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# Isolation and study of a strain of lumpy skin disease virus of cattle

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**Abstract**. The identification of new virus strains will prevent the development of outbreaks thanks to the development and use of vaccines. The aim of the work was to isolate and sequence the genome of lumpy skin disease virus from an epizootic in the Kostanay region. The genetic material of virions was identified by polymerase chain reaction (PCR); viral antigens and antibodies to them have been determined by enzyme-linked immunosorbent assay (ELISA) or diffusion test methods; to accumulate the material, the virus has been cultivated in lamb testicle monoclonal cell; new generation sequencing has been performed using MiSeq System and FastQ software; strain affiliation has been established by the BLASTN-alignment method. Specific

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amplicons of the virus with a length of 347 bp. were detected in skin samples but not found in blood. Antigens in 1:5-1:320 dilutions were identified in skin material and 2-3 passages of monoclonals; less antigenic activity was found in the blood in a 1:2 dilution. In response to the pathogen, specific immunoglobulins were synthesized in the serum of 67% of the studied animals and were detected in dilutions of 1:100-1:400. The viral material was accumulated in monoclonals and isolated in a sucrose gradient. The whole-genome sequence of the obtained material confirmed the isolation of a new strain of nodular dermatitis virus with a percentage of similarity to the closest homologues of 99.66%. The strain was named *Dermatitis nodularis bovum/2018/Kostanay/KZ*; the sequence has been submitted to GeneBank, and the object has been deposited in the Collection of Microorganisms under accession number M-9-21/D. The obtained information can be used to prevent the spread of foci of cattle infection

**Keywords:** biomaterial; sequencing; assembly; polymerase chain reaction; virus; deoxyribonucleic acid

## INTRODUCTION

Due to the globalisation of economic relations in many countries of the world, which rapidly includes the Republic of Kazakhstan, the epizootic situation in their territories is becoming increasingly vulnerable to the penetration and spread of dangerous and especially dangerous infectious diseases, including diseases of farm animals. Taking into account the wide dissemination of the lumpy skin disease in the direction from the south to the northeast, the migration of this pathogen can be dangerous for Kazakhstan territory. Epizootic outbreaks harm not only the health of animals, but also the economic situation of the region. Therefore, it is important to identify pathogens in time to prevent their spread and implement a vaccination program.

Infectious lumpy skin disease of cattle is an acute particularly dangerous infectious viral disease, accompanied by fever, nodular lesions and necrosis of the skin, swelling of subcutaneous connective tissue and organs, damage to the eyes, mucous membranes of the respiratory and digestive tracts (Issimov *et al.*, 2022). The causative agent is a virus containing deoxyribonucleic acid belonging to the *Poxviridae* family, the genus *Capripoxvirus*. Currently, three varieties are known – *Orphlins*, *Allerton* and *Neethling* – distinguished by the degree of virulence, immunogenicity and cytopathogenic action (Bayantassova *et al.*, 2023). It has been proven that only the virus of the third group causes infectious lumpy skin disease. It has an antigenic relationship with the sheep pox virus. It reproduces in chicken embryos, in primary cell cultures of calves, lambs, rabbits (Whittle *et al.*, 2023; World Animal Health Information System, n.d.). In natural conditions, cattle are most susceptible to this disease. At a temperature of 4°C, the virus remains active for 6 months. It spreads at a speed of 70 to 100 km/day (Namazi & Khodakaram Tafti, 2021).

Lumpy skin disease virus (LSDV) is an African endemic that had spread to Kazakhstan, Serbia, Greece, Bulgaria, Thailand, India and China. As indicated by P.A. Desingu *et al.* (2023), the main risk factor associated with LSDV is its microevolution: the genome constantly undergoes frameshift and nonsense mutations that cause new disease outbreaks in different regions. The wild-type strain has been found only in Africa, as well as only recombinant viruses have been found in Asia. M.B. Orynbayev *et al.* (2021) investigated an epizootic of nodular dermatitis in the region of Atyrau (Makash), Kazakhstan. The first cases of cow disease were found in 2016 (Vygovska *et al.*, 2023). The researchers managed to isolate the virus from animal material, cultivate it in a lamb testicle line monolayer, identify the provoker by polymerase chain reaction (PCR) (receiving a specific amplicon with a length of 192 bp) and sequence the genome of the pathogen.

Amplicons of the virus were detected in all samples of blood, spleen, lymph nodes and lungs of dead animals, as well as in the material of vectors – ticks and ticks. The strain was archived as *Nodulares/Dermatitis/Atyrau-2016*. Based on this strain, O. Chervyakova *et al.* (2022) constructed an attenuated vaccine by knocking out four genes (encoding interleukin-like protein, interferon-gamma receptor, thymidine kinase, and LSDV142). The modified virus was genetically stable for ten passages in monoclonals; in high doses, the one had not caused the disease, but stimulated the synthesis of virus-specific immunoglobulins in vaccinated animals.

As indicated by F. Vandenbussche *et al.* (2022), several vaccinia-like recombinant LSDVs were identified in Kazakhstan and the neighbouring region of China in 2017-2019. Previously, mass vaccination of animals against nodular dermatitis (Lumpivax) was carried out in Kazakhstan (Kropyvka *et al.*, 2024). LSDV was transmitted between animals. Vaccination caused the appearance of new strains, which are conventionally divided into four groups and differ in a number of genetic recombination's (126-146 exchanges). Therefore, it is important to search and identify such recombinants.

The tasks of the work were isolation and identification of the nodular dermatitis virus strain from the focus of infection in the Kostanay region.

## MATERIALS AND METHODS

The work was performed on the basis of on the basis of the RSE Research Institute of Biological Safety Problems of the Scientific Committee of the Ministry of Education and Science of the Republic of Kazakhstan. Biomaterials from sick and dead animals from the outbreak of epizootics were collected in September 2018 in the Kostanay region. Treatment of animals took place

in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986). To detect the lumpy skin disease virus in cattle, 4 samples of affected skin parts and 4 samples of animal blood were tested in real time by PCR with a pair of specific viral primers LSDV-2-f and LSDV-2-r and *Taq* polymerase. Amplification was carried out in 35 cycles (94ºC 3 min-((94ºC 20 sec-50ºC 20 sec-72ºC 40 sec)×35)-72ºC 7 min-4ºC storage) (Zeedan *et al*., 2019).

To determine the activity and specificity of the antigen, the method of enzyme-linked immunosorbent assay (ELISA) was used. It was taken 8 samples of animal skin and blood as well as 10 samples of virus passages. For this purpose, polystyrene flat-bottomed dies were used, which were previously sensitised with a specific immunoglobulin of the lumpy skin disease virus of cattle. Control antigens (antigen C, antigen H) were used to perform enzyme-linked immunosorbent assay. The test biomaterials were diluted in a start ratio of 1:5, and culture samples in a start ratio of 1:2. Then, control antigens C, H and test samples of 0.1 $cm^3$ were introduced into the wells. The plate with antigens was incubated (17 h 4°C), washed, contacted with conjugate (1 h 37°C), washed again and then the substrate solution was introduced (30-60 min, room temperature). The reaction results were taken into account visually or on a photometer at a wavelength of 405 nm.

To detect antibodies to the lumpy skin disease virus of cattle the ELISA was performed on a tablet with a recombinant protein of the nodular dermatitis virus of cattle. The 9 samples of blood serum were taken to analysis. Control antibodies (CC, CH) and test serums were introduced into the wells (start dilution 1:100) and incubated for (1 h, 37°C), washed, contacted with analytic serum (1 h, 37°C), washed, dried and then reacted with substrate solution. The photometric reading was performed at 405 nm (Liang *et al*., 2022). To detect the antigen of this virus or antibodies to virus after disease the diffuse precipitation reaction in 1% agar gel Difco has been performed (27 samples; blood, skin and cell cultures). Control samples with C, H, CC and CH antigens were used in the working titer, dilutions were prepared in saline solution. Wells were made in the agar using a star-shaped stencil with one central tube and 6 peripheral tubes. The central holes were filled with control samples, and the side holes – with test. The reaction kept in wet chamber for 24-48 hours at room temperature (22°C) (Colorio *et al*., 2021).

To cultivate the virus in monolayer cells, the cell cultures of lamb tests and green monkey kidneys (*Vero*) have been contaminated with skin isolates (4 samples). The cells were pretreated with benzylpenicillin and streptomycin (18 h, 4°C), infected with 0.2 ml of samples (1.5 h, 37°C), washed and grown in inactivated 2% serum (7 d, 37°C). Three passages have been performed. The test tubes were examined daily under a microscope to assess the virus (Vidanović *et al*., 2016). Samples were then taken from each vial to test for the presence of lumpy skin disease virus in a polymerase chain reaction test system (Agianniotaki *et al*., 2017). To purify the virus from cell culture, the sedimentation (10737 g, 180 min) in sucrose gradient (30%, 45%, 60%) has been performed.

Genome-wide sequencing of the virus isolate was carried out by high-performance sequencing of the next generation and subsequent genome assembly. The measure of deoxyribonucleic acid concentration was performed by spectrophotometric and fluorimetric methods (NanoDrop1000 and Qubit®.2.0 Fluorometer; Qubit dsDNA HS Assay Kits) (Nakayama *et al*., 2016). The preparation of the deoxyribonucleic acid library was carried out according to Nextera DNA Flex Library Prep Kit. The sample of deoxyribonucleic acid was subjected to tagmentation, amplification with labelling and normalisation of the library concentration (Illumina Proprietary, 2020). Sequencing was carried out using a set of reagents MiSeq Reagent Kitv3 (600 cycle), which allows sequencing 20 million readings with a length equal to 300 bp with a paired-end (Namazi & Khodakaram Tafti, 2021). The analysis of the obtained data was carried out using FastQC programmes Trimming of low-quality bases was carried out in SeqTK v1.3-r106 and Sickle v1.33. Genome assembly was carried out in SPAdes 3.13.2 programme.

Basic Local Alignment Search Tool (BLAST) software (both the web service and the local version) was used for comparative identification of the resulting contigs. The search was conducted relative to the database "nt" (nucleotide) with standard settings.

## RESULTS

To confirm the identification of nodular dermatitis virus, an amplification reaction was performed with specific primers from skin and blood samples. The results PCR-screening are shown in Figure 1.

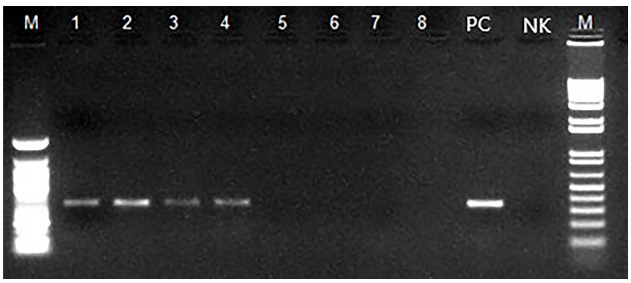


***Figure 1***. *Results of the polymerase chain reaction for the detection of deoxyribonucleic acid of the lumpy skin disease virus of cattle*
***Note****: M – marker; from 1 to 4 affected parts of animal skin; from 5 to 8 – selected animal blood; PC – positive control (347 bp); NC – negative control*
***Source:*** *compiled by the authors*

Four samples of affected skin taken from diseased and fallen animals were found to contain lumpy skin

disease virus in this test, while no virus was detected in blood. The amplicon size of the tested samples coincided with the amplicon size of the virus of the positive control sample (347 bp). Afterwards, to determine the activity and specificity of the nodular dermatitis virus antigen, an ELISA with control antigens C and H has been performed. 18 samples of biomaterials were tested, as indicated in Table 1.

***Table 1**. Test samples*

| No. | Name of the biomaterial | No. | Name of the biomaterial |
|---|---|---|---|
| 1 | Concentrated VCS test after the 3rd passage level on lamb testicles (Affected part of the skin, cow, black colour) | 10 | VCS after the 1st passage level (Affected part of the skin, heifer) |
| 2 | 20% suspension of the affected part of the skin, heifer | 11 | VCS after the 1st passage level (Affected part of the skin, cow, red colour) |
| 3 | 20% suspension of the affected part of the skin, cow, suit colour | 12 | VCS after the 1st passage level (Affected part of the skin, cow, white colour) |
| 4 | 20% suspension of the affected part of the skin, cow, white colour | 13 | VCS after the 1st passage level (Affected part of the skin, cow, black colour) |
| 5 | 20% suspension of the affected part of the skin, cow, black colour | 14 | VCS after the 2nd passage level (Affected part of the skin, heifer) |
| 6 | Blood from a cow, white colour, No. 1 | 15 | VCS after the 2nd passage level (Affected part of the skin, cow, red colour) |
| 7 | Blood from a cow, white head, No. 2 | 16 | VCS after the 2nd passage level (Affected part of the skin, cow, white colour) |
| 8 | Blood from a cow, white muzzle | 17 | VCS after the 2nd passage level (Affected part of the skin, cow, black colour) |
| 9 | Blood from a bull, white colour, No. 1 | 18 | VCS after the 3rd passage level (Affected part of the skin, cow, black colour) |

***Note:** VCS is a virus-containing suspension*
***Source:** compiled by the authors*

The results of the enzyme-linked immunosorbent assay samples for detecting the antigen of the lumpy skin disease virus of cattle are shown in Figures 2, 3. During visual evaluation, staining should be observed in wells with antigen C, weak staining is allowed in wells with antigen H, decreasing with the dilution of the antigen. There should be no staining in wells with a solution for enzyme-linked immunosorbent assay (horizontal line A). On the photometer, the reaction is indicated by the difference in optical density between the test wells and the antigen H. The result is considered positive if the optical density in the test wells is 2 or more times higher than the density in the corresponding wells of the control antigen H in two or more subsequent dilutions.

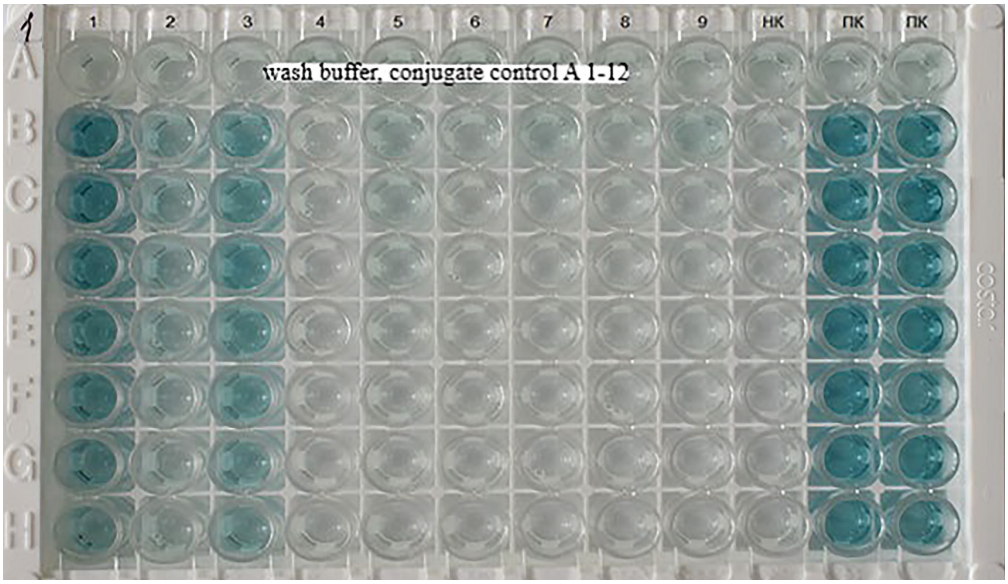


***Figure 2**. The results of tests to detect the antigen of the lumpy skin disease virus of cattle*
***Source:** compiled by the authors*

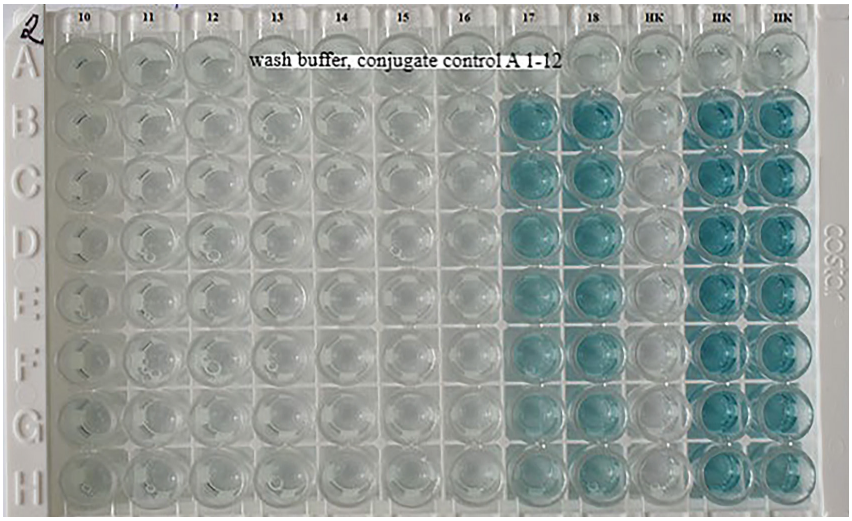


***Figure 3**. The results of tests to detect the antigen of the lumpy skin disease virus of cattle*
***Source:** compiled by the authors*

As a result, antigens of the nodular dermatitis virus of cattle with an activity of 1:5-1:320 were detected in the initial materials No. 2, 3 and 5, and in the blood No. 7 and 9 with an activity of 1:2. After carrying out viral isolation in monolayers of lamb testicle cell cultures, the antigen of the lumpy skin disease virus of cattle was detected only in one sample No. 1, 17 and 18 with an activity of 1:128-1:320 after the second and third passages of skin material from black cow. In other samples during cultivation, it was not possible to isolate this virus. An ELISA was also performed to detect protective antibodies to the lumpy skin disease virus of cattle after contact with provoker. 9 samples of animal blood serum were tested for enzyme-linked immunosorbent assay, as indicated in Table 2. Figure 4 shows the results of the samples.

***Table 2**. The studied samples for the detection of antibodies of the lumpy skin disease virus of cattle*

| No. | Name of the biomaterial | Unit of measurement | Volume | No. | Name of the biomaterial | Units of measurement | Volume |
|---|---|---|---|---|---|---|---|
| 1 | Blood serum No. 1 | ml | 0 | 6 | Blood serum No. 6 | ml | 7.21 |
| 2 | Blood serum No. 2 | ml | 3.55 | 7 | Blood serum No. 7 | ml | 1.8 |
| 3 | Blood serum No. 3 | ml | 4.8 | 8 | Blood serum No. 8 | ml | 6.7 |
| 4 | Blood serum No. 4 | ml | 6.3 | 9 | Blood serum No. 9 | ml | 3.4 |
| 5 | Blood serum No. 5 | ml | 2.45 | 10 | Blood serum No. 10 | ml | 4.8 |

***Source:** compiled by the authors*

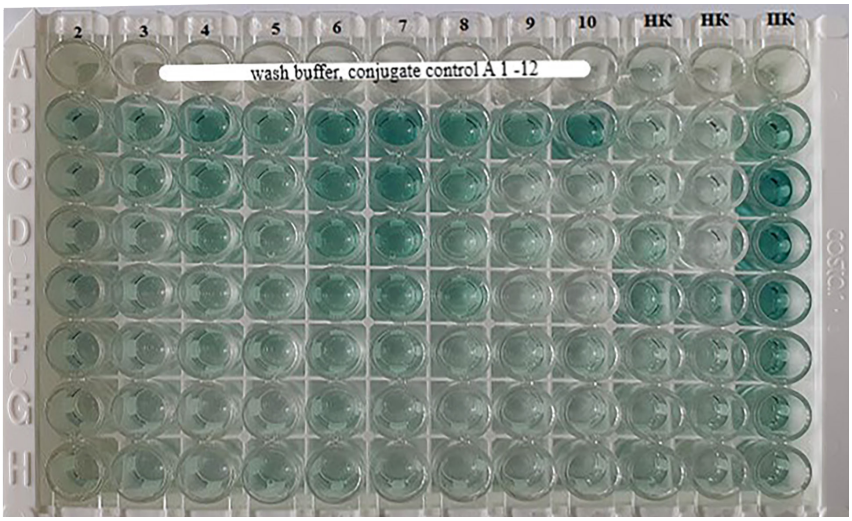


***Figure 4**. Results of tests for detection of antibodies of the lumpy skin disease virus of cattle*
***Source:** compiled by the authors*

During visual assessment, staining should be observed in wells with control antibodies CC, weak staining is allowed in wells with CH or there should be no staining. There should be no staining in wells with buffer solution (horizontal line A). The reaction is considered positive if staining is observed in wells with a test sample at a dilution of 1:400, with weak staining in similar dilutions in wells with the CH antigen. On the photometer, the reaction is taken into account by the difference in optical density in the wells with the tested samples and the CH antigen. The result is considered positive if the optical density in the wells with the tested samples is 2 or more times higher than in the corresponding wells with the control CH antigen in two or more subsequent dilutions. In samples No. 3, 5, 6, 7, 8, 9 antibodies to the lumpy skin disease virus of cattle with an activity of 1:100-1:400 were detected (67%). To set up the diffuse precipitation reaction, for antigens and antibodies identification, 18 samples of virus-containing suspensions and 9 samples of animal blood serum were tested, the results are presented in Table 3.

***Table 3**. Results of the diffuse precipitation reaction*

| No. | Name of samples | Results | No. | Name of samples | Results | No. | Name of samples | Results |
|---|---|---|---|---|---|---|---|---|
| 1 | Concentrated VCS sample after the 3rd passage level (Affected part of the skin, cow, black colour) | + | 10 | VCS after the 1st passage level (Affected part of the skin, heifer) | - | 19 | Blood serum No. 2 | - |
| 2 | 20% suspension of the affected part of the skin, heifer | - | 11 | VCS after the 1st passage level (Affected part of the skin, cow, red colour) | - | 20 | Blood serum No. 3 | - |
| 3 | 20% suspension of the affected part of the skin, cow, suit colour | + | 12 | VCS after the 1st passage level (Affected part of the skin, cow, white colour) | - | 21 | Blood serum No. 4 | + |
| 4 | 20% suspension of the affected part of the skin, cow, white colour | + | 13 | VCS after the 1st passage level (Affected part of the skin, cow, black colour) | - | 22 | Blood serum No. 5 | - |
| 5 | 20% suspension of the affected part of the skin, cow, black colour | - | 14 | VCS after the 2nd passage level (Affected part of the skin, heifer) | - | 23 | Blood serum No. 6 | - |
| 6 | Blood from a cow, white colour, No. 1 | - | 15 | VCS after the 2nd passage level (Affected part of the skin, cow, red colour) | - | 24 | Blood serum No. 7 | + |
| 7 | Blood from a cow, white head, No. 2 | - | 16 | VCS after the 2nd passage level (Affected part of the skin, cow, white colour) | - | 25 | Blood serum No. 8 | - |
| 8 | Blood from a cow, white muzzle | - | 17 | VCS after the 2nd passage level (Affected part of the skin, cow, black colour) | - | 26 | Blood serum No. 9 | - |
| 9 | Blood from a bull, white colour, No. 1 | - | 18 | VCS after the 3rd passage level (Affected part of the skin, cow, black colour) | - | 27 | Blood serum No. 10 | - |

***Note:** VCS is a virus-containing suspension*
***Source:** compiled by the authors*

The reaction results were taken into account with control antigens or antibodies, in which there must necessarily be precipitation lines between C and CC in the absence of them between C and CH or CC antibodies and antigen H. The reaction is considered positive if there are precipitation lines identical to the lines in the control between the wells with CC or C antigen and the pathological materials or sera, respectively, after the above time, there are precipitation lines in character identical to the lines in the control. In the absence of precipitation lines between these components, the reaction is considered negative. As a result, antigens of the lumpy skin disease virus of cattle were detected in samples No. 1, 3, 4 in the diffuse precipitation reaction, and antibodies of the same virus were detected in two samples with blood serum No. 21 and 24. The virus has been cultivated in animal cells monolayer. Experiments were carried out to isolate the lumpy skin disease virus of cattle from four samples of the affected parts of the skin of diseased and sick animals in monolayers of primary trypsinized cultures of lamb testicle cells and transplanted lines of green monkey kidney cell cultures (*Vero*). The results of the first passage for virus release from skin samples are shown in Figure 5 and Table 4. On day 7, in samples No. 1 and 4, changes were detected in the monolayers of lamb testicle cell cultures. Obviously, according to the PCR-signal, the titer of virus in sample 1 (from heifer cow) was lower, than in sample 4 (from black cow). Then the second passage was carried out. Its results are presented in Figure 6 and Table 5.

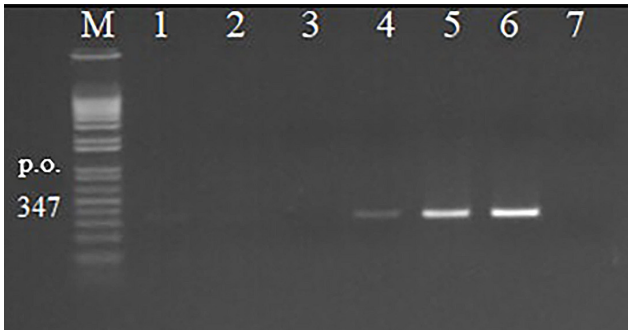


***Figure 5**. Results of the first passage for virus release from skin samples*
***Note:** M – marker; No. 1, 2, 3, 4 – test samples, 5 and 6 positive controls; 7 – negative control*
***Source:** compiled by the authors*

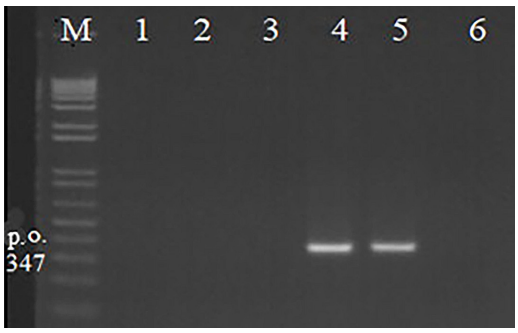


***Figure 6**. Results of the second passage for virus release from skin samples*
***Note:** M – marker; No.1, 2, 3, 4 – test samples, 5 and 6 positive controls*
***Source:** compiled by the authors*

***Table 4**. Name of samples and results*

| No. | Name of samples | Results |
|---|---|---|
| 1 | VCS after the 1st passage level (Affected part of the skin, heifer) | ± |
| 2 | VCS after the 1st passage level (Affected part of the skin, cow, red colour) | ± |
| 3 | VCS after the 1stt passage level (Affected part of the skin, cow, white colour) | - |
| 4 | VCS after the 1st passage level (Affected part of the skin, cow, black colour) | + |
| 5 | Positive control | + |
| 6 | Positive control | + |
| 7 | Negative control | - |

***Note:** VCS is a virus-containing suspension*
***Source:** compiled by the authors*

***Table 5**. Name of samples and results*

| No. | Name of samples | Results |
|---|---|---|
| 1 | VCS after the 2nd passage level (Affected part of the skin, heifer) | - |
| 2 | VCS after the 2nd passage level (Affected part of the skin, cow, red colour) | - |
| 3 | VCS after the 2nd passage level (Affected part of the skin, cow, white colour) | - |
| 4 | VCS after the 2nd passage level (Affected part of the skin, cow, black colour) | + |
| 5 | Positive control | + |
| 6 | Negative control | - |

***Note:** VCS is a virus-containing suspension*
***Source:** compiled by the authors*

After the second passage level, the virus was detected only in one sample No. 4, the suspension, from the affected part of the skin of a black cow, contains the lumpy skin disease virus. The PCR-signal was on the level of positive control; this fact proves the active reproduction of virions in host testicular cells. A third passage was then carried out to confirm the presence of this virus. The polymerase chain reaction test was positive. Thus, it was possible to isolate a lumpy skin disease virus isolate from the biomaterial, which was named *Dermatitis nodularis bovum/2018/Kostanay/KZ*. Figure 7 shows an isolate of this virus.

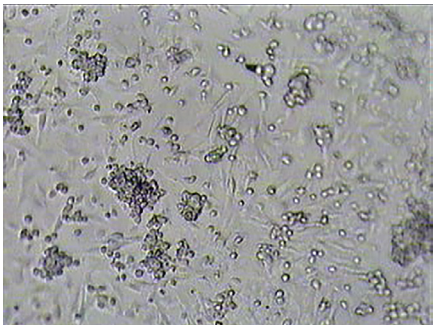

***Figure 7**. Isolate Dermatitis nodularis bovum/2018/Kostanay/KZ of the lumpy skin disease virus of cattle in a monolayer of lamb testicle cell culture*
***Source:** compiled by the authors*

The cultivation of resulting strain in lamb testicle cells caused destructive changes in the monolayer in the form of shiny light-refracting cells with a round shape, with clear outlines of the shells of the nucle-

us and cytoplasm. Virus isolation from biomaterials in monolayers of *Vero* cell cultures has not been successful. To optimize the way of virions purification, the samples were taken from each sucrose layer (at the procedure of extraction with different sucrose amount) and examined using a polymerase chain reaction to detect deoxyribonucleic acid of the lumpy skin disease virus of cattle. The results are shown in Figure 8.

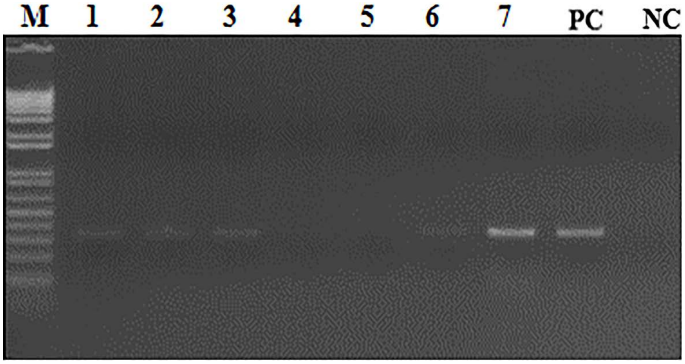


***Figure 8**. Results of polymerase chain reaction after purification of lumpy skin disease virus*

***Note:** M – marker; method 1 (cleaning in 30% sucrose at 10737 g for 180 min): No. 1 – sediment; No. 2 – supernatant; method 2 (clean in 30, 45 and 60% sucrose at 10737 g for 180 min): No. 3 – protein of over 30% sucrose; No. 4 – protein between 30 and 45% sucrose; No. 5 – protein between 45 and 60% sucrose; No. 6 – precipitate; No. 7 – the original virus isolate Dermatitis nodularis bovum/2018/Kostanai/KZ virus nodular dermatitis of cattle; PC – positive control; NC – negative control*

***Source:** compiled by the authors*

The product of deoxyribonucleic acid isolate *Dermatitis nodularis bovum/2018/Kostanay/KZ* of this virus was detected in samples No. 1, 2, 3, 6, 7. As can be seen from Figure 8, the optimal way to clean virus particles is applying of 30% sucrose solution; at the same time, virions are equally distributed in the supernatant, sediment, and protein fraction above the sucrose solution. This pure material has been taken for sequence. Upon completion of the sequencing process, the following launch metrics were obtained, shown in Figure 9.

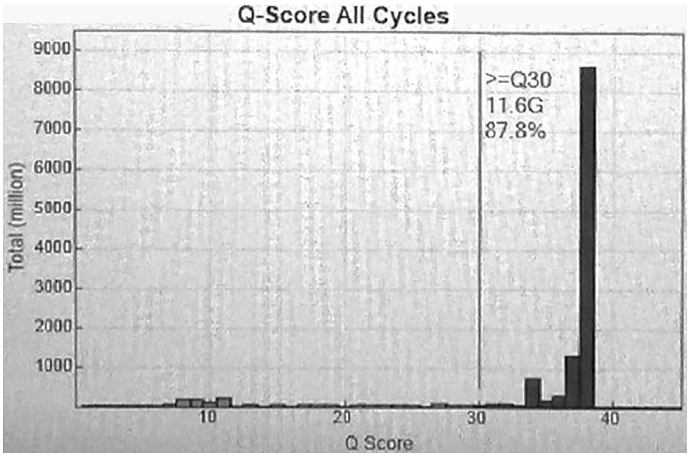


***Figure 9**. Launch metrics at the end of sequencing stage*

***Note:** clustering density: 1143 thousand/mm$^2$; number of clusters that passed the Q30 threshold: 94.4%; expected output: 16236 megabases*

***Source:** compiled by the authors*

According to the metrics, the clustering density is close to the optimal value (1200-1400 thousand/mm$^2$), the expected yield is also close to the optimal value stated by the model manufacturer (19 million bases per cycle), the threshold Q30 indicates that 87.8% of the obtained bases have a 99.9 % probability of reliability. The GC content of the sequence was 41%. According to the comparison of BLAST analysis by the nucleotide database, this contig is identified as a LSDV with an identity percentage equal to 99.36% (Fig. 10).

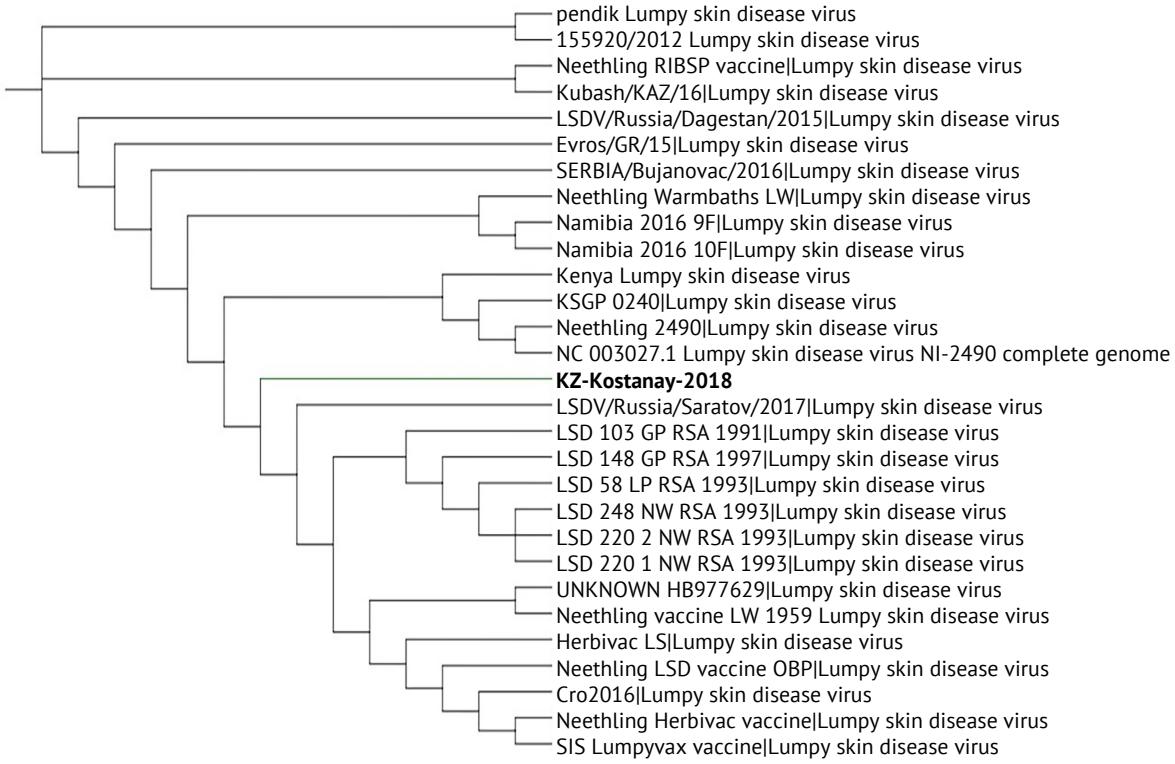


***Figure 10**. Phylogenetic tree as result of BLASTN alignment*
***Note:** tree scale – 0.001*
***Source:** compiled by the authors*

The closest homologues of the identified nodular dermatitis virus were Kenya LSDV, KSGP 0240, NI-2490 and strains of the LSD RSA clade. The remaining assembly contigs were less than 2800 bp and according to *BLAST* analysis were both *Ovis aries* genome fragments with 99.66% identity and smaller (<1000 bp) genome fragments of nodular dermatitis virus.

## DISCUSSION

The result of the work was the isolation and sequencing of the complete genome of a new strain of bovine nodular dermatitis virus derived from an epizootic in the Kostanay region. The obtained sequence has been included to the publicly available GeneBank bioinformatics data base. The strain was named *Dermatitis nodularis bovum/2018/Kostanay/KZ* M-9-21/D and was deposited in the Microorganisms Collection of the Scientific Research Institute of Biological Safety Problems.

For identification, the new generation high-throughput sequencing method with genome "collection" by Illumina's FastQC software has been performed. The advantage of this approach is the generation of hundreds of millions of sequences during one cycle. The FastQC programme makes it possible to evaluate the sequencing run process based on the generated FastQ files of individual samples for such multiple characteristics as the number and length of reads, GC% the degree of duplication, base quality, overrepresented sequences and adapters. Perhaps, existing next-generation sequencing platforms have a number of disadvantages. These include errors and assumptions in the data obtained. Such disadvantages for the Illumina platform include a declining "trend" in the base quality assessment chart and a larger range of reading lengths. Therefore, according to the generally accepted methodology for analysing next-generation sequencing data, the readings were "trimmed" to a state satisfying the reliability of accuracy (Q30). Trimming of low-quality bases was carried out in SeqTK v1.3-r106 and Sickle v1.33. The evaluation of the processed data was also carried out in FastQC. The genome was assembled in the SPAdes v3.13.2 programme with a k-measure size equal to 127 (-k 127) and a more precise assembly function (-careful). The use of a paired-end sequencing reagent with a reading length equal to 300 bp allows for correct assembly in regions of the genome with repeats less than the reading length (i.e., 300 bp) (Badhy *et al.*, 2021).

Two cell lines were used for monolayer cultivation: testicular lamb line and monkey kidney line *Vero*. If it was possible to pass three passages in the cells of the first line successfully, it was not possible to isolate the virus from the second line. Instead, N. Kumar *et al.* (2021) describe a successful method for isolating a new strain of nodular dermatitis virus from the *Vero* line. According to the phylogenetic analysis, the

identified strain was a close homolog of LSDV Kenya, like in the current experiment. The researchers managed to conduct four passages without a cytotoxic effect and obtain a high titer of viral particles. The peak of viral DNA synthesis occurred at 96 hours after infection, and the peak of virion assembly – at 120 hours. For this performance, *Vero* monolayer cultivation was adapted by plating of 500 µl of inoculum after culturing the virus in goat kidney cell culture.

Instead, S. Pervin *et al.* (2023) noted the phenomenon of cytopathic effect of LSDV on the *Vero* line. Despite this, they were able to isolate the virus from pathogen-free fertilized chicken eggs in *Vero* culture. To do this, adaptation of the virus was carried out in a minimal environment in an atmosphere with 5% carbon dioxide. The cytopathic effect had been achieved in two-three days after infection, so the threshold of 75% cytolysis has been fixed microscopically, so cultivation has been stopped after 5-6 passages. Perhaps, in the future, this approach will allow the isolation of other viruses with a higher titer. Identification has been carried out by the gene encoding a binding protein with an amplicon length of 192 bp. Phylogenetic analysis was performed by the same amplicons after sequencing and uploading the sequence to the GeneBank database. Such marker is species-specific and amplifies quickly; in addition, its sequencing is performed faster and at lower costs than whole-genome sequencing. Therefore, in the future, it can be used to identify LSDV isolated from other epizootics. The identified strain (Bangladesh) had a high degree of similarity (more than 99%) with LSDV strains from outbreaks in Kenya, Serbia, Greece and Kazakhstan.

It was not possible to detect virus antigens in all analysed skin and blood samples of sick animals. According to the histopathological study of D.M. Amin *et al.* (2021), the disease was manifested by different skin lesions. In particular, in some cases, the proliferation of the epidermis with the formation of a mesh border has been observed; in others, the lesion was accompanied by a degeneration and necrotic process of the epidermis with exfoliation of cells and the formation of deep ulcers. Part of the ulcers has been characterized by immune granulomatosis: lymphocytes, macrophages, fibrocytes and plasma cells accumulated inside. These cells were also found in muscles and around blood vessels. Viral particles were localized in the cytoplasm of the basal layer of the epithelium and hair follicles. In the mentioned experiment, it was also not possible to confirm the virus in all samples: viral particles were identified by PCR in 22 samples out of 73. It is possible that the absence of viral antigens in some samples caused by either too low titer of the virus, or a late stage of the disease, or a non-specific selection of material that did not capture a sufficient amount of basal epithelium, but included more "protective" cells of the immune response (Zaviriukha *et al.*, 2024).

Contact with the virus promotes the synthesis of specific antibodies. The ones can be detected by neutralization of virions or by immunoenzymatic analysis (Korotetsky *et al.*, 2010). The ELISA method has advantages over the virus neutralization method, because it has high sensitivity, specificity, requires less time and resources, and can "process" 96 samples at the same time. At the same time, the quantitative determination is carried out spectrophotometrically (Sthitmatee *et al.*, 2023). In particular, in 6 out of 9 serum samples of sick animals, immunoglobulins against LSDV, active in dilutions of 1:400-1:100, have been detected by ELISA. As can be seen from the results, in more than 30% of cases in sick animals, the natural immune response had not activated after the initial contact with the pathogen. This fact raises the issue of vaccinating cattle to reduce mortality risks from LSDV.

## CONCLUSIONS

In this research, a new strain of bovine nodular dermatitis virus was isolated and identified. It was found in Kostanay region inflammation focus. The highest titer of the virus was revealed in one of the skin samples of a sick animal. This isolate was selected for further cultivation in monolayer cells, purification and sequencing. Belonging to the species has been proven by the PCR method with the obtaining of an amplicon of 347 bp, which corresponded to the positive control band. The virus antigens have been detected in three samples of 20% skin homogenate suspension (in dilutions from 1:320 to 1:5), as well as in two blood samples (in dilutions of 1:2). In the culture of lamb testicle cells, the activity of the viral antigen was higher and was read in dilutions of 1:320-1:128. Antibodies have been identified in 6 blood samples out of 9 analysed (in dilutions of 1:400-1:100). The presence of virus antigens in the infected material as well as antibodies in blood serum has additionally been confirmed by a diffuse ELISA test. It was possible to cultivate the viral particles in a monolayer of lamb testicular cells. The virus titer increased with each subsequent passage. To purify the virus, the conditions of gradient centrifugation in a 30% sucrose solution have been optimized.

Thus, it was possible to isolate a lumpy skin disease virus isolate from the biomaterial of diseased, fallen animals, which was named *Dermatitis nodularis bovum/2018/Kostanay/KZ*. An isolate of lumpy skin disease virus was sequenced by genome-wide sequencing on the Illumina MiSeq platform. The collected sequence of lumpy skin disease virus is characterised by a length equal to 145865 bp and a coating of 80.86. BLASTN alignment resulted in a separate clade with contig identification as nodular dermatitis virus with a homologous similarity percentage of 99.36%. The resulting sequence was deposited in the GeneBank international database. At the last step strain *Dermatitis nodularis bovum/2018/Kostanay/KZ* of the lumpy skin disease

virus of cattle deposited under the number M-9-21/D in the Collection of microorganisms of the Research Institute of Biological Safety Problems of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Виділення та дослідження штаму вірусу бугристої хвороби великої рогатої худоби

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**Анотація**. Виявлення нових штамів вірусу дозволить запобігти розвитку спалахів завдяки розробці та використанню вакцин. Метою роботи було виділення та секвенування генома вірусу бугристої шкіри з епізоотії в Костанайській області. Генетичний матеріал віріонів ідентифіковано методом полімеразної ланцюгової реакції (ПЛР); вірусні антигени та антитіла до них визначені методом імуноферментного аналізу (ІФА) або дифузійного тесту; для накопичення матеріалу вірус культивували в моноклонах клітин яєчок ягняти; секвенування нового покоління виконано за допомогою системи MiSeq System та програмного забезпечення FastQ; приналежність штамів встановлено методом BLASTN-вирівнювання. Специфічні амплікони вірусу довжиною 347 п.н. були виявлені в зразках шкіри, але не виявлені в крові. У шкірному матеріалі та 2-3 пасажах моноклоналів ідентифікували антигени в розведеннях 1:5-1:320; меншу антигенну активність виявлено в крові в розведенні 1:2. У відповідь на збудника у 67 % досліджених тварин у сироватці крові синтезувалися специфічні імуноглобуліни, які виявлялися в розведеннях 1:100-1:400. Вірусний матеріал накопичували в моноклонах і виділяли в градієнті сахарози. Повногеномна послідовність отриманого матеріалу підтвердила виділення нового штаму вірусу нодулярного дерматиту з відсотком подібності до найближчих гомологів 99,66 %. Штам отримав назву *Dermatitis nodularis bovum/2018/Kostanay/KZ*; послідовність була подана до GeneBank, і об'єкт був депонований у Колекції мікроорганізмів під інвентарним номером М-9-21/D. Отримана інформація може бути використана для запобігання поширенню вогнищ інфекції великої рогатої худоби

**Ключові слова:** біоматеріал; секвенування; збірка; полімеразна ланцюгова реакція; вірус; дезоксирибонуклеїнова кислота