REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

RISK ASSESSMENT OF RABIES DISEASE IN AZERBAIJAN AND STUDY OF GENETIC ASPECTS OF THE VIRUS

Speciality: 3109.01 - Veterinary microbiology, virology, epizootology, mycotoxicology and mycology and immunology

Field of science: Biology

Applicant: **Chichak Vali Aliyeva**

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The dissertation work was carried out at the Veterinary Scientific-Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan, the Central Veterinary Laboratory of the Azerbaijan Food Safety Institute, the 3rd Biosafety Level Laboratory of the Veterinary Scientific-Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan, the Royal Vet Veterinary Clinic, and the Letgen Biotechnology Laboratory located in Izmir, Turkey.

Scientific consultant: PhD in Veterinary Sciences, Assoc., Professor
Shalala Karam Zeynalova

Official opponents:

Doctor of biological sciences, Professor
Konul Farrukh Bakhshaliyeva
Doctor of biological sciences, assoc., prof.
Mahir Ali Huseynov
PhD in veterinary
Maryam Rza Musayeva

Dissertation council BED 3.19 operating at the Institute of Veterinary Scientific Research, Ministry of Agriculture Republic of Azerbaijan

Chairman of the Dissertation council: Corresponding Member of the ANAS, doct. of biol. sciences, professor
Panah Zulfigar Muradov

Scientific secretary of the Dissertation council:
PhD in biology, assoc., professor
Kubra Yusif Yusifova

Chairman of the scientific seminar:
Doctor of biological sciences
Fuad Mamay Gulubayov

INTRODUCTION

Relevance of the topic and level of study. Rabies is a particularly dangerous viral infectious disease of warm-blooded animals and humans, it is a zoonotic disease characterized by severe disorders of the nervous system, including symptoms of encephalomyelitis. *The causative agent of rabies is a neurotropic RNA-containing virus belonging to the genus Lyssavirus of the Rabdoviridae family, which causes damage to nerve cells*[1].

Since ancient times, rabies, a disease known to humans, has spread throughout all continents of the world except Antarctica. The primary mode of transmission of the rabies virus to humans is through their being bitten by infected animals. Additionally, the disease can also enter the human body through damaged skin or mucous membranes.

Due to the fact that rabies infection results in 100% mortality, it is considered a particularly dangerous disease. *According to WHO and OIE data, approximately 160 people die from rabies worldwide every day, with one person dying every 10 minutes, resulting in nearly 60,000 deaths annually*[2].

The incubation period of rabies disease varies from several days to 12 months, typically lasting between 3 to 6 weeks on average. In areas where population density is high, continuous natural foci of the disease are formed.

The spread of rabies disease is mainly attributed to stray dogs, accounting for 99% of human deaths from rabies[3].

In European countries, the main reservoir of rabies disease is red foxes (Vulpes), while in Ukraine, Belarus, and Russia, as well as

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[1] Epizootology and infectious diseases / E.A. Aliyev, I.M. Azimov, U.M. Valiyev [etc.]. - Baku: UniPrint, - 2013. - 1070 p.

[2]The Irish Times -[Electron resource] / - Rabies transmitted by dogs kills 60,000 people a year, study finds / Report finds average of 160 people a day die of the almost completely preventable disease – 2015 .

[3] Bingham, J. The epidemiology of rabies in Zimbabwe. 1. Rabies in dogs (Canis familiaris) / J.Bingham, C.M.Foggin, A.I.Wandeler [et al.] // Onderstepoort. J. Vet. Res., 1999. 66(1), – p. 1-10.

in African, Asian, and Middle Eastern countries, dogs (Canis) are also implicated. In Turkey, both foxes and dogs play a role as reservoirs of rabies disease.

Rabies disease transmission is influenced by age and socio-economic factors. *Statistical analyses conducted worldwide have observed that rabies disease is most commonly found in children under the age of 15, particularly in impoverished families who lack access to hospitals or clinics*[4].

Rabies disease's main reservoir, considered to be dogs, is regarded as the primary preventive measure against the transmission of the disease and the elimination of rabies cases among both dogs and humans[5].

In Azerbaijan, rabies is a mandatory notifiable disease and is endemic across the entire country. Wild carnivores, stray dogs, and cats are considered the main sources of rabies and natural rabies transmission.

The characteristics of the populations of dogs, considered as the main source of the disease, such as density and growth dynamics, are unclear. Therefore, there is a need for scientific and epidemiological research on this matter. Azerbaijan has a large population of stray dogs. Current management of these animals involves programs for sterilization and vaccination against rabies. Azerbaijan has adopted the European Convention for the Protection of Pet Animals, which prohibits the killing of animals without a valid reason.

The management of stray and wild dog populations, which are influenced by various socio-economic, religious, ecological, and political factors, affects the spread of zoonotic diseases, including rabies, across the country. Every year, cases of rabies transmission among animals and incidents of human bites are recorded in the country. The primary etiological factor in bite incidents has been

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[4] Awareness – Rabies – [Electron resource] / – WHO, 5 June 2024. – URL: https://www.who.int/news-room/fact-sheets/detail/rabies, 2024

[5] Jemberu, W.T. Incidence of rabies in humans and domestic animals and people's awareness in North Gondar Zone, Ethiopia / W.T.Jemberu, W.Molla, G.Almaw, [et al.] // PLoS Negl. Trop. Dis., – 2013. 7(5). article ID e2216. – p. 1-6.

identified as dogs.

Based on the data provided over a period of five years: In 2016, there were 48 positive cases of rabies among animals, in 2017 there were 73, in 2018 there were 68, in 2019 there were 63, and in 2020 there were 29.

Regarding human cases, in 2016 there were 18,702 incidents of animal bites, in 2017 there were 18,470, in 2018 there were 31,060, in 2019 there were 40,234, and in 2020 there were 21,671. In 2016, 7 deaths were recorded due to rabies, in 2017 there were 3, in 2018 there were 5, in 2019 there were 5, and in 2020 there were 2 deaths (information provided by the Center for Specific Infectious Disease Control).

Although a small-scale project was previously carried out to assess the feasibility of oral vaccination in wildlife, the vaccine baits were distributed manually. *While the effectiveness of this method of vaccine distribution was not evaluated at the time, the scale of the process was later expanded and continued through the use of helicopters*[6].

During the execution of this project, the distribution of baits was done manually, and the effectiveness of this measure was not evaluated.

The fluctuation in the rapidity of rabies transmission among animals over the years (both increasing and decreasing), the limited understanding of the virus at the molecular biological level, the unknown migration of wild animal and stray dog populations, and the incomplete preparation of a strategy for the vaccination of wild animals and stray dogs highlight the need for broader scientific research into the deadly rabies disease.

With the joint collaboration of WHO and other international organizations such as FAO through United Against Rabies, the goal has been set to eliminate human rabies transmission by dogs by 2030. While there is extensive information available on rabies disease

[6] Aliyeva, Ch. Oral vaccination of wildlife using a vaccine - Rabistav // Agricultural and Veterinary Sciences, – 2023. 7(1), – p. 35-39.

through mass media, there is still a need for expansion of educational efforts among people.

The designation of September 28th as "World Rabies Day" each year is a significant public and political action aimed at promoting global efforts to combat rabies disease.

The object and subject of the study. During the years 2018 to 2023, brain samples collected for examination for suspected rabies cases and rabies vaccines administered to dogs have been the main subjects and objects of research.

The purpose and objectives of the study. In the most affected districts of the Republic of Azerbaijan, research has focused on studying the genetic characteristics of the rabies virus among domestic and wild animals, identifying geographical and climatic zones most prone to the disease, and determining the antibody levels after rabies vaccination.

The research objectives related to the stated goals include:

- ✓ Study of the rabies disease epizootiology and epidemiology in the Republic of Azerbaijan during the years 2018-2021, as well as the virus spread across geographic-climatic zones;
- ✓ Examination of brain samples suspected of rabies, which were submitted to the laboratory during the research years (2018-2021), using FAT and PCR methods;
- ✓ Investigation of the molecular-biological characteristics of the rabies virus isolated from the country's territory based on key genetic markers;
- ✓ Risk assessment of rabies disease and mapping of its spread among animals across the country using GIS (Geographic Information System);
- ✓ Conducting research on the immunization of wildlife;
- ✓ Comparative study and evaluation of the effectiveness of the most commonly imported vaccines in Azerbaijan.

Research methods. During laboratory examinations conducted on brain samples, both FAT and PCR analysis methods were used. Additionally, genetic analyses of the virus were conducted for the purpose of phylogenetic analysis. ELISA testing was utilized for rabies risk assessment mapping in the QGIS system and comparative analysis of vaccines.

Main provisions submitted for defense.

- ✓ Understanding the epizootic characteristics, clinical signs, pathoanatomical changes, and assessing the risk of rabies is essential in the fight against the disease.
- ✓ The application of FAT and PCR diagnostic methods on brain samples obtained from animals is fundamental for diagnosing the disease.
- ✓ Studying the molecular-biological characteristics of the virus and conducting phylogenetic analyses are crucial for selecting vaccines against the disease by comparing virus types spread in the country and neighboring countries.
- ✓ Comparative analysis of vaccines administered among dogs in recent years and determining antibody titers play a significant role in disease prevention, economic efficiency, and international transport of pet animals.

Scientific novelty of the study. For the first time in the country, the relationship between rabies seasonality and factors, as well as its transmission indicators, has been studied. The methods of oral vaccination among street dogs and wild animals have been investigated. Additionally, for the first time in the country, maps of rabies outbreak locations have been drawn using the QGIS system, and risk assessment has been conducted. Molecular genetic analyses have been carried out on rabies virus strains isolated from positive brain samples collected between 2018 and 2021, and a phylogenetic tree of the virus has been constructed. During the research, ELISA testing was conducted for the first time to measure the ability of vaccines to induce antibodies.

Theoretical and practical value of the study. As a result of the conducted research, the molecular-biological characteristics of

rabies virus circulating in various geographical and climatic zones of Azerbaijan have been studied. Based on the obtained results, proposals for the immunization of wild and domestic animals have been developed and prepared.

The epizootic analysis and modeling dynamics of the structure of areas, as well as the identification of risk factors, have been carried out. Additionally, the potential effectiveness of measures against possible ecological and socio-economic damage and measures against epizootics has been evaluated.

Scientifically grounded information can be used in forecasting, developing preventive measures, and preparing measures against epizootics. Furthermore, the data can be utilized in the development and preparation of long-term state programs encompassing communication and preventive measures against dangerous infections.

Approbation and application. Thirteen scientific works reflecting the main points of the dissertation have been published. Eight of them are articles, and five are theses and conference materials. The materials of the dissertation have been presented at the following conferences: the International Scientific-Practical Conference on Application of Innovations in the Development of Veterinary Science (Baku, 2019), 12th Edition of International Conference on Infectious Diseases (Rome, 2019), the Conference of Research Workers in Animal Diseases (USA, Chicago, 2023), and the 3rd International Conference on One Health, under the theme "One Health: Problems & Solutions" (Baku, 2023).

The name of the organization where the dissertation work was completed. From 2018 to 2023, scientific research activities were conducted in several institutions including the Central Veterinary Laboratory of the Azerbaijan Food Safety Institute, the 3rd Biosafety Level Laboratory of the Veterinary Scientific Research Institute of the Ministry of Agriculture, the Royal Vet Veterinary Clinic, and the Letgen Biotechnology Laboratory located in Izmir, Turkey.

Volume and structure of work. The dissertation consists of an introduction, six chapters, conclusions, result, suggestions and

recommendations, a list of references, appendices and a list of abbreviations. The total length of the dissertation is 156 pages of computer text, comprising 278513 characters.

CHAPTER I. LITERATURE REVIEW

In the literature review chapter, the history of rabies, characteristics of the disease, structure of the virus, molecular-genetic features, transcription of the rabies virus genes, genome replication, and protein synthesis properties are discussed. Additionally, the chapter covers the global spread of the disease across various countries. Furthermore, it touches upon vaccination measures against rabies among animals and regulations for the oral vaccination of wildlife.

CHAPTER II. MATERIALS AND METHODS OF RESEARCH

2.1. Description of the materials used in the study

In this section of the dissertation, information has been found that contradicts its own assumptions. Specifically, brain samples suspected of rabies were examined from various regions of Azerbaijan under the auspices of the Central Veterinary Laboratory of the Azerbaijan Food Safety Institute from 2018 to 2021. Records of these samples, including pathological-anatomical dissections, were analyzed. For the purpose of diagnosing rabies, brain samples were subjected to gold-standard tests, including Fluorescent Antibody Test (FAT) and Polymerase Chain Reaction (PCR). Samples confirmed positive for rabies were extracted and stored at -80ºC in specialized freezers for subsequent molecular research in RNA form. Following completion of the research, samples were safely disposed of in accordance with biosecurity protocols.

During the processing of samples and the performance of tests, biosafety regulations were strictly followed. A laboratory coat was worn, double gloves and protective goggles were used. In order to prevent contamination in the laboratory, thorough disinfection, cleaning procedures, and all relevant protocols were strictly observed.

Materials used in the laboratory were disinfected for 30 minutes using 0.5% sodium hypochlorite, 0.03% formalin, or 0.08% sodium hydroxide.

2.2. Information regarding samples submitted to the laboratory with suspected rabies from 2018 to 2021

In total, during the year 2018, 78 brain samples were submitted to the Central Veterinary Laboratory (CVL) of the Azerbaijan Food Safety Institute with suspected rabies. Out of these samples, 68 tested positive for rabies, 4 were negative, and 6 were deemed unsuitable for testing due to deterioration (old specimens). In 2019, the laboratory received 80 samples for rabies testing, out of which 63 tested positive. In 2020, there were 49 samples, with 29 testing positive for rabies. For the most recent year considered in the study, 2021, a total of 31 samples were tested, with 20 testing positive for rabies.

Overall, these figures reflect the number of brain samples tested annually at the laboratory for rabies suspicion, along with the proportion that tested positive each year from 2018 to 2021.

2.3. Extraction of cDNA product from RNA suspensions

In this section, the information regarding the extraction of cDNA products from RNA suspensions using the "Grisp Xpert One-Step RT-PCR Kit" and the gel electrophoresis of prepared PCR products has contradicted its assumptions.

CHAPTER III
EPIDEMIOLOGY OF RABIES

3.1. General Characteristics of Azerbaijan's Climate Indicators

In this section, the evaluation of geographical location and area for studying the disease as the first in the country has been conducted.

In the rocky highland regions of our country, bezoar goats and mouflons have settled. In the mountain-forest zone, species such as the stone marten, pine marten, bear, lynx, forest cat, occasionally leopard, mouflon (mountain sheep), roe deer, and wild boar (sus scrofa) can be found.

Furthermore, in this chapter, comprehensive information on the study of rabies in Azerbaijan up to this research has also been found to contradict its assumptions.

3.2. Epidemiology of rabies in Azerbaijan

Monitoring the epidemiology of rabies is crucial in Azerbaijan for analyzing the current situation related to this disease, preparing and implementing eradication programs, and evaluating other activities. Ensuring effective communication between relevant institutions is essential when there is a risk of rabies transmission or outbreak among animals and humans nationwide.

The epidemiological surveillance of rabies primarily relies on laboratory activities. In this regard, special attention should be given to the observation of suspected animals (both domestic and wild, owned and stray). Epidemiological monitoring among these animals should be intensified, and this process must be carried out continuously throughout the year across the entire country.

3.3. Conducting public awareness campaigns on rabies

Another essential part of the chapter is the public awareness campaigns conducted over the years related to the disease. Given that rabies is a zoonotic disease, continuous awareness campaigns among the public are crucial. The endemic nature of rabies in our country necessitates a serious approach to these awareness campaigns. Therefore, considering these factors, various awareness campaigns have been conducted over the years as part of our research activities. Examples of these campaigns include articles and appearances in mass media, dissemination of information to international platforms, and educational events held in schools, all aimed at raising awareness

about rabies.

CHAPTER IV
MOLECULAR BIOLOGY STUDY OF RABIES VIRUS

The sequences of the samples were obtained from Microsynth company in Switzerland (Table 1).

Table 1. Characteristics of the field samples selected for phylogeny analysis.

Sample ID	Species of origin	Location	Sampling year
Azer 1	Dog	Baku	2021
Azer 2	Jackal	Aghdash	2019
Azer 3	Cattle	Aghstafa	2020
Azer 4	Jackal	Lerik	2019
Azer 5	Jackal	Lachin	2020
Azer 6	Dog	Astara	2019
Azer 7	Yearling cattle	Göychay	2019
Azer 8	Dog	Salyan	2021
Azer 9	Horse	Fuzuli	2019
Azer 10	Fox	Gandja	2021
Azer 11	Horse	Tovuz	2020
Azer 12	Cattle	Qakh	2019
Azer 13	Cat	Sheki	2021

Analyses indicated that the majority of the samples tested during this research belong to the Central Asian subgroup CA4, associated with rabies virus groupings formed with the representative dog rabies virus from Azerbaijan (LN879480) (Table 2). Strains affiliated with the CA2 clade, originating from Turkey, Iran, and Georgia, belonging to the Balkan countries group, were observed with Middle Asian clades with sequenced Central Asia isolates. Similarly, genetic similarities were noted with rabies viruses

Table 2. Evolutionary Analysis: Evolutionary Assessment of the N-gene

The new sequence (this study)	Defined cluster	Similar sequence (GenBank Acc. No)	Origine	Nucleotide identity (%)	Collec-tion date
Azer-1	CA4	LN879480.1*	Azerbaijan	98,98	2002
		MW055108.1*	Georgia	98,98	2016
		KT965733.1	South Kazakhstan	98,76	2014
		KX148166.1	Turkiye	96,73	1993
Azer-3	ME1	MK760742.1*	Iran	99,19	2014
		KJ081443.1	Turkiye	97,96	2013
Azer-4	ME1	MK760742.1*	İran	99,80	2014
		DQ837466.1	Israel	98,58	2002
		KJ081443.1	Türkiyə	98,57	2013
Azer-5	CA4	LN879480.1*	Azerbaijan	98,99	2002
		MW177597.1	Azerbaijan	96,15	2012
		OL515137.1	Romania	96,15	2012
		OM542196.1	Poland	96,15	2001
Azer-8	CA4	LN879480.1*	Azerbaijan	98,58	2002
		KT965733.1	South Kazakhstan	98,34	2014
		KX148166.1	Turkiye	95,94	1993
Azer-10	CA4	LN879480.1*	Azerbaijan	99,39	2002
		KT965733.1	South Kazakhstan	99,17	2014
		MW035720.1	Russia	96,35	2018
Azer-11	CA2	MT079950.1*	Georgia	99,80	2016
		LN879480.1	Azerbaijan	96,15	2002
		KY002889.1	Dagestan (Rus)	99,34	2008
		KX148167.1	Turkiye	96,15	1993

circulating in Kazakhstan and the CA4 clade was formed with six

sequences from this study and an Azerbaijani sequence from Genbank (LN879480).

In general, research conducted on rabies in Eurasia has demonstrated a similar pattern to that observed in Georgia, where multiple strains have been identified. Additionally, in recent years, research in this field has also been conducted in Georgia, and comparative analyses have taken into account studies conducted there. Based on conducted phylogenetic analyses, the rabies virus currently prevalent in Georgia shares common genetic roots with RABV strains found in Azerbaijan, as well as in Europe (Estonia, Yugoslavia, Serbia) and Asia (Tajikistan, Kazakhstan, China, Russia). Furthermore, analyses of samples from various regions have provided evidence that these strains also share genetic aspects with RABV strains found in Russia, Iran, and Turkey.

Based on Azerbaijan's geographical position, it is evident that several different rabies strains circulate in the country simultaneously, often without control or prevention measures in place. The connection between Azerbaijan's rabies strains and those found in other countries in the Caucasus region suggests that multiple distinct strains coexist in the country. Analysis of samples taken from both wild and domestic animals has shown that two strains, Azer-1 and Azer-2, partially share N gene sequences with 100% homology, indicating active transmission between wild and domestic animals (Figure 1).

Genetic analyses have been and will continue to be conducted across countries worldwide. This is because the occurrence of a disease within a specific country necessitates broader and more in-depth research regarding that disease. To gain accurate and comprehensive insight into the spread of a virus within a country, the importance of genetic analyses cannot be disregarded.

As mentioned earlier, the study of transboundary pathogens and the implementation of control measures at a regional level require collaborative research, especially among neighboring countries. Moreover, conducting oral immunization campaigns for wild animals in close time intervals across countries can ensure a synchronized immunization effect at the regional level before

wildlife migration occurs.

In order to reduce the incidence of rabies within a country, it is crucial to develop and implement regional-level control plans, and to regularly organize joint training programs and initiatives. The findings of our research once again underscore the importance of these issues.

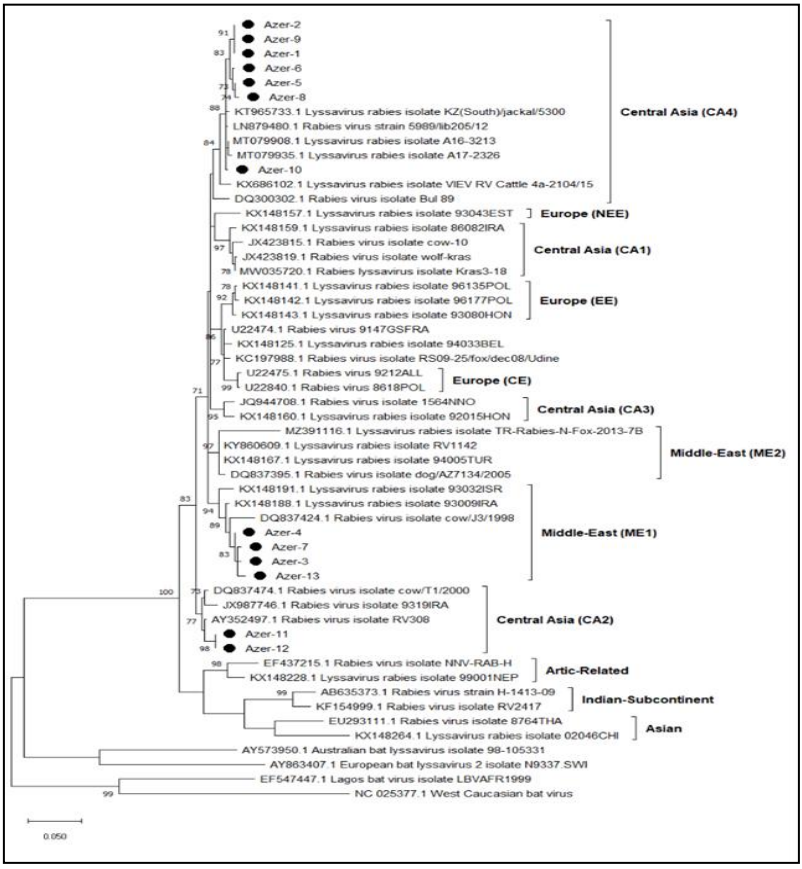


Figure 1. An overview of the phylogenetic tree. The sequences reported in this study are marked. The reference sequences are obtained from the GenBank records and their accession numbers are submitted in the figure.

CHAPTER V
RABIES RISK ASSESSMENT

5.1. Assessing the risk of rabies by country

During the country-wide risk assessment of rabies disease, the characteristics of the disease, as well as the causative agent, and possible threats were taken into account. Rabies is one of the high-risk diseases in Azerbaijan, as it is all over the world. This is due to the fact that in the case of human infection, if vaccination is not carried out quickly, it will result in death, as well as in the case of infection, death occurs among animals.

The purpose of risk assessment for rabies is to prevent the spread of rabies in the country and play an important role in protecting the health of people and animals as well as tourists traveling to the country.

During the risk assessment for our country, along with considering the general factors, the geographical location of the country, the uncontrolled animal population under occupation and the situation related to the rabies disease in the neighboring states were taken into account.

In general, conducting rabies risk assessments across different countries plays a key role in preventing the emergence and spread of the disease. In European countries in particular, the primary step taken to prevent the spread of rabies among domestic animals is to ensure that animals imported into the country are vaccinated against rabies. Additionally, the level of rabies antibodies in the animal's blood serum is tested. According to established regulations and standards, a serological immunity level of at least 0.5 IU (International Units) is considered acceptable.

5.2. Conducting risk assessment in rabies disease taking into account different infection indicators

In this part, information about the risk level of the dangers that play a role in the spread of rabies disease between humans and

animals is reflected. As a result of scientific studies conducted by researchers, it is predicted that more Lyssavirus species will be identified and classified among bat populations. Rabies virus (RABV) is the most widely distributed virus of the Lyssavirus genus among animals worldwide. Other viruses belonging to the Lyssavirus genus have more limited geographical distributions. The rabies virus is sensitive to various concentrations of formalin, phenol, halogens, mercury compounds, mineral acids, soap solutions, and other disinfectants. The virus remains stable in environments with a pH range of 5 to 10; however, it becomes unstable at pH levels below 4 or above 10. Desiccation, ultraviolet radiation, and sunlight are lethal to the virus, and it does not survive long in the environment. Laboratory experiments have shown that the virus can survive for up to 2 hours at 37°C, but 90% of the virus is inactivated within 4 to 6 hours under these conditions. During risk assessments, animals are classified into three categories based on their susceptibility to rabies: no risk, low risk, and high risk. Wild animals and bats are listed among the high-risk group. The United Kingdom has conducted a global rabies risk potential assessment, categorizing countries into three groups: no risk, moderate risk, and high risk. According to these assessments, countries such as Azerbaijan, Turkey, Armenia, Georgia, Iran, and Russia, among many others, are classified as high-risk countries.

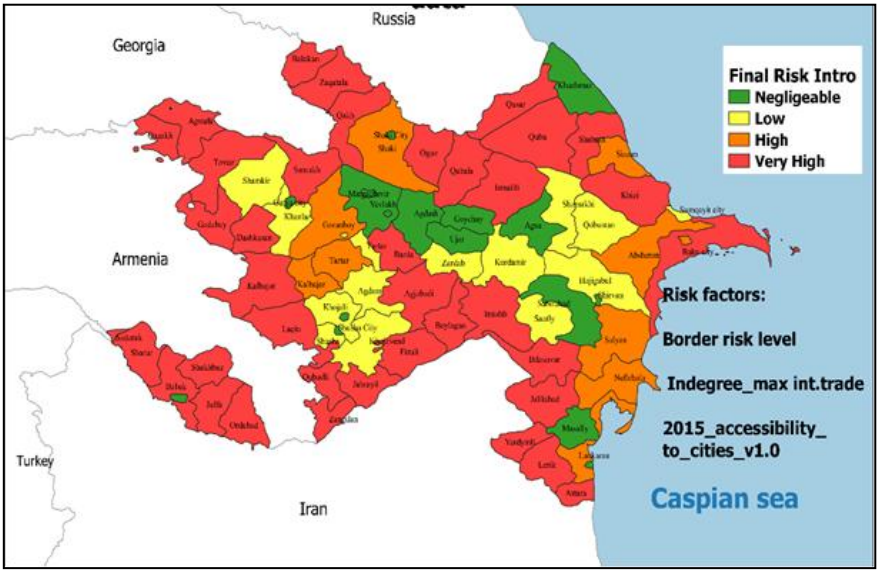
5.3. Annual risk assessment and mapping of rabies by means of GIS (QGIS)

It has been determined that in order to establish an effective rabies control mechanism at the national level, there is a need for highly sensitive surveys, a comprehensive database, and a robust monitoring system. To achieve this, accurate data were first collected on the population of animals susceptible to rabies, as well as on the occurrence and spread of the disease.

QGIS (Quantum Geographic Information System), a type of GIS, is currently considered one of the most advanced tools for conducting epidemiological research on diseases. One of the main

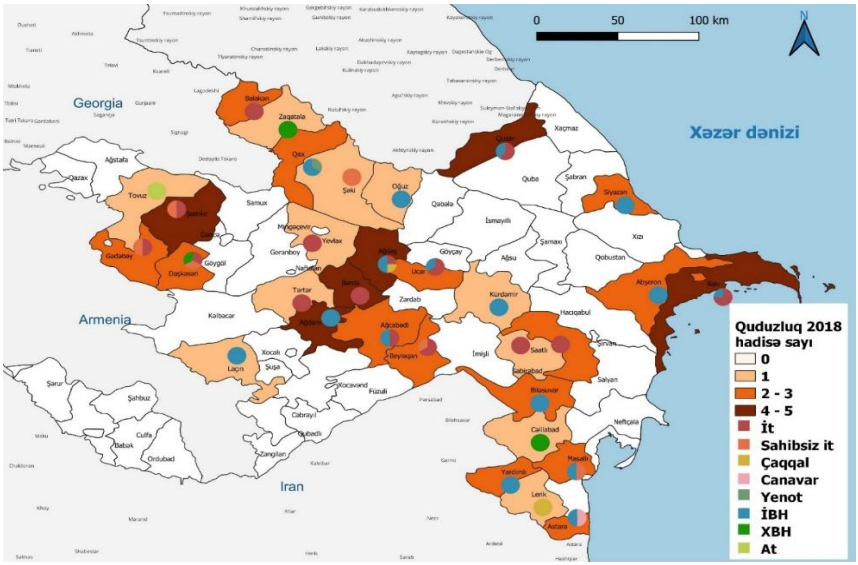
advantages of GIS is its free accessibility, along with its ability to utilize both vector and raster data.

In the neighboring countries, Turkey, Russia, Iran and Georgia, rabies is also endemic and occurs with different frequencies in almost the entire territory. In these countries, rabies is observed every year, and the number of stray animals is considered one of the main problems in the mentioned countries. Therefore, this factor was initially taken into account during the risk assessment mapping (Map 1). This means that the uncontrolled migration of animals due to weather conditions, hunger and other environmental factors in the areas near the border creates favorable conditions for the spread of rabies.



Map 1. Risk factors (bordering neighboring countries).

In 2018, the observation of the regions where rabies occurred occurred mainly in the cities of Gusar, Shamkir, Aghdash, Barda, Aghdam, Baku and Absheron with positive cases of rabies (Map 2). The number of animals with positive cases was 10 dogs, 30 stray



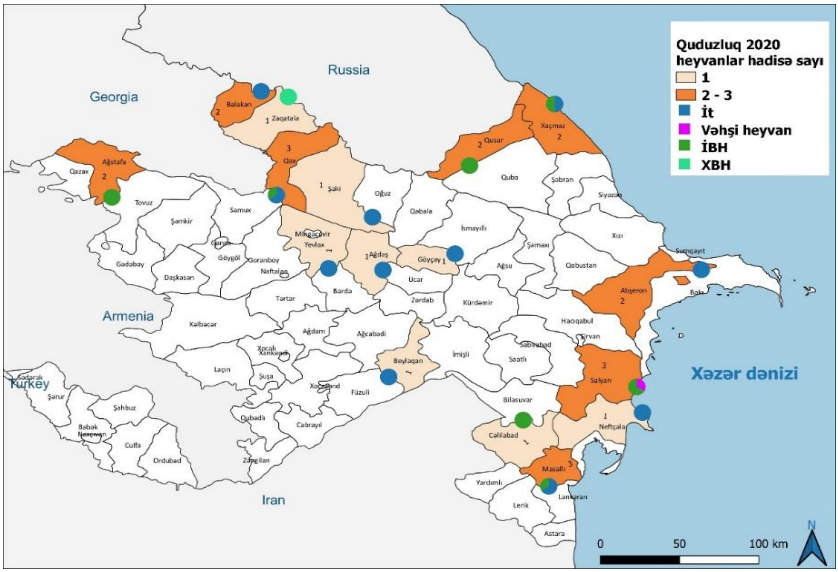
Map 2. Cases of rabies among animals in 2018 (by animal species).

dogs, 21 LR, 3 LR, 2 jackals and other animal species.

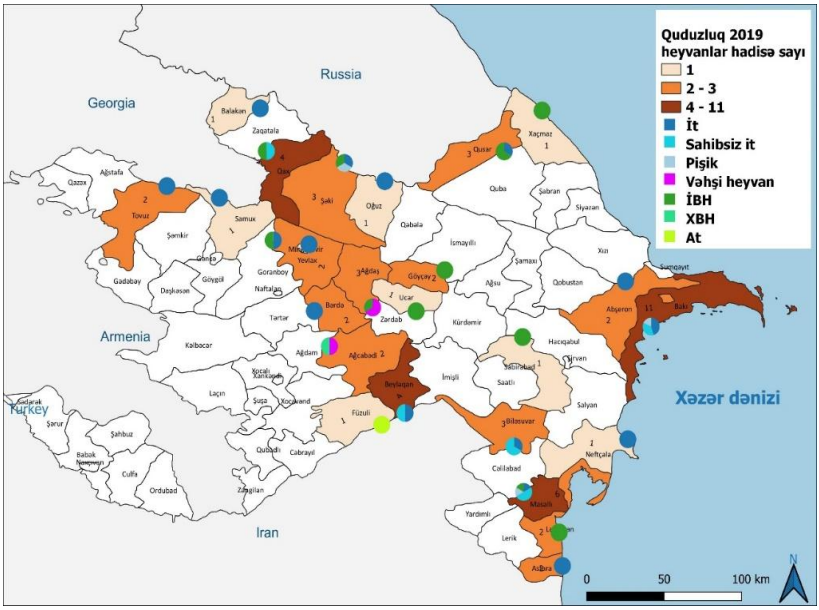
According to the indicators of the regions where rabies cases occur, it is possible to see the border regions of our lands occupied by Armenia, as well as the border areas with Russia and Georgia.

During 2019, there were 63 cases of rabies among animals across the country, and the number of positive cases was more in Baku, Absheron, Gakh, Beylagan and Masalli. Due to the geographical location of the areas, it is possible to see the presence of border areas. Of the positive samples, 39 were from dogs, 14 from stray dogs, 1 from a stray cat, 1 from a wildcat, 2 from jackals, 1 from a donkey, 2 from horses, and 3 from domestic cats. (Map 3).

Among the samples that entered the laboratory in 2020, 29 were positive for rabies, 23 were negative, and 1 sample gave a suspicious result. The samples with a positive result for rabies were identified



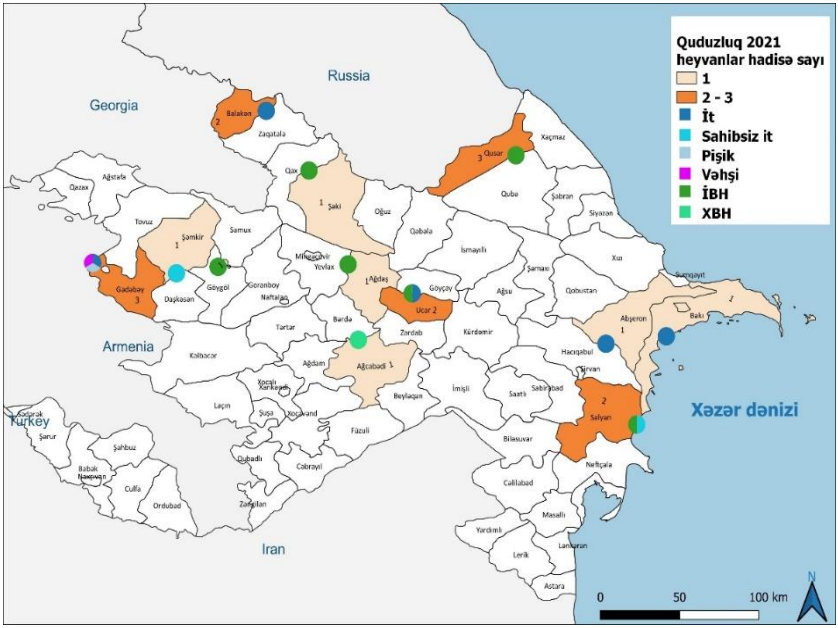
Map 3. Animal rabies cases in 2019 (by animal species).



Map 4. Animal rabies cases in 2020 (by animal species).

mainly among the samples sent from the areas including Agstafa, Gusar, Salyan, Khachmaz, Absheron economic regions and cities (Map 4). 14 of the positive samples were dogs, 10 were LR, 1 were SR, 2 were cats and 2 were jackals.

During the year 2021, the samples with a positive result for rabies were mainly determined among the samples sent from the areas including Balakan, Ujar, Absheron, Gadabay, Salyan economic regions and cities. Of the positive samples, 9 were dogs, 9 were Ibh, 1 was xbh, and 1 was a fox. The areas with the highest incidence of rabies in the years are border areas, occupied lands (these lands are now free, however, the analysis of the epidemiological situation in those lands has not been completed), and due to urbanization, stray dogs have created a large population due to their foraging and mating instinct. Baku-Absheron (Map 5)



Map 5. Animal rabies cases in 2021 (by animal species).

During the research years, dogs accounted for 61 percent of total rabies cases in 2018, 68 percent in 2019, 52 percent in 2020, and 44 percent in 2021.

Thus, according to the results of a 4-year study, the direct role of dogs (especially stray dogs) in the occurrence and spread of rabies cases across the country has been confirmed. For this reason, it is no coincidence that all the results during the sequencing of the samples are similar to the dog sequencing results. Based on the risk assessment carried out during the study, it was determined that the main risk factors for the occurrence of rabies in the country are the fact that the disease is historically endemic (also at present), the neighboring countries are unhealthy due to rabies, animal movements, usually due to hunger in the winter months. long-range movement in fauna (transition from country to country), uncertainty in the number of stray dogs (population size unknown) and overpopulation.

At the same time, it is likely that bats, which are considered the main carriers of rabies around the world, living in our country may cause the disease to spread. Therefore, blood-sucking bats are mainly found in abandoned areas, caves, and regions, and the lack of research on bats is one of the factors that make the presence of rabies in the country and the risk of spreading it to large areas relevant.

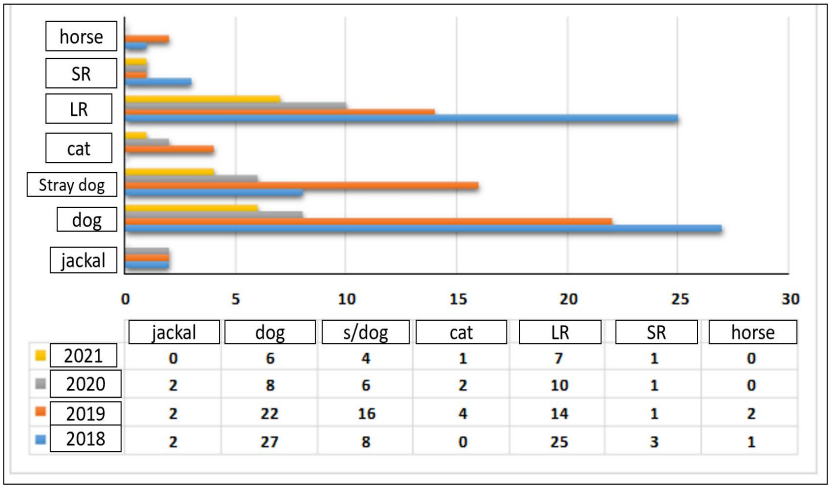
5.4. Study of the seasonal factor in the spread of rabies

The seasonality and socio-economic effects of the disease are also reflected in the dissertation.

According to the results of the studies conducted during the research years, according to the frequency of occurrence of the disease by season, the decrease in the incidence of the disease was towards the spring, winter, summer and autumn months. At the same time, statistical analyzes were conducted on which species of animals the observed rabies cases occurred during the mentioned years (Table 3, Graph 1).

Table 3. Seasonal dynamics of rabies occurrence among animals by year

Year	December-Feburary	March-May	June-August	September-November
2018	16	21	17	14
2019	18	24	8	13
2020	17	5	3	4
2021	4	8	4	4



Graph 1. Incidence of rabies among animal species

CHAPTER VI

COMPARATIVE ANALYSIS OF VACCINES USED AGAINST RABIES AND ASSESSMENT OF THE SOCIO-ECONOMIC EFFECTS OF RABIES

Comparative analysis of rabies vaccines. As a result of research conducted on rabies, it was determined that the main spreader of the disease in the country is dogs. Stray dogs usually

transmit the disease from wild animals to humans. Also, they themselves become infected with rabies and spread the disease to large areas. As the main source of infection, a comparative study of the most widely used vaccines in the country was conducted on dogs. The main purpose of this study is to determine the immunity induced by the administered vaccines and to monitor the changes in blood parameters among dogs after vaccination. For this purpose, antibody was determined after vaccination by means of ELISA. This phase of the research work was carried out at VSRI and Royal Vet Veterinary Clinic.

Initially, the most commonly used vaccines for dogs were selected. Thus, in making this selection, annual vaccination reports sent by veterinarians to AFSA were reviewed and individual veterinarians were consulted. In addition, it was taken into account during the selection of vaccinations administered to stray dogs at the expense of the state. As a conclusion of these works, three most used vaccines - Multikan 8, Biocan R and Nobivac Rabies - were selected for the purpose of research.

The results of the conducted studies were analyzed separately. Based on the average number of the titer level created by each vaccine in animals after application, the average titration number of the Nobivac Rabies vaccine administered to animals in the form of a single dose was 102.2, the Biocan-R vaccine was 95.2, and finally the average titration number of the Multikan-8 vaccine was 74. In general, a titration obtained above 50 is considered to be higher than 0.5 in normal international units, i.e. a valid quantity. The mean titration number of all three vaccines indicated a high probability that animals would develop anti-rabies antibodies during their administration. Separate analyzes by vaccine showed high antibody titers in 15 animals after Nobivac Rabies and Biocan-R administration, but 5 out of 15 animals had lower titers after vaccination with Multican-8. Although more than 50% (67%) of the 15 animals we vaccinated with Multikan-8 had antibody titers within the guidelines, Multikan 8 was not recommended compared to Nobivac Rabies and Biocan-R. This was due to the determination of a suitable antibody titer in each of the total 30 animals vaccinated

with Nobivac Rabies and Biocan-R, as opposed to Multikan-8.

In addition, when comparing the amount of antibody titers in the blood of animals vaccinated with Nobivac Rabies and Biocan-R, it was determined that the level of antibodies in the blood of animals vaccinated with Nobivac Rabies was higher than the amount of antibodies in the blood of animals vaccinated with Biocan-R.

Assessment of the socio-economic effects of rabies. If we look at the economic losses caused by rabies in the country, we should first pay attention to the number of productive animals that died due to this disease. Thus, the damage caused to agricultural animals due to rabies was as follows:

– 21 LR and 3 SR in 2018
– 14 LR and 1 SR in 2019
– 10 LR and 1 SR in 2020
– In 2021, 9 LR and 1 SR were destroyed.

Since the main losses among farm animals occurred in cattle, the calculation of economic damage was primarily conducted based on these animals. Nevertheless, throughout the research years, attention was also drawn to the economic losses caused by the disease among other animals, particularly small ruminants and equines.

Since the dynamics of the rabies disease show uncertainty, the increase and decrease in the number of positive results over the years replace each other. For this reason, it was determined that the decrease in the dynamics for certain years did not lead to the spread of the disease across the country and the continuation of the trend towards the reduction of economic damage.

In case of rabies disease, the economic damage caused in animal husbandry is classified as follows:

- Damage caused by the death of animals or forced sterilization (due to loss of live weight, meat, milk and other products).

The damage caused by the death of animals was calculated using the following formula:

$$\mathbf{ED=D \times W \times P}$$

ED - economic damage, D - the number of dead (forced slaughtered) animals, W - live weight of dead (forced slaughtered) animals, P -

price of live weight of animals (Tables 4, 5, 6, 7).

Table 4. Livestock

Year	Dead (forcibly slaughtered) animals		Live weight loss total (kg)	The cost of live weight (manat)	Total economic loss (manat)
	head	Waight (kg)			
2018	21	350	7350	4,5	33075
2019	14	350	4900	4,6	22540
2020	10	350	3500	4,8	16800
2021	9	350	3150	5	15750
2022	4	350	1400	5,2	7280
Total amount					**95445**

Table 5. Milk loss in cattle

Year	Dead (forcibly slaughtered) animals		Product loss total (litres)	Selling price of 1 liter of milk (manats)	Total economic loss (manat)
	head	average annual milk yield (liters)			
2018	21	2500	52500	0,7	36750
2019	14	2500	35000	0,7	24500
2020	10	2500	25000	0,7	17500
2021	9	2500	22500	0,9	20250
2022	4	2500	10000	1	10000
Total amount					**109000**

Table 6. Small ruminants

Year	Dead (forcibly slaughtered) animals		Live weight loss total (kg)	The cost of live weight (manat)	Total economic loss (manat)
	head	Waight (kg)			
2018	3	60	180	7	1260
2019	1	60	60	7	4200
2020	1	60	60	7	420
2021	1	60	60	7,5	450
2022	9	60	540	7,5	4050
Total amount					**10.380**

Table 7. Equines

Year	Dead (forcibly slaughtered) animals	Average price of an animal (manats)	Total economic loss (manat)
2018	2	500	1000
2019	3	500	1500
2020	3	500	1500
2021	2	500	1000
2022	3	500	1500
Total amount	13		**6500**
Total amount			**42500**

CONCLUSION

Based on the literature and the results of our research, rabies remains relevant nationwide and should be monitored in all regions. While rabies has become a historical issue in many developed countries, Azerbaijan still ranks among high-risk countries.

As a comprehensive outcome of our research efforts, the selection of vaccines imported into the country should consider the genetic relatedness of rabies viruses circulating in neighboring countries such as Georgia, Russia, Iran, and Turkey. Choosing vaccines prepared from effective strains specific to the region is crucial.

Among vaccines applied among dogs, the Netherlands-produced Nobivac-Rabies is recommended due to its high antibody titer levels and safety parameters.

The characteristics of the "Rabistav" vaccine, administered for the first time via helicopter for oral vaccination of wildlife in the country, have been studied, and its effectiveness has been determined post-application.

RESULTS

1. To assess the probability of rabies disease occurrence, a scientific risk analysis of rabies transmission between animals and humans was conducted, and maps of the disease foci across the country were created using GIS. The risk analysis of rabies disease has served as a tool in implementing necessary measures in the fight against rabies among animals, including the development of guidelines for managing stray dog populations [4, 6, 7].
2. As part of the preventive vaccination against rabies in domestic animals, a comparative analysis of the most used vaccines was carried out for the first time. Among them, the 'Nobivac Rabies' vaccine, which was distinguished by its high vaccination titer, was considered the most effective [8].
3. Based on the results of phylogenetic analysis, a genetic similarity was found between the rabies virus circulating in Azerbaijan and those circulating in Georgia, Russia, Iran, and Turkey. Additionally, the analysis showed that most of the samples tested during this study were related to the Central Asia subgroup CA4, formed by the representative dog rabies virus from Azerbaijan (LN879480) [2].
4. For the first time, oral vaccination of wildlife was conducted using a helicopter, and the Rabistav vaccine was applied [3].

5. The investigation of the disease occurrence dynamics revealed that the disease most occurred between March and May (21 positive cases in 2018 – 31%, 24 positive cases in 2019 – 38%, 8 positive cases in 2021 – 40%). In contrast, in 2020, the highest number of cases occurred between December and February (17 positive cases – 59%) [1, 5].

RECOMMENDATIONS

1. The characterization of the population of dogs, considered the main source of rabies in Azerbaijan (density, growth dynamics), remains unclear. Given the significant population of stray dogs across the country, there is a need for broader scientific and epidemiological research in this regard.
2. Risk assessment for rabies transmission between animals and humans should be conducted with a more precise scientific approach to evaluate the likelihood of emergence of rabies sources.
3. Prevention of rabies should focus on preventing the disease from spreading among animals and humans. Therefore, registration of all domestic dogs and cats should be conducted, and their preventive vaccination implemented. International guidelines should be followed when transporting animals from one country to another. The presence of stray dogs and cats in residential areas should be minimized. Animals that have bitten people or other animals should be brought to animal shelters and kept under observation for 10 days.
4. Collection and analysis of relevant data on the population of wild carnivores should be carried out.
5. Evaluation of the effectiveness of ongoing preventive vaccination measures against rabies should be conducted regularly. For this purpose, traces of the tetracycline marker included in the composition of the vaccine should be calculated from the canine teeth of captured wild

animals to determine the effectiveness of vaccination.

6. Experts should be trained to collect samples from wild animals (dead or suspected wild animals) for the purpose of rabies control. In combating rabies, mass parenteral vaccination of dogs should be carried out in parallel with oral vaccination of foxes, considering the epidemiological role of fauna in rabies transmission.
7. Laboratory methods including virological and molecular biological methods are suitable for application. For rabies diagnosis, the fluorescent antibody test (FAT) should be used as a confirmatory test, and polymerase chain reaction (PCR) should be used as a diagnostic test.
8. Due to their ability to induce high antibody titers in dogs, the use of Nobivac Rabies and the oral vaccine Rabistav in the form of bait for wild animals are recommended.

List of published scientific works related to the dissertation topics

1. Aliyeva, Ch., Aliyeva, T. The importance of risk assessment in the fight against rabies // Proceedings of the International Scientific-Practical Conference on "Application of Innovations in the Development of Veterinary Science", – Baku: – November 25-27, – 2019, – pp. 140-145.
2. Aliyeva, Ch., Aliyeva, T. Study of rabies virus circulating in Azerbaijan // 12th Edition of International Conference on Infectious Diseases. – Rome, Italy: – April 22-23, – 2019, Vol. 5, – p. 33.
3. Aliyeva, Ch.V., Zeynalova, Sh.K. Monitoring of seasonal dynamics in rabies disease // – Russia: Veterinary Medicine Today, – 2023, 12 (2), – pp. 154-157.
4. Aliyeva, Ch. Phylogeny analysis reveals the circulation of three geographical lineages of rabies virus in Azerbaijan / Ch. Aliyeva, T. Aliyeva, Kh. Bayramov [et al] // – London: Clinical Microbiology, – 2023, 12 (2), Article No. 1000333, – pp. 1-7.

5. Aliyeva, Ch. Oral vaccination of wildlife using a vaccine – Rabistav // Agricultural and Veterinary Sciences, – 2023, 7 (1), – pp. 35-39.
6. Aliyeva, Ch., Zeynalova, Sh. Scientific risk assessment of rabies // One Health Journal (Ukraine), – 2023, 23 (1), – pp. 47-52.
7. Epizootiology and risk of rabies spread in different natural climatic zones of the Republic of Azerbaijan / Ch. Aliyeva, A. Gojayev, T. Aliyeva [et al.] // – Nakhchivan: Scientific Works of Nakhchivan State University, Series of Natural and Medical Sciences, – 2023, No. 3 (124), – pp. 133-137.
8. Aliyeva, Ch. A retrospective analysis of a feline rabies case in Baku, Azerbaijan // – Baku: Khazar Journal of Science and Technology, – 2023, No. 2, – pp. 5-10.
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12. Aliyeva, Ch. The tracking of seasonal dynamics in rabies disease // One Health Conference, Khazar University. – Baku: – June 1-2, – 2023, 7 (2), – pp.58.
13. Aliyeva, Ch., Zeynalova, Sh., Aliyeva, T. Study of molecular characteristics of rabies virus spread in Azerbaijan (2018–2021) // Conference of Research Workers in Animal Diseases. – Chicago, USA, – 2023, – p. 169.

The defense will be held on 20 June 2025 at 11⁰⁰ at the meeting of the Dissertation council BED 3.19 operating at the Institute of Veterinary Scientific Research of the Ministry of Agriculture of the Republic of Azerbaijan.

Address: Baku city, Nizami district, Boyuk Shor settlement, 8th Kondalan Street.

Dissertation is accessible at the library of the Veterinary Scientific Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan.

The electronic versions of the dissertation and abstract are available on the official website of the Veterinary Scientific Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan (https://www.beti.az//).

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