagnostics of isolated cultures but also for assessing the epidemiological state of specific areas within the Ustyurt natural focus.

Epizootiological Zoning of the Autonomous Natural Ustyurt Plague Focus

The composition of rodent hosts, which are plague carriers, gives Ustyurt a distinct epizootiological profile. The plague epizootic spreads from year to year, giving this focus a real potential to become endemic. Based on the nature of the Ustyurt terrain, nine physical-geographic districts can be distinguished [60; p. 248]: Northern Ustyurt, Sameno-Osmantoy, Central Ustyurt, Borsakelmas, Urru-Iltedjin, Assake-Audan, Zaaudan, Southwestern (Kazalin), and Musbel-Qorabaur.

Ustyurt is not epizootiologically uniform; this is due to the uneven distribution of the main host and ectoparasites. The zoning of the focus is based on:

1. characteristics of plague microbe strains (Leu^+ and Leu^-),
2. abundance and diversity of carriers and ectoparasites,
3. the landscape of the territory, which collectively define the nature of epizootics in the region [47; pp. 20–29, 48; pp. 36–38].

The Ustyurt natural focus can be conditionally divided into four main zones:

1. **Northern Ustyurt**
2. **Western Ustyurt**
3. **Southern Ustyurt**
4. **Central Ustyurt**

And by landscape-ecological zones:

- Northern Ustyurt lowland
- Northern Ustyurt plateau
- Sam sands
- Qoratuley depression
- Plakorno plain
- Central Uvalist area
- Assake-Audan depression
- Southern Uvalist zone

According to specialists from the Karakalpakstan Anti-Plague Service, Ustyurt is conditionally divided into Northern, Central, and Southern zones.

For zoning, we used the following as criteria:

- characteristics of plague strains,
- number and types of carriers and ectoparasites,
- landscape of the region,
- nature of the epizootic process.

We concluded that leucine-dependent (Leu^-) strains circulate in northern and central Ustyurt, while leucine-independent (Leu^+) strains, likely influenced by natural genetic processes, dominate in southern Ustyurt. This difference is significantly influenced by the environment.

Since leucine dependency is directly related to the availability of leucine, it is appropriate to divide Ustyurt into two primary regions:

- Northern and Central Ustyurt with Leu^- strains
- Southern Ustyurt with Leu^+ strains

This distinction can serve as a useful marker for tracking plague epizootic dynamics.

Regions with Leucine-Dependent (Leu^-) Strains:
a) Northern Ustyurt Plateau
b) Northeastern Ustyurt
c) Northern Ustyurt Basin-Plain
d) Sam
e) Jaurinquduq
f) Qoratul
g) Central Plain
h) Borsakelmas
i) Muzbel-Qorabaur
j) Assake-Audan

Regions with Leucine-Independent (Leu^+) Strains:
a) Zaaudan
b) Southwestern Ustyurt

Regions Not Considered Enzootic for Plague:
a) Eastern Ustyurt
b) Southeastern Ustyurt
Note: This does not mean that plague epizootics cannot arise in these areas.

In addition to the regions listed above, there are also smaller areas—such as Chuqurqoq, Risboy, and Qoraqidir—that differ in their geographical location and epizootic characteristics. These designations may certainly be revised with appropriate adjustments during further study of the epizootic properties. However, the above information can serve as a basis for practical activities within the anti-plague system.

Due to the steady increase in the population of Ustyurt and the fact that the Silk Road highway and transport connections with nearby Ustyurt districts pass through this area, numerous geological exploration expeditions are being conducted here. To implement sanitary and epidemiological measures, the activity of the epizootic process is monitored, the number of carriers and ectoparasites is recorded, and disinfection (disinsection) and rodent control (deratization) measures are carried out regularly. Additionally, public health education is conducted among medical personnel and the local population.

When we analyzed the characteristics of plague microbe strains isolated from the natural foci of the Kyzylkum and Ustyurt regions of the Republic of Uzbekistan, we found that these pathogens were isolated from various wild rodents and their ectoparasites in the natural foci (see Table 1.5).

1.5-table

Volume of Work (%) on the Study of Y. pestis Pathogens Isolated from Natural Foci

No.	Foci	Ectoparasites (%)	Rodents (%)	Camels (%)	Plants (%)
1	Kyzylkum	32.9	35.7	0.3	0.3
2	Ustyurt	15.5	15.3	–	–
Total: 100%		48.4	51.0	0.3	0.3

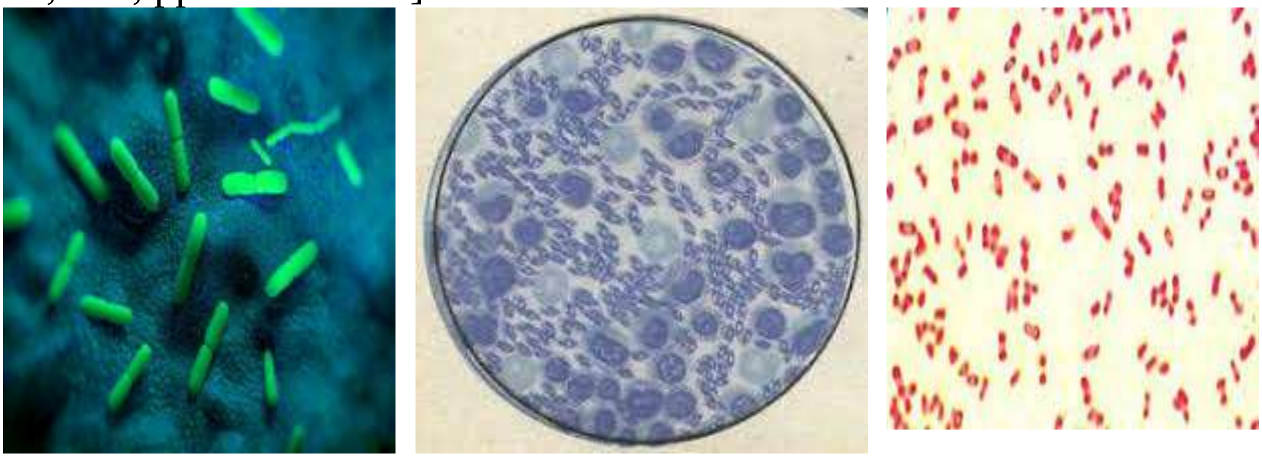
All designated isolated strains were examined within a period ranging from two to six months. The study of the strains was carried out using laboratory and microbiological diagnostic methods specific to the plague pathogen, including:
a) cultural and morphological characteristics;
b) fermentative activity;
c) sensitivity to plague and pseudotuberculosis phages;
d) motility;
e) virulence of the isolated strains in white mice and guinea pigs;
f) amino acid requirements;
g) sensitivity to antibiotics;
h) sensitivity to pesticin I;
i) calcium dependence at 37°C;
j) pigment formation;
k) ability to produce Fraction 1 (F1) antigen [69; p. 39, 72; pp. 25–35, 104; pp. 137–145].

Cultural and morphological characteristics of the strains: To study the cultural and morphological characteristics of all Y. pestis strains isolated at 28°C, the following nutrient media were used: Nutrient agar (HiMedia M001, HiMedia Laboratories Pvt. Ltd., India), pH 7.2 ± 0.2; neutral agar and broth, neutral agars (Russian Federation, Serpukhov and Makhachkala cities), pH 7.1–7.2.

Description of the cultural-morphological features of the plague pathogen:

It appears as a rod-shaped bacterium with rounded ends, measuring 1–2 microns in length and 0.3–0.7 microns in width. It stains well with all aniline dyes and is Gram-negative. At 28°C, it grows and produces a capsule. It does not form spores and is non-flagellated [4; pp. 71–76, 24; pp. 75–78, 68; pp. 9–13].

The plague microbe is oval-shaped and displays characteristic **bipolar staining**—with the ends of the cells staining darker due to the accumulation of stain-absorbing granules at the poles, which is distinctive of the plague bacterium. One of its key traits is **polymorphism**, meaning that under the microscope, strains appear in various sizes and shapes—ranging from elongated to nearly round forms and from lightly to intensely stained bacteria. Thus, **bipolar staining and polymorphism** are important cultural and morphological traits of plague pathogens. This is illustrated in **Figure 1.2**. However, these characteristics must be confirmed by further bacteriological research, clinical observations, and epidemiological data [58; pp. 145–152, 73; p. 22, 110; pp. 1110–1122].



1.2.– Figure. Microscopic Appearance of the Plague Pathogen.

The plague pathogen is highly demanding of nutrient media. Unlike many microorganisms, its optimal growth does not occur at 37°C, but rather at 28°C on nutrient media with a pH of 7.1–7.2. The plague pathogen displays several distinctive morphological features when grown on solid and liquid media. Specifically, on nutrient agar after 24 hours, a thin, grayish film-like growth is observed. After 48 hours at 28°C, gray-white colonies with bluish-hued halos begin to appear. Under the microscope, initially elongated filamentous colonies are visible—resembling 'broken glass.' These filaments then clump together into delicate light-colored rods without a dark center, forming a crumpled 'fringed napkin' appearance. Over time, these transform into typical colonies with a brown center, convex shape, and fine granularity.

Colonies are bordered by clear, transparent edges. The characteristic growth of the plague pathogen is one of the most important diagnostic signs. However, the pathogen can also exhibit atypical growth on nutrient agar, with R-forms transitioning to S-forms [45; pp. 140–144, 84; p. 514, 117; p. 15]. Among all the strains we studied, atypical growth was observed in 1.5% of the total.

When the plague pathogen is cultured in liquid medium, the broth remains clear, and a cotton-like sediment forms at the bottom of the test tube. Sometimes, cotton-like microbial accumulations appear along the walls of the tube. A thin film may form on the surface, which can be barely noticeable, especially in young cultures. A distinguishing characteristic of growth in broth is the appearance of clumps or fragments resembling autoagglutination (agglutinative growth).

Thus, when all strains were studied in solid agar and liquid broth, primarily typical growth patterns were observed.

Fermentative Properties:

To carry out metabolism, the plague pathogen produces several enzymes used in diagnostics within the classification of pathogenic species. The bacteria oxidize large amounts of organic substances to form acid but do not produce gas. The most active oxidation occurs with hexoses (glucose, galactose, fructose, mannose). Less active oxidation is seen with pentoses (arabinose, xylose). Not all pathogens oxidize arabinose. Despite the intense breakdown of maltose in most strains, they do not ferment rhamnose, lactose, or sucrose.

The pathogen does not oxidize one-atom (methanol, ethanol, propanol) or two-atom (ethylene glycol) alcohols, and only some strains can break down the three-atom alcohol glycerol. Four-atom (erythritol) and five-atom alcohols (arabitol, adenitol, xylitol) are not broken down. Among hexitols, glucose and certain six-atom sugar alcohols (mannitol, sorbitol) are fermented. The fermentative properties of the studied strains are presented in Table 1.6.

Thus, with acid formation but without gas, the strains ferment glucose, maltose, and mannitol; some strains broke down rhamnose on days 6–10, while sucrose, lactose, and arabinose were not fermented at all. Urea was completely hydrolyzed.

1. Determining the Sensitivity of Plague Strains to Plague Phage: We tested the effect of phages on plague pathogens isolated from

humans, camels, rodents, ectoparasites, and plants. The activity of the plague phage followed a similar pattern to that of other phages. The process of phagolysis includes three stages:

1. Adsorption of the phage to the bacteria
2. Latent phase
3. Lysis (destruction) of the bacterial cell

Characteristics of the plague phage include:

a. Formation of round lysis zones around colonies, with diameters of 3–5 mm.

1.6- table

Analysis Results of the Fermentative Properties of Y. pestis Strains (%)

Maltose	Rhamnose	Lactose	Glucose	Mannitol	Sucrose	Glycerin Agar
99.5 (+) / 0.5 (–)	100 (–)	97.8 (–) / 2.2 (+)	100 (+)	98.5 (+) / 1.5 (–)	98.3 (–) / 1.7 (+)	100 (+)
Control (+)	(–)	(–)	(+)	(+)	(–)	(+)

Commment: Positive result- (+), negative result – (-).

b) 70–80% of phage particles are adsorbed onto the bacteria within five minutes;
c) the duration of the latent phase is 20–25 minutes;
d) an average of 100–110 phage particles are adsorbed per infected bacterial cell.

All plague phages lyse the strains of plague pathogens, as they exhibit high specificity. However, many strains of pseudotuberculosis pathogens, which are closely related to the plague microbe, are not lysed. Still, the specificity of the plague phage is not absolute, as some strains of pseudotuberculosis pathogens are well lysed by plague phages. The pseudotuberculosis phage can lyse both plague and pseudotuberculosis cultures. This has significant practical value in bacteriology.

Thus, all studied strains produced **positive phagolysis reactions** when tested with plague phages.

Plague and pseudotuberculosis phages possess distinct antigenic properties [68; pp. 9–13]. Their antigenic profiles differ: plague antiphage serum neutralizes only plague phage but has no effect on pseudotuberculosis phage—and vice versa. Antiphage sera are used in practice to suppress the activity of bacteriophages.

Motility

We tested the motility of all isolated plague strains on 0.3% semi-solid neutral agar (pH 7.1–7.2). Two-day-old cultures grown at 28°C on agar were inoculated into 5 ml columns of semi-solid agar by stab injection. The cultures were incubated for 18–20 hours. Growth along the stab line without lateral diffusion was observed, which is characteristic of non-motile bacteria.

To enhance result clarity, 0.01% of 2,3,5-triphenyltetrazolium chloride was added to the agar before sterilization. This dye stained growing bacteria bright red.

Thus, all strains of the plague pathogen showed clear non-motile results. Determining motility is one of the primary indicators for differentiating between plague and pseudotuberculosis pathogens.

Virulence in White Mice and Guinea Pigs

A key individual characteristic of the plague microbe is **virulence**, which reflects the degree of pathogenicity and can vary significantly between strains of the same microbial species [25; pp. 85–89; 52; pp. 18–25]. Virulence is one of the most important properties of a parasitic microorganism and is directly related to the challenge of developing live vaccines [18; pp. 38–49; 53; pp. 504–514; 89; pp. 56–57; 114; pp. 123–129; 116; pp. 1888–1892].

The high virulence of the plague pathogen is primarily due to its toxicity [105; p. 374]. However, not only virulent strains, but also many avirulent strains may retain harmful traits.

To study the virulence of plague strains, we used white mice and guinea pigs, as these animals are highly sensitive to pathogens from many natural plague foci. Freshly isolated cultures were used for this purpose.

Before conducting the virulence experiment, all cultures were re-inoculated onto slanted agar tubes and incubated at 28°C for 48 hours. The resulting cultures were suspended in 0.9% saline and adjusted to the desired turbidity using OSO standards from the Tarasevich GISK.

Each strain was tested on 4 white mice (infected with 100 microbial cells each) and 2 guinea pigs (infected with 1000 microbial cells each).

The data are presented in **Table 1.7**.

1.7-table

Virulence Indicators (%) of Y. pestis Cultures Isolated from Natural Plague Foci

Kyzylkum Natural Foci					Ustyurt Natural Foci			
Rodents		Ectoparasites		Camel	Rodents		Ectoparasites	
High virulence	Low virulence	High virulence	Low virulence	High virulence	High virulence	Low virulence	High virulence	Low virulence
63.6	36.4	70.3	29.7	100	65	35.0	77.0	23.0
100		100			100		100	

Figure 1. Laboratory Animal – Guinea Pig

Thus, based on the biological research conducted to assess the virulence of strains isolated from the Kyzylkum natural focus, our findings show that among the tested strains: 63.6% were highly virulent and 36.4% weakly virulent in rodents; 70.3% were highly virulent and

29.7% weakly virulent in ectoparasites. For the Ustyurt natural focus, 65.0% of the strains from rodents were highly virulent and 35.0% weakly virulent; 77.0% of the strains from ectoparasites were highly virulent and 23.0% weakly virulent.

Figure 2. Laboratory Animal – White Mice.

Thus, our biological research aimed at determining the virulence of strains isolated from the Kyzylkum natural focus demonstrated that 63.6% of the tested strains from rodents were highly virulent, and 36.4% were weakly virulent; among ectoparasite-derived strains, 70.3% were highly virulent, and 29.7% were weakly virulent. In the Ustyurt natural focus, 65.0% of rodent-derived strains were highly virulent, and 35.0% weakly virulent; while 77.0% of ectoparasite-derived strains were highly virulent, and 23.0% weakly virulent.

Amino Acid Requirements

The plague pathogen is a **natural auxotroph** [148; pp. 356–361]. Therefore, the amino acids necessary for the growth and reproduction of Yersinia pestis form the trophic link between host and pathogen [152; pp. 259–268].

To determine the importance of each group of amino acids in the growth and reproduction of the plague bacterium, we studied these nutritional dependencies. The traditional method for identifying amino acid requirements involves using a carbohydrate-salt-based synthetic medium with added amino acids or removing them one by one until the minimum required set is established. This approach measures bacterial growth capacity [51; pp. 612–616; 77; pp. 3–12; 150; pp. 541–552].

Our research aimed to study the population heterogeneity of plague strains from Kyzylkum and Ustyurt in terms of amino acid requirements at 37°C—since this is the constant body temperature of plague carriers.

The effect of amino acids on bacterial growth was assessed using conventional media with reduced protein hydrolysate content. As the base, Nutrient Agar HiMedia M001 (HiMedia Laboratories Pvt. Ltd., India) was used, consisting of: peptone from animal tissues 5.00 g, sodium chloride 5.00 g, meat extract 1.50 g, yeast extract 1.50 g, and agar-agar 15.00 g, with a pH of 7.2 ± 0.2. The amino nitrogen content was reduced from the standard 154–160 mg% to 119–123 mg%.

The amino nitrogen concentration was adjusted so that no growth occurred when strains were inoculated into sectors of agar plates at 37°C. In some cases, growth appeared only at the first streak.

To study the amino acid requirements of each plague strain, we added only one amino acid at a time to the nutrient agar at a concentration of 4–5 mg/100 ml. Strains were inoculated into agar plate sectors using a bacteriological loop. The control group consisted of strains plated on media without amino acids. Incubation was conducted at 37°C for six days.

Growth results were recorded based on whether significant or weak stimulation of Y. pestis growth was observed during the incubation period. The primary phenotypic characteristics of the strains were determined according to the “Guidelines for Studying Plague Pathogen Strains” [69; p. 39].

As a result of our studies, all strains showed cultural and biochemical properties typical of those isolated from the Kyzylkum and Ustyurt natural foci**,** including:

a. requirement for amino acids (methionine, threonine, phenylalanine, and cysteine) at 28°C;
b. presence of the **pigment sorption marker (Pgm)** on Jackson-Burrows medium.

Growth Results by Group

Group 1 Strains

Stimulated by **8 amino acids**.

Day 1: Growth observed on media with cysteine, threonine, methionine, and lysine.
Day 1: Punctate growth on media with phenylalanine.
Day 2: Growth on valine-containing media.
Day 3: Abundant growth with cysteine, phenylalanine, threonine, methionine, glutamic acid, lysine, and leucine.
No stimulation from arginine, isoleucine, proline, or ornithine.

Group 2 Strains

Also tested with **8 amino acids**.
Day 1: Abundant growth on media with phenylalanine, cysteine, threonine, proline, methionine, and lysine.
Day 2: Growth on arginine-containing medium.
Day 3: Abundant growth on media with proline, methionine, lysine, phenylalanine, threonine, cysteine, arginine, and ornithine.

Group 3 Strains

Stimulated by **7 amino acids**.
Day 1: Colony growth on media with threonine, cysteine, proline, methionine, and phenylalanine.
Day 2: Growth observed on lysine-containing medium.
Day 3: Abundant growth with threonine, proline, phenylalanine, lysine, isoleucine, and cysteine.
No stimulation from ornithine, glutamic acid, leucine, valine, tyrosine, or arginine.

Group 4 Strains

Stimulated by **9 amino acids**.
Day 1: Abundant growth on media with cysteine, phenylalanine, threonine, methionine, and leucine.
Punctate growth observed on media with tyrosine.
Day 2: Growth on valine and glutamic acid media.
Day 3: Abundant growth on media with cysteine, phenylalanine, threonine, methionine, glutamic acid, valine, tyrosine, leucine, and arginine.
The **results are presented in Table 4**.

1.8-table

Growth of Y. pestis Strains on Nutrient Media with Amino Acids at 37°C

Strain Group	Day	Growth in nutrient media containing amino acids:												
		Glutam	Isoleuc	Proline	Methio	Lysine	Phenyl	Ornithi	Leucin	Valine	Cystein	Tyrosin	arginin	threoni
1st group	1	-	-	-	+	+		-	-	-	+	-	-	+
	2	-	-	-	+	+	+	-	-	+	+	-	-	+
	3	+	-	-	+	+	+	-	+	+	+	-	-	+
2nd group	1	-	-	+	+	+	+	-	-	-	+	-	-	+
	2	-	-	+	+	+	+	-	-	-	+	-	+	+
	3	-	-	+	+	+	+	-	-	-	+	-	+	+
3 rd group	1	-	-	+	+	-	+	+	-	-	+	-	-	+
	2	-	-	+	+	+	+	-	-	-	+	-	-	+
	3	-	+	+	+	+	+	-	-	-	+	-	-	+
4th group	1	-	-	-	+	-	+	-	+	-	+	+	-	+
	2	+	-	-	+	-	+	-	+	+	+	+	-	+
	3	+	-	-	+	-	+	-	+	+	+	+	+	+
Control (at 28°C)					+		+		±		+		±	

Note: (+) – presence of culture growth, (–) – no culture growth, (+) – two different outcomes observed.

During the epizootic period from 1952 to 1988, more than 5000 strains of plague pathogen isolated from the natural foci of Kyzylkum and Ustyurt were studied. It was shown that their growth at 28 °C depends on methionine, threonine, phenylalanine, and cysteine, with some cultures additionally requiring arginine [74; pp. 139–143, 79; pp. 52–62, 95; pp. 138–147, 109; pp. 3673–3681]. According to our

research, these features were found in all strains isolated from Kyzylkum and Ustyurt natural foci, indicating that they are auxotrophic for methionine, threonine, phenylalanine, and cysteine. Assessment of the polyauxotrophy of *Y. pestis* based on data from S.T. Nurtazin [74; pp. 139–143] found that among 31 strains isolated from Central Asian desert foci (including Kyzylkum and Ustyurt) up to 1979, growth at 37°C also required valine and isoleucine. In contrast, strains from the Muyunkum focus, unlike Kyzylkum and Ustyurt strains, did not require leucine at 37°C. Our study revealed that the number of amino acids required for the growth of various *Y. pestis* strains at 37°C varied from 7 to 9. While consistent with Nurtazin's findings, our analysis additionally showed that Kyzylkum and Ustyurt strains require valine and isoleucine. We also observed dependence on leucine for growth—previously a characteristic only of Muyunkum strains. Furthermore, strains from Kyzylkum and Ustyurt required glutamic acid, proline, lysine, and tyrosine at 37°C. Additional requirements for ornithine and arginine were found only in some strains isolated from the central and northern areas of the Kyzylkum focus. Most strains isolated from Ustyurt required methionine, threonine, cysteine, phenylalanine, and valine, and some showed a need for isoleucine and leucine.
The findings on the amino acid requirements of *Y. pestis* strains isolated from Kyzylkum and Ustyurt natural foci during the study period indicate the need for deeper investigation using a broader amino acid set. These data could help distinguish separate ecological and geographical groups of strains circulating in the indicated regions of Uzbekistan.

Antibiotic Sensitivity.

Studying the properties of plague pathogens isolated from different natural foci, especially their sensitivity to antibiotics, is crucial for treating plague infections and suppressing microbial activity and replication in the body. As with other diseases, the bactericidal and bacteriostatic effects of antibiotics are key to treatment success. Determining the effective doses and combinations of narrow- and broad-spectrum antibiotics helps minimize post-infection complications.
Y. pestis strains isolated from rodents and ectoparasites in the desert natural foci of Ustyurt and Kyzylkum were obtained through animal experiments using suspensions from internal organs of rodents and ectoparasite homogenates, following the "Manual on Plague Prevention

in Central Asian Desert Foci."
Upon isolating the bacteria, antibiotic susceptibility testing is essential for planning treatment and prevention. Resistant strains require alternative antibiotic combinations.

We used the disk diffusion method with antibiotic-impregnated discs produced by the Russian Federation's Plague Research Institute. Suspensions (10^9 CFU/ml) were prepared and plated thinly on agar plates. *Y. pestis* cultures were seeded in a lawn method, and discs were placed on the agar surface using sterile forceps. After incubation at 28°C for 18–20 hours, the zone of inhibition around each disc was measured.

Modern antibiotic discs include sensitivity zone diameter standards (sensitive, intermediate, resistant). Readings were interpreted using standard charts and measured with special rulers. A disk dispenser ensured sterility, accurate placement, and saved time. All antibiotic discs contained equal concentrations.
The following antibiotics were tested: streptomycin, tetracycline, chloramphenicol, gentamicin, ciprofloxacin, oleandomycin, lincomycin, and penicillin.

Our study found *Y. pestis* strains were:

- 78.4% sensitive to streptomycin, 17.8% moderately sensitive, 3.8% resistant.
- 50.2% sensitive to tetracycline, 33.4% moderately sensitive, 16.4% resistant.
- 74.6% sensitive to chloramphenicol and gentamicin; 18.5%–20.1% moderately sensitive; 5.2%–6.9% resistant.
- 93.7% sensitive to ciprofloxacin; 5.9% moderately sensitive; 6.2% resistant.
- 15.8% sensitive to oleandomycin; 53.4% moderately sensitive; 30.8% resistant.
- 40.1% or more sensitive/moderately sensitive to lincomycin; 19.4% resistant.
- 48.1% sensitive to penicillin; 27.0% moderately sensitive; 24.9% resistant.

These results highlight the importance of streptomycin in treating plague and the potential emergence of resistant strains. Antibiotic sensitivity is influenced by numerous factors, including improper use, suboptimal dosing, and failure to conduct sensitivity testing prior to treatment.

The detection of resistant strains underscores the need for continued genetic research. Based on our findings, the growth of *Y. pestis* in Ustyurt and Kyzylkum may be influenced by specific factors, which has both theoretical and practical value.

Tables 1.9 and 1.10 summarize the antibiotic sensitivity analysis of *Yersinia pestis* strains.

Although 78.4% of strains were sensitive to streptomycin, 3.8% were resistant. Sensitivity was also confirmed for oleandomycin (15.8%), lincomycin (40.1%), penicillin (48.1%), tetracycline (50.2%), chloramphenicol (74.6%), and gentamicin (74.6%). The findings indicate natural variation in antibiotic susceptibility, emphasizing the importance of this variability in plague epizootiology and the need for deeper DNA-level studies.

Sensitivity to Pesticin.

The plague pathogen has the ability to produce antibiotic-like substances called **bacteriocins** (specifically, *pesticins*), which, upon contact with sensitive bacteria, lead to their cell death. However, the producing bacteria themselves are not lysed. Bacteriocins differ from true antibiotics in the following key features:

- Bacteriocins are **protein-based substances**, whereas antibiotics do **not** contain protein molecules.
- They exhibit **lethal effects only against bacteria of the same species or closely related species**.

The action of antibiotics on microorganisms is generally broader and can affect microorganisms located far from the antibiotic-producing organism.

The production of bacteriocins is **regulated by episomal genetic elements** (i.e., extrachromosomal factors of inheritance) [71; pp. 22–27, 144; p. 291].

1.9-table

Results of the analysis of *Yersinia pestis* strains' sensitivity to antibiotics (%)

№	Streptomycin			Tetracycline			Chloramphenicol			Gentamicin		
	Sensitive	Moderately sensitive	Resistant	Sensitive	Moderatyely sensitive	Resistant	Sensitive	Moderately sensitive.	Resistant	Sensible	Moderately sensible	Resistant
1.	**(67,6±8,0)**	**(20,6±6,9)**	**(11,8±5,5)**	**(20,6±6,9)**	**(26,5±7,5)**	**(52,9±8,5)**	**(67,6±8,0)**	**(20,6±6,9)**	**(11,8±5,5)**	**(85,3±6,0)**	**(14,7±6,0)**	-
2.	**(22,7±8,9)**	**(77,3±8,9)**	-	**(22,7±8,9)**	**(77,3±8,9)**	-	**(18,2±8,2)**	**(68,2±9,9)**	**(13,6±7,3)**	**(22,7±8,9)**	**(77,3±8,9)**	-
3.	**(93,7±4,2)**	**(6,3±4,2)**	-	**(87,5±5,8)**	**(12,5±5,8)**	-	**(93,7±4,2)**	-	**(6,3±4,2)**	**(93,7±4,2)**	**(6,3±4,2)**	-
4.	**(100%)**	-	-	-	**(100%)**	-	-	**(100%)**	-	**(84,2±8,3)**	**(10,5±7,0)**	**(5,3±5,1)**
5.	**(82,7±2,7)**	**(17,3±2,7)**	-	**(56,5±3,5)**	**(27,8±3,2)**	**(15,7±2,6)**	**(79,1±2,9)**	**(13,1±2,4)**	**(7,8±1,9)**	**(76,9±3,0)**	**(21,5±2,9)**	**(1,6±0,9)**
6	**(97,6±1,6)**	**(2,4±1,6)**	-	**(61,2±5,2)**	**(32,9±5,0)**	**(5,9±2,5)**	**(100%)**	-	-	**(88,2±3,4)**	**(11,8±3,4)**	-
7.	**(5,9±5,7)**	**(23,5±10,2**	**(70,6±11)**	**(5,9±5,7)**	-	**(94,1±5,7)**	**(64,7±5,1)**	**(5,9±5,7)**	**(29,4±11)**	**(5,9±5,7)**	**(11,8±7,8)**	**(82,3±9,2)**
8.	**(57,9± 11,3)**	**(42,1±11,3**	-	**(57,9±11,3**	**(42,1±11,3**	-	**(52,6±11,3)**	**(47,4±11,4**	-	**(52,6±11,**	**(26,3±10,1**	**(21,1±9,3)**
9.	**(33,3±27,2)**	**(66,7±27,2**	-	-	**(100%)**	-	**(33,3±27,2)**	**(66,7±27,2**	-	**(66,7±27,**	**(33,3±27,2**	-
10.	**78,4%**	**17,8%**	**3,8%**	**50,2%**	**33,4%**	**16,4%**	**74,6%**	**18,5%**	**6,9%**	**74,6%**	**20,1%**	**5,2%**
Total:	**100%**			**100%**			**100%**			**100%**		

1.10-tabel

Analysis results of *Yersinia pestis* strains' antibiotic sensitivity (%)

№	Ciprofloxacin			Oleandomycin			Lincomycin			Penicillin		
	Sensible	**Moderately sensitive**	**Resistant**	**Sensible**	**Moderately sensitive**	**Resistant**	**Sensible**	**Moderately sensitive**	**Resistant**	**Sensible**	**Modertely sensitive.**	**Resistant**
1.	**(64,7±8,1)**	**(35,3±8,1)**	**-**	**-**	**-**	**(100%)**	**-**	**-**	**(100%)**	**-**	**-**	**(100%)**
2.	**(18,2±8,2)**	**(63,6±10,2)**	**(18,2±8,2)**	**-**	**-**	**(100%)**	**-**	**-**	**(100%)**	**(45,5± 10,5)**	**(50±10,6)**	**(4,5±4,4)**
3.	**(93,7±4,2)**	**(6,3±4,2)**	**-**	**-**	**-**	**(100%)**	**(65,6±8,3)**	**(28,1±7,9)**	**(6,3±4,2)**	**(78,1±7,3)**	**(15,6±6,4)**	**(6,3±4,2)**
4.	**(100%)**	**-**	**-**	**(5,3±5,1)**	**(36,8±11)**	**(57,8± 11,2)**	**(63,1±11)**	**(15,8±8,3)**	**(21,1±9,3)**	**(52,6±11,4)**	**(31,6±10,6)**	**(15,8±8,3)**
5.	**(70,7±3,2)**	**(20,4±2,9)**	**(8,9±2,0)**	**(11±2,2)**	**(78,5±2,9)**	**(10,5±2,2)**	**(52,9±3,6)**	**(43,4±3,5)**	**(3,7±1,3)**	**(57,6±3,5)**	**(25,1±3,1)**	**(17,3±2,7)**
6.	**(94,1±6,3)**	**(5,9±2,5)**	**-**	**(34,1±5,1)**	**(61,2±5,2)**	**(4,7±2,2)**	**(24,7±4,6)**	**(62,4±5,2)**	**(12,9±3,6)**	**(42,3±5,3)**	**(41,2±5,3)**	**(16,5±4)**
7.	**(5,9±5,7)**	**(88,2±7,8)**	**(5,9±5,7)**	**(53±12,1)**	**(29,4±11)**	**(17,6±9,2)**	**(23,5±10,2)**	**(64,7±11,5)**	**(11,8±7,8)**	**(70,6±11)**	**(11,8±7,8)**	**(17,6±9,2)**
8.	**(57,8±11,3)**	**(21,1±9,3)**	**(21,1±9,3)**	**(36,9±11)**	**(57,8±11,3)**	**(5,3±5,1)**	**(47,4±11,4)**	**(52,6±11,4)**	**-**	**-**	**(31,6±10,6)**	**(68,4±10,6)**
9.	**(66,7±27,2)**	**(33,3±27,2)**	**-**	**-**	**-**	**(100%)**	**(33,3±27,2)**	**(66,7±27,2)**	**-**	**-**	**(33,3±27,2)**	**(66,7±27,2)**
10.	**72,0%**	**21,8%**	**6,2%**	**15,8%**	**53,4%**	**30,8%**	**40,1%**	**40,5%**	**19,4%**	**48,1%**	**27,0%**	**24,9%**
Total.	100%			**100%**			**100%**			**100%**		

Sensitivity to Pesticin.

The plague pathogen has the ability to produce an antibiotic substance—bacteriocins—that cause cell death in susceptible bacteria upon contact but do not result in their lysis. Bacteriocins differ from true antibiotics by the following main features:

- Bacteriocins are protein-based substances, meaning that protein molecules are present in their composition, unlike antibiotics.
- They exhibit a lethal effect primarily on microorganisms of the same or closely related species.

Antibiotics typically affect microorganisms more broadly and systematically, including those distantly related to the producing organism. The production of bacteriocins is regulated by episomal genetic factors [71; p. 22–27, 144; p. 291].

To detect bacteriocins, indicator strains (bacterial forms) that are sensitive to the agent in question are required. However, some serotypes of pseudotuberculosis bacteria are not sensitive to pesticin [155; p. 677–686, 160; p. 55–64]. Pesticin is produced by many plague bacterial strains, but it does not affect the producing strain itself. Thus, the plague pathogen population includes both pesticin-sensitive and pesticin-resistant cells. Pesticin-like substances are also produced by many enteric bacteria. Pesticinogenic strains simultaneously carry both coagulase and fibrinolytic activities.

Calcium dependence at 37°C. The calcium dependence trait is closely associated with the virulence of the plague pathogen. Virulent strains are calcium-dependent. Loss of calcium dependence is accompanied by a significant decline in virulence for white mice and guinea pigs [17; p. 46–51, 87; p. 72–78, 163; p. 5147–6152].

To determine bacterial calcium ion requirements, we used Higuchi-Smith oxalate-magnesium nutrient medium, and a modification of a medium using defibrinated blood treated with hemolysis or sodium sulfite developed by B.M. Sulaymanov. Cultures were incubated simultaneously at 28°C and 37°C. Strains grown at 28°C were then cultured on Higuchi-Smith medium at a dose of 200 CFU and incubated at 37°C for 40–42 hours.

The number of calcium-independent colonies was counted. Additionally, after incubation at 28°C for 30–48 hours, the number of calcium-dependent bacterial colonies was also determined.

As a result, no calcium-independent strains were found among those isolated from the Kyzylkum and Ustyurt natural foci. Predominantly, newly isolated cultures displayed calcium dependence, with those requiring higher calcium concentrations outgrowing others.

Pigment Production (R) by the Plague Pathogen. High-level pigment production (R trait) indicates virulence in the isolated strain. Within a virulent strain population, pigment-producing cells (R+) predominate quantitatively. Loss of pigment production typically results in a sharp decline in virulence.

Daily agar cultures of the studied strains grown at 28°C were inoculated at a dose of 200 CFU onto Jackson-Burrows medium. Inoculated plates were incubated at 28°C, and after 4–5 days, the number of pigmented and non-pigmented colonies was counted. On Jackson-Burrows medium, pigment-positive colonies (R+) had more distinguishable pigmentation. The characteristics of newly isolated strains reflected the percentage composition of R+ and R− cells in the population.

Thus, most strains isolated from the Kyzylkum and Ustyurt foci possessed the pigmentation trait. However, in 7% of cases, entirely R− (non-pigmented) strains were also observed.

Detection of Fraction 1 (F1) Antigen Production in Bacteria. The antigenic structure of the plague pathogen includes two major antigens: a thermolabile capsular antigen and a thermostable somatic antigen, with one additional surface somatic antigen [38; p. 38–40, 65; p. 25–33, 117; p. 15]. According to researchers, the plague pathogen may possess between 12 and 28 antigens [75; p. 79–83, 111; p. 513, 154; p. 73–89, 159; p. 1–8]. However, most of these antigens remain uncharacterized.

A specific antigenic marker is the Fraction 1 (F1) capsular substance [156; p. 301–308]. F1 synthesis is mainly influenced by the incubation temperature, with maximal production occurring around 37°C. Small amounts of intracellular F1 may also form at lower temperatures.

In our study, F1 detection was carried out using the following methods:

a) Double diffusion in gel (Ouchterlony method)
b) Indirect hemagglutination reaction (IHA) using plague immunoglobulin erythrocyte diagnostic reagent and antibody neutralization reaction (ANR) with plague antigen erythrocyte diagnostic reagent

c) Enzyme-linked immunosorbent assay (ELISA)
d) Serological detection of antibodies to F1 in rodent serum [30; p. 735–740, 153; p. 1–9, 161; p. 1226–1234]

In our research, a 3-day agar culture grown at 37°C in Hottinger medium supplemented with 0.5–1% hemolyzed blood and 0.5% calcium chloride was used. A suspension containing 2 x 10^10 CFU was prepared and heat-treated in a water bath for 15 minutes.

We used IHA to detect F1 due to its high sensitivity and simplicity, especially for large-scale strain screening. If IHA results were negative, ANR was also performed to verify capsule antibody specificity.

Plague cultures were grown on 0.5–1% hemolyzed blood-neutral agar. Suspensions of 10^9 CFU/ml in 1–2% formalin were prepared. After 12 hours of inactivation, controls were plated on neutral agar. Suspensions were diluted to 5 x 10^6 CFU/ml and tested using IHA, ANR, and passive hemagglutination test (PHAT). Reactions were set in at least 8 wells of a polystyrene microplate using Takachi-type micro-methods (0.2 ml well volume).

In our study, all cases with positive IHA and ANR results were considered noteworthy. Serological reactions were carried out using sera and emulsions derived from patients, rodents, and ectoparasites. Results: IHA positive in 66.7% at dilutions from 1:40 to 1:320; ANR positive in 73.3%; rodent samples IHA positive in 47.0%, ANR in 45.0%; ectoparasite samples PHAT positive in 28.1%, ANR in 37.2%.

Results were recorded 2–3 to 5 hours after the reaction. A dense ring-shaped precipitation was considered a negative result but still indicative of antigen presence. IHA, ANR, and PHAT were performed simultaneously.

In conclusion, the presence of highly seropositive titers confirms that the epizootic process is ongoing in the studied regions, necessitating continued epidemiological surveillance and preventive measures

§1.5. Main epidemiological and clinical characteristics of plague disease

Plague in humans belongs to a group of infections with various transmission mechanisms. This epidemiological definition of plague infection allows for consideration of potential sources and modes of transmission in humans [28; p. 23, 29; pp. 48–53, 59; pp. 9–16]. In humans, plague is characterized by diverse clinical manifestations.

Taking into account the specific epidemiological severity, clinical forms can be grouped into three categories:

- **Group A** includes forms with the lowest epidemiological risk, primarily occurring when transmitted by ectoparasites (e.g., fleas) [41; pp. 201–218]. These include the pure bubonic form, cutaneous form, and cutaneous-bubonic form.
- **Group B** includes internal or generalized forms—primary septicemic and secondary septicemic—which occupy an intermediate position between groups A and C.
- **Group C** includes externally spreading, central forms, often highly contagious—primary pneumonic, secondary pneumonic, and intestinal forms.

The abundance of the pathogen in the body, internal organs, and blood makes Group B and C forms more epidemiologically dangerous than Group A. This classification is conditional since a patient may shift from one group to another during the disease course (except for primary septicemic and primary pneumonic forms).

The conditional nature of this scheme is due to the fact that the release of a virulent pathogen from the patient's body can occur not only through the lesion sites but also via other means (urine, feces, etc.). The pneumonic form, as a possible complication, is not only dangerous to the patient but also to others [59; pp. 9–16, 108; pp. 10–24]. When an infected individual transitions between groups, it necessitates specific epidemiological measures in the context of plague control [82; pp. 5–10, 64; pp. 66–73, 107; pp. 5–12]. Thus, grouping clinical forms by their epidemiological role helps define strategic control measures. In the classification and diagnosis of plague, the localization of each form plays a vital role—whether it begins in the buboes, skin, or lungs [71; pp. 22–27, 101; pp. 2–20, 102; pp. 220–228]. However, there may not always be a strict correlation between clinical features and epidemiology. Therefore, clinical classification should reflect the epidemiological significance of each form.

Ectoparasites as Plague Vectors

Three elements are essential for plague transmission: the plague microbe (pathogen), rodents (infection reservoir), and fleas (infection vector). Fleas have the ability to serve as temporary reservoirs of the infection [81; pp. 43–50, 94; pp. 121–122]. The transmission cycle is as follows: *Y. pestis* → rodent → flea—this interaction within specific zoogeographical and climatic conditions determines the emergence of plague epizootics and epidemics [12; pp. 108–111, 32; pp. 50–53, 35; pp. 387–397, 46; pp. 25–29, 67; pp. 136–145].

Flea development consists of four stages: egg, larva, pupa, and adult. The process depends on microclimatic conditions of the habitat, which affects emergence from the pupa. Young fleas do not feed immediately after emergence and can remain unfed for a long time [129; pp. 1932–1940]. Fleas can live from 60 to 370 days, and under basement conditions up to 1,725 days. Rodent fleas differ from household fleas—they are less mobile and have weaker jumping ability [20; pp. 92–103, 47; pp. 20–29].

The infection mechanism is carried out by adult fleas through several modes:

1. Ingestion of infected fleas or their feces by animals;
2. Infection through flea bites or wounds contaminated with crushed fleas or their feces;
3. Mechanical transmission via the flea's piercing apparatus during biting;
4. Ingestion of regurgitated infectious material from the digestive tract into the bite wound;
5. Direct injection of the pathogen via saliva during a flea bite.

The most important mode is infection through regurgitated contents from the flea's digestive tract into the wound during a bite.

Flea infection does not last long; elevated temperatures reduce this period. Infected fleas are not immediately contagious and need time before becoming infectious [86; pp. 129–134, 88; pp. 52–53]. The infectious period extends from the beginning of infection until the flea's death. During this time, transmission can occur via bite or contamination of the animal's skin with flea feces [61; pp. 95–106]. The duration of infectivity varies widely among flea species—some sources indicate from 18 to 358 days. Despite many adverse environmental factors, a sufficient proportion of fleas maintain enzootic transmission in the natural foci [60; p. 248, 80; pp. 50–55].

It is known that many flea species abandon the host's corpse in search of a new host after death, which has great importance in both epizootiology and plague epidemiology. Multiple factors affect the speed of epizootics, one of which is the interaction between the pathogen and vector [22; pp. 57–59, 93; pp. 148–153].

Experimental data have confirmed that great gerbils (Rhombomys opimus) in different parts of the Kyzylkum and Ustyurt natural foci

exhibit varying susceptibility to plague infection [63; p. 215]. These factors are key in activating epizootic processes, with the involvement of reservoir hosts, vectors, and their ectoparasites in different parts of these natural foci. Most *Y. pestis* strains isolated from mammals in the region were associated with the great gerbil. In the Kyzylkum focus, thin-fingered gerbils may also serve as primary vectors—especially since environmental conditions are frequently altered by human activity.
Studies of the Kyzylkum and Ustyurt foci revealed not only vector species but also the periodicity of ectoparasite epizootic activity.

Plague pathogen carriers – wild rodents

Figure 3. Great gerbil (*Rhombomys opimus*)

Figure 4. Small gerbil

Figure 5. Red-tailed gerbil (*Meriones libycus*)

Figure 6. Southern gerbil (*Marmota menzbieri*)

7. Yellow ground squirrel (*Psammomys obesus*)

Figure 8. Common weasel (Mustela nivalis)

Figure 9. Polecat (Mustela erminea Lunnaeus, 1758)

Figure 10. Hamster.

Figure 11. Jerboa. Dipodidae Fischer von Waldheim, 1817

Figure 12. Field mouse. Sicista subtilis

Figure 13. Hedgehog. Erinaceus europaeus

Figure 14. Steppe marmot

Figure 15. Camel.

The Plague Pathogen – Ectoparasites

Figure 16. Ixodid tick

Figure 17. Ixodid tick Hyalomma mardinatum

Figure 18 Ixodes scapularis

Figure 19. Rhipicephalus_sanguineus

Figure 20. Hyalomma-anatolicum-excavatum-female-male

Figure 21. Xenopsylla_cheopis

Figure 22. Xenopsylla_ *hirtipes*

Figure 23. Flea. Pulex irritans

Figure 24. Southern rat flea

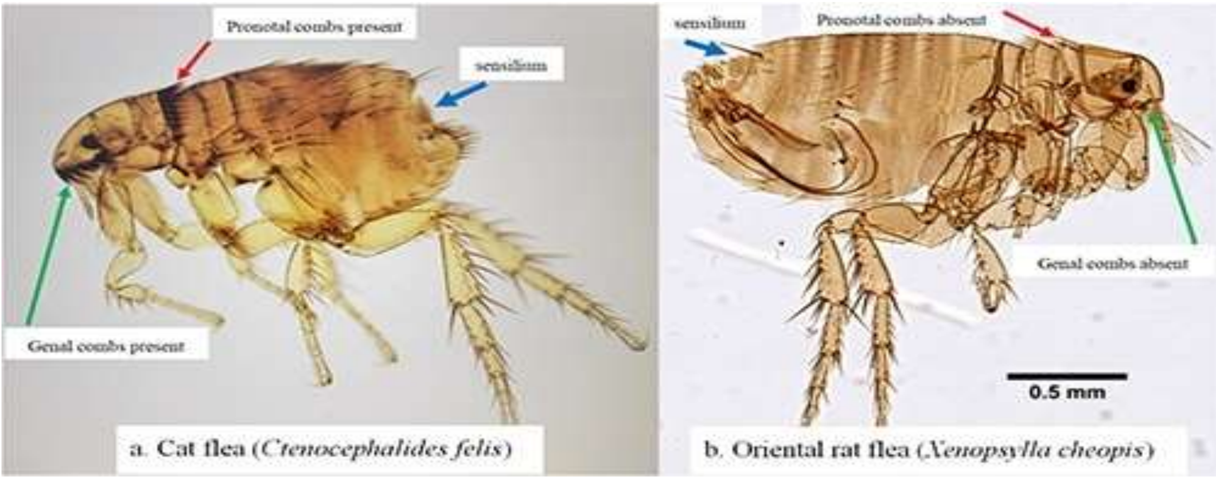


Figure 25. Ctenocephalides felis

The investigated carriers include: Great gerbil, red-tailed gerbil, diurnal gerbil, yellow marmot, thin-fingered marmot, woolly-footed jerboa, hamster, hedgehog, weasel, field mouse, camel, and ectoparasites (Figures 3–25):

Hyalomma, *Ixodes*, *Nosopsyllus*, *Xenopsylla*, and several unidentified ticks and fleas, including species such as: *X. skrjabini*, *X. gerbilli*, *C. lamellifer*, *X. conformis*, *C. tartus*, *Ye. oschanini*, *X. hirtipes*, and *C. vamas.*

Quantitative data expressed as percentages are presented in Table 6, showing that the largest proportion of *Y. pestis* strains (39.1%) were isolated from great gerbils.

- Second place: *X. hirtipes* (11.9%)
- Third place: *X. gerbilli* (10.5%)
- Fourth place: *X. conformis* (5.2%)
- Camel ectoparasites accounted for 0.2%.

Before the onset of sepsis, microbial accumulation is primarily observed within the bubo. During generalization, the entire body is affected by the direct impact of bacteria, and by the terminal stage of the disease, the plague pathogen accumulates throughout the body. Death from plague almost always occurs in the form of rapidly progressing sepsis, depending on the route of dissemination [98; pp. 17837–17842, 119; p. 10, 157; pp. 2206–2214]. Primary changes in bubonic plague occur in specific groups of lymph nodes [120; pp. 549–559, 122; pp. 1079–1085]. During the generalization stage, pathological changes can develop in all organs, involving the hematogenous spread of plague bacilli. This phenomenon may result from the disruption of the hematoparenchymal barrier, allowing bacteria to enter the bloodstream from the primary site and accumulate in tissues. The vessel walls and capillaries undergo pathological changes, facilitating the penetration of microbes [128; pp. 15–17].

Pathological changes are found not only in the vascular and lymphatic systems but also in internal organs, such as:

- Necrosis
- Accumulation of serous exudate
- Infiltrates
- Proliferation
- Endothelial necrosis and necrobiosis [133; p. 52, 134; pp. 16–25].

Sepsis develops as a result of bacteremia. However, the plague pathogen does not persist in the bloodstream for long periods [131; p. 1106, 132; pp. 531–538]. Thus, early blood culture testing often yields positive results, while later samples may return negative. According to Albrecht and Gohn, plague cultures were isolated:

- 5 days before death – 1 case
- 2 days before death – 4 cases
- 1 day before death – 24 cases
- On the day of death – 22 cases

1.11-table.

Analytical Indicators (%) of the Isolated Y. pestis Strains

№	**Carriers**	**%**	**№**	**Ectoparasites**	**%**
1.	Great gerbil – *Rhombomys opimus*	**39,2**	**1.**	*Hyolomma*	**1**
2.	Red-tailed gerbil – *Meriones libycus*	**2,2**	**2.**	*Nosopterus*	**0,6**
3.	Southern gerbil – *Meriones meridianus*	**5**	**3.**	Xenopsvlla	**0,6**
4.	Narrow-*fingered ground squirrel*	**0,6**	**4.**	*Xenopsylla skrjabini*	**4,2**
5.	Weasel – *Mustela nivalis*	**0,6**	**5.**	Mites of unspecified type	**0,6**
6.	Yellow ground squirrel – *Citellus maximus*	**2,2**	**6.**	*Ixcodes*	**0,3**
7.	Badger	**0,3**	**7.**	Fleas of unspecified type	**4,7**
8.	Hedgehog	**0,3**	**8.**	*Xenopsylla gerbilli*	**10,5**
9.	Field mouse	**0,3**	**9.**	*Coptopsylla* lamellifer	**5,0**
10.	Long-*footed jerboa*	**0,3**	**10.**	*Xenopsylla* comformis	**4,2**
11.	Camel – *Camelus dromedarius*	**0,3**	**11.**	*Echidnophaga oschanini*	**0,9**
12.	Saxaul (plant)	**0,3**	**12.**	*Xenopsylla hirtfpes*	**11,9**
			13.	*P.Yamas*	**0,6**
			14.	*Ceratophyllus tarsus*	**3,3**

Bacteremia in Plague Is a Common Phenomenon, but It Has Limited Significance in the Early Stages of the Disease

Routes of Transmission of Plague:

In nature, rodents serve as the reservoir for infection. Infected fleas parasitizing rodents act as vectors. Zoonotic infections among rodents can be found on all continents except Australia [147; pp. 874–881]. Outbreaks among rodents often lead to human infections. This is the primary route of transmission — from rodents to humans via fleas [78; pp. 35–42; 163; pp. 5147–5152].

Another route is direct transmission from infected animals to humans.

The third transmission mode is through damaged skin that allows pathogen entry.

A further route is airborne transmission from infected animals or humans via droplets.

Thus, sporadic natural cases in endemic zones are characterized as follows:

- Source (Reservoir): infected animals
- Transmission Routes:
  1. Transmissive: flea bites, less often ticks
  2. Contact (wound-based): slaughtering infected animals, rarely animal bites
  3. Alimentary: through contaminated food
  4. Aerosol (airborne/dust): inhalation of contaminated dust [151; pp. 223–234]

Most authors report that transmissive routes account for 61–82%, followed by contact-wound routes (18–25%) [130; pp. 2281–2290]. The alimentary route is less common (3–11%), while the aerosol route accounts for 5–15%. The lower percentage of aerosol cases reflects the fact that exposure requires closer contact with infected individuals.

The process of plague epizootics — involving microbe → flea → rodent — is a complex biological system influenced by many factors, including:

- **Presence of the pathogen (source of infection)**
- **Infected animals (donors)**
- **Transmission mechanisms (vectors)**
- **Susceptible hosts (recipients)**

This is a self-regulating ecological system, influenced by local climate, soil, terrain, etc., which in turn affect animal population behavior, movement, food availability, predators, and competition [8; p. 186; 85; p. 513]. The unpredictability of some plague epizootics stems from this ecological variability. Nevertheless, homeostasis through self-regulation forms the foundation of anti-epidemic strategies [64; pp. 66–73; 89; pp. 56–57].

Plague epizootics, while sharing common features, differ in their transmission dynamics between natural endemic zones [55; pp. 38–43; 56; pp. 19–22].

In the Kyzylkum and Ustyurt desert plague foci, the epizootic process shows two seasonal peaks, reflecting seasonal plant development, summer drought, and stress on host animals. A sudden drop in flea gonotrophic activity (egg development) is typically observed in spring, caused by temperature fluctuations and heavy rain. During April–June, flea populations capable of transmitting plague dominate.

Mass flea reproduction generally peaks in late June to early July, coinciding with intensified solar radiation.

On other continents, plague outbreaks typically occur during autumn-winter or winter-spring seasons. The study of plague pathogen strains isolated from Kyzylkum and Ustyurt natural foci revealed their susceptibility to antibiotics and increased need for certain amino acids, highlighting natural variability and differences in virulence levels. This underscores the necessity of further molecular-genetic studies to assess the importance of such changes.

CHAPTER II. PREVENTION OF PLAGUE INFECTION IN RESERVOIR ANIMALS IN LIVESTOCK FARMS AND ASSESSMENT OF THE IMPORTANCE OF MASS VACCINATION

In epidemiological control of plague (Y. pestis), special importance is given to camels, which act as reservoirs of the infection. Sick camels, as well as raw products obtained from them (milk, meat, hide), are considered primary sources of infection. In the Republic, about 17,000 camels are vaccinated annually by the veterinary service [63; p. 215]. However, it is difficult to obtain an accurate count of the camel population, as this species is composed of free-ranging animals whose herds are constantly changing. In the literature, several cases of plague transmission to humans from camels are described, usually occurring when infected or deceased animals are slaughtered [26; p. 421, 66; pp. 6–16, 112; p. 161].

Many studies show that camel organisms are generally resistant to Y. pestis, but under certain conditions—such as fatigue or accompanying diseases—they become susceptible to infection [65; pp. 25–33, 131; p. 1106]. In such cases, the infection may generalize, increasing the risk of human infection.

In nature, camels typically become infected through the bites of migratory fleas carrying plague. These fleas often originate from predators that come into contact with great gerbil habitats. In desert natural foci within Uzbekistan, the main vectors of plague are rodents that expand their range in search of food.

During population surges, great gerbils may invade deep predator burrows (e.g., foxes, small carnivores), creating conditions for interaction between their fleas. Notably, the flea species *Pulex irritans* is identified as one of the most common vectors of camel plague [138; pp. 38–43]. Fleas themselves become infected with plague bacteria through such interactions.

During gerbil population declines, ectoparasites searching for hosts may attack other animals, including camels. The presence of these factors supports literature reports confirming plague-infected camels even during periods of low rodent numbers [125; pp. 311–316, 141; pp. 237–245].

To quantify the infection mechanism in camels, data from various years described in literature can be presented as empirical indicators. Not every camel bitten by ectoparasites becomes ill, and in most cases, infected camels have been observed to recover [26; p. 421, 120; pp. 549–559].

Clinical signs such as fever and painful buboes were observed for periods ranging from one day to 27–32 days. In camels showing symptoms, 76.9% yielded positive *Y. pestis* cultures from regional lymph nodes. Postmortem examination of lymph nodes and internal organs yielded 17.3% positive results. Septicemic plague caused by bacteria entering the bloodstream results in fever, weakness, and fatigue, which intensify over 4–5 days, typically leading to the animal's death by the 7th day.

However, based on some researchers' findings, septicemic plague in camels may develop only in isolated cases during natural infections.

Under natural conditions, A.N. Matrosov's studies [64; pp. 66–73] from 1990–1998 revealed that among 97 deceased camels in the Altai region, plague was diagnosed bacteriologically in 3 animals (3.3%). According to data from Z.J. Abdel [1; pp. 25–33], between 1975 and 1988, 896 camels from the natural foci of Kyzylkum and Ustyurt were studied, and pathogenic cultures were isolated from 8 animals (0.9%). According to more recent data, during epizootic intensification, plague pathogens were isolated in the laboratory from 1 camel (0.09%) in affected areas. The resistance of camels to plague has been confirmed through the detection of specific antibodies to Y. pestis in the blood serum of some animals that had contact with the microbe but did not develop infection. These findings are supported by [4; pp. 71–76, 118; pp. 197–209, 158; pp. 1–8]. Nevertheless, comparative data confirm that camels are significantly more susceptible to plague than other animal species.

In epizootic areas, the presence of plague-specific antibodies in the blood of infected camels can reach 0.7%, according to N.V. Popov et al. [79; pp. 52–62]. These figures closely align with the results of serological studies of camel serum from epizootic regions reported by N.R. Khabalova et al. [85; p. 513], where specific antibodies were detected in 1.2% of camels.

Thus, it can be assumed that fewer than 1% of camels in regions with intense plague epizootics may become infected. The seasonal distribution of plague cases in camels is as follows: 9.4% of cases occur between December and February, 10.5% in March–May, 45.8% in June–August, and 34.2% in September–November. Therefore, the highest relative incidence of plague in camels occurs in summer–

autumn. Currently, many camels are raised in the private sector. Without laboratory testing, it is usually impossible to distinguish plague in camels from other diseases. The manifestation of plague in camels often includes loss of appetite and weight loss. Typically, sick animals show signs of weakness and lie on their sides. In generalized cases, the disease may present more clearly through enlargement of lymph nodes—usually in the groin, thighs, or axilla, and less commonly in the neck or other areas—or as pulmonary involvement. Periodic symptoms include labored breathing and bloody discharge from the nose and mouth. As the disease progresses, plague microbes enter the bloodstream, multiply, and cause rapid deterioration leading to death. Fever rises significantly, resulting in severe complications. The unique role of camels in plague epidemiology is linked to the fact that slaughtering often involves multiple individuals, increasing the risk of group infection if the animal harbors plague bacteria. Additionally, the meat from a slaughtered camel may be distributed among multiple families or even transported to other regions, potentially creating new foci of infection.
Human infection with camel plague typically occurs through damaged skin—usually the hands—leading to inflammation of regional lymph nodes (buboes). The manifestation of the disease in humans depends on the route of transmission, physical condition at the time of infection, bacterial load, the timing of diagnosis, and other factors. Descriptions of plague in humans from different sources reflect the specific outbreak conditions in various years [98; pp. 17837–17842, 100; pp. 2–20, 116; pp. 1888–1892].

In recent decades, due to the intensification of public sanitary education campaigns, the slaughter of camels infected with plague has significantly decreased, thereby minimizing the risk of epidemic outbreaks in such conditions.
The main goal of plague prevention in camels is to prevent the emergence of the disease among them. This includes a full range of medical, sanitary-epidemiological, and veterinary-sanitary measures [56; pp. 19–22, 64; pp. 66–73, 67; pp. 136–145]. In the first group of measures, special attention is given to thorough microbiological investigation of areas where camels reside, especially in enzootic plague zones. The sole purpose of studying these areas should be to objectively assess the epizootic situation related to plague, to differentiate the territories under investigation by anti-plague institutions from an

epizootological point of view, and to identify locations where epizootic processes occur and where camels tend to congregate.

If epizootics are detected, complete preventive measures should be implemented in the areas posing an epidemiological threat based on the data obtained. In this regard, the most critical measures involve disinfection of habitats of wild rodents, which serve as natural plague reservoirs. This method of prevention is often considered the most effective in camel-populated areas due to its high efficiency. To assess the specific epidemiological role of camels in the process, it is important to classify the areas where they graze into the following categories based on the ongoing epizootic activity among wild rodents in natural plague foci:

1. High-risk areas
2. Medium-risk areas
3. Low-risk areas

Based on this risk classification, measures must be taken to prevent camels from being taken to graze in these zones. In addition, it is crucial to maintain continuous monitoring of the epizootic-epidemiological situation, conduct microbiological studies, and carry out such activities in coordination with the Plague Prevention Center and veterinary services.

Veterinary-sanitary measures also play an essential role [55; pp. 38–43, 76; p. 195]. These include comprehensive registration of camels both on farms and in the private sector, and most importantly, strict veterinary oversight of camels grazing in plague-enzootic areas. Any animal suspected of having plague should be promptly identified and isolated after informing anti-plague institution personnel. If infected animals are found, immediate laboratory analysis (serological, microbiological, and biological) must be conducted on lymph nodes, wounds, and—if applicable—blood samples.

In bacterioscopic examination, the cultures appear as Gram-negative, ovoid-shaped bacteria surrounded by a thin capsule, and stain bipolarly using the Loeffler method.

Study of the isolated cultural-morphological characteristics showed that the strain is well adapted to meat-peptone broth and agar, and when incubated on selective media at 28°C for 18–24 hours, it grows well. On solid nutrient media in Petri dishes, the colonies typically form compact R-type shapes with a dense center and a fringed border resembling embroidery.

Analysis of the strain's cultural and morphological features, sensitivity to plague and pseudotuberculosis phages, enzymatic properties, growth requirements at 37°C, need for calcium ions, ability to produce pigment on haemin media (R type), formation of fraction 1 antigen, fibrinolytic and coagulase activities, toxigenicity, virulence, antibiotic sensitivity, and lack of motility all confirm its identity as the plague pathogen.

Subcutaneous and intraperitoneal injection of the cultural suspension into guinea pigs and white mice led to the death of all animals. Microbiological and serological examination of the blood and internal organs of laboratory animals confirmed that the isolated pathogen definitively belongs to *Yersinia pestis*, the causative agent of plague.

§2.1. Studying specific plague prophylaxis in reservoirs.

Based on our scientific research, we believe that vaccinating camels cannot guarantee protection against this infection. To vaccinate one camel, a vaccine dose equivalent to what would be used for 40 people is required, amounting to 234,940 UZS. In the absence of an epizootic, vaccinating healthy camels in these regions is considered economically unjustifiable, and instead of the above costs, we propose conducting disinsection once a year, which would require 136,000 UZS per camel annually. So far, we have not encountered any data in the literature on the effectiveness of camel vaccination. In this regard, it is necessary to conduct continuous monitoring using accurate methods for detecting plague in camels, which will undoubtedly help improve the level of protection for our population and public health, and aid in implementing preventive measures.

Veterinary-sanitary measures should also include systematic processing of camel wool on farms located in regions where plague-carrying ectoparasite migration is at its highest. In some cases, one of the most important measures in identifying plague-infected camels is to screen their blood serum for plague-specific antibodies (serological testing), involving the resources and personnel of anti-plague institutions. Isolated animals should be placed under daily supervision (temperature checks, visual inspection). Treatment of sick camels is not anticipated. If the condition of monitored camels improves, their future is determined based on the results of laboratory analyses, especially blood tests. If their condition does not improve, they are slaughtered in specially designated areas with full compliance with anti-epidemic safety measures.

Mandatory slaughtered or fallen camels undergo necropsy in the presence of anti-plague institution specialists. According to relevant regulations, organ samples are taken for testing in the laboratories of the anti-plague service. The site of the camel's necropsy is disinfected with a 4% solution of chlorinated lime or Lysoformin. The carcasses are incinerated.

The wool obtained from healthy camels raised by farming households in plague-endemic areas is treated with insecticides, placed in sealed containers, and sent to special wool-washing machines for appropriate processing. A veterinary certificate is issued with a corresponding mark. Hides and wool taken from fallen camels without prior inspection are disinfected or incinerated. Veterinary-sanitary measures in enzootic areas include a prohibition on the unsupervised slaughter of camels. Even in the absence of a plague epizootic, slaughtering camels with diseases of unknown origin is also prohibited.

The population also plays an important role in preventing plague among camels, and their active participation in preventive measures significantly enhances their effectiveness. In this regard, it is necessary to strengthen public health education campaigns. At the same time, special attention should be paid to preventing the slaughter of sick camels, especially those with illnesses of unknown origin, without prior examination by veterinary personnel or, if necessary, specialists from anti-plague institutions.

Specific immunization is one of the preventive measures against certain infectious diseases [145; p. 484, 149; pp. 313–360]. Various studies have been conducted on the specific prevention of plague to evaluate the effectiveness of vaccination in camels and to determine the level of plague-specific antibodies as an indicator of immune response [146; p. 484]. To assess the immunity of camels vaccinated with the EV plague vaccine, PGAR and AgNR systems were used to measure plague-specific antibody titers. Analysis of 40 such animals yielded the following results:

- Specific antibody formation was detected in 32% of the animals;
- On day 10, the proportion of animals with a geometric mean titer of 1:90 was 33.3% in PGAR and 40% at 1:160 in RNAg;
- By day 21, titers increased to 1:160 and 1:170 in PGAR and RNAg respectively, but by day 30, only 15% of animals still had

detectable specific antibodies, and by 3 months, only 5%. Moreover, a significant decrease in antibody titers was observed by day 60 (1:70 and 1:90), and by 3 months the titers fell to 1:40 in both tests. After 6 months, no specific antibodies were found in the blood serum of any animals.

As a result of the studies, plague-specific antibodies were detected in 29.5% of all examined camels. On day 14, 39% of animals had PGAR titers of 1:110 and 47% had RNAg titers of 1:160. The next day, slight increases were observed — PGAR at 1:120 and RNAg at 1:170 — but antibody levels in the blood serum began to decline afterward. By day 32, only 17% of animals still had detectable antibodies. After 5 months, only 2 animals had detectable antibodies with PGAR and RNAg titers of 1:20/1:40 and 1:40/1:40, respectively. A decrease in antibody titers from the second month after vaccination was also recorded, with titers dropping to 1:40 by the fourth month. These results confirm previous experimental findings on the low effectiveness of specific plague prophylaxis in camels (specific antibodies persisted in only 29.5% of tested animals during the first month). This raises doubts about the feasibility of mass vaccination of camels for plague prevention. Moreover, the complexity and significant economic costs of these measures cannot be overlooked.

Thus, the problem of preventing plague in relation to the medical significance of camels remains urgent, as analytical data indicate a high frequency of contact between these animals and the plague pathogen. If veterinary inspections are not carried out before slaughter and specific prophylaxis is ineffective, the risk of human infection from camels increases considerably. All of the above, combined with the relatively large camel population and recent economic conditions and changes in property ownership, highlight the need to revise some aspects of camel disease prevention. It is of great importance to develop and legally formalize state-level regulations regarding financial compensation for owners of camels that die of plague.

Cost calculation for one vaccination course:
One vaccine vial (2 ml), designed for 10 human doses, requires 4 ampoules (40 doses) per camel, totaling 234,940 UZS. One box of vaccine (10 ampoules of 2 ml) costs 3455 rubles. The ruble-to-soum exchange rate used was 170 (based on 2021 rates for vaccines imported from the Russian Federation).

Calculation of one course of insecticide treatment: Most commercially available antiparasitic products for cattle or camels are based on cypermethrin or deltamethrin. These are sprayed on animals using a 0.005–0.008% aqueous emulsion.

The average price of 1 liter of 5% insecticides (such as Delsid, Butox, Biorex, Creolin) in the Russian Federation is approximately 4500 rubles. Taking into account customs fees and 10% VAT, the cost can rise to 5000 rubles per liter. At an exchange rate of 170 UZS per ruble, the price per liter becomes 850,000 UZS. For spraying treatment of one large animal (camel), an average of 4–5 liters of a 0.005–0.008% emulsion is required — this corresponds to 480–520 ml of the product or 160 ml of the 5% concentrate per animal, costing approximately 136,000 UZS.

Therefore, it is recommended to regularly disinfect camels (once a year) instead of vaccinating them. This measure is being practically implemented as part of epizootiological and epidemiological research carried out in natural foci. These efforts are organized in a cost-effective manner by deploying field specialists on a reduced staffing basis, without incurring significant economic losses

CHAPTER III. RESULTS OF STUDYING THE ECONOMIC EFFICIENCY OF USING THE POLYMERASE CHAIN REACTION (PCR) METHOD IN THE MOLECULAR-GENETIC STUDY OF *Yersinia pestis*

Determining the typical species characteristics using microbiological and serological methods forms the basis for diagnosing the plague pathogen (*Yersinia pestis*) [10; pp. 34–42, 135; p. 776]. Identifying the pathogen of epizootics and conducting epidemiological studies are complicated due to various situational changes (e.g., ecology). Two types of variability in microbial populations have been studied:

1. Interclonal variability
2. Intraclonal variability

The first is based on the proliferation of different populations and is supported by genetic exchange. The second is based on processes that ensure the generation of different generations due to genetic rearrangements. To study the intensity of genetic exchange in plague bacteria, the pathogen must be examined outside the host organism, in water and soil environments [5; pp. 1012–1020, 70; pp. 122–129, 107; pp. 5–12, 111; pp. 3–12].

The most dangerous “lineages” of pathogens become more potent under favorable conditions, which leads to adverse epidemic situations and an increase in disease incidence. The antibiotic resistance traits of strains are also transferred through genes in pathogenic microorganisms, and genetic rearrangements may occur during adaptation to a new host, though these remain within certain biological limits. In such cases, the balance of the genotype is not disrupted. These events are associated with the movement of elements and regulatory structures in various parts of the genome [95; pp. 138–147, 122; pp. 1079–1085, 140; pp. 13–27].

The above-mentioned mechanisms are crucial for observing the early stages of epidemic processes, during which microorganisms can acquire important additional genes via genetic exchange, such as those associated with antigenicity, oxygen utilization, and others. One of the key tasks of molecular epidemiology, which studies mechanisms of population redistribution, is to reveal the hidden mechanisms behind the development of epidemics and the spread of pathogens.

The current monitoring system includes isolating pure cultures of pathogens and creating microorganism collections to monitor natural infection foci, which requires deeper identification of microorganisms. Identifying species and subtyping within species allows detection of epidemiologically significant strains. General forecasting assessments are based primarily on the biological and molecular genetic characteristics of pathogenic strains.

Microbiological confirmation of especially dangerous and quarantinable infections is carried out by specialized microbiological laboratories [76; p. 195, 139; pp. 269–275]. The study process should determine:

1. Clonal distribution (mostly in one clone);
2. Its dominance over other clones;
3. The possibility of reconstructing the direction through internal clonal variability.

Typing Methods:

1. Phenotypic studies
2. Genotypic studies of DNA structure

Phenotypic methods include:
a) Biotyping
b) Serotyping
c) Phage typing
d) Resistance to antimicrobial agents
e) Typing and subtyping with monoclonal antibodies

Genetic (molecular) typing is more specialized and is based on detecting differences in DNA structure. One of the most widely used and effective genotypic methods is PCR (Polymerase Chain Reaction) and the analysis of DNA fragment length polymorphism (RFLP), particularly plasmid analysis [10; pp. 34–42, 36; pp. 49–56, 115; pp. 4601–4611, 123; pp. 2911–2923].

Currently, plasmid analysis of *Y. pestis* strains is conducted in nearly all natural foci. As a result, differences in plasmid structure among various foci have been identified, which allow determining the host species distribution associated with specific natural plague foci [33; pp. 37–40].

The calcium-dependent plasmid rCad of *Y. pestis* carries structural genes of major virulence factors, including the V antigen [104; pp. 137–145]. Outer membrane proteins, which regulate bacterial interactions and determine survival and reproduction in host lymphoid tissues, as well as having antifagocytic and cytotoxic activity, are absent in non-pathogenic strains [108; pp. 5–12, 113; pp. 18–30].

Studies of cellular immune responses have shown that these proteins participate in the immunobiological restructuring of the human immune system [109; pp. 3673–3681]. The mechanism of signal transduction and regulation of Yop (Yersinia outer membrane proteins) expression is conserved [115; pp. 4601–4611, 161; pp. 1226–1234]. There is a distinction between in vitro and in vivo conditions, represented by Yop secretion and modulation of their expression in the interaction between the pathogen and host cells [162; pp. 5138–5146].

For virulence expression, *Y. pestis* must synthesize F1 antigen (Fraction-1). Laboratory strains and naturally circulating strains in rodents can show reduced virulence if they lose the ability to produce this antigen. The F1 antigen is the primary immunogen of *Y. pestis* and its removal from molecular or live vaccines may cause a significant reduction in immunogenicity [49; pp. 62–67, 124; pp. 69–78, 152; pp. 259–268].

The production of F1 capsule antigen is dependent on the bacterial host, and its synthesis and secretion phases correlate with host interaction.

A heat-stable protein specific to *Y. pestis*, identified as fibrinolysin, has been shown using diagnostic fluorescent immunoglobulins [146; p. 484, 160; pp. 55–64].

Some authors link hemolytic activity of *Y. pestis* to its virulence, as hemolysis is observed in infected individuals. However, not all pathogens from different foci exhibit hemolytic activity due to the absence of pesticinogenic plasmids. Both hemolytic and fibrinolytic activity are detected at 37°C and are closely related to virulence [138; pp. 38–43, 148; pp. 356–361].

Functional and restriction analysis of plasmids has enabled the construction of physical and genetic maps and determination of operon sequences, which include extended open reading frames for several proteins [43; pp. 140–144].

An important trait of virulent *Y. pestis* strains is their ability to synthesize purines (Pur^+). Loss of this function results in loss of virulence without affecting other microbial properties. This is explained by the absence of free purines in the blood of animals, which limits replication of mutants. If animals receive purines together with Pur^- strains, virulence is restored [1; pp. 25–33].

Insertion sequence (IS) elements play a significant role in the activity of pathogenic genes by contributing to the production of virulence factors and essential antigens in many bacterial chromosomes and plasmids [94; pp. 121–122].

Pathogenic genes can be transferred between bacterial phages and chromosomes or plasmids via conjugation, transduction, and transformation, spreading to other bacteria [99; pp. 113–145]. These manipulations occur due to transposons formed from two copies of IS elements in direct or inverted orientations.

Identification and molecular typing of *Y. pestis* using IS elements is performed through hybridization and PCR (polymerase chain reaction). Highly efficient IS typing of *Y. pestis* has been confirmed by PCR using primers specific to these IS elements [103; pp. 7324–7331].

The Polymerase Chain Reaction (PCR) method was first used at the U.S. Army Medical Research Institute of Infectious Diseases (by Mark Walcott). Since most DNA polymerases become denatured at temperatures above 56°C (up to 95°C), it was decided to use a thermostable enzyme obtained from outside the human body, found in a hot environment such as the geysers of Yellowstone National Park [106; pp. 11–28].

The DNA chain is held together by weak hydrogen bonds and consists of two complementary strands bound by nitrogenous bases: adenine–thymine and cytosine–guanine. These strands are parallel to one another.

In this context, the functions and relevant enzymes are as follows:

1. DNA strand separation:
    - a) Helicases
    - b) SSB proteins (single-strand binding proteins)
    - c) Topoisomerases
2. DNA polymerization:

    - a) DNA polymerase
3. Primer distribution:
    - a) Primases
4. Gap sealing:
    - a) Ligases [34; pp. 51–55]

During PCR, the replication process takes place through the following steps:

1. DNA denaturation – by heating
2. DNA polymerization – using Taq DNA polymerase
3. Primer annealing – primers are added to the mixture
4. Ligation of gaps does not occur because the fragments are very short

PCR begins the amplification (duplication) of millions of copies of any DNA sequence using simple, inexpensive ingredients and a DNA template, which includes:

- a DNA template,
- primers (short DNA fragments),
- DNA polymerase,
- dNTPs (deoxynucleotide triphosphates),
- Mg^{2+}, and
- a buffer [161; pp. 1226–1234].

Capabilities of PCR:

- It is used to create multiple copies of the DNA template.
- The amplified DNA fragments can be sequenced to identify the coding region of a specific gene [152; pp. 69–78].
- It allows diagnosis of various diseases by amplifying specific genes [126; p. 20].
- The amplified fragments are unique, like human fingerprints, and can be used to distinguish between different organisms.

PCR uses DNA replication. DNA polymerase synthesizes a new complementary strand using a single-stranded DNA template, while cyclic temperature changes drive the reaction. The newly synthesized double-stranded DNA then serves as the template for the next round of replication [142; pp. 35–78].

PCR consists of three main steps:

1. Denaturation – heating the double-stranded DNA to produce single-stranded templates
2. Annealing – a small DNA fragment (primer) binds to the single-stranded template to initiate amplification
3. Extension – DNA polymerase replicates the DNA

These steps are repeated to obtain a sufficient quantity of DNA – usually through 25 to 45 cycles.

The dependence of PCR stages on duplex temperature separation:

1. Denaturation: 30 seconds – at 93°C to 95°C for 1 minute.
2. Primer annealing: 30 seconds – at 37°C to 65°C for 1 minute.
3. Extension (polymerization): 30 seconds – at 72°C for 1 minute.

§3.1. Method for extracting DNA from test materials

To do this, an extract of crushed organs or insects (ticks, flies, fleas, etc.) in distilled water is required. A specific amount of ATL Buffer and Proteinase K is added to this extract. The mixture is vortexed for a few seconds and then incubated in a dry incubator or water bath at 56°C for 1 hour. The extract obtained from fleas must be centrifuged at maximum speed, and the clear supernatant must be separated from the sediment. AL buffer is added to the centrifuged liquid and mixed again in a vortex. It is then processed again in a water bath and centrifuged, so that the droplets flow down to the bottom of the test tube.

Ethanol is added to the tube, mixed in a vortex, and centrifuged. The contents of the tube are transferred to another filter-equipped tube without wetting the edges for further centrifugation. The remaining portion of the mixture is removed, and AW-1 buffer is added. The sample is centrifuged again and transferred to another tube. AW-2 buffer is then added, centrifuged, and drained into the next tube. AE buffer stored at room temperature is added. Afterward, the centrifuge tube is left without the column and either placed in PCR or stored in a freezer at -20°C without the column [112; p. 161].

§3.2. Types of Polymerase Chain Reaction (PCR) methods

Detailed Stages of PCR

I. Stage – Denaturation:

1. Mix the test sample, DNA primers, templates, nucleotides, and Taq polymerase.
2. The nucleotide sequence in the sample is determined by:
   a) The specific pairing of DNA primers – usually oligonucleotides about 20 bp long.
3. Heat up to 95°C to break the DNA strands:
   a) Increasing the duration of denaturation prolongs the process, but reduces the stability of DNA template enzymes

II. Stage – Annealing:

1. Temperature is lowered to 15°C–25°C;
2. Primers attach to complementary nucleotide sequences;
3. Knowing the target sequence is required.

Primers:

1. Primer selection is based on sequences that match both sides of the target DNA;
2. Two primers should be designed for each DNA strand;
3. 18–24 variable range (bp);
4. Both primers should have similar melting temperatures (Tm);
5. The purine:pyrimidine content should be close to 1:1 if possible;
6. Magnesium ions ("Mg") aid primer binding to the DNA template and stabilize the replication complex with polymerase.

III. Stage – Polymerization:

1. As Taq polymerase works best at 75°C, heat the tube to 72°C–75°C;
2. DNA polymerase recognizes the primer and synthesizes a complementary copy from the template, forming a new single-stranded DNA;

3. The product synthesis rate is approximately 150 nucleotides per second.

Amplification:

1. Taq DNA polymerase uses the available individual deoxyribonucleotides (dATP, dCTP, dTTP, dGTP) to synthesize the new complementary DNA strand;
   Note: Taq polymerase has a high error rate (proofreading by exonucleases from 3' to 5' direction is not observed) [138; pp. 38–43].

PCR Process Consists of:

- Sample extraction and preparation
- Amplification
- Electrophoresis (in classical PCR)
- Visualization
- Data analysis

Detection of PCR Products

1. Agarose gel electrophoresis:
   a) Provides good resolution and reliable detection (from 200 bp to 50 bp);
   b) Buffer is required
2. TAE (Tris-acetate) – low buffering capacity (ionic strength)
3. TVE (Tris-borate) and TRE (Tris-phosphate) – high buffering capacity

Isolation of PCR Products – Visualization of DNA via Fluorescence

1. Ethidium bromide (EtBr):
   a) Inserts between DNA bases;
   b) The EtBr-DNA complex gives strong fluorescence;
   c) The result is weaker with single-stranded DNA.

Potential issues with Taq polymerase:

1. A disadvantage is that it confirms the verification of newly synthesized DNA;

2. Potential incorporation of dNTPs (deoxynucleotide triphosphates), which may not be complementary to the original strand [121; pp. 53–64];
3. Errors in coding results;
4. Recently discovered DNA polymerases TLI and Pfu are less efficient, but have higher accuracy;
5. Contamination of DNA with foreign microflora;
6. Correct selection of the required polymerase is essential.

Taking all of the above into account, we encountered the task of isolating bacterial cell DNA and viral cell RNA. For bacterial DNA extraction, we used one of the methods from the mini kits provided by QIAGEN, which are intended for the study of various materials: blood, plasma, serum, lymphocytes, sperm, urine, any organ fluids, pure culture, tissues, dried blood for forensic purposes, and more.

Currently, there are two types of PCR:

1. Classical PCR
2. Real-time PCR:
   a) Portable PCR
   b) Stationary PCR

In our research, depending on the location, both types were used—real-time PCR was applied both in the field and laboratory.

Medical equipment used:

1. Real-time stationary PCR apparatus Light Cycler 2.0 – Roche company;
   Centrifuge LC Carousel Centrifuge 2.0 – Roche company;
   A laptop with special software and an HP Deskjet 5740 printer.
2. Portable R.A.P.I.D. – Idaho Technology Inc.
3. Laminar cabinet with Class II biosafety cabinets for PCR, and Class III biosafety cabinet – Air Clean 600 PCR Workstation brand [41; pp. 201–218].

PCR method application was conducted over three years in both field expeditions and laboratory conditions of the Anti-Plague Institution. PCR training was conducted by international specialists.

Skill enhancement and practical training were carried out at the Centers for Disease Control and Prevention (CDC) in Tashkent.

For testing Y. pestis strains by PCR, reagents from *Bio Chim, a Division of IDAHO Technology*, were used, including Target 1 and 2 kits, 40 nests/20 vials. All collected samples were tested using PCR. Of these, 70.9% were from rodents, and 29.1% from ectoparasites. Material obtained from rodent organs was inoculated into appropriate nutrient media, and from the suspicious colonies that grew, real-time PCR was performed. For direct PCR, emulsions were prepared from fleas and ticks. In parallel, the same emulsions were also cultured on appropriate nutrient media for microbiological analysis.

Out of the total samples, 2.5% gave positive results. All strains isolated from the natural focus were subjected to PCR, and all tested positive, accounting for 15.3% of the studied strains.

We present to your attention illustrations of the PCR stages and the formation of DNA strands in Figures 3.1–3.2.

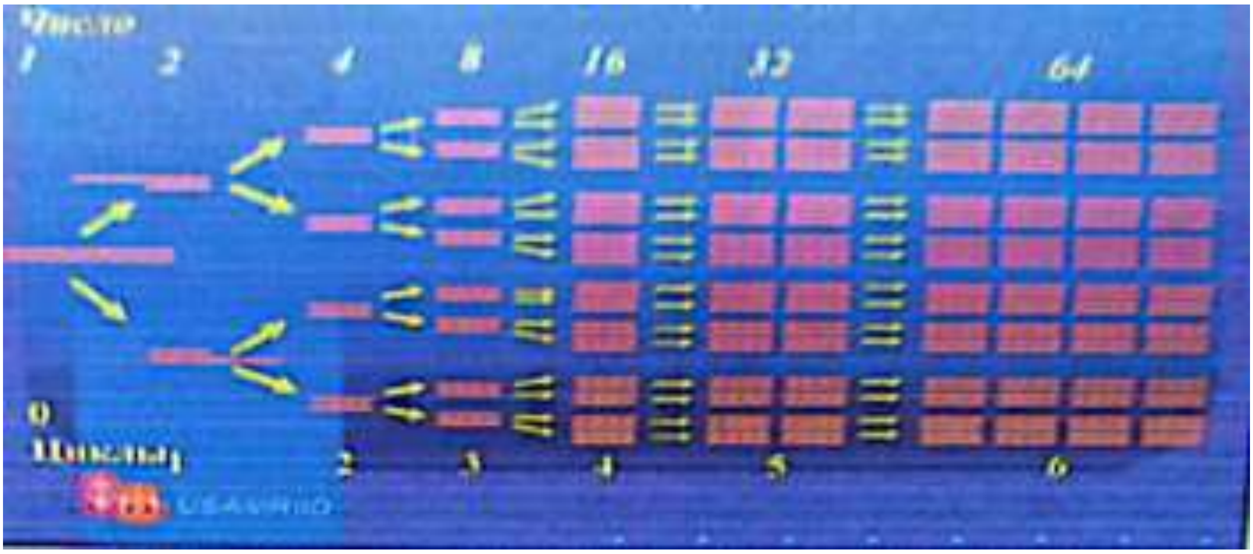
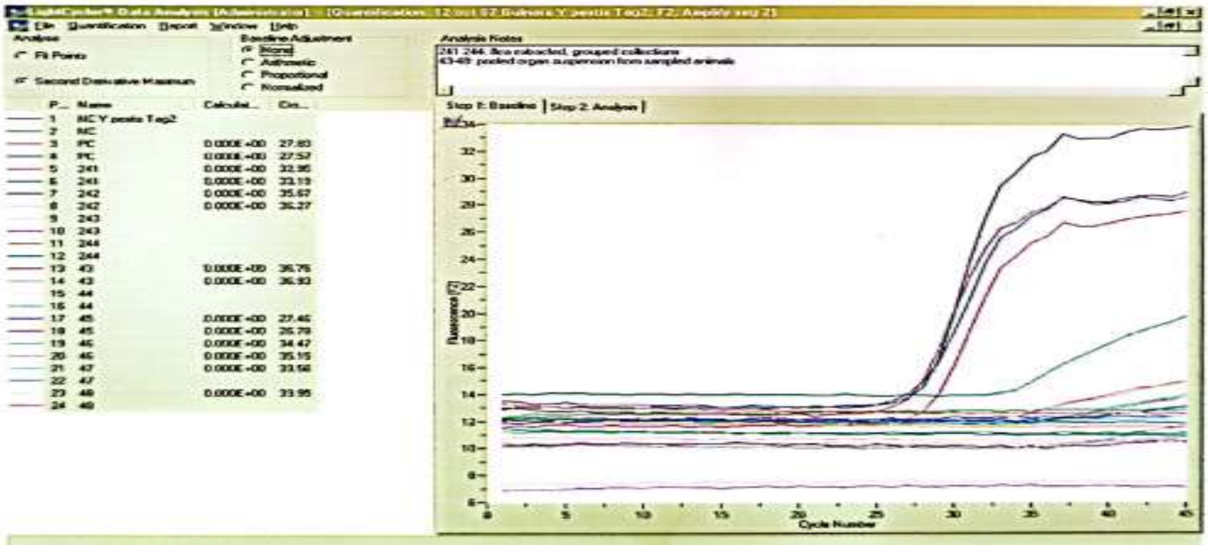


Figure 3.1. Stages of PCR: Exponential growth of DNA



3.2 .-Figure Amplification curve

The expenses for our microbiological investigations and molecular-genetic studies conducted within the framework of our scientific work were calculated from an economic perspective (based on calculations made in 2021). The cost estimates are presented in Tables 3.1 and 3.2 below.

3.1-table

Information on the necessary expenses for bacteriological analysis to detect one plague pathogen.

Item Name	Quantity	Unit	Unit Price	Total Price
Alcohol	500	g	120	60000
Agar ChPS	50	g	3150	157500
Ingredients for diagnosticum	1	box	840000	840000
Erythrocyte a/g diagnosticum	1	vial	840000	840000
Erythrocyte a/t diagnosticum	1	vial	420000	420000
Pokrovsky phage	1	ampoule	100000	100000
L-413S Larina phage	1	ampoule	100000	100000
Pseudotuberculosis phage	1	ampoule	100000	100000
MPB meat-peptone broth	10	g	1700	17000
Set of 15 types of antibiotics	1	set	130000	130000
Luum-serum	1	ampoule	230000	230000
Bioprobe	2	pcs	25000	50000
Gloves	20	pairs	1000	20000
Rhamnose	1	g	4000	4000
Sucrose	1	g	1200	1200
			Total:	3,069,700

3.2 –Table

Information on the necessary expenses for PCR analysis to detect one plague pathogen.

Item Name	Quantity	Unit	Unit Price	Total Price
Immunoassay test system for plague microbe detection (IFA-PEST-F1-M), for 25 tests	1	package	1800000	72000
Immunoassay test system for plague antibody detection, for 25 tests	1	package	1190000	47600
For detection of plague DNA, for 100 tests	1	kit	2230000	22300
Reagent kits for PCR DNA detection, for 100 tests	1	kit	1000000	10000
Estimated cost per test:				
Eppendorf 1.5 ml	2	pcs		
Thin-walled flat-cap tube	1	pcs		
Filtered pipette tip 10 µl	2	pcs		
Filtered pipette tip 100 µl	2	pcs		
Filtered pipette tip 200 µl	1	pcs		
Filtered pipette tip 1000 µl	3	pcs		
Non-filtered pipette tip 200 µl	3	pcs		
Alcohol	100	ml	100	10000
Gauze napkin 15x45	10	pcs		
0.6% Mega Chlor Active disinfectant	1500	ml		
			Total:	161900

Thus, the conducted analyses revealed that traditional microbiological examination methods used under field expedition conditions in natural plague foci in Uzbekistan are comparable to real-time PCR analysis methods, differing only in the required cost and time expenditure.

Accordingly, molecular epidemiology methods make it possible to conduct a comprehensive epidemiological monitoring of the spread of various variants of the plague pathogen in natural foci, allowing differentiation of strains not only from different plague foci but also within a single focus based on specific traits or characteristics. Naturally, this is of great importance in the context of the real threat of bioterrorism and the emergence of antibiotic-resistant variants of the plague pathogen. As a molecular method, PCR, alongside traditional methods, enables the resolution of fundamental tasks in microbiology and epidemiology, and, for the first time in Uzbekistan, allows for the study of evolutionary patterns of the plague microbe.

CHAPTER IV. ELECTRONIC REPORTING AND MONITORING OF QUARANTINE AND HIGHLY DANGEROUS INFECTIOUS DISEASE CULTURES AND PATHOGENIC MATERIALS IN UZBEKISTAN THROUGH THE CONTROL SYSTEM (PAKS)

In the Republic of Uzbekistan, the movement of pathogenic materials belonging to Group I-II bacterial agents can be tracked, monitored, and controlled using the Pathogen Asset Control System (PACS) software, developed by analyst A.Kh. Zhilokov from the Black & Veatch company.

PACS is a computer-based system developed and used for recording and controlling isolated strains stored in centralized repositories and collections. The system provides a secure, reliable, and user-friendly method for registering and tracking pathogenic materials [37; p. 53].

Before the development of the PACS program, after each investigation, all strain passports were recorded manually in logbooks. The completed documents were then archived. Specialists spent a lot of time filling out all those journals. The implementation of the PACS program has significantly saved time in this process.

The PACS system package consists of the following hardware and software components:

- Computer workstation
- Printer for printing documents
- Printer and scanner equipment
- Supporting software
- PACS computer application

System Description and Key Advantages

We highlight a number of key advantages offered by the system:

1. The system was specifically developed to meet the needs of microbiological culture collections, i.e., it was designed and developed in accordance with regulatory documents governing microbiological collections in Uzbekistan and other CIS countries.

It meets the demands and expectations of many scientific centers in the CIS, including specialists working in quarantine and highly dangerous infections. During PACS training sessions, specialists working directly with such infections participated based on their specific needs, many of which were later integrated into the program.

The system allows automatic generation of reports and documents (e.g., microorganism passports, reports, logs) in various formats based on the data entered.

The main unit of account in the system is the strain and its passport. The electronic passport of a strain replicates the current microorganism journal passport format. The system uses internationally recognized taxonomic classifications of microorganisms.

2. Barcode technology is used to track containers holding strains, which provides the following advantages:
   - Increases accuracy and speed of data entry, allowing for rapid and error-free input;
   - The barcodes used for container labeling are resistant to mechanical, thermal, and chemical effects, and can be used within a temperature range of +80°C to -196°C;
   - The creation of barcode numbers and printing of stickers is carried out in accordance with the "Object Numbering System," which ensures uniqueness of numbers and allows staff to include all necessary data in the barcodes;
   - It prevents unauthorized changes to container information using markers or other tools.
   - An example of barcode-labeled test tubes used for registration is shown in Figure 4.1.

Figure 4.1. Barcode

Special equipment was used for generating, applying, and scanning barcodes — the Zebra R2844-Z thermal transfer printer and the Symbol LS 4008 barcode scanner. This equipment is shown in Figure 4.2.

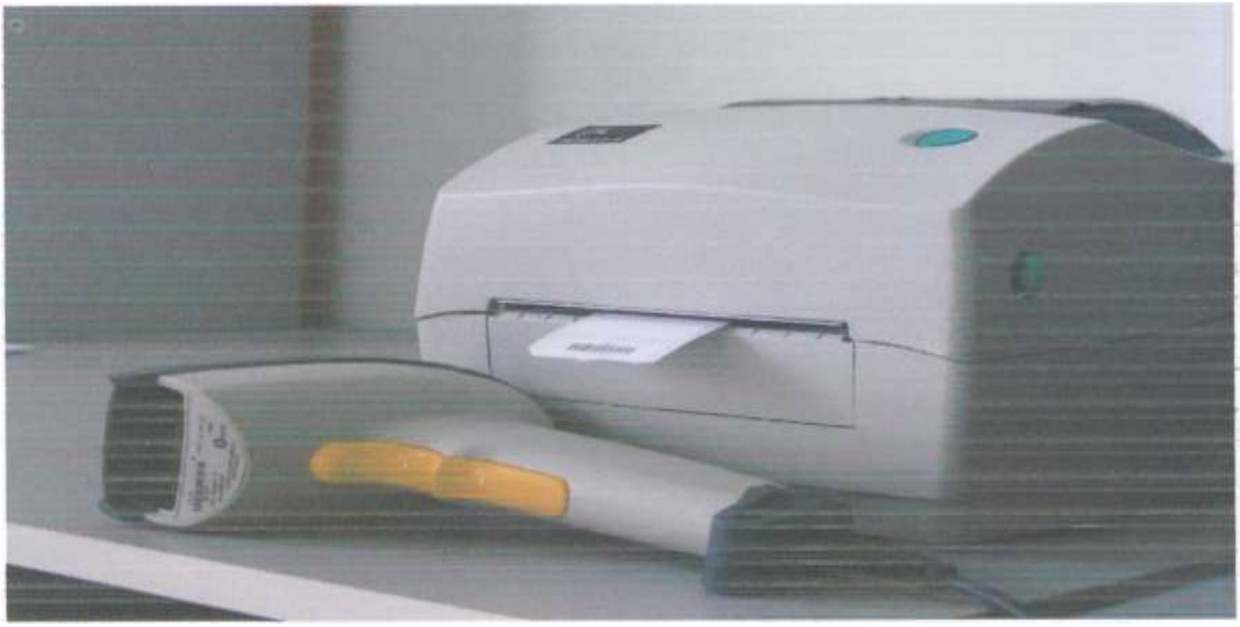


Figure 4.2. Barcode Printer and Scanner

In ensuring the security of data storage and processing:

- Access to the system is granted only to specialists with a username and password assigned by the institution's management;
- Data access is carried out based on user permissions, allowing each employee to access only the data segments relevant to their official authority;
- The system is not connected to any external communication channels, thus ensuring that information does not leak;
- Data is automatically backed up to the hard disk, which helps prevent data loss in the event of computer equipment damage or malfunction.

In addition, the system provides the following functionalities for detailed tracking and control of strains and their movements:

- Most operations are carried out step-by-step and sequentially, so the processes are automated and well-documented;

- Comprehensive information about all strains in the collection can be obtained in one place. Catalogs and registration logs contain detailed data on test tube strains registered in the system;
- The system’s event log records all performed actions, such as strain registration, transfer, subculturing, collection inventory, etc. It automatically logs the date and time of the operation, type of operation, and the name of the responsible staff member;
- The inventory function enables comparison between stored strains and the PACS database contents, allowing verification of data accuracy;
- The system stores information about the exact location of each test tube (ampoule), including which shelf in the refrigerator or freezer it is stored on.

PACS uses localization tools installed on the Windows XR operating system and features a multilingual interface. The displayed information automatically changes based on the selected language’s hotkeys.

Despite all these advantages, the system remains very simple and easy to use.

All functions of the PACS computer system can be grouped into several categories: operations, catalogs, documents, reports, and administrative functions.

Operations include: “Receiving objects for storage,” “Issuing objects,” “Transferring objects,” “Inventory,” etc. These represent actual operations performed with containers and are used by the system for registration purposes.

Catalogs contain detailed information about all registered strains and containers in the system. This information can be edited and updated at any time.

Documents and reports are provided in printed format and include data on performed actions, such as Destruction Acts of microorganisms, Inventory Book of Stored Cultures, Strain Registration Logs, and others.

Administrative functions are intended for configuring the system according to the parameters of the institution where the program is installed. Typically, these settings are managed by system administrators who have more in-depth knowledge of the program's functions than the end users.

§4.1. Strain Cataloging System

The system was implemented by collection specialists who had completed an advanced training course on PACS management. The following tasks were carried out:

- An electronic scheme of the collection was created;
- Detailed electronic passports for various microbiological cultures were developed (an example of such an electronic passport is shown in Figure 4.3);
- User reports and roles were configured to restrict access to system data based on the job responsibilities of the staff. Additionally, each specialist was assigned a specific role in the program according to their duties. The usernames and passwords granting access to the program were provided separately from the system data.

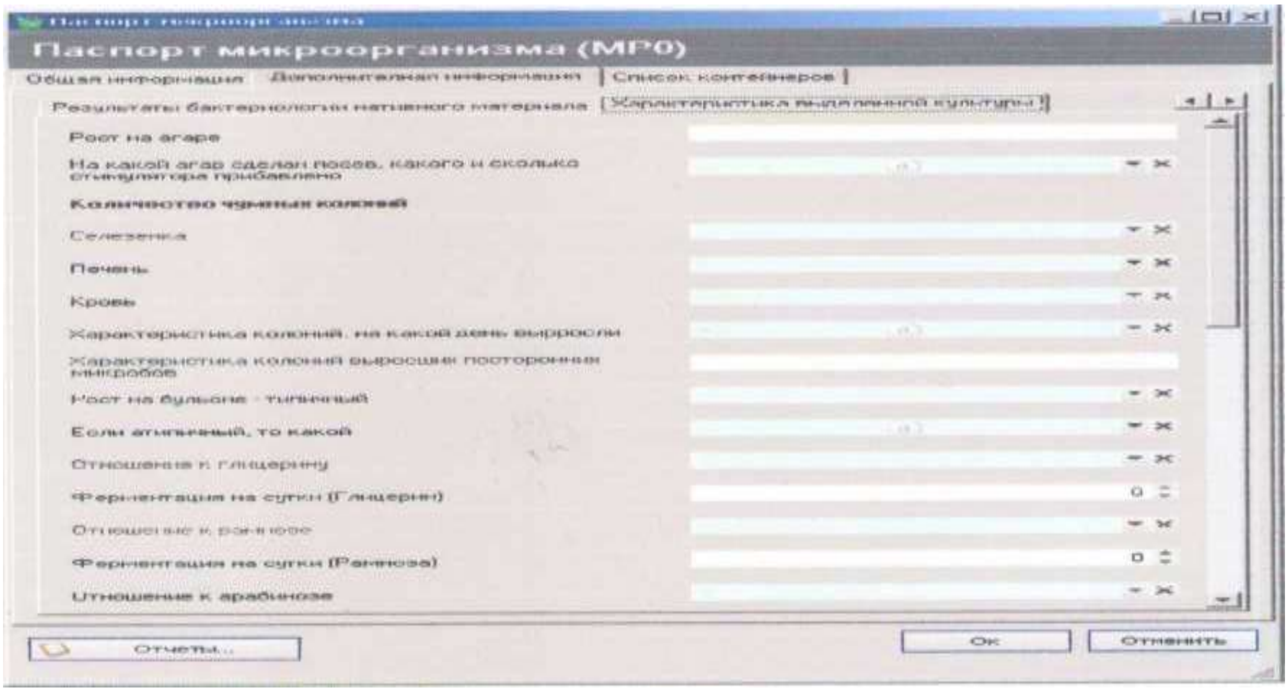


Figure 4.3. Microorganism Passport

- The structure of the microorganism tree was developed, containing all necessary information about the cultures;

- A strain passport coding system was established. According to this system, the codes are individually assigned to each strain's container.

After the initial setup of the system was completed, strains were placed into the catalog. Strain cataloging involves registering existing passports within the system, which includes detailed information about the strains' characteristics and the location of the culture tubes. A barcode is attached to each strain's test tube, facilitating future tracking and easy access to data about the tubes.

Each day, a certain number of strain records are entered into the system, including all data specified in the passports.

When registering new strains in the system, the "object receipt master" function is used. This tool allows the operator to:

- Enter all required information sequentially (who is registering the objects, their source, etc.),
- Print barcode labels for test tubes in several steps,
- Complete the passport details, and
- Assign the storage location for the test tubes.

Conducting the initial cataloging has allowed specialists to work efficiently with the strain catalog and perform the following actions:

- Search for required strains using one or more parameters such as strain number, test tube numbers, date of receipt, isolation date, etc. In particular, data can be searched using a custom-designed search form;
- Add or modify information for any strain;
- Print documents such as the strain passport, the location of the strain's test tube (e.g., which shelf in the refrigerator or freezer), and more.

The external view of the strain catalog can be seen in Figure 4.4. The test tube catalog, which contains information on individual tubes, provides specialists with full details. The test tube details window includes data such as location, strain number, microorganism lineage, registration date, and more. This window is shown in Figure 4.5.

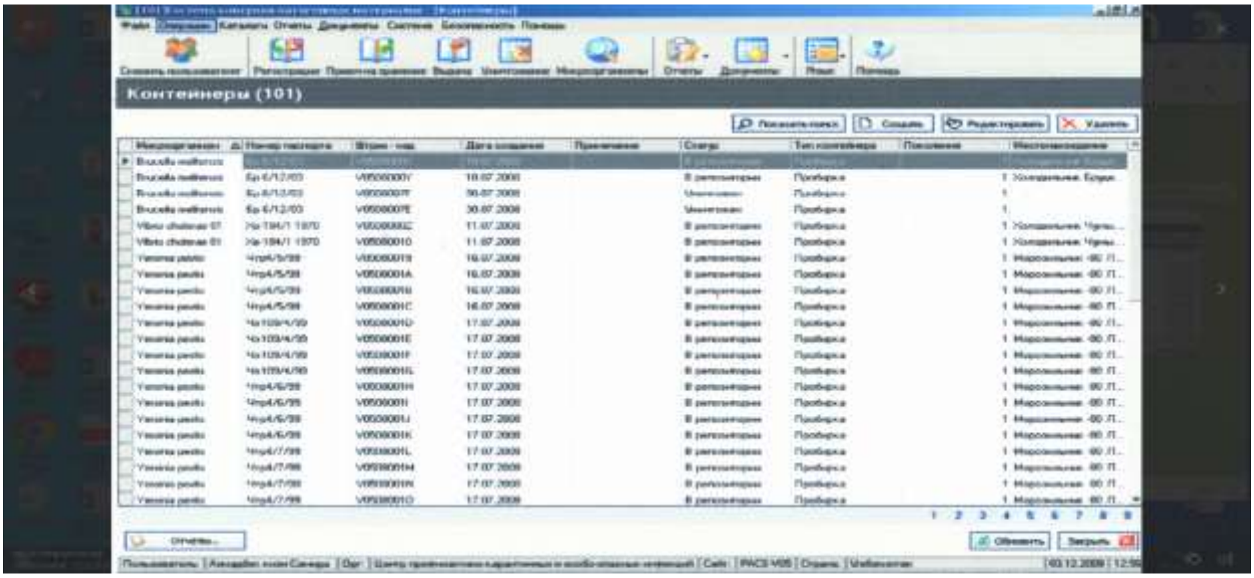


Figure 4.4. Catalog

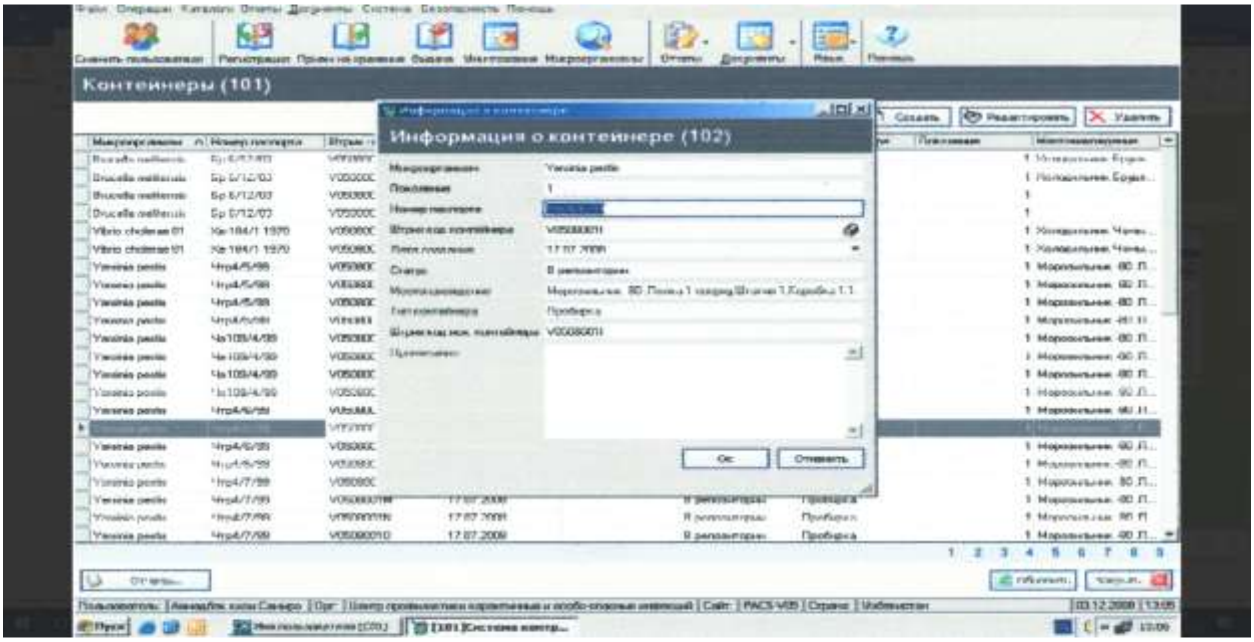


Figure 4.5. Detailed Information About the Test Tube Containing the Microorganism

The re-cultivation function in the program is widely and frequently used to record all re-cultivation activities of strains stored in the collection. The system allows for the registration of an unlimited number of different strains in a single re-cultivation process. At the same time, the system automatically tracks container types, generations, and the number of re-cultivated strains.

At the end of each re-cultivation, the specialist only needs to indicate the new storage location for the obtained strains and record the disposal of the previous test tubes. Barcode labels for the new test tubes are printed, and the corresponding passport is automatically generated.

To monitor all cultivation, purification, and other operations performed with the strains in the collection, the Pathogenic Materials Movement Log is used—this is the electronic equivalent of a traditional journal. This interactive log displays quantitative data and reflects processes such as receipt, issuance, destruction, re-cultivation, and splitting of microbiological cultures. Reports can be printed upon request for a specified time range as needed by the user.

The system's export functions allow specialists responsible for data management to work with PACS information in other applications such as Microsoft Word, Excel, Adobe Acrobat Reader, and more. Exported PACS documents can be edited and used for various purposes.

When working with the system, catalog specialists use two main sources as references:

1. The context-sensitive help built into the program
2. The PACS Version 1 User Manual

This manual provides a detailed description of all functions and operations available in the system. According to the usage procedures for the PACS system for registering and monitoring strains, the following key advantages have been achieved:

- Increased accuracy in identifying and characterizing strains using barcode technology;
- Enhanced security by storing descriptive information on barcode-labeled test tubes identified through barcode technology and electronic data systems;
- Improved control over the movement and relocation of strains;
- Significant advantages in data entry, storage, and interaction with a structured strain database, opening up new opportunities for scientific research on strains;
- Simplified administrative processes such as strain movement monitoring and inventory management, with improved efficiency through the electronic reporting system;
- Time savings in completing documentation and preparing reports on completed work.

Thus, the PACS system is currently being actively used, with ongoing efforts to expand its application scope. The implementation of this system facilitates easier report preparation for medical personnel and, most importantly, saves time.

CHAPTER V. EPIDEMIC CONTROL MEASURES AND IMPROVEMENT OF PREVENTION STRATEGIES FOR PLAGUE

To qualitatively and quantitatively assess the risk of human infection with plague, the term "epidemiological potential of natural foci" is currently considered the most accurate. It expresses the potential epidemic hazard level for the population over a certain period, based on the interaction of natural and social factors [11; pp. 85–94, 21; pp. 105–109, 50; pp. 62–67].

We studied the components of the epidemic potential of the Kyzylkum and Ustyurt natural desert plague foci, as well as the natural and social factors involved and their interactions. The epidemiological potential of natural foci is constantly in motion, with natural and social factors interacting continuously. Among these, the epizootic condition of the focal area—i.e., natural factors—is the most variable.

Studying epidemic potential allows for the identification of areas posing the greatest epidemic threat to the population and supports the implementation of targeted measures. The results of the research enable cost-effective epidemiological monitoring and the development of an improved and effective complex of preventive measures.

Epidemiological measures are organized in accordance with Order No. 96 of the Ministry of Health of the Republic of Uzbekistan dated February 18, 1999, titled *"On improving epidemic protection of the population of the Republic of Uzbekistan from quarantine and other highly dangerous infections."* Based on this resolution, an anti-epidemic task force is formed with the involvement of leading specialists.

In accordance with this order, responsible specialists are assigned to organize epidemic and preventive measures in the designated area. During the preparatory phase, the structure of the natural focus is determined. It should be noted that a large part of Uzbekistan lies within the largest and most active Central Asian desert plague focus zone in the world.

Within the territory of the Republic, there are two relatively large autonomous desert plague foci—Kyzylkum and Ustyurt—covering almost the entire territory of Karakalpakstan, as well as the desert-steppe regions of Bukhara, Navoi, Khorezm, Kashkadarya, and Surkhandarya provinces.

Anti-epidemic measures consist of a set of rational recommendations aimed at preventing the spread of plague among the population, reducing incidence, and eliminating foci [51; pp. 612–616, 66; pp. 6–16].

Until 1992, control of the natural plague foci in Central Asia was carried out by an anti-plague institution operating throughout the territory of the former Soviet Union, allowing for the use of substantial resources for reconnaissance and elimination of natural foci.

In the 1990s, economic difficulties during the transition period negatively affected public health, including a weakening of epizootic and epidemiological control of plague. Due to limited resources, only certain grid squares of natural foci could be monitored as planned. As a result, the system for managing enzootic and epizootic issues was improved to ensure reliable protection of the population from plague.

For example, in the spring of 2019, a group of four specialists was sent on a one-month field expedition to identify epizootological plague zones. A total of 1,163 mertiolate-impregnated papers were delivered for testing rodent sera, of which 129 yielded positive results.

Previously, such field missions consisted of 8–10 people, with each person’s expenses exceeding 10 million UZS. By reducing the number of specialists to 4, expenses per person were reduced to 5.5–7 million UZS, cutting costs by half. The economic efficiency of the operation amounted to 50–60%.

The main anti-epidemic measures are directed toward:

1. Identifying the source of infection;
2. Determining the transmission mechanism of the pathogen;
3. Assessing the susceptibility of the human body.

During an epidemic, staff of the regional sanitary-epidemiological centers conduct door-to-door visits, organize medical examinations involving healthcare workers, isolate contacts in special wards, perform epidemiological analysis, and carry out body temperature measurements (three times daily) and daily medical supervision. For urgent prophylaxis, antibiotics (e.g., tetracycline 0.5 g twice daily for each contact) are prescribed. Medical staff must maintain constant surveillance of those who had contact with infected individuals.

Considering the high contagiousness of plague, its lethality, and potential for epidemic spread, effective prevention includes establishing a robust anti-epidemic system in epizootic areas and sanitary border control.

This complex of measures includes:

1. Monitoring the health of the population (door-to-door visits);
2. Conducting epizootological surveillance to detect plague outbreaks in time;
3. Based on local administrative decisions and in accordance with recommendations from anti-plague institutions, carrying out camel monitoring by veterinary services, including counting camels and other related activities;
4. In enzootic areas, considering the epizootic situation, vaccinating the population against plague with live EV vaccines (J. Girard and T. Robic strains);
5. Accelerating sanitary-educational outreach by healthcare and epidemiological institutions, including mandatory involvement of doctors and mid-level health personnel;
6. Deratization and disinsection: This involves eliminating rodent vectors (deratization) and fleas (disinsection) in both field and residential zones. Although complete eradication of fleas and rodents is not feasible, regulating their population is essential to prevent outbreaks.
    - Destruction of carriers and ectoparasites in their nests is conducted by anti-plague institutions using special techniques.
    - The goal is to prevent the development of plague epizootics in specific areas or to halt active epizootic processes by drastically reducing rodent and flea populations.
    - Flea control helps prevent the transmission of the pathogen from infected to healthy rodents.
    - Vector control is one of the most effective anti-epidemic measures, using substances like DDT, hexachlorane, and others in 10% powder form with various fillers (e.g., kaolin, chalked talc).

- Where possible, vector control devices like ADP-66 are used to inject powder into rodent nests using a compressor, ensuring nearly 100% flea elimination throughout the colony.
- In hard-to-reach places, AL-1 pistols and handheld aerosol grenades are used to deliver insecticides.

Use of DDT requires special one-time permission from the Ministry of Health of the Republic of Uzbekistan, due to environmental risks such as runoff into water sources. In place of DDT, following [60; p. 248], we successfully used effective and guaranteed alternatives like Ektocid and Ivermectin (including Cypermethrin, Deltamethrin, Deltacid, Butox, Biorex, Creolin) for disinsection and deratization.

These were applied in dosages of 0.5–1 ml per 50 kg of body weight, especially in camel-breeding areas of the Kyzylkum natural focus.

On average, 50 camels per designated area were treated with these agents. Within three days, no fleas were found on treated animals. Moreover, untreated camels in the same herd also benefitted indirectly from the intervention.

This effective pest control showed no negative impact on humans or camel owners. In residential areas, low socioeconomic conditions, rodent intrusion into buildings, and accumulation of waste contribute to flea proliferation. Raising public awareness, conducting flea and rodent extermination campaigns, and delivering sanitary education play an important role in controlling the problem.

In this context:

- Indoor disinfection includes incubating rooms for 2–3 hours with 1–3% chlorophos solution, followed by wet cleaning.
- Dead insects are swept up and burned to destroy plague-infected microbial cells.
- A thorough room cleaning is then performed.
- Rodent extermination should be performed regularly in all locations as a preventive measure.
- In the case of an epizootic outbreak in a residential area, emergency deratization is conducted alongside simultaneous disinsection, because as rodents die en masse, fleas leave their hosts and may attack humans, potentially spreading infection.

There are four methods used in rodent control:

1. Chemical method – use of toxic gases and poisoned bait (e.g., 8–12% zinc phosphide, zookumarin);
2. Mechanical method – trapping devices (e.g., traps, snares, hooks, etc.);
3. Biological method – use of predatory animals (cats, dogs, "rat wolves," bait barrels);
4. Gas method – use of fumigants (e.g., chloropicrin, sulfur dioxide) or automobile exhaust gases.

§5.1. Anti-Epidemic Measures Against Plague

To ensure modern and fully evaluated localization of the outbreak, the epidemic preparedness of the medical-epidemiological service must be maintained at the required level [14; pp. 18–21]. In accordance with this principle, medical, epidemiological, and sanitary services include the following activities:

a) Notification of the **first cases** of highly dangerous infectious diseases;
b) Medical examination;
v) Sanitary control of facilities;
d) Isolation of patients and referral of contacts for further observation;
ye) Medical supervision;
f) Bacteriological examinations;
g) Preventive vaccination;
x) Disinfection;
i) Deratization and disinsection.

All anti-epidemic measures must be implemented in accordance with Order No. 1 dated 02.01.2002**,** titled *“On improving the system of prevention and control of plague outbreaks in the Republic”* which outlines the main aspects of a comprehensive plan [83; p. 86].

For this purpose, comprehensive anti-epidemic plans for the republic, regions, cities, and districts are developed for a five-year period and updated annually.

The main sections of such plans include:

- The procedure for reporting information about the identified patient by higher authorities, healthcare institutions, local government bodies, anti-plague institutions, and other stakeholders;

- The procedure for transporting the patient (or deceased) to a pre-designated hospital, including the formation and composition of the evacuation team;
- Assigning a hospital for the admission of suspected or confirmed plague patients and equipping it according to anti-epidemic regime requirements;
- Allocating, vacating, and reequipping facilities for targeted or temporary hospitalization of patients, and isolating those who had contact with infected individuals;
- Identifying the laboratory base for conducting research and diagnostic tests;
- Allocating and training personnel to work within the epidemic focus;
- Ensuring the supply of treatment, preventive, diagnostic, and disinfectant tools, medications, and protective clothing;
- Designating mortuary facilities for postmortem examinations of plague victims;
- Organizing and providing transport vehicles for the focus area;
- Allocating accommodation facilities for personnel due to the emergency transition to epidemic conditions;
- Providing food to patients, their contacts, and personnel working within the focus area;
- Ensuring the material resources necessary to implement measures for containing and eliminating the plague focus;
- Assigning consultants, including a therapist, infectious disease specialist, epidemiologist, and pathologist;
- Conducting vaccination and medical supervision of the population;
- Organizing management of operations in the outbreak zone (Emergency Anti-Epidemic Commission, medical headquarters);
- Conducting sanitary-educational outreach among the population.

When the first cases of acute infectious diseases emerge, the response tactics are outlined in Order No. 1 of the Ministry of Health of the Republic, as previously mentioned. The order includes a notification scheme for suspected plague patients (or deceased individuals).
(See Figure 5.1 for the notification sequence when a case is detected.)

- Focus localization refers to a set of measures aimed at preventing person-to-person transmission of the infection, which includes:
- a) Identifying plague-infected patients and admitting them to the hospital;
  b) Identifying deceased individuals who died from this disease and burying them together with preliminary pathological-anatomical materials examined in a laboratory;
  v) Identifying and isolating persons who had contact with infected individuals;
  g) Identifying individuals who participated in the slaughter of plague-infected camels and those who had contact with the raw meat of such animals;
  d) Carrying out initial disinfection of contaminated objects;
  e) Hospitalizing patients with fever.

When a patient diagnosed with plague seeks medical care at a treatment and prevention facility:

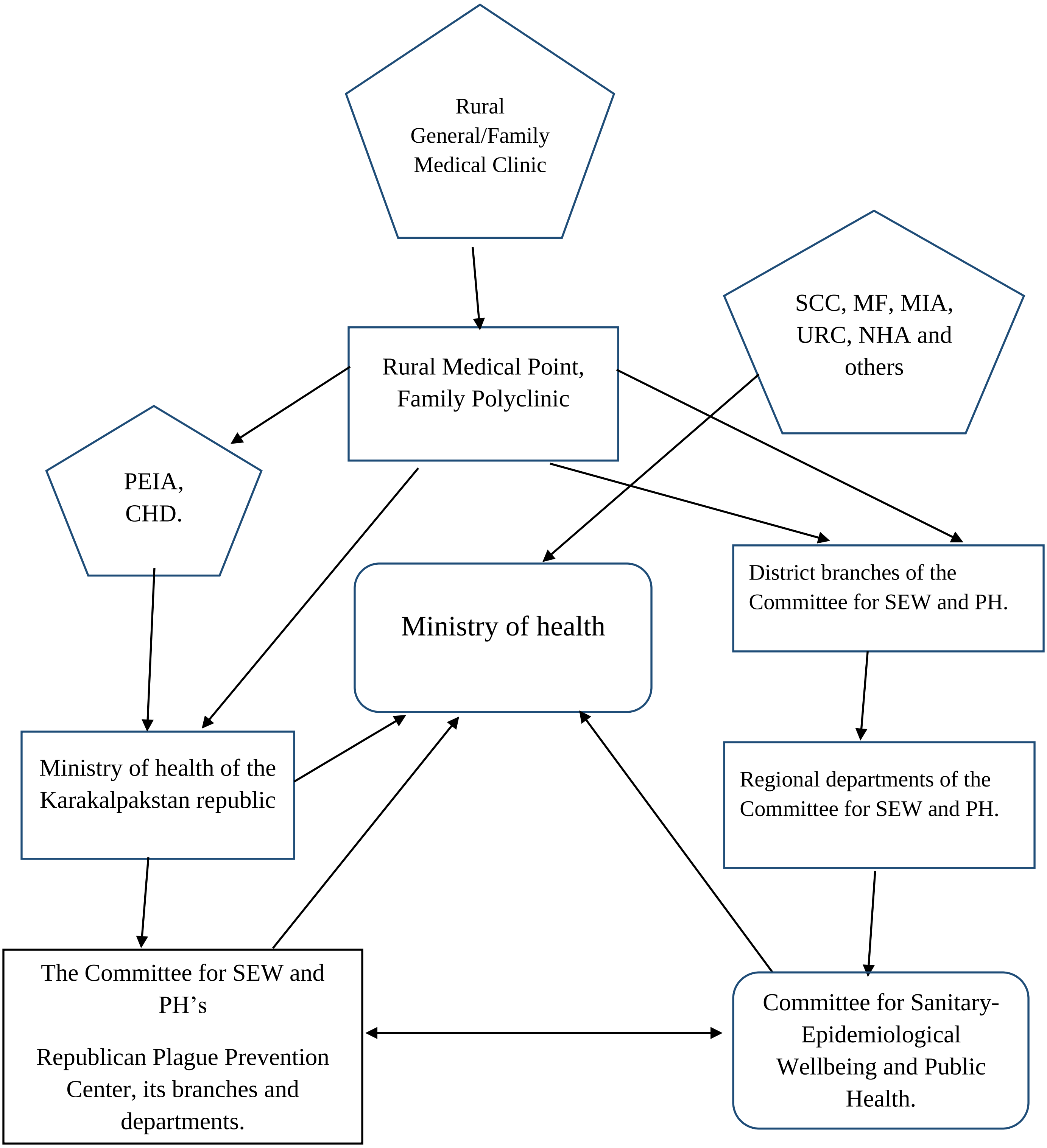


Figure 5.1. Notification Scheme When a Suspected Plague Patient (or Deceased) Is Identified

j) Medical monitoring of the population to identify new cases of the disease;
z) Determining the scope and types of quarantine measures and implementing them promptly.

The decisive moment in localizing a plague focus is the identification of a sick or deceased person with signs of infection and the immediate implementation of anti-epidemic measures. For this, a notification system must be in place, as previously described, along with information about locations storing protective equipment and procedures for collecting materials for laboratory testing, clearly displayed in visible areas of treatment and prevention institutions.

The following scheme must be known by all responsible persons and medical personnel:

1. Notify the territorial state sanitary-epidemiological control institution about the identified patient;
2. Inform higher authorities in the chain of command;
3. The head or deputy head of the designated hospital calls in consultants and an evacuation team to hospitalize the patient;
4. Establish internal control posts, hospitalize the patient in a specialized hospital, and isolate them;
5. Follow procedures for wearing anti-plague protective clothing;
6. Collect material for laboratory examination;
7. Depending on the patient's condition, begin antibiotic treatment;
8. List all individuals who had contact with the patient; immediately suspend admissions and discharges at the facility and completely and temporarily isolate all occupants of the building where the suspected patient was found;
9. Conduct current disinfection using appropriate concentrations of slaked lime, hydrogen peroxide solution, or chloramine;
10. Designate a special work procedure in the morgue. If a body is present, it is recommended not to perform an autopsy until the arrival of a consultant. Burial must be conducted under strictly controlled high-risk conditions, with the participation of a specialist in infectious diseases and support from the evacuation team;

11. If the patient resides in a hotel or temporary accommodation, they must be isolated in compliance with all epidemiological procedures if plague is suspected;
12. Hospitalize the suspected patient using a specialized emergency medical team;
13. If necessary, provide emergency medical assistance to the patient while applying all precautionary measures;
14. Monitor individuals who had contact with the patient;
15. Begin anti-epidemic measures after making a preliminary diagnosis based on clinical presentation and epidemiological history;
16. Properly collect samples for laboratory examination and ensure their correct delivery to the laboratory;
17. A final diagnosis is made only after laboratory tests confirm a positive result for plague;
18. As additional confirmation, conduct the antibody titer increase reaction to the plague microbe antigen, and utilize biological testing.

When the final diagnosis of plague is made based on laboratory data, combined with characteristic clinical presentation and epidemiological history, the Emergency Anti-Epidemic Commission (ChPK) officially declares a plague focus. According to this decision, a medical headquarters for epidemic control is established under the leadership of a representative from the anti-plague institution. During its first meeting, the following actions are taken:

1. The quarantine zone is defined, with boundaries approved by the ChPK for each individual case.
2. Entry and exit to and from the quarantine zone are allowed only with the permission of the head of the medical headquarters, and only for local residents and personnel working in the epidemic focus.
3. Conduct sanitary awareness campaigns among the population.
4. Quarantine can be lifted from the settlement only by the decision of the ChPK.
5. Identify the source of infection, the sick, and individuals who had contact with them, and evacuate them to infectious disease or isolation wards.

6. Carry out primary and final disinfection of contaminated areas with appropriate disinfectant solutions, and decontaminate all soft items in special chambers.
7. Disinfect auxiliary buildings and additional premises.
8. Form inspection teams to monitor the implementation of the anti-epidemic regime in specialized institutions.
9. Monitor the population and carry out preventive measures, including plague vaccination.
10. Conduct epizootological assessments in the region and adjacent areas to the affected locality.
11. Establish a zooparasitological group to determine the presence and intensity of plague epizootics in natural foci, and assess the number of rodents and ectoparasites.
12. A veterinary inspection team is tasked with recording the number of camels.
13. Use active outreach workers to conduct door-to-door educational visits and distribute sanitary and educational materials.
14. Medical personnel shall immunize the population through prophylactic plague vaccinations. Recently, during the period of epidemic response, the local population in the focus area has been vaccinated to protect them in the post-epidemic phase. The EV vaccine causes few post-vaccination complications due to its low reactogenicity and dermal application.
15. Perform full laboratory examinations of the patient or corpse—including serological, bacteriological, clinical, and biochemical tests—and analyze materials collected during the surrounding epizootological investigation. Laboratory infrastructure is organized by anti-plague personnel, and category I pathogen specialists handle the sample collection. For treating plague patients, isolation facilities are organized with separate infectious and non-infectious units, and buildings are equipped according to official guidelines.
16. In collaboration with the consultative group, organize treatment services, quarantine services (focusing on containment of the epidemic zone and protection of critical infrastructure), and administrative-logistical support (transport, communication, food).
17. Conduct daily staff meetings to identify shortcomings and resolve issues.

18. Provide daily reports on patient conditions and activities carried out to the regional administration and the Ministry of Health of the Republic.
19. Once all above epidemic control measures have been implemented, a decision on closure of the outbreak is made based on the recommendation of the medical headquarters and the conclusion of the Emergency Anti-Epidemic Commission. The Ministry of Health and the anti-plague institution submit reports on all activities conducted and the performance of all units involved in the containment and elimination of the focus.

Prevention of Camel Infection

Due to fluctuations in the epizootic situation in the Kyzylkum natural focus, special attention must be paid to the prevention of camel infections. All camels belonging to the local population must be monitored by veterinary service personnel. If there is any suspicion of infection, the necessary examinations must be carried out. If the plague pathogen is isolated as a result of the examination, slaughtering of the camel is not permitted. A comprehensive anti-plague response must then be implemented.

1. The room where the camel is kept, its surrounding area, and all rooms of the household must undergo disinfection, disinsection, and deratization carried out by personnel of the Central State Sanitary-Epidemiological Service, anti-plague services, and veterinary services.
2. All healthy camels in the area must remain under constant veterinary supervision, with temperature monitoring and general health assessments.
3. During quarantine, slaughtering camels, distribution of meat, and collection of camel milk and wool are strictly prohibited.
4. If the disease is confirmed, hunting of wild animals in the area is also prohibited.
5. Enhanced control must be implemented in border areas with Kazakhstan and other neighboring countries.
6. Livestock owners and all animal caretakers in the area must be provided with special protective clothing.
7. Grazing livestock on common pastures must be temporarily prohibited.

Special Prophylactic Measures for Plague

During the onset of the disease and the period of anti-epidemic interventions, vaccination of the population is not recommended, as immunity typically develops within approximately one month. It is assumed that vaccination, due to additional antigenic stimulation in the body, might trigger the progression of the infectious process [18; pp. 38–49, 41; pp. 201–218]. However, in critical cases, administering vaccines in epidemic zones is considered appropriate because it protects people during the post-elimination period of the focus. This is linked to a specific stage of immunity that forms within a week after vaccination, during which rapid development of the disease in vaccinated individuals is unlikely.

Moreover, the EV live vaccine, which is applied to the skin, has low reactogenicity, resulting in minimal post-vaccination complications. This method is also economically efficient, as only the vaccine is required, without additional medications, making it a cost-effective preventive strategy.

In such cases, the following individuals should be included in the vaccination campaign:

- Medical and veterinary personnel,
- All identified contacts,
- Ministry of Health specialists involved directly,
- Security and internal affairs officers,
- Representatives of local district and regional organizations,
- Residents of the affected area.

Vaccination is performed intradermally using the live EV vaccine at a dose of 3 billion microbial cells. As a result of the first targeted preventive vaccination, a stable immune response is developed, and the antigen–antibody ratio is balanced, leading the entire immunized population to feel well.

Measures for the Complete Elimination of a Plague Focus

To eliminate the plague focus, the following are required:

1. Complete treatment of plague-infected patients.

2. Prophylactic treatment of individuals who had contact with plague patients.
3. Discharge of plague patients from the infectious disease hospital according to guidelines:
   a. Depending on the form of plague, after maintaining normal body temperature for 6–10 days;
   b. After 2–4–6 days of receiving negative laboratory test results following treatment.
4. Implementation of anti-epidemic measures by the sanitary-epidemiological service.
5. Official liquidation of the plague focus is carried out only after submission of the medical headquarters' report to the Emergency Anti-Epidemic Commission (ChPK), which then issues a decision.

In conclusion, studying natural plague foci helps identify epizootic imbalances, which are supported by the ecological and geographical features of these regions. This is reflected in the uneven distribution of Y. pestis carriers. As ecological conditions continue to change, new epizootic foci may emerge, requiring more comprehensive and intensified anti-epidemic and preventive measures among the population.

Final summary

The natural plague foci in Uzbekistan cover an area of 19.8 million hectares, mainly located in desert and mountainous regions. From an epidemiological standpoint, the most important are the Kyzylkum and Ustyurt natural foci. As the global situation regarding quarantine and highly dangerous infections, especially plague, periodically intensifies, Uzbekistan's healthcare system is undertaking serious preventive measures [82; pp. 5–10].

According to Order No. 1 of the Ministry of Health of the Republic of Uzbekistan dated 02.01.2002**,** the decision *"On improving measures for preventing the emergence and spread of plague in the Republic"* establishes that epidemiological surveillance for plague is carried out by the Republican Center for Plague Prevention**,** along with its regional branches and departments.

In the country, continuous monitoring of natural foci is carried out according to the "Comprehensive Five-Year Plague Prevention Action Plan of the Republic of Uzbekistan" [42; pp. 5–8].

The mission of the anti-plague service in implementing this plan is to ensure the safe and uninterrupted operation of industrial facilities in plague conditions without violating quarantine measures. To address this challenge, epidemiological surveillance must be improved according to specific regional conditions**.**

Comprehensive research continues on the Kyzylkum and Ustyurt natural plague foci**.** The drying of the Aral Sea has had a noticeable impact on the flora and fauna of Uzbekistan.

The Kyzylkum natural zone, with a total area of 38.5 million hectares, consists of 15 landscape-ecological regions (LERs). The primary plague carrier in Kyzylkum is the great gerbil (Rhombomys opimus)**,** characterized by a relatively high and stable population**,** robust burrows**,** high resilience**,** and moderate susceptibility to plague **[**63; p. 215].

In various ecological regions of Kyzylkum, the distribution of great gerbils is uneven**.** Several types of settlements are observed: continuous residential types**,** diffuse-mosaic types**,** and cliff-type settlements [92; pp. 96–101]. Overall, for the Kyzylkum area, the psammophilous (sand-loving) rodent community plays the most significant role in the natural maintenance of plague foci. The formation of plague foci can also be associated with mountain plains and low mountain regions [91; pp. 122–126].

The periodicity of epizootics is greatly influenced by a combination of factors, one of the most important being the relationship between the pathogen and the host [7; pp. 59–72, 23; pp. 19–26]. Great gerbils exhibit varying susceptibility and predisposition to plague infection [74; pp. 75–78, 82; pp. 5–10]. The gerbils in Kyzylkum are characterized by more intense and prolonged bacteremia, which promotes the activation of the epizootic process in the Kyzylkum natural focus through the involvement of both carriers and transmitters [19; pp. 7–17].

The Ustyurt natural plague focus consists of 11 landscape-epizootiological regions [6; p. 29], bordering the Pre-Caspian lowlands in the north and northwest, and covering 200,000 square kilometers. The eastern (Prioral) part lies within Karakalpakstan, while the southwestern part is located in the territory of the Republic of Turkmenistan. The boundary of the lowland is sharply defined by high (150–200 m) dissected cliffs. Its total area is 15.8 million hectares [63; p. 215].

The fauna includes more than 20 species of rodents [38; pp. 38–40; 62; pp. 116–123]. In the north of Ustyurt, besides great gerbils, marmots are common and widespread. Ogman rats, lesser jerboas, and two-toed jerboas are also found. Murine rodents are rare. Among predators: wolves, corsac foxes, red foxes, wild cats, light-colored polecats, weasels, and sables [39; p. 23]. Hunting animals throughout the region is prohibited, as they are considered potential sources of plague transmission to humans.

The typical primary carrier of plague in this focus is the great gerbil, which is unmatched in its abundance and distribution among other rodents. They mainly inhabit small hilly sand dunes, depressions between sand ridges, bases of sandy ridges, and moist, saline riverbanks. In the eastern part of Ustyurt, their burrows are primarily found in interridge lowlands with coarse, hilly sands. The secondary carriers include comb-tailed and red-tailed gerbils, and some small marmots [35; pp. 387–397; 40; pp. 34–38]. Occasional carriers include: yellow marmots, two-toed jerboas, tarbagans, house mice, and moles.

In southern Ustyurt, due to the drying of the Aral Sea, favorable conditions are being created for maintaining and forming new epizootics, and this region is also considered a zone for plague elimination and epizootiological study. As a result of the drying and retreat of the sea, the Aral Sea area has shrunk by nearly three-fourths of its original size.

One of the most urgent challenges in plague prevention is human contact with camels, as our analyses show a high frequency of plague pathogen infection in these animals.

The literature describes several plague outbreaks among humans linked to infected camels, often during or after slaughtering the animals [26; p. 421; 66; pp. 6–16; 112; p. 161]. Many studies have shown that, overall, camels exhibit resistance to plague bacteria. However, under certain conditions—such as fatigue or coexisting diseases—camels may become susceptible [65; pp. 25–33; 131; p. 1106].

To vaccinate a single camel, a dose equivalent to that used for 40 humans is needed, costing 176,205 UZS. One ampoule of vaccine costs 58,745 UZS. In the absence of epizootics, we consider vaccinating healthy camels impractical due to the economic burden. Instead, we recommend conducting annual disinsection, which costs approximately 136,000 UZS per camel per year. To date, we have not found literature confirming the effectiveness of camel vaccination.

In nature, camels contract plague when bitten by infected migratory fleas. These fleas are often hosted by predators that can enter great gerbil habitats. In Uzbekistan’s desert plague foci, main carriers extend their range while searching for food and interacting with rodent colonies. In other cases, great gerbils—especially during population booms—enter deep predator burrows, facilitating close contact between gerbil and predator fleas. The flea species Pulex irritans is especially associated with camel plague cases [138; pp. 38–43]. These fleas become infected through such interactions.

During gerbil population declines, ectoparasites searching for hosts may also attack other animals, including camels. The presence of these factors is supported by literature documenting plague-infected camels during periods of low rodent numbers [125; pp. 311–316; 141; pp. 237–245].

To quantitatively identify the infection mechanism in camels, data from various years described in the literature can be used as empirical indicators. It is noted that not every flea bite results in illness, and in most cases, infected camels recover [26; p. 421; 120; pp. 549–559].

Under natural conditions, A.N. Matrosov’s research [64; pp. 66–73] found that 3 out of 97 dead camels (3.3%) in the Altai region (1990–1998) tested positive for plague via bacteriological analysis. According to Z.J. Abdel [1; pp. 25–33], among 896 camels studied in the Kyzylkum and Ustyurt natural foci (1975–1988), pathogenic cultures were isolated in 8 cases (0.9%).

Camel resistance to plague is also reflected in the presence of plague-specific antibodies in their blood serum, indicating previous exposure without disease progression, which has been confirmed in multiple sources [4; pp. 71–76; 118; pp. 197–209; 158; pp. 1–8].

Nevertheless, comparative data confirm that camels infected with plague are significantly more susceptible to the disease than many other animals.

In the epizootic area, the presence of plague-specific antibodies in the blood of infected camels may reach 0.7%, according to data by N.V. Popov et al. [79; p. 52–62]. These data are close to the serological analysis results of camel serum from epizootic areas reported by N.R. Khabalova et al. [85; p. 513], where specific antibodies were detected in 1.2% of camels.

Therefore, it can be assumed that in regions with a high prevalence of plague epizootics, fewer than 1% of camels may become infected.

Based on the study of the Kyzylkum and Ustyurt natural plague foci, we can conclude that both foci remain active and retain high epizootic potential. Cultures have been identified in these foci, and the characteristics of the strains found show that 94.9 ± 1.1% of the signs are typical of plague pathogens, while 5.1 ± 1.1% are atypical, possibly due to amino acid differences or other components. This fact indicates that regardless of the stage of the epizootic process, source of isolation, or storage period, the plague microbe is evolving, and its specific ecotypes are emerging.

Each natural focus contains plague carriers that act as reservoirs of infection, although they may not be of high epidemiological significance due to irregular contact with humans. In both Kyzylkum and Ustyurt, the primary carrier is the great gerbil.

When determining the epidemic potential of Yersinia pestis strains, it is essential to constantly monitor their virulence and improve their applicability in differential diagnostics using new modern express methods such as PCR. This is especially important in field conditions and compatible with classical methods using specialized laboratory equipment.

Currently, plasmid detection in Y. pestis strains is being conducted in almost all natural foci. As a result, differences in plasmid structures from various foci have been identified, enabling the identification of specific plague carriers within a particular natural focus. For the manifestation of virulence, the F1 antigen (fraction 1) of Y. pestis is required — whether obtained in the laboratory or developed under natural conditions in rodent organisms. Variants of F1 antigen with reduced virulence are distinguishable. This antigen is a key immunogen of the plague bacterium; if removed from molecular or live vaccines, their immunogenicity decreases significantly [5; p. 1012–1020, 70; p. 122–129, 107; p. 5–12, 111; p. 3–12].

In our studies, both types of real-time PCR were used depending on location — in the field and laboratory. All collected samples were tested via PCR: 70.9% from rodents and 29.1% from ectoparasites. Materials collected from rodent organs were cultured on nutrient media and the resulting suspicious colonies were tested with real-time PCR. For direct PCR, emulsions were prepared from fleas and ticks. In parallel, microbiological studies used the same emulsions cultured on appropriate media. Out of the total, 2.5% showed positive results. All strains isolated from the natural focus tested positive by PCR, accounting for 15.3% of all studied strains.

In the Republic of Uzbekistan, for the first time in Central Asia, efforts were made to monitor and control the movement of pathogenic agents from group I–II bacterial infections. A computer-based system (PACS) was developed and applied for recording and monitoring pathogens in centralized infectious disease storage laboratories and collections. This system provides a secure, reliable, and user-friendly method for tracking pathogenic materials [37; p. 53].

The functions of the PACS computer system are divided into several groups: operations, catalogs, documents, reports, and administrative functions.

Ensuring safe and uninterrupted operations without violating plague quarantine regulations is the primary task of the anti-plague control service in implementing the plans of the Ministry of Health of the Republic of Uzbekistan. To properly plan and assess preventive measures, and to carry out anti-epidemic interventions, it is necessary to understand the theoretical aspects of epidemiology, which include biocenotic structures characterized by features of plague strains, carrier species, and the composition and abundance of ectoparasites [66; p. 6–16].

Based on the analysis of many years of data, we determined the epizootic index and the characteristics of the studied areas as plague foci for most of the epizootic sectors in the Kyzylkum and Ustyurt desert natural foci. Detailed landscape and epizootological characteristics were given for each region, indicating areas where plague might take root — i.e., microfoci — and showing the specific patterns of epizootic progression.

To achieve cost-effective implementation, we propose reducing the number of specialists in initial inspection field missions from 8–10 to 4 people, which would reduce previous expenses (10,000,000 UZS) by 50–60%.

The microbiological properties of all isolated strains were studied. The data indicate their variability based on natural sensitivity to antibiotics and additional amino acid requirements, which aids in understanding the mutability of the plague pathogen.

All of the above were encountered to varying degrees during our research. However, we have reached some conclusions not yet reflected in the literature, such as:

- Study of the streptomycin resistance traits of specific Y. pestis strains;
- Broad use of modern molecular genetic diagnostic methods (PCR) for plague under field conditions;
- Development of a computerized management system;
- Recommendation to replace costly camel vaccinations with regular annual vector control instead;
- Reduction in the number of specialists sent on field missions by half without compromising practical or economic efficiency.

CONCLUSION

1. Taking into account the danger posed by the natural plague foci in the epizootic zones of the active desert foci in the Kyzylkum and Ustyurt regions, it has been prescribed to include them in the action plan and implement appropriate control measures.
2. The detection of streptomycin-resistant strains isolated from the natural plague foci within the Republic has been noted, and the importance of the information obtained through their microbiological identification has been emphasized.
3. It was established that traditional laboratory testing methods for diagnosing plague are comparable and interrelated with real-time PCR diagnostics.
4. The Pathogen Accounting and Control System (PACS) software enables the entry, storage, and analysis of data in a systematized database, offering advantages such as tracking the movement of tubes containing strains, simplifying inventory management, and facilitating control operations. As a result, reporting has become easier and, most importantly, time-saving. This has opened new opportunities for working with scientific data related to strains.
5. Reducing by half the number of specialists dispatched on expeditions to plague natural foci makes it possible to develop and apply criteria for assessing and responding to potential risks of plague outbreaks, without compromising the effectiveness of the planned objectives.
6. In evaluating the specific role of camels in epidemic processes, it is critical to classify the grazing areas in plague-endemic zones—considering ongoing epizootics among wild rodents—into high-risk, moderate-risk, and low-risk zones. It is essential to prohibit the movement of camels to high-risk areas for grazing, identify the primary risks, and continuously monitor the epizootic-epidemiological situation in coordination with the Plague Prevention Center and veterinary services.

Abbreviations

- **SSRISC** – State Scientific Research Institute of Standardization and Control
- **ha** – hectare
- **DNA** – deoxyribonucleic acid
- **SPHSC** – Sanitary-Epidemiological and Public Health Committee
- **WHO** – World Health Organization
- **ELISA** – Enzyme-Linked Immunosorbent Assay
- **kg** – kilogram
- **LER** – Landscape-Ecological Regions
- **CIS** – Commonwealth of Independent States
- **mln** – million
- **ISS** – Industry Standard Sample (Otraslevoy Standartniy Obrazets)
- **PCR** – Polymerase Chain Reaction
- **IHA** – Indirect Hemagglutination Reaction
- **AgNR** – Antigen Neutralization Reaction
- **AbNR** – Antibody Neutralization Reaction
- **IHIA** – Inhibition of Passive Hemagglutination Reaction
- **RF** – Russian Federation
- **RAS** – Russian Academy of Sciences
- **Psb** – Pigment Absorption
- **R** – Pigment Formation
- **R+** – Pigment Positive
- **Pur+** – Ability to Synthesize Purines
- **Pgm- Psb** – Pigment Absorption Variant
- **MoH** – Ministry of Health
- **SES** – Sanitary-Epidemiological Service
- **EI** – Epizootic Index
- **ESC** – Emergency Situations Commission
- **HDI** – Highly Dangerous Infections
- **APC** – Anti-Plague Center
- **F1** – Fraction 1
- **Leu–** – Leucine Dependent
- **Leu+** – Leucine Independent

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