REPUBLIC OF AZERBAIJAN

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ABSTRACT

EPIDEMIOLOGICAL CHARACTERISTICS AND MOLECULAR STUDY OF NODULAR DERMATITIS, SCHMALLENBERG, AND RABIES DISEASES IN ANIMALS IN AZERBAIJAN

Speciality: 3109.01 – "Veterinary microbiology, virology, epizootology, mycotoxicology with mycology and immunology"

Field of science: Biology
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INTRODUCTION

Relevance of the Topic. Controlling the spread of dangerous infectious diseases remains a pressing issue worldwide. The group of hazardous diseases includes rabies, newly emerging and understudied Schmallenberg viruses, and nodular dermatitis. The absence of proper epidemiological surveillance and multiplex diagnostics creates conditions for the wider spread of these diseases. Changes in the environment directly impact the emergence, spread, and evolution of infectious diseases, particularly those transmitted by vectors. Global change refers to ecological shifts resulting from human activities that affect the fundamental mechanisms operating within the biosphere. Pathogens, which are the causative agents of infectious diseases, are distinguished by their high diversity and complexity.

Azerbaijan is considered endemic for Schmallenberg, nodular dermatitis, and rabies, yet currently, no characterization (molecular-biological) of the viruses responsible for these diseases is being conducted.

Over time, microorganisms undergo changes in adaptive processes that determine their evolution, which, in turn, leads to the emergence of new infectious diseases, the elimination of existing ones, or their transformation. Therefore, the mutations and adaptations exhibited by viruses are considered normal. The pathogens of the dangerous infectious diseases listed below (nodular dermatitis and Schmallenberg) belong to previously unstudied viruses and newly emerging infectious diseases: a known infection spreading to a new geographical region or population; a new infection arising from evolution or change in an existing pathogen; or

a previously unknown disease or a pathogen diagnosed for the first time.

In diseases transmitted through a vector (mosquitoes—*Culicoides spp.*), in addition to the pathogen and the host, a third party is required during the infection cycle—the vectors. This fact leads to more complex eco-epidemiological consequences. The habitats of arthropod vectors depend on environmental conditions, including temperature variability, humidity, the location of water bodies, and more. *"Arthropod vectors are capable of spreading the disease to new hosts"*[1].

Since November 2011, the Schmallenberg virus has spread widely among large and small ruminants in Germany, the Netherlands, Belgium, the United Kingdom, France, Luxembourg, Italy, and later Spain. Shortly thereafter, the virus spread to many parts of the continent and the British Isles. The virus infects both domestic and wild large and small ruminants, but there is no evidence that humans are susceptible to the disease. The Schmallenberg virus is transmitted by vector carriers, primarily biting midges of the *Culicoides* genus (*C. obsoletus sensu stricto*, *C. scoticus*, *C. chiopterus*, *C. dewulfi*, *C. nubeculosus*). Infected young animals typically do not show clinical signs (subclinical infection), exhibiting only mild, non-specific symptoms such as fever, diarrhea, or reduced milk production for a few days. The virus's ability to cross the placenta leads to premature births and stillbirths.

"*Congenitally infected lambs or calves may exhibit varying degrees of arthrogryposis, hydranencephaly, torticollis, kyphosis, lordosis,*

[1] Afonso A, Abrahantes J.C, et al. The Schmallenberg virus epidemic in Europe-2011–2013 // Prev Vet Med. 2014;116: pp. 391–403

[2] Chowdary R., Street C., et. al., Genetic characterization of the Wyeomyia group of orthobunyaviruses and their phylogenetic relationships// J Gen Virol.2012, 93 (5), 1023

scoliosis, ankylosis, and brachygnathia, reflecting the gestational age of the fetus during infection."[2]. The negative impact of the Schmallenberg virus infection is greater in sheep compared to large ruminants.

In 2012, the virus affected 31 districts in Azerbaijan, causing an outbreak across the entire country. As a result, the rate of abortion among animals reached up to 90%. No vaccination against the disease is carried out in Azerbaijan.

Nodular dermatitis (LSD) is a viral disease of large ruminants and "*can be compared to foot-and-mouth disease in terms of the economic damage it causes*"[3].

Mortality rates of 60% or higher have been observed in some cases, though generally, they are lower. Nodular dermatitis (ND) is a transboundary viral disease of large ruminants. The virus belongs to the *Capripoxvirus* genus within the *Poxviridae* family. Nodular dermatitis is prevalent in southern and eastern Africa, but since the 1970s, the virus has spread northwest across the continent to the western Sahara. From 2000 onward, it spread to several Middle Eastern countries, and in 2013, cases were confirmed in Turkey.

The causative virus is linked to sheep pox. The Neethling poxvirus is recognized as the prototype of the ND virus. The disease spreads in epidemic or sporadic forms. New infection outbreaks are often recorded in areas far from the previous spread zone. The risk of spread is higher during humid summer months, though it can occur

[3] https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/LUMPY_SKIN_DISEASE_FINAL.pdf.

in winter as well. It is most common along water basins and in low-lying areas. Quarantine restrictions designed to limit the spread of the infection have been unsuccessful, leading to the assumption that insects serve as vectors. Experiments have shown that three species of hard ticks found in Africa are biological transmitters of the virus. African buffaloes are known carriers of the disease.

In affected animals, painful subcutaneous swelling develops, followed by fever, nasal discharge, hypersalivation, and later, characteristic nodules on the skin and other parts of the body in 50% of susceptible cattle. The incubation period of the disease is 4–14 days. Control and prevention of ND involve vaccination, quarantine, and movement control of cattle; vector control, slaughter of infected and exposed animals, and area sanitation through disinfection. Vaccination is reported to be the most effective method of controlling ND in both endemic and non-endemic regions. Currently, only live vaccines are available for ND, and while different vaccines are licensed for use in various countries, the most commonly used strain in attenuated vaccines is Neethling. According to reports, this vaccine has been highly effective in controlling epidemics in the Balkans. In countries where goat pox is prevalent, the attenuated Gorgan goat poxvirus vaccine can be used.

"In 2014–2015, the mortality rate of animals due to nodular dermatitis in Azerbaijan was 22.5%"[4].

Rabies is a global public health problem. The World Health Organization estimates that more than 15 million people worldwide receive post-exposure vaccinations each year. Rabies is an infectious disease that is almost always fatal once clinical symptoms appear. In approximately 99% of cases, the rabies virus is transmitted to

[4] Franka R.T., Smith, Dyer J. L., Wu X., Niezgoda M., Rupprecht C. E., 2013: Current and future tools for global canine rabies elimination. Antiviral Res. 100, 220–225.

humans from domestic dogs. In this context, rabies can affect both domestic and wild animals.

The rabies virus (RABV), a member of the Lyssavirus genus in the Rhabdoviridae family, is a neurotropic virus that causes fatal encephalitis in warm-blooded animals. RABV has a single-stranded RNA genome of approximately 12 kb with negative polarity, encoding five genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and an RNA-dependent RNA polymerase (L). The virus is transmitted to humans through bites or scratches, typically via saliva.

Rabies is present on all continents except Antarctica, with 95% of fatalities occurring in Asia and Africa. Rabies requires constant large-scale monitoring as part of a system of anti-epizootic and anti-epidemiological measures in animals. The results of rabies monitoring studies are regularly published in specialized international journals such as the *Rabies Bulletin Europe (RBE)* and the *World Rabies Service (WSR)*.

Modern rabies viruses (RABV) are divided into two main groups with distinct evolutionary trajectories based on their diversity, history, and recent research. Based on new genomic sequencing, isolates are grouped into two large clusters with several sub-clades. The two main clades are bat-associated RABV clusters: one clade consists of isolates from bats in the Americas. Researchers report *"a very large clade within the dog-associated RABV group: two clades in Africa, an Arctic clade, clades in Asia and the Indian subcontinent, and the more widespread 'Cosmopolitan' clade"*[5].

Rabies is widespread in Azerbaijan. Each year, cases of the disease occur among humans and animals, resulting in deaths of

[5] Vos, A., Freuling C., Eskiizmirliler S., Un H., Aylan O., et al, Rabies in foxes, Aegean region, Turkey// Emerg. Infect. Dis. 15, 2009, pp.1620–1622.

individuals who either did not receive timely vaccination or were not vaccinated at all. Between 2014 and 2018, 220 positive cases were recorded among animals. Currently, PCR technologies are used in conjunction with sequence analysis, making it an invaluable method for detecting the genetic relationships of rabies viruses and their strains. *"Restriction analysis of PCR products is conducted for the comparative characterization of the genomes of virus strains"* [6] .

Thus, limited information about the disease in domestic and wild animals, the lack of study on the molecular-biological properties of viruses, and the genetics of isolated strains make this issue highly relevant.

Based on the above, the objectives and tasks of the dissertation are outlined below.

Objectives and Tasks of Research

1. Study of the epidemiological characteristics of Schmallenberg, nodular dermatitis, and rabies viruses;
2. Investigation of the impact of ecological factors on the spread of viruses.
3. Examination of the causative agent of Schmallenberg disease and its vector carriers, *Culicoides*;
4. Molecular-genetic characterization of the Schmallenberg disease virus;
5. Study of the seroprevalence of Schmallenberg disease;

[6] Marston DA, McElhinney LM, Johnson N, Müller T, Konzelmann KK və başqaları. (2007) Avropada 1 və 2-ci növ yarasa lissavirusunun tam genom ardıcıllığının digər lissaviruslarla müqayisəli təhlili və G-L 3' translayisa edilməyən regionda saxlanılan transkripsiya ləğvi və pliadenilləşmə motivi üzrə sübut.

6. Molecular epidemiology of nodular dermatitis disease and molecular-genetic analysis of the nodular dermatitis virus using modern diagnostic methods;
7. Investigation of the immune status of animals immunized against nodular dermatitis with various vaccines, considering that the nodular dermatitis virus belongs to the same family as the sheep pox virus;
8. Study of the seroprevalence of nodular dermatitis disease;
9. Molecular-genetic analysis of the rabies virus;
10. Molecular-genetic study of rabies virus strains;
11. Selection of specific primers for amplifying different segments of the rabies virus genome and optimization of PCR;
12. Differentiation of vaccine strains and street isolates of the rabies virus based on restriction analysis of PCR products;
13. Submission of nucleotide sequences of Azerbaijan isolates to the National Center for Biotechnology Information (NCBI).

Research Methods.

Samples were collected from 31 districts of Azerbaijan. The collected materials included aborted fetuses, tissues, and serum samples. To detect viral RNA in extracts, the reverse transcription quantitative PCR (RT-qPCR) method was used. For the examination, the LSI VetMAX™ Schmallenberg Virus (SBV) – S Gene – TaqMan™ RT-qPCR kit was employed to detect SBV. Tests were conducted on the RotorGene 6000 (Qiagen, Germany) until 2016 and subsequently on the BioRad CF96 (Bio-Rad Laboratories, USA). For the study of nodular dermatitis, the ID Screen® Capripox Double Antigen Multi-species ELISA kit from IDvet was used. Blood samples collected before vaccination and one month after were utilized the test. For molecular-biological analyses, a 10% suspension prepared from skin, nodules, and internal organs was used. Samples were extracted using a DNA extraction kit manufactured by Qiagen (USA).

The Mastermix Capripox Virus Triplex PCR kit was employed. After amplification, PCR products were sequenced by Eurofins Genomics (Ebersberg, Germany) using the same M13 primers (M13 Forward and M13 Reverse (-29)) used in the PCR, along with three internal forward and reverse primers (JW662-684F, JW938) in both directions.

Sequences were assembled using the ContigExpress program in Vector NTI software, version 11 (Invitrogen, France). The BIOEDIT program was used to interpret gene sequences. Identity percentages and similarity scores were determined in BIOEDIT. Phylogenetic analyses were performed using the PhyML method (GTR model, 1000 replicates) with the SeaView software. The graphical PhyML tree of the N gene was visualized using Evolview (http://www.evolgenius.info/evolview), with each node calculated using 1000 replicates.

Provisions Submitted for Dissertation Defense

- The change of seasons and ecological factors in the spread of the Schmallenberg virus indicate that the disease does not have a seasonal character.
- Culicoides are possible vectors in the transmission of Schmallenberg disease.
- The epizootiological characteristics of nodular dermatitis confirm that the virus entered Azerbaijan from Iran.
- The detection of new clinical symptoms in the virus is an indicator of an increased mortality rate.
- Rabies strains circulating in Azerbaijan indicate that the virus belongs to three subclades.

Scientific Novelty of the Research. As a result of the conducted research, it was determined for the first time in Azerbaijan that the active season of Schmallenberg disease vectors leads to the active spread of the virus. In this case, the likelihood of exposure to SBV was higher in young animals before the growing season. Naturally

acquired SBV immunity, being long-lasting, helped prevent infection in adult animals. The epizootiological characteristics of the Schmallenberg virus were studied, and it was established that the virus was introduced into the country through imported animals. In the early diagnosis of the disease, molecular-genetic characterization revealed that the virus predominantly localizes in brain tissues.

It was determined that the clinical symptoms and pathoanatomical changes of nodular dermatitis in cattle in Azerbaijan were more severe compared to observations in other countries, resulting in a higher mortality rate (1.2%). Molecular and serological diagnostics of the nodular dermatitis virus were conducted to determine the specificity of clinical samples. The ELISA method, applied for the first time, assessed the effectiveness of vaccination. A total of 1,500 serum samples were collected from animals. To identify risk factors for seropositivity, univariate and multivariable mixed-effect logistic regression models were used, determining an overall seroprevalence of 4.35% at the animal and herd levels (95% CI: 5.6–9.4). Grazing type, animal gender, and age were identified as statistically significant risk factors for antibody formation against the disease. Gender, region, herd size, contact with buffaloes or other wildlife, and the introduction of new cattle breeds were found to be statistically unrelated to virus spread. The overall seroprevalence was 4.3% at the animal level and 36% at the herd level.

Analysis of the results showed that eight isolates in a monophyletic rabies group indicate they belong to a single epidemiological cycle. The average nucleotide identity within the CA4 subclade, formed by six sequences from the study samples and one “Azer” sequence extracted from GenBank (LN879480), was determined to be 96%. To analyze the spatiotemporal dynamics of rabies in Azerbaijan, a Bayesian MCC tree was constructed using BEAST v1.7.4 with 69 N gene sequences. The study of the initial

structure of selected fragments confirmed two main groups of the rabies virus in Azerbaijan based on N and G genes. Comparison of the N and G genes and fragments of isolates from Azerbaijan with European and Asian nucleotide sequences showed that, in most cases, isolates group according to their geographic origin. In some instances, Azerbaijan isolates clustered with isolates from Russia, Turkey, Georgia, and Iran. The nucleotide sequences of Azerbaijan isolates were submitted to the National Center for Biotechnology Information (NCBI).

It was clarified that out of ten isolates collected from different regions over the years, seven tested positive using real-time RT-PCR and conventional RT-PCR for partial and full N gene amplification. Phylogenetic analysis based on a comparison of the full N gene sequences of seven Azerbaijan isolates with 62 reference *Lyssavirus* sequences showed that all seven samples belong to the Cosmopolitan clade. The study determined that six of the seven analyzed samples belong to the CA4 subclade and one to the CA2 subclade of Central Asia.

Theoretical and Practical Significance of the Research. The reasons for mass abortions among animals imported to large farms operating in the country were investigated, and differentiation from other infectious diseases was performed. For the first time globally, the causes of significant damage caused by an emerging virus to local farms were studied. The determination that naturally acquired immunity is long-lasting helped prevent infection in adult animals. Nodular dermatitis was differentiated from other infectious diseases, and the reasons for its spread were identified, allowing the identification of risks that farm owners should address and improving the vaccination process. The absence of statistically significant risk factors such as grazing type, gender, and age in antibody formation was significant in preventing the disease.
As a result of the research, samples from animals spreading the

rabies virus were analyzed within the "One Health" framework, confirming two main groups of the rabies virus in Azerbaijan. It was determined that the disease is primarily spread by stray dogs, proving their danger as virus carriers.

Validation and Application. A total of 27 works related to the dissertation topic have been published, including 17 scientific articles. The dissertation materials were presented at the following conferences: the International Scientific-Practical Conference on "Innovative Development of Agricultural Science and Education: Global Experience and Modern Problems" (Ganja, 2016), the International Conference dedicated to the 90th anniversary of T. Taghizade's birth (Baku, 2016), "ISDS 2016 Conference" (Atlanta, USA, 2016), the 1st International Scientific Conference on "One Health: Problems and Solutions" (Baku, 2018), "1st International Conference on One Health: Problems and Solutions" (Baku, 2016), "Conference of Research Workers in Animal Diseases Live Streaming of Featured" (Chicago, 2019), and "Conference of Research Workers in Animal Diseases Live Streaming of Featured" (Chicago, 2020).

Volume and Structure of the Dissertation. The dissertation spans 302 computer-typed pages and consists of an introduction, literature review, methods used, examination materials, experimental results and their analyses, conclusions, recommendations, guidelines, and a bibliography. The results are presented with 70 figures and 31 tables. The total character count of the dissertation is 433,422.

LITERATURE REVIEW
CHAPTER I

GENERAL CHARACTERISTICS OF VIRUSES DURING THE STUDY OF EPIDEMIOLOGICAL FEATURES AND MOLECULAR RESEARCH OF NODULAR DERMATITIS, SCHMALLENBERG, AND RABIES IN ANIMALS IN AZERBAIJAN AND WORLDWIDE

In the first chapter of the dissertation, the characteristics of Schmallenberg disease and a general overview of studies dedicated to the evaluation of the virus are analyzed. In the second chapter, the spread of nodular dermatitis disease, the overall epidemiological situation in Azerbaijan, and the results of studies devoted to its investigation are examined. In the third chapter, a general characterization of rabies disease, an analysis of the results of studies dedicated to assessing the situation in Azerbaijan, and the level of investigation of the problem planned for research are reviewed, along with the identification of the tasks necessary for its implementation.
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MATERIALS AND METHODS

CHAPTER II

AREAS WHERE THE RESEARCH WAS CONDUCTED, METHODS AND MATERIALS USED IN THE ANALYSIS OF RESEARCH SAMPLES

2.1. Characteristics of Research Objects

The research was conducted between 2013 and 2021 at the Virology Department of the Republic Veterinary Laboratory,

between 2018 and 2020 at the Department of "Infectious Diseases of Animals and Veterinary Clinic" of the Veterinary Scientific Research Institute, at the Virology Section of the Level 3 Biosafety Central Reference Laboratory, at the "ANSES" rabies reference laboratory in France, and at the "AHVLA" reference laboratory for rabies and poxvirus research in the United Kingdom.

2.2. General Characteristics of Methods and Approaches Used for Sample Collection and Laboratory Analysis of Samples

Samples were collected from 31 districts of Azerbaijan. The collected materials included aborted fetuses, tissues, and serum samples.

All samples were stored at -80°C without any medium until they were directly processed. For the diagnosis of Schmallenberg disease, tissues from aborted fetuses of large and small ruminants were extracted. "*The reverse transcription quantitative PCR (RT-qPCR) method was used to detect viral RNA in the extracts".*[7] The LSI VetMAX™ Schmallenberg Virus – S Gene – TaqMan™ RT-qPCR kit was utilized for SBV detection. Tests were performed on the RotorGene 6000 (Qiagen, Germany) until 2016, and subsequently on the BioRad CF96 (Bio-Rad Laboratories, USA).

The IDEXX Schmallenberg Ab Test™ was used to detect antibodies against Schmallenberg, following the manufacturer's protocol.

"For the study of nodular dermatitis, the ID Screen® Capripox Double Antigen Multi-species kit was used for ELISA testing"[8].

[7] Regge N, Deblauwe I, Rd D, Vantieghem P, Madder M, Geysen D, et al. Detection of Schmallenberg virus in different Culicoides spp. by real-time RT-PCR// Transbound Emer Dis. 2012; 59(6): pp.471–5.

[8] Babiuk S, Wallace DB, Smith SJ, Bowden TR, Dalman B, Parkyn G, et al. Detection of antibodies against capripoxviruses using an inactivated sheeppox virus ELISA// Transbound Emerg Dis. 2009; 6(4):pp.132–41.

This test, produced by IDvet, utilized blood samples collected before vaccination and one month after.
For molecular-biological analyses, a 10% suspension prepared from skin, nodules, and internal organs was used. Samples were extracted using a DNA extraction kit manufactured by Qiagen (USA). The Mastermix Capripox Virus Triplex PCR kit was employed. For sequence analysis, samples were sent to the "Poxvirus" reference laboratory in the United Kingdom.

Sequencing. "*After amplification, PCR products were sequenced by Eurofins Genomics (Ebersberg, Germany) using the same M13 primers (M13 Forward and M13 Reverse (-29)) as in the PCR, along with three internal forward and reverse primers (JW662-684F, JW938), in both directions"*[9].

Sequences were assembled using the ContigExpress program within Vector NTI software, version 11 (Invitrogen, France). The BIOEDIT program was used to interpret gene sequences. Identity percentages and similarity scores were determined in BIOEDIT. Phylogenetic analyses were conducted using the PhyML method (GTR model, 1000 replicates) with the SeaView software. The graphical PhyML tree of the N gene was visualized using Evolview, with each node calculated based on 1000 replicates.

EXPERIMENTAL SECTION

CHAPTER III

3.1. Study of the Epidemiology of Schmallenberg Disease in the Republic of Azerbaijan

[9] Johnson, N., McElhinney L. M., Smith J., Lowings P., Fooks A. R., Phylogenetic comparison of the genus Lyssavirus using distal coding sequences of the glycoprotein and nucleoprotein genes //Arch. Virol. 2002, 147, pp.2111–2123.

The emergence and spread of Schmallenberg disease are attributed to global warming, the movement of blood-sucking insects, humans, and machinery, as well as violations of veterinary sanitary regulations. Environmental changes directly affect the appearance, spread, and evolution of infectious diseases, particularly those transmitted by vectors. Global change refers to ecological shifts resulting from human activities that impact the fundamental mechanisms operating within the biosphere. Pathogens, the causative agents of infectious diseases, are distinguished by their high diversity and complexity.

In 2012, a mass abortion event among small ruminants was recorded in the Beylagan district. Gradually, abortions began to spread to other districts and later affected large ruminants. The disease's range of spread grew rapidly. The number of animals experiencing abortions increased daily in areas such as "Turyanchay" in Agdash, "Garainek" in Agdam, "Mehdili" in Barda, and others. Our investigations revealed that no acute clinical symptoms were observed in pregnant animals prior to the abortions, which is why no preventive measures were taken.

Due to the similarity of the observed general symptomatology and "the suspicion of the Schmallenberg virus as a potential cause, which has been described in European countries in recent times," this hypothesis emerged"[10].

As evident from the figure, the first confirmed case of Schmallenberg virus infection was recorded in October 2012, and by the end of the year, antibodies against the virus were detected in the blood of 1,301 animals in Azerbaijan (Figure 1). Epidemiological investigations revealed that, starting in 2010, large ruminants of various breeds were imported into the country from Europe. These

[10] (http \\ www.evolgenius.info \ evolview)

animals were bred for dairy and meat production. After being held in quarantine in Ganja for 21 days, the animals were distributed to

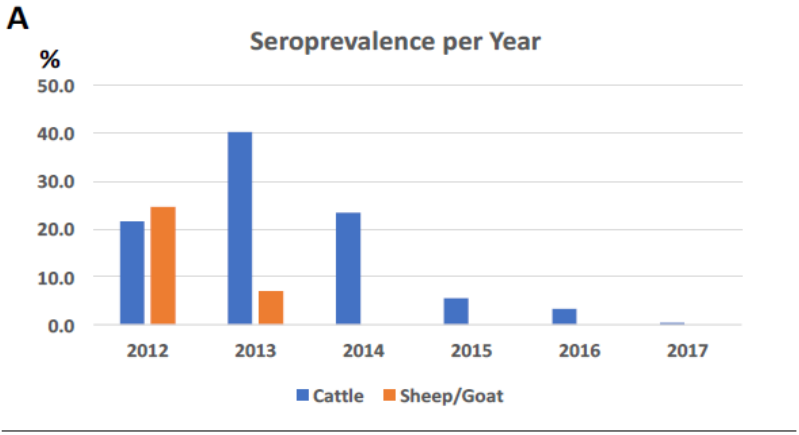


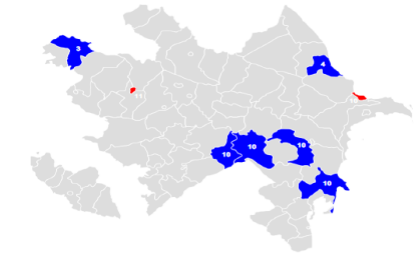
Figure 1. Results of Seromonitoring Among Large and Small Ruminants

farms in various districts. All imported animals were pregnant. In 2012, abortion incidents were recorded among sheep in the Beylagan district. Examination of the samples showed no presence of brucellosis or chlamydia.

In 2012, antibodies against Schmallenberg disease were detected in the blood of animals brought to Beylagan, Imishli, Sabirabad, and Neftchala districts in the south of the country, as well as Sumgait (east) and Ganja (west). During laboratory ELISA testing, antibodies against Schmallenberg disease were identified.

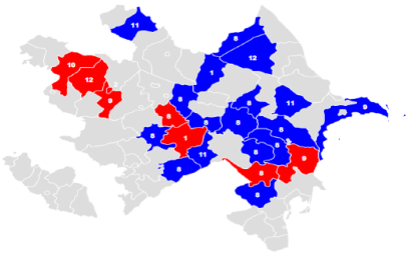
The epidemic gradually spread to other areas. By February 2014, reports from additional regions confirmed that antibodies against the Schmallenberg virus were present in the blood of an additional 2,696 local and imported animals (Figure 2). In 2013, viral RNA was detected in 22% of cattle, and the disease's spread reached nearly 60% (Figures 2B and C). Ongoing studies identified animals

with antibodies in subsequent years as well, though the prevalence was lower compared to 2012 and 2014 (Figures 2C, D, E, and F).



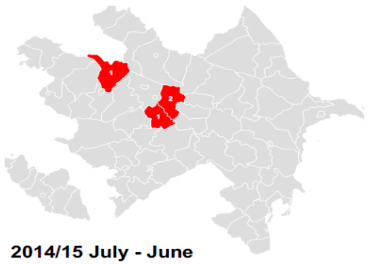
2012/13 October - June

A.



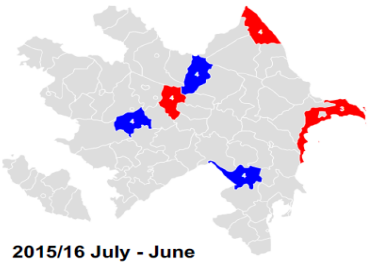
2013/14 July - June

B.



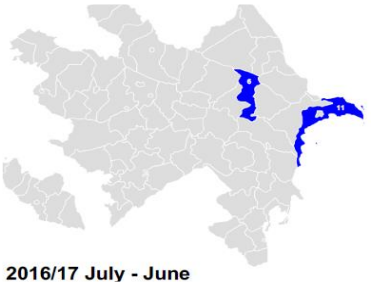
2014/15 July - June

C.



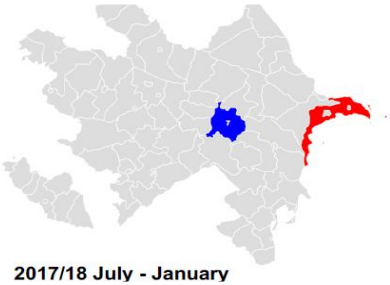
2015/16 July - June

D.



2016/17 July - June

E.



2017/18 July - January

F.

Figure 2. Geographical distribution of Schmallenberg disease

PCR-confirmed Schmallenberg virus RNA was detected between 2012 and 2014 (Figures 2A and B). Between October 2012 and January 2018, cattle or sheep with antibodies against the Schmallenberg virus were found in 42 districts of Azerbaijan (Figure 2B). Most cases occurred in autumn and winter (October–February), with fewer incidents between May and July.

The map illustrates the circulation of the Schmallenberg virus in Azerbaijan from late 2012 to early 2018.

Although the ELISA kit used for antibody detection does not distinguish SBV from other Simbu group viruses, RT-qPCR results accurately identified the virus. According to the manufacturer, RT-qPCR is specific to SBV because primers and probes were selected for regions that differ from Akabane, Simbu, and Shamonda viruses due to several mismatches. Since there is no evidence of another Simbu serogroup virus circulating in neighboring countries, the wider region, or Europe, we assume that all cases identified as antibody-positive during the study are attributable to SBV.

Thus, it appears that SBV reached Azerbaijan approximately one year after its initial appearance in Europe in 2011. Given that all tested materials involved suspected (symptomatic) cases, it was expected that the virus would enter the country during the vector season. Most likely, initial cases before October were fewer in number and either went unnoticed or unrecorded. Since the majority of early cases were detected in local sheep and cattle in southern Azerbaijan, it is hypothesized that the virus entered the country either through infected wild animals or via *Culicoides* vector species from Iran. The latter are considered the primary vectors of SBV spread in Europe.

However, at that time, there was no information regarding the presence or testing of SBV in Iran. The first report of SBV in Iran is

based on samples collected from horses in the northeast of the country between July 2014 and September 2015. At that point, the prevalence was determined to be between 5–7%, which closely resembles the causative agent we identified in Azerbaijan. An alternative scenario suggests the direct importation of viremic animals from Europe.

Investigation of the Impact of Ecological Factors and Seasonal Changes on the Spread of Schmallenberg Virus. The spread of the virus ceased with the onset of winter, leaving large parts of the country unaffected.

As a result, in 2013/2014, more animals in Azerbaijan were infected, including a significant number of naïve animals (those not previously exposed to infection or vaccination). The second wave of the disease began in the southern and central districts, with the first confirmed cases detected in August (Figure 3). It was determined that the virus survived the winter in Azerbaijan and/or beyond the southern border, re-emerging at the start of the vector season and rapidly spreading across the country. Southern Azerbaijan has a subtropical climate with average temperatures higher than those in northern and central Europe, along with more rainfall throughout the year compared to southern Europe. These factors favored a longer season—or even uninterrupted activity—for *Culicoides* vectors, which in most of Europe typically spans from April to October.

It is likely that the vast majority of susceptible cattle and wild animals in Azerbaijan were infected with SBV between 2012 and 2014, developing immunity to the disease, which explains the disruption in the sequence of outbreaks (Figure 4). We detected the virus in only 40% of cattle and 24% of sheep and goats, but testing symptomatic animals was shown not to accurately reflect the seroprevalence rate in the overall animal population. After 2014, antibodies against SBV were detected in cattle, and the spread

decreased (Figure 2C).

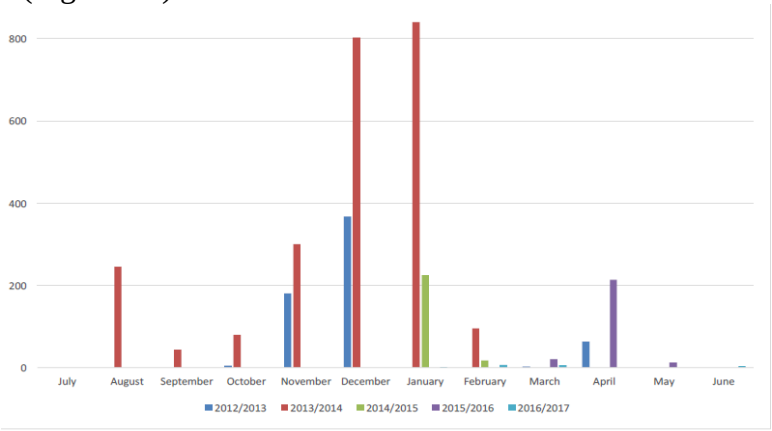


Figure 3. Impact of seasonal variation on the epidemiology of the disease in large ruminants

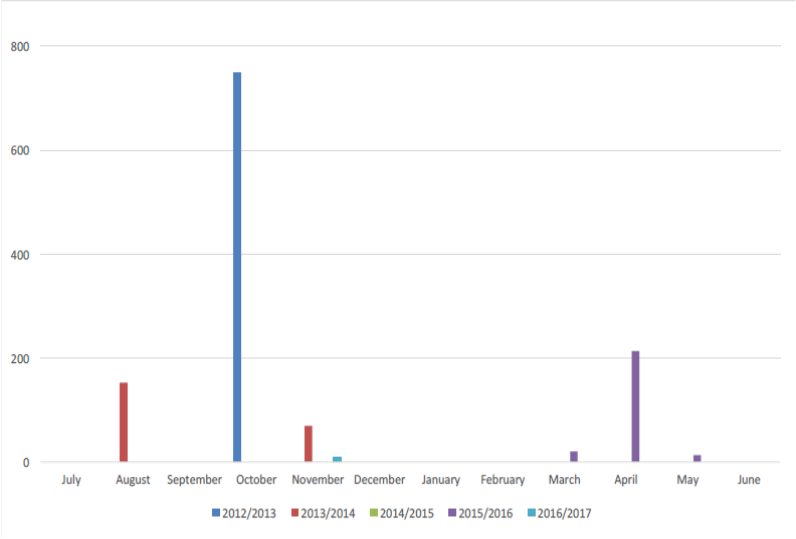


Figure 4. Impact of seasonal variation on the epidemiology of the

This could indicate a low level of virus circulation or continuous reintroduction. No clear seasonal pattern was observed among antibody-positive cases. Despite the detection of antibodies in some suspected cases, no viral RNA was detected in any samples tested since February 2014 (Figure 2D).

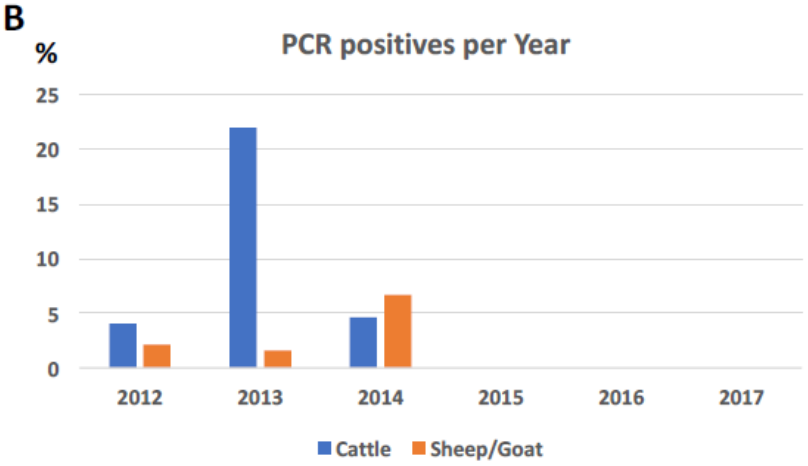


Figure 5. Annual distribution of PCR-positive samples

Although only a portion of suspected cases were tested in this manner, this serves as further evidence that SBV is not likely endemic in Azerbaijan and/or does not circulate significantly. During the second wave, most animals with confirmed antibodies upon importation never left the country. Our data suggest that the majority of these cases are unlikely to be related to SBV, and we believe the high numbers can be explained primarily by two factors: first, increased sensitivity and awareness of symptomology associated with the availability of SBV testing; second, an increase in

other diseases with similar or partially similar symptomology, such as brucellosis. Although the virus does not appear to be circulating at this point, the absence of a vaccination program and the large number of susceptible animals create the potential for its re-emergence. During validation tests, the analytical characteristics of the developed test systems were determined. Modern requirements applied to PCR-based test systems recommend the use of recombinant DNA reactions as positive controls due to their advantages, including safety in preparation and use, high specificity, and long shelf life. Several approaches exist for creating internal controls. Depending on the method of creation, the pathogen's characteristics, and the research objectives, either an exogenous or endogenous internal control is selected for the PCR test system (Figure 5).

Each has its advantages and disadvantages. During experimental validation, it was determined that an endogenous internal control based on primers added to a fragment of the β-actin-encoding gene, present in the blood and organs of all animals, was the most optimal.Examination of blood samples, serum, and organs from cattle and small ruminants revealed positive samples. Additionally, the location where the animals were infected was clarified. The earlier hypothesis that infection occurred during importation—either in quarantine or at the farm—was not substantiated. Our investigations showed that large ruminants already had antibodies against Schmallenberg disease in their blood at the time of importation. The research results enable veterinary service workers to promptly identify animals infected with Schmallenberg and prevent their entry into the country, thereby curbing the further spread of the disease within Azerbaijan.

Thus, considering the intermittent circulation of SBV in regions previously exposed to the 2011/2012 Schmallenberg epidemic in

Europe, a combination of control measures is required to reduce the risk of SBV infection in domestic animals.

CHAPTER IV

NODULAR DERMATITIS DISEASE AMONG LARGE RUMINANTS IN AZERBAIJAN

4.1. Epidemiology of Nodular Dermatitis Disease

Nodular dermatitis is a skin disease affecting agricultural animals, caused by a DNA virus—nodular dermatitis virus (NDV)—belonging to the *Capripox* genus within the *Poxviridae* family. While other *Capripox* strains infect sheep and goats, NDV is observed in large ruminants (Davies, 1981). The disease was first recorded in Zambia in 1929 and subsequently spread to southern Africa and northward toward Sudan. Outside Africa, it was first diagnosed in Israel in 1989, and in subsequent years, it was reported in Bahrain, Kuwait, Oman, Yemen, Lebanon, and Jordan (Wainwright et al., 2013). The disease is characterized by fever, nodular lesions on the skin, mucous membranes, and internal organs (World Organisation for Animal Health (OIE), 2010). It can lead to reduced milk productivity, the formation of skin nodules, temporary or permanent infertility, and/or death of infected animals, resulting in significant economic consequences in affected countries (Alemayehu et al., 2013). The severity of clinical signs depends on the virus strain and the breed of the infected large ruminants (OIE, 2010). Transmission is believed to occur primarily through insect bites, although direct contact is also possible (Majori-Cohen et al., 2012; Chihota et al., 2001; Kitching & Mellor, 1986). The average incubation period for NDV is 6–9 days.

The disease's average mortality rate is approximately 10%, though it is typically higher in cases of secondary infections (OIE, 2010).

Azerbaijan is located on the shores of the Caspian Sea, bordered by Russia to the north, Georgia to the northwest, Armenia to the west, and Iran to the south. At the end of 2013, outbreaks of ND were reported to the OIE from Turkey and Iran, with additional outbreaks in Iran in early 2014 (OIE World Animal Health Information System; Wainwright et al., 2013; European Food Safety Authority, 2015). To determine whether NDV had spread from these neighboring countries to Azerbaijan, a response team from the State Veterinary Control Service was dispatched to the southern border region of Azerbaijan to examine large ruminants and provide recommendations for control and prevention. This study describes the first confirmed cases of ND in Azerbaijan in 2014.

4.2. Affected Areas and Populations

In May 2014, an inspection of small farms with large ruminants was conducted in the Bilasuvar district. Large ruminants exhibiting clinical signs characteristic of nodular dermatitis (ND) were identified in Amankand, located near the border with Iran. bordering Iran, as well as in Ujar and Agdash, which are located more centrally in the country but connected to the southern part of Azerbaijan via major highways (Figure 6).

Infected large ruminants refused feed, and symptoms such as fever, purulent ocular-nasal discharge, and lethargy were observed. In some cases, red, hard nodules were noted in the neck and abdominal regions, with degenerative changes such as necrosis, edema, and exudate observed on the skin surface around the nodules (Figure 7).

In the majority of disease cases recorded in October 2014, a pulmonary form leading to dyspnea was observed. It was hypothesized that death in pulmonary cases resulted from respiratory

failure. During autopsies, congestion in the lungs and nodules in internal organs were frequently observed. A total of 2,762 disease

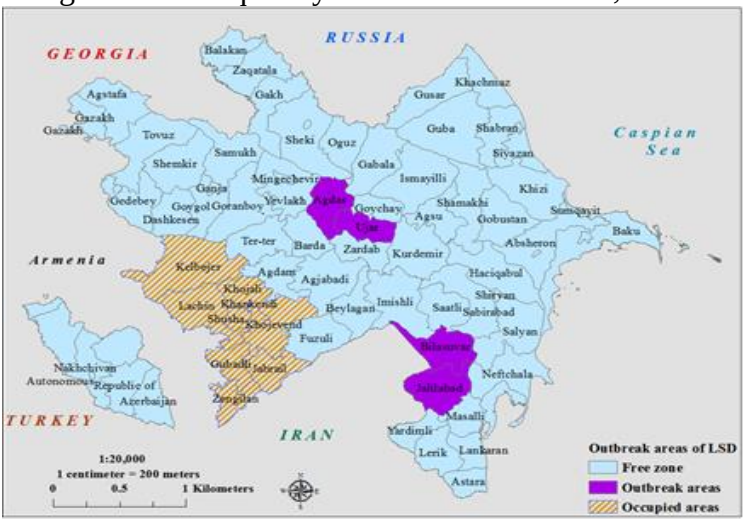


Figure 6. Regions reporting suspected or confirmed cases of Lumpy Skin Disease (LSD) in large ruminants in 2014

Figure 7. Clinical skin symptoms and sample collection

cases were recorded, with 33 large ruminants (1.2%) succumbing to the disease. Disease cases were reported in June, July, and October 2014. In October 2014, the majority of recorded cases presented the

pulmonary form, resulting in dyspnea. It was presumed that death in these cases was due to respiratory failure. Autopsies frequently revealed lung congestion and nodules in internal organs (Figure 8).

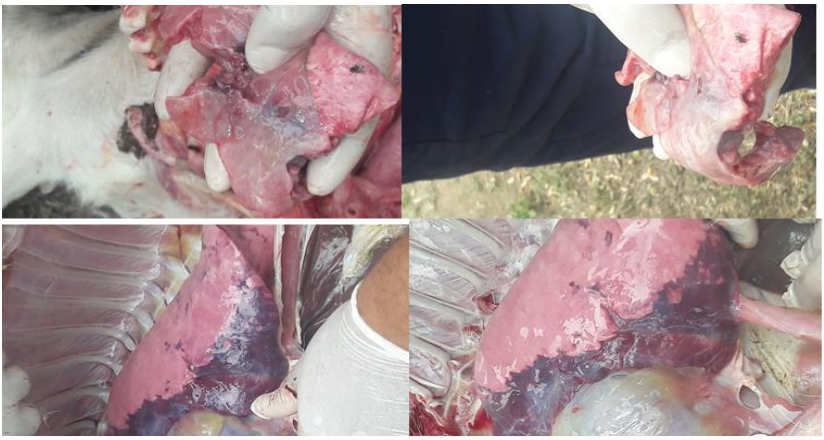


Figure 8. Pathological and anatomical changes.

Real-Time PCR Testing. A total of 385 samples were tested for NDV using real-time PCR: 130 skin samples, 106 blood samples, and 148 internal organ samples (Table 1).

Table 1. Sensitivity indicators in PCR testing of samples.

Sample type	Number of tests	Number of positive results %	Positive	Mean Sd value	Standard deviation of Sd values
Nodules	130	130	100	19.3	1.10
Blood	106	42	40	29.4	1.39
Internal organs	149	83	56	23.2	0.66
Total	**385**	**255**	**66**	-	-

Of these, 255 samples (66%) tested positive via PCR. All skin lesion samples tested positive, showing lower Ct values compared to blood or organ samples, indicating higher viral concentrations. Blood samples had the highest Ct values and were least likely to test positive, with lower viral concentrations recorded (Figure 9).

As a result, 2,567 infected cattle recovered, though it remains unclear which treatment protocols aided their recovery. All affected farms were instructed to restrict animal movement outside the farm for 30 days. Following the outbreak, two million doses of live sheep and goat pox vaccine (Poxvac, Vetal Company, Turkey) were procured. In 2015, a five-year vaccination campaign was initiated in Azerbaijan to prevent the spread of the disease. A total of 1.6 million cattle in affected districts, neighboring districts, and southern border regions of Azerbaijan were vaccinated in 2015, with a 20% reserve vaccine stock maintained in case of further outbreaks.

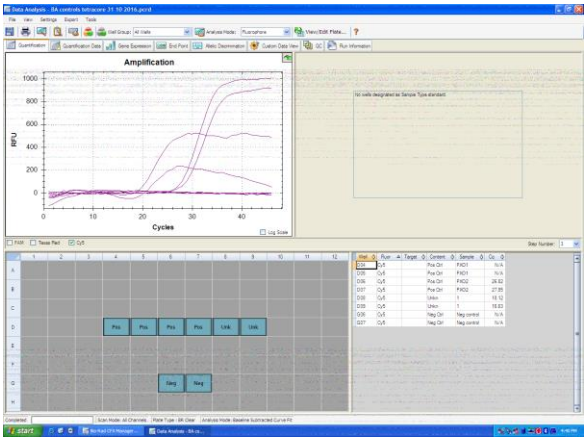


Figure 9. Results of polymerase chain reaction (PCR) testing.

4.3. Study of the Seroprevalence and Risk Factors of Nodular Dermatitis Virus Among Large Ruminants in Azerbaijan

The study aimed to determine the seroprevalence of nodular dermatitis disease and its risk factors, covering five regions of Azerbaijan.

Description of the Study Area: Samples were collected from the Agdash, Zardab, Ujar, Goychay, and Barda districts. These areas are considered part of Azerbaijan's lowland region. The selected farms practiced a mixed crop-livestock system, combining agriculture and animal husbandry. Animals in these areas are taken to summer pastures only during the summer months. Examinations were conducted at the Veterinary Scientific Research Institute. An indirect ELISA method (ID Screen® Capripox Double Antigen Multi-species, manufactured in France) was used to detect antibodies in the blood.

Sample Size and Data Collection: The objective was to assess differences in seroprevalence across five regions of the country, where average annual precipitation and livestock production systems vary. Animals vaccinated against foot-and-mouth disease in 2018–2019 were selected for sampling. Information on general vaccination practices was collected, and herds not vaccinated against nodular dermatitis were chosen. As proposed by Dohoo et al. (2009), multi-stage sampling was used, with herds in each region selected such that the probability of selection was proportional to herd size. Herds with at least 10 animals were included. Based on these criteria, 1,500 samples were collected from 35 different herds. The study included samples from local, Holstein-Friesian, and Charolais breeds, covering animals aged from 2 months to 6 years. Animals were

categorized by age: 0–12 months (calves), 13–24 months (young cattle), and over 24 months (adult cattle).

Sample Collection and Processing: Blood was collected in single-use 10 ml sterile Vacutainer SST tubes. Serum was separated from the blood and stored in a refrigerator at 4–8°C (Figure 10). The separated serum was transferred to 2 ml cryotubes, labeled with the date, animal age, sex, and district name. Antibodies against ND were detected using the Double Antigen ELISA (ID Screen®) for *Capripoxvirus* antibodies. For each sample, the percentage ratio of sample OD to OD (S/P%) was calculated using the formula:

$$S/P\% = [(ODsample - ODNC) / (ODPC - ODNC)] \times 100$$

where OD is the optical density of the sample, ODPC is the optical density of the positive control, and ODNC is the optical density of the negative control. Samples with S/P% < 30% were considered negative, while those with S/P% ≥ 30% were considered positive.

Figure 10. Sample collection and labeling for ELISA test

Data Management and Statistical Analysis. Seroprevalence was calculated by dividing the number of ND-positive animals by the total number of animals tested. Prevalence at the herd level was determined by dividing the number of positive herds by the total number of herds. A herd was considered positive if at least one animal tested seropositive for nodular dermatitis. True seroprevalence was adjusted using the apparent seroprevalence (AP) and the sensitivity (Se, 91%) and specificity (Sp, 99.7%) of the ELISA test. True prevalence was calculated using the formula proposed by Stevenson (2007)

True Prevalence = (AP + Sp − 1) / (Se + Sp − 1)

Herd seroprevalence was plotted by month to assess potential seasonality. Possible risk factors for seropositivity were analyzed using univariate mixed-effect logistic regression, with herd identifier included as a random effect to account for clustering at the herd level. All variables significant at $p < 0.2$ were further evaluated. During model development, confounding was assessed at each step by checking for changes in estimates, with changes >25% indicating confounding. All analyses used a 95% confidence level, and a statistical significance threshold of $p < 0.05$ was applied, except for fixed variables. Data analysis was conducted using EPI INFO 2016 software.

Demographic Characteristics of the Studied Herd. In 2019, blood samples from 1,500 large ruminants across five districts in Azerbaijan were tested for ND antibodies. Each region had 300 animals selected, with an average of 30 animals per herd and a minimum of 10 animals per herd. Of the sampled cows, 840 were female and 660 were male. The majority were local breeds (1,250), followed by Holstein-Friesian (120) and Charolais (130).

Results of Real-Time PCR Testing

Of the 385 samples tested via PCR, 255 (66%) were positive. All skin lesion samples tested positive, showing lower Ct values compared to blood or organ samples, indicating higher viral concentrations. Blood samples had the highest Ct values and were least likely to test positive, with lower viral concentrations recorded. Consequently, 2,567 infected cattle recovered, though it remains unclear which treatment protocols aided their recovery. All affected farms were instructed to restrict animal movement outside the farm for 30 days. Following the outbreak, two million doses of live sheep and goat pox vaccine (Poxvac, Vetal Company, Turkey) were procured. In 2015, a five-year vaccination campaign was launched in Azerbaijan to prevent the disease's spread. A total of 1.6 million cattle in affected districts, neighboring districts, and southern border regions were vaccinated in 2015, with a 20% reserve vaccine stock maintained for potential further outbreaks.

Seroprevalence Rate of Nodular Dermatitis Antibodies.

The case fatality rate during the outbreak in Azerbaijan was calculated based on reported disease cases; overall, 1.2% of the known exposed cattle succumbed. The case fatality rate varied significantly by month: 0% in June, 0% in July, and 1% in October. The reason for the differing fatality rates is unknown but may be related to reporting practices, as disease cases, rather than deaths, were primarily recorded. Variations in fatality rates and case incidence have also been observed in other countries. Prevalence and incidence rates were not calculated in this study, as the total outbreak size was based on a passive reporting system, and not all susceptible animals in affected areas were examined. Additionally, some farmers may not have reported lesions to district veterinary authorities. Our investigations identified the lack of an animal identification system as the primary reason prevalence rates could not be calculated. In farms, an accurate identification system aids in effectively tracking

disease spread when an outbreak occurs, enabling the identification of animals exposed to infection to prevent further transmission.

As cattle and large ruminants frequently change owners, a national identification system is essential for tracing an animal's journey from birth to slaughter or death.

The vaccines used in Azerbaijan's vaccination program for small ruminants were also applied against nodular dermatitis, partially providing immunity. Continuous vaccination of cattle in affected and surrounding areas was deemed necessary to protect against environmental transmission and insect bites. Nodular dermatitis virus was first detected in Azerbaijan in 2014 in the Bilasuvar district and later recorded in three other districts. Control measures, including the vaccination program, were implemented, yet disease cases were reported in various districts after October 2014.

These incidents confirmed the likelihood of the virus persisting in the environment and continuing to pose a risk to unvaccinated cattle in exposed areas. A total of 1,500 serum samples were collected from animals. Univariate and multivariable mixed-effect logistic regression models were used to identify risk factors for seropositivity. The overall seroprevalence at the animal and herd levels was 4.35% (95% CI: 5.6–9.4). Grazing type, animal sex, and age were statistically significant risk factors for ND antibody development in large ruminants. Breed, region, herd size, contact with buffaloes or other wildlife, and the introduction of new cattle breeds were not statistically associated with virus spread.

This study was the first in Azerbaijan to use ELISA testing to detect antibodies against nodular dermatitis. It investigated seropositivity and risk factors for the virus across five geographic regions of Azerbaijan, marking the first study of ND seroprevalence among large ruminants in the country. The overall seroprevalence was determined to be 4.3% at the animal level and 36% at the herd

level.

Thus, this research, conducted for the first time in Azerbaijan, demonstrated that nodular dermatitis is a highly pathogenic disease and confirmed its spread. The reasons for its dissemination were identified.

CHAPTER V
Study of the epidemiological characteristics of rabies

5. Research on Rabies Virus Conducted Between 2000 and 2010.

The Caucasus is a geopolitically significant region located between Europe and Asia. This geographic position makes the region equally important for both the epidemiology and control of transboundary infectious diseases such as rabies. Azerbaijan, the largest country in the Caucasus region, considers rabies a notable and endemic disease; however, limited information is available regarding its spread among humans and animals. To compare incidents occurring in surrounding areas, partial nucleoprotein gene sequences were obtained from samples of rabies cases among animals.

Between 2000 and 2010, recorded rabies cases among humans and animals were reviewed and analyzed by region and year. A comparison of rabies virus strains circulating in Azerbaijan with those in neighboring countries revealed the presence of multiple parallel rabies virus lineages in Azerbaijan, highlighting the need for additional sample collection and characterization. The study showed that, since 2006, official reports on rabies indicate an increase in cases among humans and animals, as well as in the number of animal bites requiring preventive measures. This occurred despite continuous vaccination of dogs and the culling of stray dogs.

The history of rabies incidents in Azerbaijan is believed to have begun with the onset of the disease in dogs, particularly stray dogs, alongside recorded cases in wildlife. Similar to the history of rabies in Europe, stray dogs and wild animals (foxes, wolves, jackals) have been identified as disease carriers in Azerbaijan.

The primary epizootiological foci of rabies exhibit characteristic features dependent on the ecology of its carriers—foxes and wolves. As foxes typically inhabit areas within a 10–30 km radius, the surrounding area exposed to their infectious influence and the location of the infection source are considered epizootic centers for rabies in wild animals, within a 30 km radius.

Key epizootic indicators include the onset and persistence of the disease. These are critical for developing principles for rabies prevention, implementing differential control measures, and forecasting epizootic dynamics.

The spread of rabies among humans is regulated by Azerbaijani legislation. Patients exhibiting rabies-specific symptoms and reports of animal bites from local hospitals are sent to one of five regional Anti-Epidemiological Stations (AES). Examinations are confirmed by the AES using clinical identification, and detailed disease information is submitted to and stored at the Republican Disease Control Center in Baku. Rabies cases among humans between 2000 and 2010 were analyzed by year, region, and patient age. The investigation of animal bites and the application of post-exposure prophylaxis (PEP) are also regulated by the AES, and thus, data on animal bite incidents and PEP administration from 2000 to 2010 were included in these analyses.

Scintigraphic investigations of animal bites are conducted by veterinary authorities. Heads of animals suspected of rabies are submitted by District Veterinary Departments to regional (or district) veterinary laboratories. Samples are then sent to the Republican Veterinary Laboratory (RVL) in Baku for confirmation using the

Direct Fluorescent Antibody Test (FAT) and Mouse Inoculation Test (MIT). When reports of attacks by wild animals or dogs on domestic animals or humans are received, active investigations are conducted by the Agricultural Services Agency. Upon receiving a bite report, stray dogs in the area are culled as much as possible, while dogs kept in yards are quarantined. District veterinarians monitor the dogs for 10 days, and those suspected of rabies are euthanized, with samples submitted to the laboratory.

Between July 2012 and March 2013, 10 such animal samples from several districts, testing positive via FAT and MIT at the RVL, were collected and selected for virus characterization. Brain homogenates (10% by weight in PBS) were applied to FTA™ cards (Whatman BK, GE Healthcare) and allowed to dry for one hour before being sent to the Rabies Reference Laboratory at the Animal and Plant Health Agency (APHA) in the UK.

Polynucleotides were eluted from FTA™ cards by incubating two 3 mm trepanobiopsies from each card in 50 µl of HPLC water on ice for one hour with periodic agitation. RNA (ribonucleic acid) was reverse-transcribed, and, as previously described, a nested Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed for each sample to amplify a 606 bp segment of the nucleoprotein gene. PCR products were purified (Qiagen) and sequenced as previously indicated. Using ClustalX2 (version 1.2), a consensus sequence over 400 bp was determined with at least one forward and one reverse primer.

A Bayesian Markov Chain Monte Carlo (MCMC) phylogenetic tree was constructed using BEAST (v1.4.8) with a GTR substitution model featuring a discrete gamma distribution of percentage differences. Additionally, as selected in Mega5 using the Best-Fit Substitution Model, invariant site proportions were applied. A chain length of 30 million was used, with the first 10% of trees

excluded as standard when selecting the maximum clade credibility tree (Tree Annotator).

PCR Components Used. The polymerase chain reaction (PCR), with the aid of specific primers, enables the identification of specific segments of the rabies virus genome. In our study, the primary purpose of PCR was to sequence samples, although its use for diagnostic purposes was also investigated.

The results of Real-Time TaqMan qRT-PCR and Conventional hnRT-PCR are presented in Table 5.2.1.2.

Of the 10 samples applied to FTA paper, 7 tested positive by both conventional and RT-PCR methods.The virus type identified in the 7 positive RT-PCR samples was the classical rabies virus. For the TaqMan RT-PCR, 7 out of 10 tested samples showed positive Ct values ranging from 19.46 (corresponding to 1.37×10^8 copies/µL RNA) to 28.97 (2.67×10^3 copies/µL RNA). The three negative samples (AZ 1, AZER 8, and AZER 10) had Ct values ranging from 35.52 to 39.16. Ct values exceeding the thresholds specified in the TaqMan RT-PCR methodology were considered negative. The TaqMan RT-PCR, using the QuantiTect probe (Qiagen, France), achieved a 95% amplification rate for the N gene with 20 RNA copies.

TaqMan RT-PCR allows for the identification of RABV by amplifying a fragment (111 bp) of the N gene across all known species in a specific RT-PCR setting, as well as partial amplification (606 bp) of the *Lyssavirus* N gene. During gel electrophoresis preparation, positive results appeared as a distinct band, while a negative result was characterized by the absence of any band. However, a diffuse region might appear at the end of the lane due to unspent primers or non-specific PCR products of varying sizes. To monitor the size of the obtained PCR products, a 2% agarose gel with a 100 bp marker (yielding a final band) was used. Horizontal electrophoresis was conducted in the presence of ethidium bromide

to analyze the PCR products. The prepared 2% agarose gel was placed in the electrophoresis chamber. Using an automatic pipette, 12 µL of PCR product was loaded into the gel wells, followed by the addition of 12 µL of DNA marker. Electrophoresis was run for 60 minutes at 100 volts. The gel was then removed and placed in a transilluminator chamber equipped with a digital camera, and the results (electrophoresis) were displayed on a computer monitor. The results of PCR for field isolates and strains are provided in Table 2.

Table 2. Results of Conventional RT-PCR for Amplification of the Entire N Gene Across All Tested FTA Samples. N gene amplification: (111 bp) and (606 bp) 2: Nb: Number; Neg: Negative; Pos: Positive.

Tested samples		N gene[1]: amplifikasiyası Detection
sample	species	
AZER 1	Cat	Neq
AZER 2	Cow	Poz
AZER 3	Cow	Poz
AZER 4	Dog	Poz
AZER 5	cat	Poz
AZER 6	Donkey	Poz
AZER 7	Cat	Poz
AZER 8	Cat	Neq
AZER 9	Jackal	Poz
AZER10 2 :—	Jackal	Neq

5.1. Statistics on Rabies Disease in Humans

Between 2000 and 2010, there were reports of 77 human rabies infections. Regarding post-exposure prophylaxis (PEP), 29% of cases (22/77) occurred in children, with a slightly higher incidence (22%) in the population group aged 14 years and younger. The known annual incidence averaged 7 cases, ranging from 2 to 15 cases over the study period. The average annual incidence (7.7 cases) across the

three years from 2008 to 2010 was higher than the average (3.3 cases) for the three years from 2005 to 2007, though this difference was not statistically significant (t-test, p = 0.07). The distribution of rabies cases in humans is observed throughout Azerbaijan (Figure 11).

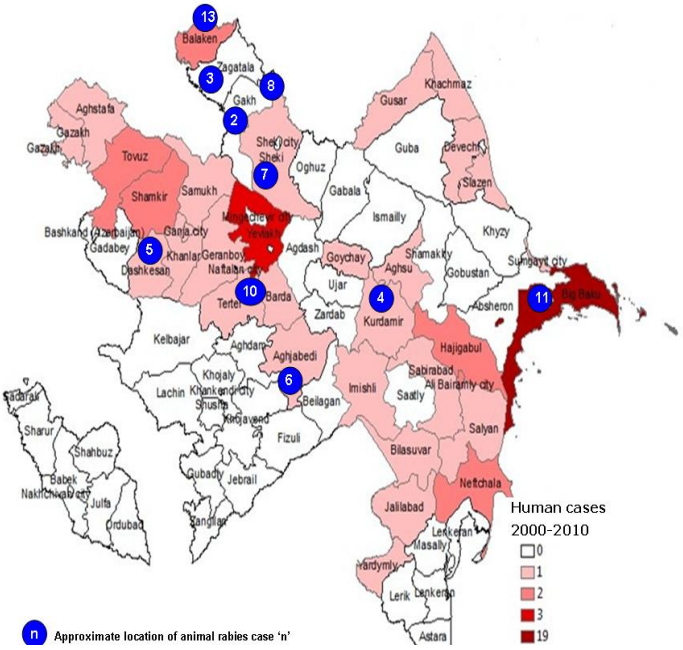


Figure 11. Map of Azerbaijan. The total number of known human rabies cases by district from 2000 to 2010 and the approximate locations of animal rabies cases sequenced in this study.

The highest number of rabies cases occurred in Baku and its surrounding areas. However, due to the high population density in Baku, the average annual incidence per 100,000 people (0.091 cases

per 100,000) is close to the national average (0.088 cases per 100,000).

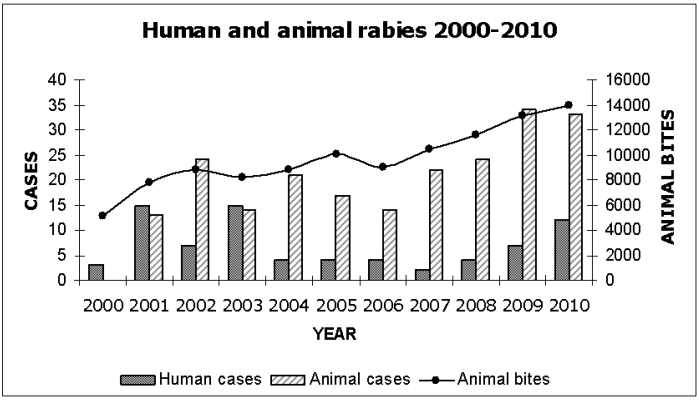


Figure 12. Rabies Disease in Humans and Animals in Azerbaijan. Annual rabies cases in humans and animals from 2000 to 2010, along with the number of animal bites reported to the Anti-Epidemiological Stations (AES) during the same period.

The number of animal bites requiring post-exposure prophylaxis (PEP) and reported to the AES increased annually from 2006, reaching a total of 13,961 cases in 2010. Using official population estimates, this translates to 155 individuals per 100,000 receiving PEP for rabies in 2010 (Figure 12).

Sample Collection for Rabies Disease in Animals. During the study period, a total of 326 animal samples were submitted for rabies testing. Of these, 216 samples tested positive, 75 tested negative, and 35 could not be tested (Table 2).

Table 3. Animal Samples Submitted to the Republican Veterinary Laboratory for Rabies Testing Between 2000 and 2010.

Table 1. Animal samples (submitted to the Republican Veterinary laboratory) for rabies diagnosis 2000–2010, and human rabies cases (reported to APS)

	Year											
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Sub total	Total
Animal samples submitted to RVL for rabies diagnosis													
Dog	n.d.	7	15	9	14	9	9	13	7	17	11	111	
Cattle	n.d.	4	5	4	3	4	1	5	9	8	15	58	
Sheep	n.d.	0	0	0	0	0	1	1	0	1	0	3	
Horse	n.d.	0	0	0	2	0	1	0	1	2	0	6	
Other	n.d.	2	4	1	2	4	2	3	7	6	7	38	
Total Positive	0	13	24	14	21	17	14	22	24	34	33		216
Negative	9	10	5	4	3	10	3	0	8	6	17		75
Un-testable	6	7	3	1	3	2	1	7	2	1	2		35
Total submitted	15	30	32	19	27	29	18	29	34	41	52		326
Human rabies cases reported to APS													
Children	3	5	1	5	0	1	1	1	1	2	2		22
Adult	0	10	6	10	4	3	3	1	3	5	10		55
Total cases	3	15	7	15	4	4	4	2	4	7	12		77
PEP	5161	7780	8857	8216	8801	10 008	9062	10 419	11 554	13 083	13 961		106 902

n.d., no data; 'Other' includes wildlife; PEP, number of animal bites reported to APS requiring post-exposure prophylaxis according to WHO guidelines; RVL, Republican Veterinary Laboratory; APS, Anti-Plague Service.

The ratio of positive to negative results remained consistent over the decade, but the number of untestable samples decreased. Over half of the samples were from dogs (n=111), with the second-largest group of positive samples from cattle (n=58) (Table 3). The remaining samples were from wild animals and other domestic animals. During the study period, Azerbaijani authorities vaccinated approximately 1.5 million dogs against rabies and culled 0.5 million (Figure 13).

Ten samples, representing various regions of Azerbaijan, were submitted to the International Organisation for Animal Health (OIE) Reference Laboratory for Epizootic Diseases at the Animal and Plant Health Agency (APHA).

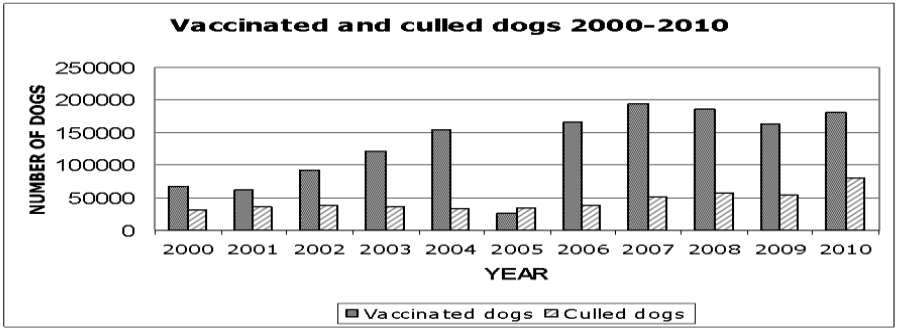


Figure 13. Vaccinated and Culled Dogs. The total number of dogs vaccinated and culled annually from 2000 to 2010.

Analysis of the results showed that all samples belong to a rabies virus strain believed to have spread globally through human migration over the past two centuries. Eight of the results within a monophyletic group indicate they belong to a single epidemiological cycle (Figure 13).

As shown in Figure 14, this group is more closely related to viruses detected in Russia and Eastern Europe. The strains collected from Dashkasan and Aghjabadi, included in this study, are genetically distinct from the main group of strains specific to Azerbaijan and from each other. The strain from Dashkasan shares a common origin with more recent strains from the Middle East and is more closely related to two historical isolates from Georgia. The strain from Aghjabadi is distinct from Middle Eastern strain groups identified between 1990 and 2004

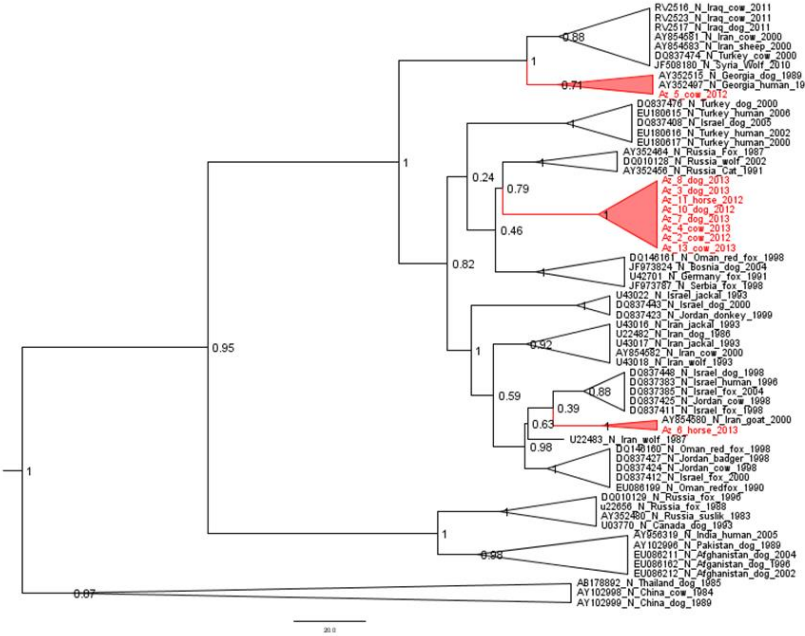


Figure 14. Phylogenetic Tree. A Bayesian phylogenetic tree using 400 bp sequences of the N gene, compared with isolates from neighboring countries.

5.2. Research on Rabies Virus Conducted Between 2014 and 2018

Over a 9-year period from 2010 to 2018, a study on the recurrence of rabies cases established varying activity levels of epizootic foci. Among 351 households, rabies was not recorded in 117 (33.33%), occurred once in 87 (24.79%), twice in 60 (17.09%), and three or more times in 87 (24.79%). The farm territories within the district were used as the accounting unit. The differing frequency of rabies cases across these farm territories allowed for a

comparative assessment of the disease risk level using statistical-cartographic analysis. To develop forecasts for the rabies situation in animals, a comparative evaluation of the region's disease risk level was deemed necessary, based on the experiences of Konstantinov (1981) and Anderson (1983). This assessment was taken into account when preparing preventive measures.

Results of Sequence Comparison Between Seven Azerbaijani FTA Sequences and Reference Sequences. The sequence comparison was conducted based on seven Azerbaijani sequences (1353 bp) from this study and reference sequence data from the following regions: Central Asia (CA1, CA2, CA3, CA4), Middle East (ME1 and ME2), and Europe (CE, EE, NEE, WE). These results are summarized in Table 4. Six sequences from this study, along with one Azerbaijani sequence extracted from GenBank (LN879480), formed the CA4 subclade, showing an average nucleotide identity of 96%. The referenced LN879480 sequence was isolated from a dog in Azerbaijan in 2002. A nucleotide identity of 99.3% was observed between the LN879480 dog sequence and the six Azerbaijani sequences from this study.

As shown in Table 4, within the CA2 subcategory, a cat sequence (AZER 5) exhibited nucleotide identities of 99.3% and 97.8% with AY352497 (human, 1989) from Georgia and AY854583 (strain V703, isolated from a sheep in Iran in 2000), respectively. The Azerbaijani cat sequence AZER 5 showed 100% amino acid identity (450/450 × 100) with the Georgian (AY352497) and Iranian (AY854583) strains. Within the CA4 subclade, amino acid identity was 96%. Analysis of the amino acid sequences revealed that all seven isolates from this study had aspartic acid (D) at position 101 of the N gene, a characteristic proposed by David et al. (2007) as typical of dog rabies virus variants.

AY35249																									
7_GEORG ID	0,95	0,98	0,95	0,98	0,95	0,96	0,95	0,95	0,95	0,95	0,99	0,95	0,95	0,95	0,95	0,95	0,95	0,95	0,95	0,95	0,98	0,95	0,96	0,99	0,95
AY85458																									
0_IRAN	ID	0,94	0,97	0,94	0,98	0,97	0,95	0,95	0,95	0,95	0,94	0,95	0,95	0,95	0,97	0,97	0,96	0,97	0,97	0,97	0,94	0,94	0,95	0,95	0,95
AY85458																									
1_IRAN		ID	0,93	0,98	0,94	0,94	0,94	0,94	0,94	0,94	0,97	0,94	0,94	0,94	0,94	0,94	0,94	0,94	0,94	0,94	0,98	0,94	0,95	0,98	0,94
AY85458																									
2 IRAN			ID	0,94	0,97	0,98	0,95	0,95	0,95	0,94	0,94	0,95	0,95	0,95	0,97	0,97	0,97	0,98	0,97	0,97	0,94	0,95	0,95	0,95	0,95
AY85458																									
3_IRAN				ID	0,94	0,95	0,94	0,94	0,94	0,94	0,98	0,94	0,94	0,95	0,94	0,94	0,94	0,94	0,94	0,94	0,99	0,94	0,95	0,98	0,95
AY85458																									
4_IRAN					ID	0,98	0,95	0,95	0,95	0,95	0,95	0,95	0,95	0,95	0,98	0,98	0,97	0,98	0,98	0,98	0,94	0,95	0,96	0,95	0,95
AY85458																									
5_IRAN						ID	0,96	0,95	0,95	0,95	0,95	0,95	0,95	0,95	0,98	0,98	0,97	0,98	0,98	0,98	0,95	0,95	0,96	0,95	0,96
AY85458																									
6_IRAN							ID	0,96	0,96	0,96	0,95	0,96	0,96	0,96	0,95	0,95	0,95	0,95	0,95	0,95	0,94	0,98	0,99	0,95	0,98
AZ2								ID	1,00	0,99	0,94	1,00	1,00	1,00	0,95	0,95	0,95	0,95	0,95	0,95	0,94	0,95	0,96	0,95	0,96
AZ3									ID	1,00	0,95	1,00	1,00	1,00	0,95	0,95	0,95	0,95	0,95	0,95	0,94	0,95	0,96	0,95	0,96
AZ4										ID	0,94	0,99	0,99	1,00	0,95	0,95	0,95	0,95	0,95	0,95	0,94	0,95	0,96	0,95	0,96
AZ5											ID	0,95	0,95	0,95	0,94	0,95	0,95	0,95	0,94	0,95	0,98	0,95	0,95	0,99	0,95
AZ6												ID	1,00	1,00	0,95	0,95	0,95	0,95	0,95	0,95	0,94	0,95	0,96	0,95	0,96
AZ7													ID	1,00	0,95	0,95	0,95	0,95	0,95	0,95	0,94	0,95	0,96	0,95	0,96
AZ9														ID	0,95	0,95	0,95	0,95	0,95	0,95	0,95	0,96	0,96	0,95	0,96
DQ83741																									
1 ISR															ID	0,99	0,97	0,99	1,00	0,99	0,94	0,95	0,96	0,95	0,95
DQ83743																									
9_ISR																ID	0,97	0,99	0,99	0,99	0,94	0,95	0,96	0,95	0,95
DQ83744																									
1_ISR																	ID	0,98	0,97	0,95	0,95	0,95	0,95	0,95	0,95
DQ83744																									
8_ISR																		ID	0,99	1,00	0,95	0,95	0,96	0,95	0,95
DQ83747																									
3 ISR																			ID	0,99	0,94	0,95	0,96	0,95	0,95
DQ83748																									
3_ISR																				ID	0,95	0,95	0,96	0,95	0,95

Table 4. Percentage Nucleotide Identity of N Gene Regions. Sequence alignment and analysis were performed using Vector NTI and BioEdit software, respectively. These results were obtained by comparing the full nucleoprotein gene (corresponding to nucleotide positions 71–1423) of seven sequences of interest with a set of 25 reference sequences

5.3. Phylogenetic Analysis of Rabies Samples

Using PhyML, two phylogenetic analyses were conducted with sequence lengths of 400 bp and 1353 bp, producing trees with identical topologies. The PhyML trees, represented in Figures 3A and 3B, demonstrated that the seven samples were characterized within the cosmopolitan lineage. The seven samples examined in this study fell into two distinct phylogenetic subclades—CA2 and CA4—within the Central Asian (CA) lineage circulating in the region, with CA2 previously described by Troupin et al. The Central Asian lineage was divided into three subclades by Troupin et al.: CA1

(viruses from China, Iran, and Russia), CA2 (viruses from Iraq, Iran, Turkey, and Russia), and CA3 (a cluster with uncertain geographic boundaries, including China, Iran, and Russia).

5.3.1. Phylogenetic Analysis of the Complete N Gene Sequences of 7 Samples with 62 Reference Sequences

The analyses indicated that the majority of samples tested in this study (6 out of 7 sequences) belonged to the Central Asian CA4 subclade, which includes a representative dog rabies virus from Azerbaijan (LN879480). The sample from Balakan city (cat, AZER 5) grouped with sequences from Iraq, Turkey, Iran, and Georgia within the Central Asian CA2 subclade. The CA4 subclade was formed by six sequences from this study and one Azerbaijani sequence extracted from GenBank (LN879480), isolated from a dog in Azerbaijan in 2002. Figures 3A and 3B illustrate the phylogenetic analysis between the full N genes of the seven Azerbaijani sequences and 62 reference sequences.

Phylogenetic Analysis of Partial N Gene Sequences of 7 Samples with 79 Reference Sequences. This analysis, depicted in Figures 4A and 4B, included 69 sequences and 10 additional Azerbaijani sequences. The majority of samples from this study (6 out of 7 sequences) and the referenced Azerbaijani sequences (8 out of 10) belonged to the CA4 subclade. The seventh isolate from this study (cat AZER 5 from Balakan) aligned with the CA2 subclade, alongside isolates from Iraq, Turkey, Iran, and Georgia, as well as an additional Azerbaijani strain (KJ645928, isolated from a cow in Dashkasan in 2012). The tenth sequence extracted from GenBank (KJ645921), isolated from a horse in Aghjabadi in 2013, belonged to the Middle East (ME1) lineage, alongside isolates from Iran, Jordan, and Israel (Figure 15).

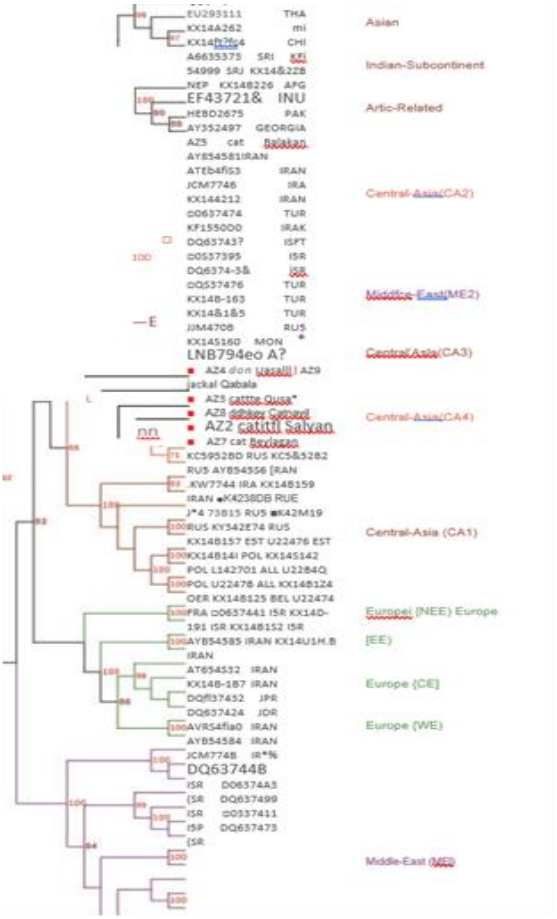


Figure 15. Phylogenetic relationships among the full N gene sequences (1353-bp) of seven samples taken from Azerbaijan, 10 rabies virus sequences representing the Indian subcontinent, Asia, and Arctic-related phylogenetic groups, 51 reference sequences representing the Middle East (ME1, ME2), Central Asia (CA1, CA2, CA3), and Europe (CE, EE, NEE, BI), and one reference sequence from Azerbaijan (LN879480).

The CA4 subcategory comprised 15 Azerbaijani strains from domestic animals (n = 14) and wild animals (n = 1), including dogs (n = 6), a cat (n = 1), cattle (n = 5), a donkey (n = 1), a horse (n = 1), and a jackal (n = 1).

Here, 7 of the Azerbaijani sequences cluster with sequences from Ukraine, Russia, and Mongolia. One Azerbaijani sequence clusters with sequences from Turkey, Iran, Israel, Georgia, and Jordan (the study was conducted by Sh. Zeynalova in 2012–2013). Phylogenetic relationships were determined for the N gene at a 136-bp ratio using SeaView (PhyML method, GTR model, 1000 replicates). GenBank accession numbers were included for each taxon in the tree.

The CA2 subclade consists of sequences from Iran (n = 4), Turkey (n = 1), Iraq (n = 1), two Azerbaijani strains isolated from a cow in 2012 (KJ645928), and a cat from this study (AZER5).

CA2 consists of two wild and six domestic animals: jackal (n = 2), cow (n=4), cat (n=1), and sheep (n=1).

The geographical localization of the seven phylogenetically linked positive rabies samples is shown in Figure 5. Each virus strain was defined by the corresponding clade (CA2 and CA4) identified in the phylogenetic analysis (Figure 16).

5.4. Evolutionary Analysis: Evolutionary Assessment of the N Gene

To analyze the spatio-temporal dynamics of rabies in Azerbaijan, a Bayesian MCC tree was constructed using the BEAST v1.7.4 software with 69 N gene sequences. Analysis of the MCC tree confirmed that all isolates from Azerbaijan belong to the Cosmopolitan subclade and are divided into the subclades CA2 (with 1 isolate) and CA4 (with 6 isolates) (Figure 15).

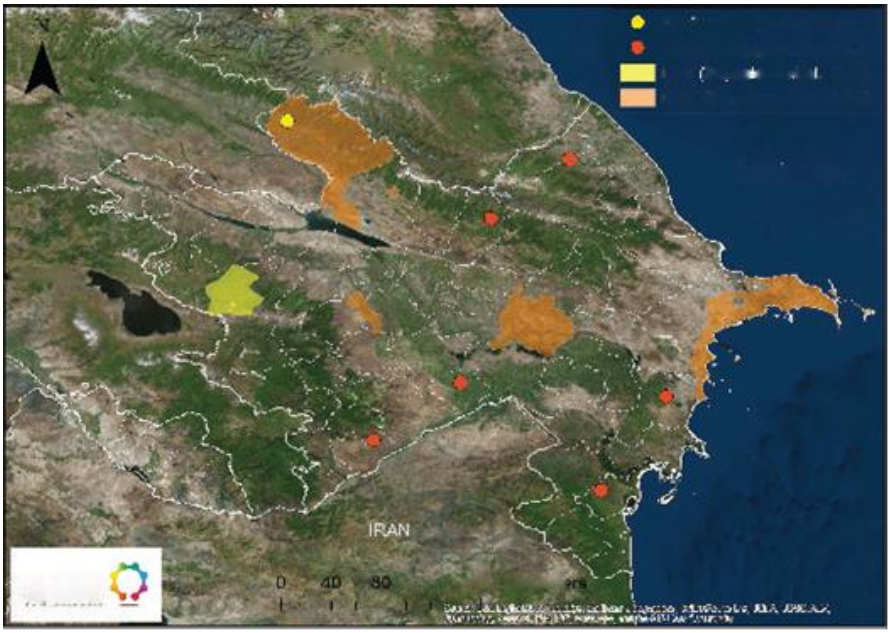


Figure 16. Geographical localization of seven rabies-positive Azerbaijani samples based on viral typing. Each viral strain on the map is identified by the corresponding phylogenetic subclade (CA2 and CA4) defined by the phylogenetic analysis.

The BEAST analysis yielded a substitution rate of 2.65 x 10^{-4} per site (95% HPD Interval [1.6167E-4, 3.9556E-4]). These rates are consistent with previous estimates in the Middle East and Africa for dog-related RABV groups from studies by David et al. Using the same Bayesian approach, we estimated the TMRCA of the two Azerbaijani clades, CA2 and CA4. Based on the study by Troopin et al., the TMRCA of CA2 was estimated to be between 1934–1976 (mean 1952), and the most recently defined node for CA4 (including six isolates and a reference sequence from 2002 [LN879480]) was estimated between 1987–2005 (mean 1994). The

node above CA4 was estimated to have a TMRCA between 1876–1945 (mean 1905). Caution is needed in describing the TMRCA of the CA4 clade, as it includes only recent isolates (2016) from this study.

Based on the data obtained, the rabies problem remains highly relevant in the regions of Qakh, Balakan, Baku, and Beylagan. Between 2014–2018, 34 cases were recorded in Baku, 13 in Balakan, 8 in Qakh, and 15 in Beylagan. The long-term persistence of concern, taking into account zonal characteristics, necessitated improved measures for disease control and prevention. For this purpose, a detailed study of the epizootic dynamics was required.

International experience has shown the relevance of establishing specialized centers that collect and analyze regular reports from disadvantaged areas to determine and localize epizootic hotspots. Mapping this information allows for rapid identification of the direction of epizootic wave progression and the associated rabies threat. The timeliness and completeness of data are crucial. Until now, reduced laboratory studies in some disadvantaged and high-risk regions of the country have posed a serious obstacle. The highly mobile carriers of the pathogen recognize neither administrative nor national borders, necessitating consistent information exchange between all administrative regions of the country.

The importance of retrospective studies cannot be underestimated. Preparing regional cadastres of dysfunction points detected over the past 15–20 years and creating cadastral (address) maps makes it possible to identify the contours of natural rabies hotspots, determine "primary" focus areas of stationary dysfunction, and reveal their connections to outbreaks in neighboring areas. When defining "risk zones," one must consider the epizootic index values (ratio of problem years to observation years) and the density of dysfunction points (number per unit area). Urban infection rates must also be taken into account during epizootic hotspot mapping, as they

depend directly on rabies cases in suburban areas. Cartographic analysis also enables an assessment of the situation in protected natural reserves, which often become "nuclei" of natural rabies hotspots.

Rabies control measures should be preventive. Xripunov E.M. and Muller W.W. identified oral vaccination of wild carnivores as a new method in rabies outbreak elimination strategies in all Western European countries.

In the first stage of the study period, a total of 326 animal samples were submitted for rabies testing. Among these, 216 tested positive, 75 negative, and 35 could not be tested. The positive-to-negative ratio has remained unchanged for a decade, although the number of untestable samples has decreased. More than half of the samples were taken from dogs (n = 111), and the second largest group of positive samples was from cattle (n = 58). The remaining samples were from wild and other domestic animals.

During the study period, Azerbaijani authorities vaccinated a total of 1.5 million dogs against rabies and culled 0.5 million.

Ten samples were selected and submitted to the Information Laboratory of the International Organization for Epizootic Diseases (OIE) by the Animal Health and Veterinary Laboratories Agency (HSBLA) to represent various regions of Azerbaijan. Analysis of the results showed that all samples corresponded to a widespread lineage of rabies virus thought to have spread globally through human migration over the past two centuries. Eight of the results formed a well-supported monophyletic group, indicating they belong to the same epidemiological cycle. This group is associated with viruses commonly found in Russia and Eastern Europe.

The strains from Dashkasan and Aghjabedi included in the study are genetically distinct from both each other and from the main strain group typical of Azerbaijan. The strain from Dashkasan shares a more recent common ancestor with historical isolates from Georgia

and is related to more recent strains from the Middle East. The strain from Aghjabedi is distinct from Middle Eastern strain groups that circulated between 1990–2004.

Despite dog vaccination and culling of stray dogs, reported rabies cases and PEP (post-exposure prophylaxis) applications increased in Azerbaijan between 2006 and 2010. This rise in human and animal cases may indicate an overall increase in rabies and suggests that current control methods may be insufficient. Culling of dogs remains a widely used method for short-term rabies control, but it has been observed to potentially worsen the situation by altering the demographic characteristics of local dog populations. In contrast, coordinated and continuous vaccination of dogs has repeatedly proven to be an effective method of reducing rabies incidence. When carried out in accordance with international standards and considering the demographic characteristics of local dog populations, vaccination programs can be more effective when combined with sterilization and other dog population management methods. In areas where domestic dogs are kept in large numbers, increasing public awareness about the benefits of vaccination may also lead to better compliance with national vaccination regulations.

In 2009, the incidence of known human rabies cases in Azerbaijan (0.07 cases per 100,000 people) was higher than in Turkey (0.025) and neighboring Iran (0.02), but similar to Georgia (0.13) and much lower than in Iraq (0.89). The recurrence of rabies among children under the age of 15 was lower compared to results from similar studies conducted in other countries where rabies among children is more common. This anomaly in Azerbaijan may be due to cultural differences that reduce children's exposure to potentially rabid animals. However, a more likely explanation is underreporting, as many children do not inform adults about animal bites, leading to rabies cases not being investigated. Therefore,

raising awareness among children about the risks of rabies remains a crucial part of rabies control.

As with all surveillance-based studies, there are certain limitations in the official data on rabies. Reporting efforts may vary by region and over time. One of the main limitations in explaining increases in rabies cases is that the effort to report them can also change over time. Additionally, current human rabies cases are often diagnosed without laboratory confirmation, leading to possible over- or underreporting, especially since clinical symptoms may overlap with those of other diseases. Furthermore, in the current surveillance system, rabies data on animals tends to focus on species in close contact with humans, while cases among wild animals may be underreported. This may explain why the number of reports related to dogs and cattle is significantly higher, which makes it difficult to fully understand the actual relative incidence of disease among domestic and wild animals.

Globally, most human rabies cases are the result of bites from domestic dogs, and historical data show that rabies resulting from dog bites was more widespread in the Caucasus region of the former Soviet Union. However, sylvatic (wildlife) rabies continues to occur regularly in neighboring countries, and these cases may involve rabies resulting from stray or wild animal bites. This epidemiology can vary regionally and is important for prioritizing control strategies. There are anecdotal reports of an increase in wolf populations in the Caucasus, but reliable census data are lacking. The rise in known wolf attacks could be attributed to an increase in wolf numbers, but it could also be driven by land use changes and increased interaction between wildlife and humans. Between 2000 and 2010, the number of cattle in the country increased from 1.7 million to 2.4 million, and land used for agriculture rose from 1 million to 1.6 million hectares. Other wildlife-related issues of concern in Azerbaijan include the increasing population of North

American raccoons (Procyon lotor) and the potential risk of lyssavirus in bats. Although there have been no reported cases of rabies in raccoons in Azerbaijan, if rabies were to spread within this population, it could pose a serious threat to control efforts, as seen in North America.

Phylogenetic analyses of selected rabies isolates from Azerbaijan show that multiple strains have circulated in the country. This is not surprising for a country like Azerbaijan, which has several large land borders, and it indicates the transboundary movement of rabies in the region. Previous studies in Eurasia have also shown multiple strains in Georgia. At the time, analysis of available samples suggested that the Caucasus Mountains acted as a barrier to the spread of rabies, but now most strains found in Azerbaijan appear to be more closely related to strains from Russia, suggesting that this geographical barrier may no longer be effective.

Between 2014 and 2018, molecular genetic studies of rabies isolates collected from various regions of Azerbaijan showed that seven out of ten tested samples were positive using real-time RT-PCR and conventional RT-PCR for partial and full amplification of the N gene. Phylogenetic analysis based on a comparison of the full N gene sequences of the seven Azerbaijani samples with 62 reference Lyssavirus sequences showed that all seven samples belong to the Cosmopolitan clade. The study further revealed that six of the seven samples and one sample belonged to the CA4 and CA2 sub-lineages of Central Asia, respectively (Figure 17).

The phylogenetic analysis based on a comparison of partial N gene sequences from seven Azerbaijani samples, 11 reference Azeri sequences, and 61 reference *Lyssavirus* sequences showed that 6 dogs, 1 jackal, and 6 domestic animals belonged to the CA4 isolated from Iran, Turkey, Iraq, and Azerbaijan, included two Azerbaijani strains—KJ645928 and AZER5 (isolated from a cat in sublineage.

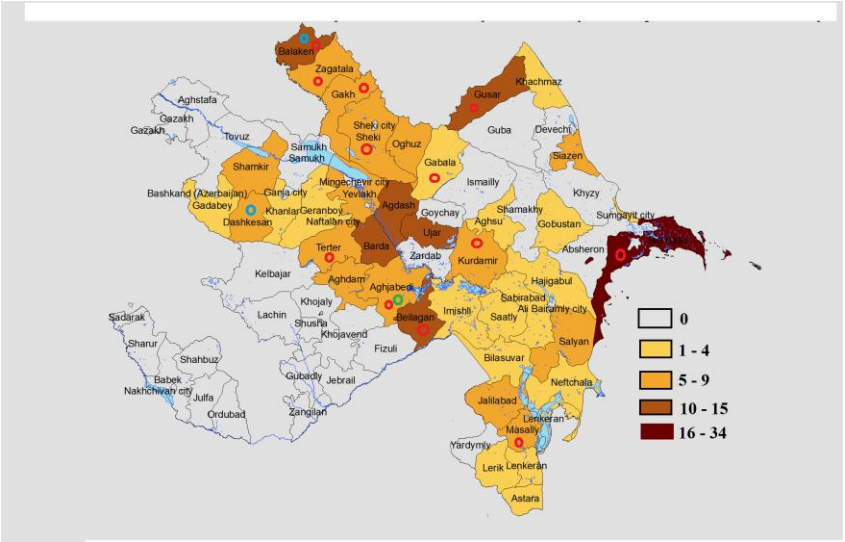


Figure 17. The distribution of rabies samples sequenced in Azerbaijan across various subclades and their spread in different regions.

In contrast, the CA2 subcategory, consisting of 9 strains Balaken, located in the central part of the country): 2 jackals and 6 domestic animals: 4 cows, 1 cat, and 1 sheep.

The phylogenetic study of the seven isolates from Azerbaijan and the reference Azerbaijani sequences indicated the presence of at least two lineages in the country, which is associated with the dominance of dogs in the CA4 subclade. Dogs and other wild canids act as vectors in neighboring countries. The presence of the ME and CA2 subclades in Azerbaijan is explained by infected animals coming from neighboring countries.

To obtain suitable PCR products for nucleotide sequencing, nRT-PCR was carried out on pathological material targeting the N genome. The studies showed that the selected PCR variant allows for the detection of field isolates with different antigenic variants and

genogroups, as well as fixed strains. The resulting products were used for nucleotide sequencing. The results obtained from pathological material containing various field isolates and vaccine strains were fully consistent when using PCR. This demonstrates the high sensitivity of the PCR method, and compared to biological testing, it is more advanced, has been optimized for routine diagnostics, and its use has been validated. It should be noted that direct detection of the antigen in the brain using IFRA is a reference method for rabies diagnosis. The results of the study of the primary structure of the selected fragments of the N and G genes allow the conclusion that there are two main groups of the rabies virus in Azerbaijan.

A comparison of the N and G genes and their fragments of isolates from Azerbaijan with European and Asian nucleotide sequences showed that, in most cases, the isolates group according to their geographical origin. In some cases, it has been proven that Azerbaijani isolates group with those from Russia, Turkey, Georgia, and Iran.

RESULTS

1. For the first time in Azerbaijan, the epizootiological characteristics of Schmallenberg virus were studied, and it was determined that the virus entered the country through imported animals [1, 12].
2. The role of ecological factors in the spread of the Schmallenberg virus was investigated, and it was observed that the virus spread regardless of those factors [13, 14, 16].
3. The molecular-genetic characterization of the Schmallenberg virus was carried out, PCR was applied, and currently, it is considered the only method. Brain tissue was found to be the best sample for virus detection [9, 16, 27].

4. The epizootiological characteristics of lumpy skin disease were studied, and it was determined that the disease entered Azerbaijan from Iran. New clinical symptoms of the virus were identified, which led to an increase in the mortality rate [5, 7, 18, 19].
4. The molecular and serological diagnostics of lumpy skin disease virus were carried out. The specificity of the samples was determined. For the first time, serological diagnostics were performed, and the effectiveness of vaccination was evaluated [4, 6, 26].
5. The epizootiological characteristics of the rabies virus were studied, and it was determined which regions had higher incidence rates [2, 3, 15, 20, 22].
6. The circulating rabies strains in Azerbaijan were identified. Their next-generation sequencing was carried out, and 17 samples were placed in a phylogenetic tree [8, 10, 25].
7. PCR optimization was performed by selecting specific primers to amplify various parts of the rabies virus genome [11, 17, 23, 24].
8. The nucleotide sequences of Azerbaijani isolates were submitted to the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) [20, 21].

RECOMMENDATIONS:

Before importing animals into the country, serological and PCR diagnostics should be performed to ensure they are disease-free.

To determine the effectiveness of vaccination, seromonitoring should be carried out, and laboratory tests should be conducted to select appropriate vaccines. Proper vaccination will ensure protection against diseases.

In combating rabies, the main threat-vaccination, control, and identification of stray dogs-must be addressed. After preventive measures, they should be sent to shelters.

List of Scientific Publications Related to the Dissertation Work

1. Zeynalova Sh., Asadov K., Vatani M., Biological observation of Schmallenberg's disease in Azerbaijan // Scientific works of the Institute of Microbiology of ANAS, 2015, v.13, №1, p.205-207
2. Zeynalova Sh., Epidemiology and genotyping of rabies in Azerbaijan 2010-2013 years // Materials of the International scientific-practical conference on the topic "Innovative development of agricultural science and education: world experience and modern problems". - Ganja, 2015, p.511-15.
3. Agaeva E.M., Zeynalova Sh.K., Narimanov V.A. Innovative methods of molecular diagnostics in medicine// Biomedicine, -2015, No. 2, p 16-19.
4. Zeynalova Sh., Asadov K., Vatani M., Epidemiology and molecular diagnostics of nodular dermatitis among large horned animals in Azerbaijan//Scientific works of the Institute of Microbiology of the Academy of Sciences of Azerbaijan, 2016, v.14, No.1, p.188-195.
5. Zeynalova Sh. Adaptation of rabies diagnostics to biosafety rules and selection of an effective method// Azerbaijan Agricultural Science, 2016, III edition, p.81-84.
6. Zeynalova Sh et al. Epizootology and Molecular Diagnosis of Lumpy Skin Disease among Livestock in Azerbaijan// Frontiers Microbiology(İsveçrə), 2016, V 29; N7: p.1022.
7. Zeynalova Sh, Epizootology and Molecular Diagnosis of Lumpy Skin Diesease Among Livestock in Azerbaijan// Online J Public Health Inform. 2016; 8(1): e178.
8. Agayeva E., Zeynalova Sh., Molecular-genetic methods in microbiological diagnostics//Azərbaycan Tibb Universiteti, elmi-praktik konfrans, Baku 2016. s. 95
9. Safi N., Asadov K., Zeynalova Sh et al., The prevalence of rabies cases in the territory of Azerbaijan//ISDS Conference, 2017, 1 (9) 1.

10. Hasanov E., Zeynalova Sh. et al., Assessing the impact of public education on a preventable zoonotic disease: rabies // Epidemiol. Infect., Cambridge University Press, 2017, pp.227-235.
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