Foods and Raw Materials. 2027;15(2)

ISSN 2308-4057 (Print)
ISSN 2310-9599 (Online)

Research Article
Open Access

Available online at http://jfrm.ru/en
https://doi.org/10.21603/2308-4057-2027-2-707
https://elibrary.ru/QNXKHD

Genetic heterogeneity and pathogenetic mechanisms of bovine leukemia virus

Maxim V. Petropavlovsky[1,*], Irina M. Donnik[1], Anna S. Krivonogova[2], Albina G. Isaeva[1], Alisa S. Badretdinova[1], Nikolay A. Martynov[1]

[1] Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia
[2] Ural State Agrarian University, Yekaterinburg, Russia

* e-mail: petropavlovsky_m@mail.ru

Received 20.11.2025; Revised 01.12.2025; Accepted 08.12.2025; Published online 05.06.2026

Abstract:
Bovine leukemia virus is an RNA virus of the *Retrovivridae* family. It causes a chronic infectious disease in farm animals that poses a serious threat to global cattle farming. According to phylogenetic studies, the pathogen is classified into 12 genotypes.
This comprehensive bioinformatic analysis featured new bovine leukemia virus isolates (n = 57) sampled from various regions of the Russian Federation with different epizootic situations. The analysis featured both new sequences and old samples from Russia available in the National Center for Biotechnology Information. This large-scale approach made it possible to cover all known genotypes.
G4 genotype was associated with major amino acid substitutions in gp51 (T47A, A73P, R121H in epitope G; S56F in epitope H; I144T in ND2; D166G in $CD8^+$). These substitutions provide a selective advantage through immune evasion mechanisms, including escape from humoral immunity, impaired T-cell recognition, and increased receptor affinity. G4 also correlated with rapid progression.
The rapid replacement of G7 by G4 in the Tyumen Region and the general dominance of G4 in Russia were due to a more aggressive course of infection resulting from the genetic ability of the pathogen to adapt to the host immune system. The obtained data underscore the necessity of developing genotype-specific diagnostic systems and implementing routine molecular-genetic monitoring to control the spread of bovine leukemia virus. New data on genetic variability and its impact on genotype-phenotype correlations may help curb the infection.

Keywords: Bovine leukemia virus, bioinformatic analysis, genotype G4, gp51, amino acid substitutions, *env* gene, epitope, epizootiology, virus evolution, diagnostics

Funding: The study was supported by the Russian Science Foundation, project No. 23-16-00103, https://rscf.ru/project/23-16-00103/

Please cite this article in press as: Petropavlovsky MV, Donnik IM, Krivonogova AS, Isaeva AG, Badretdinova AS, *et al.* Genetic heterogeneity and pathogenetic mechanisms of bovine leukemia virus. Foods and Raw Materials. 2027;15(2):257–269. https://doi.org/10.21603/2308-4057-2027-2-707

INTRODUCTION

Bovine leukemia virus (BLV) belongs to the *Deltaretrovirus* genus of the *Retroviridae* family. It causes enzootic bovine leukemia (EBL) [1–4]. The infection has a prolonged latent stage with the virus triggering polyclonal expansion of infected B-lymphocytes that express the phenotypic markers $CD5^+$ and IgM^+. This proliferation clinically manifests as persistent lymphocytosis which progresses to malignant B-cell lymphoma or leukemia in the terminal stage [5–7].

The surface glycoprotein gp51 (SU) is crucial for BLV pathogenesis. It is encoded by the *env* gene. The receptor binding domain (RBD, 1–173 amino acids) interacts with the CAT-1 receptor of B-cells [8, 9]. Neutralizing domains ND1 (97–106 amino acids), ND2 (131–150), and ND3 (210–225) are targets for antibodies that block the infection [10, 11]. T-cell epitopes (gp51N5, gp51N11, gp51N12) induce the $CD4^+/CD8^+$ cytotoxic response [12].

The pathogenic adaptation of *env* is driven by its genetic variability, particularly in the N-terminal region [13] of gp51 (1–155 amino acids). Substitutions in the neutralizing domains disrupt the conformation of ND1 epitopes, thus reducing the affinity of neutralizing anti-

bodies [14, 15]. N-glycosylation mutations enhance the fusion of viral and cellular membranes, increasing the infectivity [13]. Recombination between G4/G7 genotypes in the *env* region may result in chimeric strains [16]. Apart from affecting laboratory diagnostics, such quasispecies with their altered pathogenicity pose risks of interspecies transmission [17–19].

Mutations in *env* can alter the infectivity, replicative capacity, and pathogenicity of BLV. Studying them is important for new diagnostic and preventive strategies [15, 20–22].

In industrial cattle farming, effective diagnostic and preventive measures depend on the severity and the rate of pathological and clinical symptoms. These two factors are important from a practical perspective [1, 2, 23, 24]. Faulty laboratory control data to correlate with the genetic variability of individual pathogen isolates. Genetic variability in the ND of gp51 directly compromises the accuracy of standard serological tests, e.g., ELISA (enzyme-linked immunosorbent assay) or AGID (agar gel immunodiffusion) kits. It happens as a result of a mismatch between the epitopes of the test system and the circulating strain. For some BLV genotypes, the sensitivity of AGID drops by 15–30% [1, 20, 25]. For natural epizootic, the polymerase chain reaction (PCR)-based identification often fails due to the genetic variability of the pathogen mutations in the target sites of primers.

Early studies of the *env* gene were limited to 444 base pairs, failing to detect many recombinations of the glycoprotein gp51 and quasispecies. Current strategies include a larger fragment when assessing important amino acid substitutions in the BLV glycoprotein gp51 (SU). The whole-genome sequencing covers 8,714 base pairs with subsequent classification into phylogroups (A, B-1, B-2, C) [26]. The deep sequencing includes 970 base pairs. It amplifies the region that encompasses all functional domains of gp51 (RBD, ND1-ND3, T-epitopes) [20, 25, 27–29]. Currently, researchers continue to accumulate more data on nucleotide substitutions in the *env* regions associated with BLV infectivity, replication, and pathogenesis [15, 20].

Our study featured the genetic heterogeneity and pathogenetic mechanisms of BLV. It relied on the molecular genetic determinants (amino acid substitutions in the gp51 glycoprotein of the *env* gene) from samples obtained across Russia.

The EBL epidemic safety differs from region to region. Our research is the first all-Russian comprehensive genetic analysis of BLV. For G4 strains, we established the range of specific amino acid substitutions in the domains of the gp51 glycoprotein that determined its antigenic properties (T47A, A73P, R121H in the G epitope; S56F in H; I144T in ND2; D166G in $CD8^+$). As a result, we modelled a molecular mechanism that explains the selective advantage and increased virulence of the G4 genotype. The identified substitutions cause a complex impairment of the immune response. Modified G and H epitopes were the primary drivers of the evasion of humoral immunity while the changes in the $CD8^+$ domain resulted in impaired T-cell recognition. We established a direct correlation between the circulation of genotype G4 and unfavorable epizootic indicators, including the prompt clinical manifestations. This fact confirms that G4 is more virulent than G7. The new fundamental data substantiate the need to improve the diagnostic and monitoring system for BLV. Genotype-specific diagnostic systems and regular molecular genetic monitoring may track the genetic changes in BLV and curb the spread of EBL.

STUDY OBJECTS AND METHODS

The study was conducted at the Ural Scientific Research Veterinary Institute – structural subdivision of the Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences (Yekaterinburg, Russia). The clinical trials took place on agricultural premises in the regions of Sverdlovsk, Tyumen, and Krasnodar. The subjects included local cattle of various age and sex, as well as experimental sheep.

The animal studies followed the primary International Guiding Principles for Biomedical Research Involving Animals (Council for International Scientific Organizations, Geneva, 1985); the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986); the Position on the Ethics of Animal Use in Research (Russian Science Foundation). The animal experiment protocol was approved by the Ethics Committee of the Ural Scientific Research Veterinary Institute – structural subdivision of the Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences (Yekaterinburg, Russia). All experiments followed the procedures for virological bioassays (Gulyukin, 2008). All animal manipulations were performed by qualified veterinarians. Animal housing and nutrition followed standard physiological and veterinary-sanitary guidelines.

The serological screening relied on a solid-phase enzyme-linked immunosorbent assay (ELISA) using the ID Screen® bovine leukemia virus (BLV) Competition reagent kit (Vet Factor LLC, Russia). The results were recorded using an iMark TM photometer (Bio-RAD, USA).

The hematological studies involved an Abacus Junior Vet hematology analyzer (Diatron, Hungary) and a Micros MCX100 microscope (Micros Austria, Austria).

The immunological parameters in animals were determined by assessing the content of T- and B-lymphocytes in a spontaneous e-rosette test with sheep erythrocytes for T-lymphocytes (E-ROL), T-helper lymphocytes ($CD4^+$), and T-suppressor lymphocytes ($CD8^+$), as well as with murine erythrocytes for B-lymphocytes (M-ROL).

The phagocytic activity and neutrophil index were obtained by the opsonophagocytic reaction using latex phagocytosis as proposed in Smirnov's methodologies.

The circulating immune complexes were described by selective precipitation with polyethylene glycol on a Bio-Rad iMark microplate photometer (Bio-Rad Laboratories, USA).

The blood microscopy was imaged with a microscope equipped with an Axiocam 208 digital camera (Carl Zeiss, Germany).

The DNA extraction and quality control involved a commercial AmpliPrime RIBO-prep VET kit (Nekst-Bio LLC, Russia).

The DNA concentration was measured using a Qubit4 Fluorometer (Thermo Fisher Scientific, USA) with a Qu-Dye dsDNA HS reagent kit (200×; Lumiprobe, Russia).

The molecular genetic screening relied on a realtime PCR using the commercial Ampli Sens Leukosis reagent kits (InterLabServis LLC, Russia) and a CFX96 Touch thermal cycler (Bio-Rad, USA).

The procedure of targeted amplification was as follows. We amplified 970 base pairs of *env* using a modified semi-nested PCR method. The mix consisted of 100 mM Tris-HCl (pH 8.5 at 25°C); 100 mM KCl; 0.4 mM of each dNTP; 4 mM $MgCl_2$; 0.06 units/μL Taq DNA polymerase, 0.2% Tween 20; and HS-Taq DNA polymerase stabilizers, 0.35 μM of each primer.

The primers and amplification conditions were replicated from a study of Pluta *et al.* [27] with minor modifications. Round 1 involved AP_4762 and ZM2_5786 at the following temperature conditions: 95°C – 5 min; 40 cycles: 95°C – 25 s, 62°C – 25 s, 72°C – 50 s, 72°C – 10 min. Round 2 featured AP_4762 and ZM5_5733 at the same temperature conditions, except for the higher annealing temperature (66°C).

The Sanger sequencing was performed on an Applied Biosystems 3500 Genetic Analyzer in two directions, using forward (AP_4762) and reverse (ZM5_5733) primers. This test provided sequences up to 900 nucleotide pairs. The bioinformatics analysis included such software as FinchTV, SeqScape 3.0, and megax 10.0.5, as well as the BLAST web service. The analysis started with a primary alignment to the BLV reference whole-genome sequence from the National Center for Biotechnology Information (NC_001414.1) followed by manual editing (Fig. 1).

The analysis included a fetal lamb kidney (FLK) BLV sample obtained from a virus-producing cell line that was prepared and cryopreserved in the laboratory in advance.

RESULTS AND DISCUSSION

The molecular-genetic studies featured biological material of bovine leukemia virus (BLV)-seropositive animals from three Russian regions. According to routine serological and hematological studies as of early 2023, these regions had the following enzootic bovine leukemia (EBL) situations. Krasnodar Region: 823 clusters, 472 animals with hematological stage; Tyumen Region: 282 clusters, 152 animals with hematological stage; Sverdlovsk Region: 26 clusters, no animals with hematological stage [30].

Previously, the following BLV genotypes were reported in these areas. Krasnodar Region: G4 (Belgian) and G7 (Australian), with G4 predominating; Sverdlovsk Region: G7; Tyumen Region: G4 in all samples, with G7 predominating in 2010. These results indicated a shift in the BLV genotype circulating in these cattle populations.

Based on clinical and immunological studies, subleukemic (leukocyte concentration 25.1–40.0×10^9/L) and leukemic stages (≥ 40.0×10^9/L) were more frequent in the regions with G4 genotype. Subleukemic cows exhibited severe endogenous intoxication with an active inflammation, stressed hematopoietic system, and imbalanced reactions in the cellular and humoral parts of the immune system. They also had varying degrees of deviations in the T-cell and B-cell mediated immunity. This fact indicated a persistent epizootic situation in the region and a severe course of the disease.

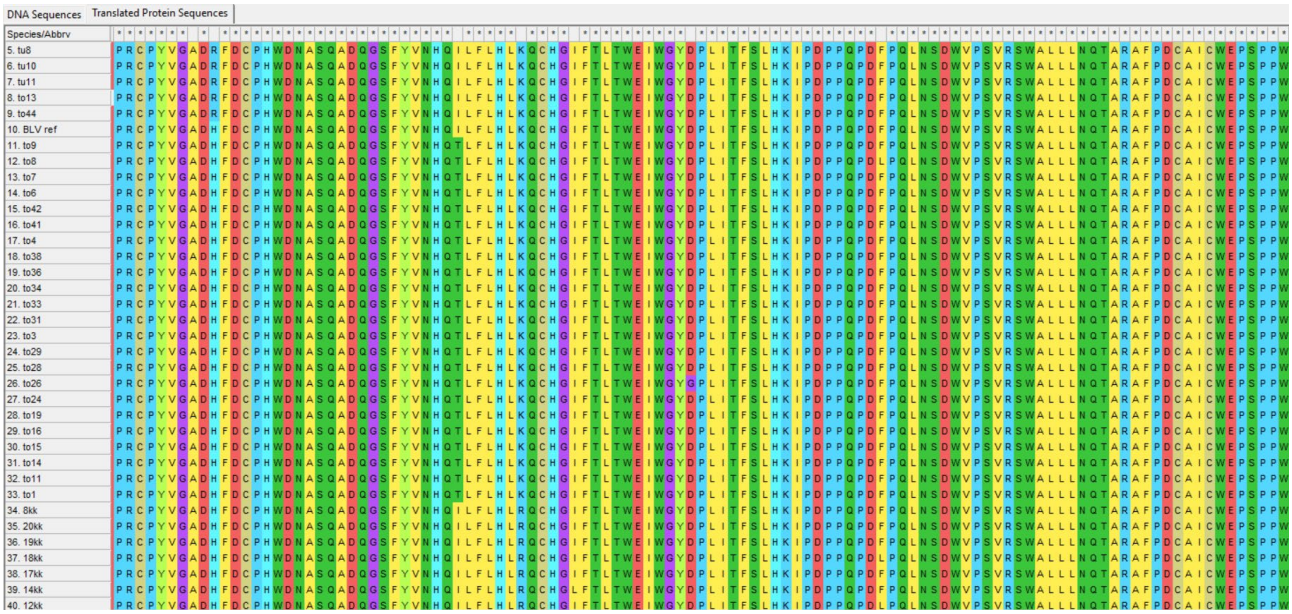

Figure 1 Aligning amino acid sequences of bovine leukemia virus glycoprotein gp 51 from different Russian regions in megax 10.0.5

We studied the pathogenetic characteristics by infecting laboratory sheep with biomaterial from cattle with different BLV genotypes.

The sheep (7–8 months; n = 6) were injected abdominally with virus-containing leukocyte material from whole bovine blood. Group 1 received G4, Group 2 received G7, and Group 3 remained intact. The sheep stayed under clinical observation for 70 days. Samples were first collected on day 1 for baseline values, and then on days 10, 30, 40, and 70 after infection. The test parameters included: peripheral blood, relative and absolute counts of T-lymphocytes and B-lymphocytes, phagocytic activity of neutrophil cells, and circulating immune complexes. On days 1 and 2, the experimental animals revealed no immediate or delayed hyperimmune reactions to the virus-containing leukocyte material.

Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) tests were used to diagnose BLV infection in sheep from both experimental groups on days 14–27, i.e., the interspecies barrier was overcome during parenteral inoculation of infected biological material. The T-cell and B-cell immunity demonstrated a higher count of T-lymphocytes in both experimental groups on day 10. The count of T-lymphocytes increased by a factor of 1.2–1.3 (3.48–3.77×10^9/L), compared to the background values (2.90 ± 0.13×10^9/L). The increase was quite natural, associated with antibody response to the antigen. On day 30, we observed an almost identical increase in the count of B-cells: in was 2.01–2.34×10^9/L in the infected animals, with background values of 1.81 ± 0.09×10^9/L. These results confirmed the adequacy of the immune response to BLV antigens in the experimental animals.

The Group 1 sheep infected with BLV genotype G4 developed a more severe pathological process.

The semi-nested PCR method [27] yielded 57 amplicons of various origins.

To determine the BLV genotype, the amplicons were aligned with isolates from the NCBI GenBank database. The isolates were of different geographic origins. According to phylogenetic analysis, they belonged to genotypes G1 to G12. The analysis involved sequences from other regions of Russia (including old data). The total number of isolates in the multiple alignment and subsequent bioinformatics analysis was 166 (Table 1).

A separate stage of the bioinformatic research was the study of amino acid substitutions in the epitopes or functional domains of gp51 (SU) using the megax software package (Fig. 2–4).

Figure 2 shows the differences from the reference sequence FLK-BLV M35242.1 (G1), which, together with NC_001414.1/BLV ref (isolate G4), served as a control. Sequence numbers are on the left. The colored horizontal bands above the alignment show the antigenic determinants in the structure of the surface glycoprotein gp51: Signal peptide (pink), conformational epitopes F (orange), G (blue), H (purple), ND1 – neutralization domain (light green), and $CD4^+$ and $CD8^+$ T-cell epitopes (green).

Figure 3 illustrates the differences from reference sequence FLK-BLV M35242.1 (G1). It was sequenced together with other isolates and included in the multiple alignment data as a control together with NC_001414.1/BLV ref (isolate G4). Sequence numbers are on the left. The colored horizontal bands above the alignment show the antigenic determinants in the structure of the surface glycoprotein gp51: Conformational epitope G (blue), linear epitopes E, E' (gray), ND 1,2 – neutralization domains (light green), and $CD4^+$ and $CD8^+$ T-cell epitopes (green). The zinc-binding peptide region is marked with light blue, and the hinge region of GYDP is yellow.

Figure 4 shows the differences from reference sequence FLK-BLV M35242.1 (G1). It was sequenced with the isolates to serve as a control together with reference NC_001414.1/BLV ref (isolate G4). Sequence numbers are on the left. The colored horizontal bands above the alignment show the antigenic determinants in the structure of the surface glycoprotein gp51: Linear epitopes A, B, B' and D, D' (gray), ND 3 (light green), and transmembrane hydrophobic region (TMHR) (red).

The major amino acid substitutions were localized in an N-terminal part of gp51 (SU) polyprotein: T47A, A73P, A119T, and R121H in epitope G; S56F in epitope H; D81N, N84D; ND2 in epitope of $CD8^+$ T-cells (N5); I144T, L149V, K150R, L155I in epitope of $CD8^+$ T-cells (N11 and N12), in the zinc-binding region of the peptide; and D166G in $CD8^+$ epitope, in the hinge region β-turn structural motif of GYPD (Table 2).

Some of the identified amino acid substitutions were reported in [25, 27, 31–35], but some were unique in Russia (T47A, A119T, D81N, N84D, L155I, D166G), maybe, even random.

For the first time, we identified samples of genotype G4 with H121R substitution, which are more typical of G7 isolates (samples kk1, kk7).

The highest number of amino acid substitutions belonged to G4 isolates. However, sequences of genotype G7 were more conservative.

The nucleotide substitutions in the glycoprotein gp51 (SU) are responsible for infectivity, replication, and pathogenesis, i.e., they may lead to a more malignant course of EBL.

Major amino acid substitutions and their functional impact. ***Epitope G (T47A, P73A, A119T, R121H).*** Epitope G (48, 73, 74, 82, 121 amino acids) is the key immunodominant B-cell determinant of gp51 (SU) [11].

Antibodies that target this glycoprotein domain actively neutralize viral particles. Epitopes F, G, and H of gp51 (SU) are conformational, thus the shape of the macromolecule depends on disulfide bonds and glycosylation [36].

T47A is a region near G epitope. We detected its substitution in G4 samples from the Tyumen Region (to3-to41). Threonine at position 47 can form hydrogen bonds due to its hydroxyl group. Substituting it with alanine (a hydrophobic amino acid) can disrupt the local hydrophilicity and lead to the loss of the glycosylation site, disrupting protein folding and stability.

Table 1 Bovine leukemia virus isolates in multiple alignment and amino acid substitution analysis of glycoprotein gp51 (n = 166)

Region of origin	Genotype	Name/ NCBI code	Number
Sverdlovsk Region (this study)	G7	tu3, tu5, tu6, tu8, tu9, tu10, tu11	7
Tyumen Region (this study)	G4	to1, to3, to4, to5, to6, to7, to8, to9, to11, to14, to15, to16, to17, to19, to21, to24, to26, to28, to29, to31, to32, to33, to34, to36, to38, to39, to41, to42	28
	G7	to10, to13, to18, to44	4
Krasnodar Region (this study)	G4	1kk, 2kk, 3kk, 4kk, 6kk, 7kk, 8kk, 9kk, 10kk, 11kk, 12kk, 13kk, 14kk, 16kk, 17kk, 18kk, 19kk, 20kk	18
Bashkortostan Republic, 2021	G4	bash3, bash11, bash16, bash18, bash20, bash27	6
Tyumen Region, 2020	G4	OK283585.1/1CH, OK283599.1/2CH, OK283602.1/3CH, OK283614.1/9CH, OK283570.1/10CH, OK283580.1/17CH, OK283582.1/18CH, OK283588.1/21CH, OK283591.1/23CH, OK283595.1/25CH, OK283598.1/28CH, OK283586.1/1F, OK283587.1/20F	13
	G7	OK283612.1/8CH	1
Historical samples, 2010	G4	1.Russia (Krasnodar), 2.Russia (Krasnodar), 2k.Russia (Kurgan), 6T.Russia (Tyumen), JF720358.2/10C (Chelyabinsk), 4Z.Russia (Chelyabinsk), 5Z.Russia (Chelyabinsk)	7
	G7	3k.Russia (Kurgan), 4k.Russia (Kurgan), 5T.Russia (Tyumen), 5T.Russia (Tyumen), JQ353633.1/1S-c6 (Tyumen), JF720350.2/2S (Tyumen), JF720351.2/3S (Tyumen), JF720352.2/4S (Tyumen), 4_Russia (Sverdlovsk), 5_Russia (Sverdlovsk Region), HM563749.3/5_ (Sverdlovsk Region), HM563750.2/2 (Sverdlovsk), HM563760.2/3-3 (Sverdlovsk Region)	13
Novosibirsk Region	G4	OP850751.1/Tatarsk58, ON799104.1/57_Bol, OL660389.1/51_Koch, OP850795.1/Toguchin55, OL660248.1/75_Razd	5
	G7	OL660401.1/53_Koch, OL660358.1/49m_Koch_, JQ686119.1/NK13	3
Elsewhere in Russia	G4	JQ686091.1/MC45 (Moscow Region)	1
	G7	JN695879.1/9V (Vologda Region)	1
	G8	JQ675760.1/MKC3511 (Moscow Region)	1
Foreign references	G1	flk, M35242.1/FLK-BLV, D00647.1/pBLV-A1 (Australia), EU266063.1/ Tehran (Iran)	4
	G2	AF257515.1 (Argentina), LC080654.1/lima40 (Peru)	2
	G3	AF033818.1/G4 (USA), KP201465.1/GBGS-12 (Korea)	2
	G4	MG204552.1/IR-Esfehan (Iran), EF065648.1/USCA-2 (USA), U87872.1/3 (Germany), MN966688.1/K1170 (SAR), M35240.1/LB285 (Belgium), M35238.1/LB59 (France), LC498580.1/DZ1.4 (Egypt), LC193462.1/ Zambia01-2016 (Zambia), LC060795/1_4 (Mongolia), KF801459.2/8MD (Moldova), HQ902258.1/1 (Belarus), HM563781.2/4-2 (Ukraine), HM563778.2/70 (Poland), HM563774.3/68 (Poland), EU262575.2/301 (Poland), S83530.1/I2 (Italy), AY515273.1/96 (Chili), FJ808596.1/LS-SFB (Argentina), MK820044.1/B3008 (China), OK945989.1/26AT (Kazakhstan), OK945958.1/K5 (Kazakhstan), OK945979.1/8D_BKO (Kazakhstan), (Kazakhstan), OK945972.1/50K_CKO (Kazakhstan), OK945959.1/K8 (Kazakhstan), OK631885.1/1PZ (Kazakhstan), NC_001414.1/BLV ref	26
	G5	EF065645.1/CRAG-1 (Costa-Rika), EF065643.1/CRLV (Costa-Rika)	2
	G6	FJ808582.1/PL-1238 (Argentina), AY185360.2/151 (Brazil), LC080656.1/ par62_PAR (Paraguay)	3
	G7	LC060801/1_10 (Mongolia), KF801467.2/1MD (Moldova), KF801464.2/14MD (Moldova), KF801457.1/13MD (Moldova), HM563763.2/160 (Poland), EU262555.2/151 (Poland), OK945983.1/11be (Kazakhstan), OK631887.1/12PP (Kazakhstan), OK945970.1/12K_CKO (Kazakhstan)	9
	G8	HM563764.3/4-6 (Ukraine)	1
	G9	LC080659.1/mon1_BOL (Bolivia), LC080668.1/por28_BOL (Bolivia)	2
	G10	LC154064.1/L2 (Myanmar), KU233561.1/Sa8-H1 (Thailand)	2
	G11	KU764747.1/E102 (China), KU764746.1/E101 (China)	2
	G12	OK945975.1/9S_BKO (Kazakhstan), OK945974.1/7S_BKO (Kazakhstan), OK945977.1/15D_BKO (Kazakhstan)	3

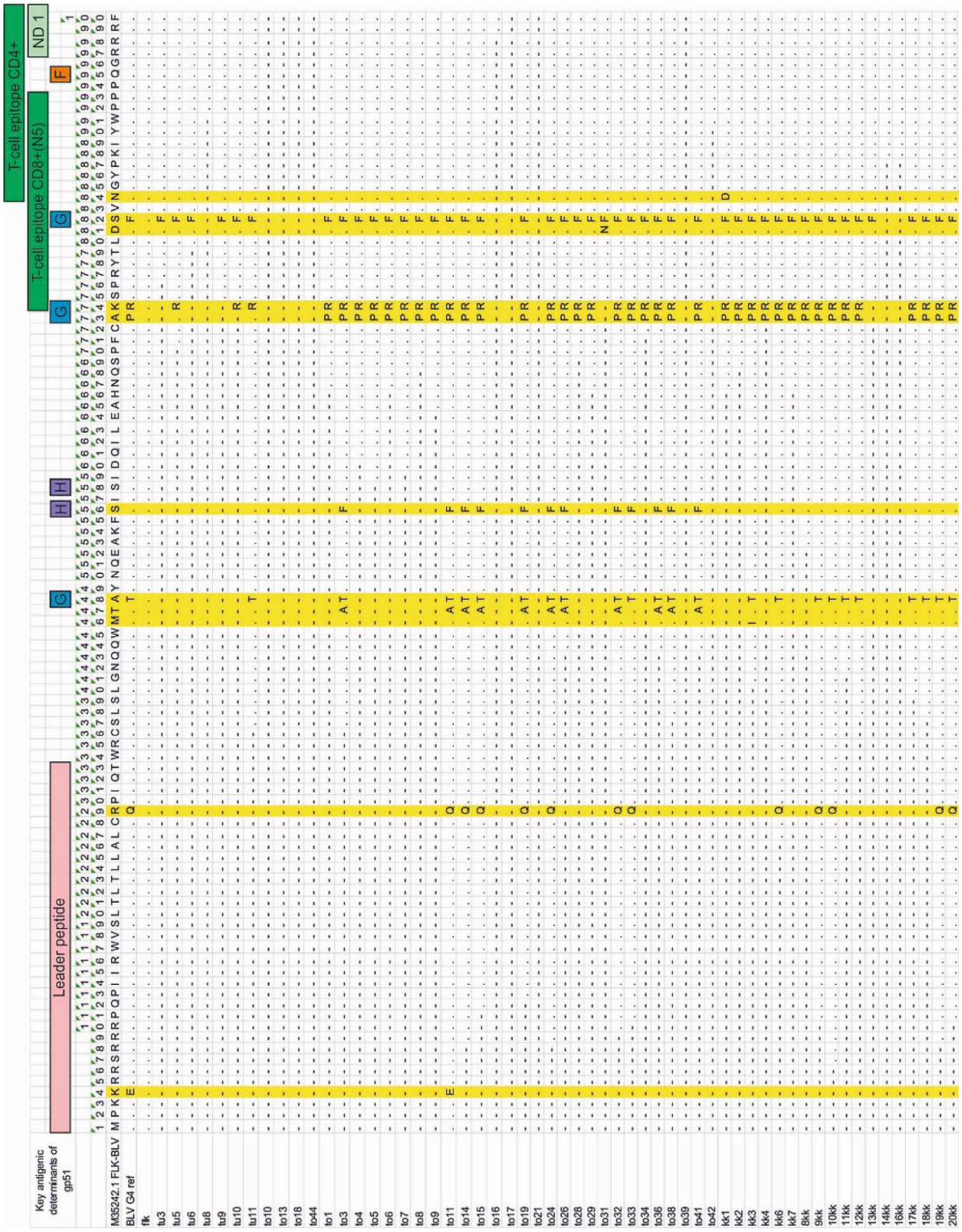

Figure 2 Aligned amino acid sequences (1–100 amino acids) of bovine leukemia virus glycoprotein gp51 (n = 57).

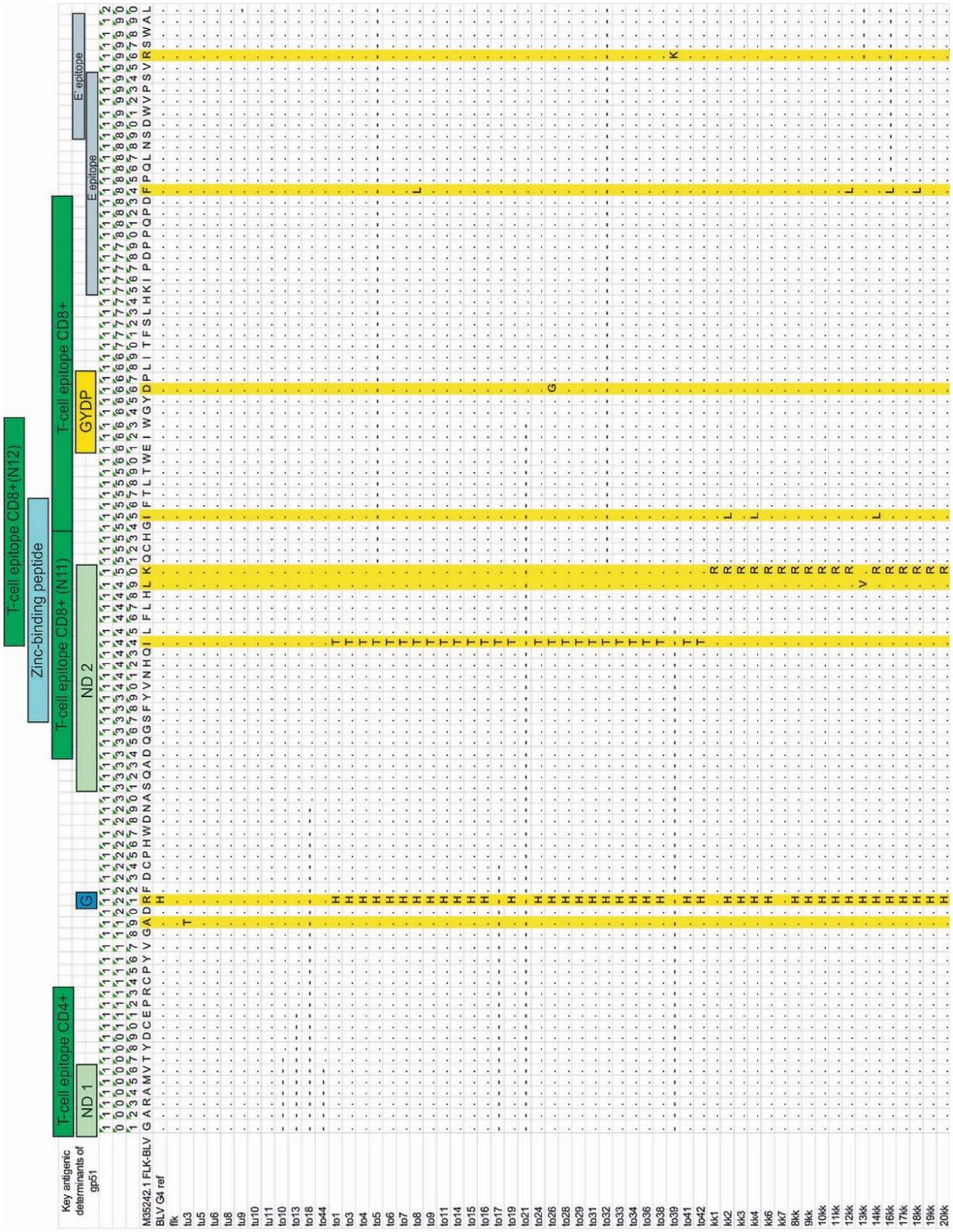

Figure 3 Aligned amino acid sequences (101–200 amino acids) of bovine leukemia virus glycoprotein gp51 (SU) (n = 57)

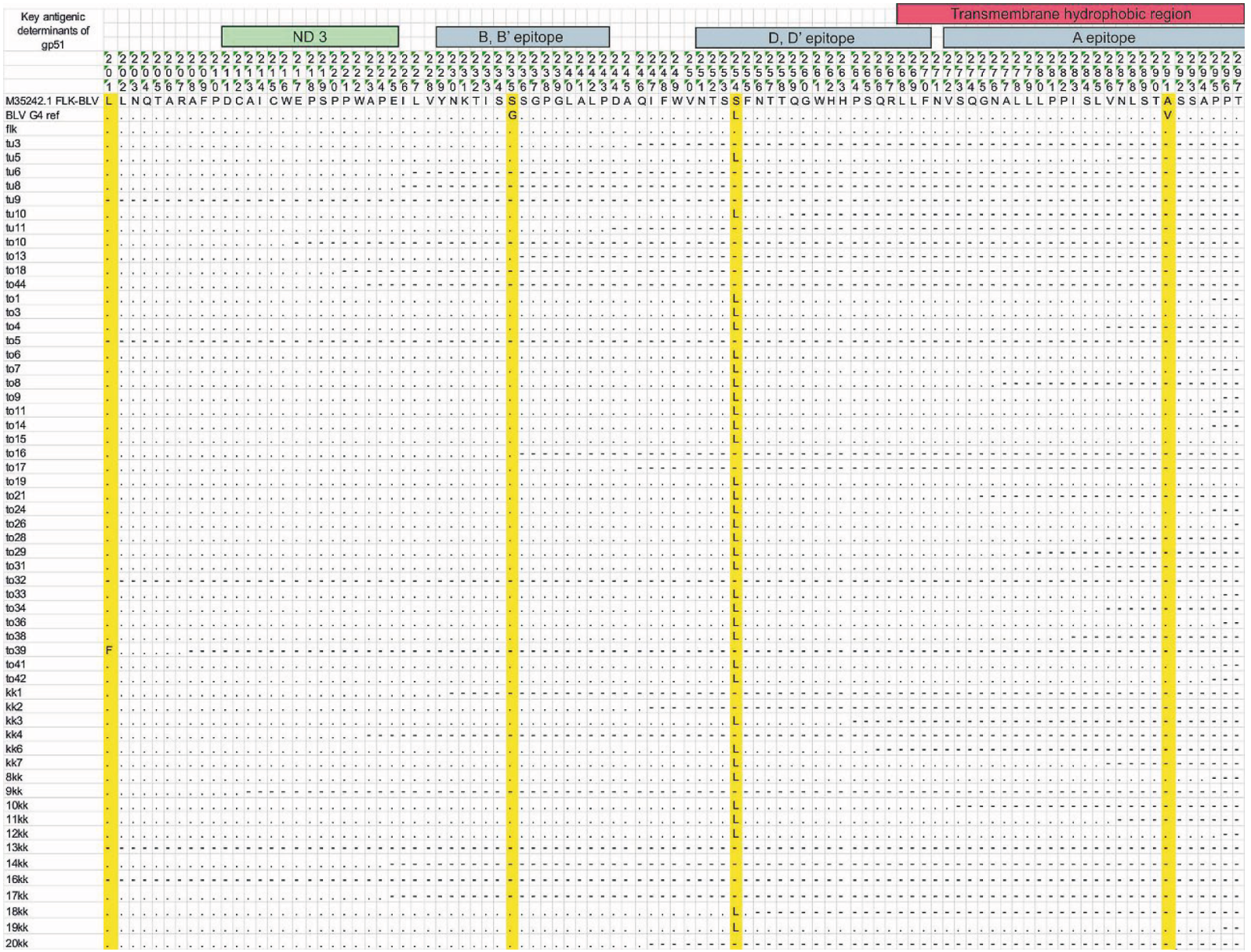

Figure 4 Aligned amino acid sequences (201–297 amino acids) of bovine leukemia virus glycoprotein gp51 (SU) (n = 57)

Table 2 Amino acid substitutions in bovine leukemia virus glycoprotein gp51 (SU) (n = 57)

Epitope or region	Mutation	Biochemical changes	Possible structural effects	Possible immune effects	Genotype
G	T47A	–OH group lost (Thr → Ala)	Hydrogen bond disrupted	Lower antibody affinity	G4
	A73P	More rigid (Ala →Pro)	*β*-hinge changed	Poor recognition of conformational epitopes	G4
	A119T	–OH group added (Ala → Thr)	Possible glycosylation	Epitope masking	G4, G7
	R121H	Different charge (Arg → His)	Hydrogen bonds with other amino acids disrupted	Poor recognition of epitopes; poor neutralization; bonds with cellular receptors disrupted	G4, G7
H	S56F	Hydrophobization (Ser → Phe)	Immersion in lipid bilayer	Loss of antibody affinity	G4
$CD8^+$ T-cell N5	D81N	Negative charge lost (Asp → Asn)	TCR bonds disrupted	Low CTL activation (↓ proliferation)	G4
	N84D	Negative charge added (Asn → Asp)	Orientation in MHC-I changed	Impaired recognition of TCR (↓ IFN-*γ*)	G4
ND 2, $CD8^+$ T-cell (N11, N12), zinc finger	I144T	Hydrophobe → polar (Ile → Thr)	Hydrophobic core disrupted; zinc finger destabilized	Poor neutralization of antibodies	G4
	L149V	Smaller size (Leu → Val)	Denser structure; zinc finger destabilized	Low antibody avidity	G4
	K150R	Longer chain (Lys → Arg)	Stronger ionic interactions; zinc finger destabilized	Resistance to antibodies	G4
$CD8^+$, zinc finger	L155I	Neutral change (Leu → Ile)	Minor local compactions of protein structure; zinc finger destabilized	–	G4
$CD8^+$ (GYPD hinge)	D166G	COO^- group lost (Asp → Gly)	Hinge disrupted; conformation changed	Poor recognition	G4

The A73P substitution was detected in all isolates of G4 genotype. Alanine at position 73 imparts flexibility to the local polypeptide chain. Proline has a cyclic structure (pyrrolidine ring). Substitution with proline can increase chain rigidity by affecting its shape or hinge, which potentially correlate with the conformational dynamics of the entire region.

We detected the A119T substitution in the G7 isolate from the Sverdlovsk Region (tu3). This substitution was not random, as it had been detected in the same region in 2020 (3CH, 17CH, 23CH, 1F, 20F), but it was identified for G4 genotype. Alanine at position 119 is a hydrophobic amino acid whereas threonine is polar. This substitution may increase the hydrophilicity of this region of the N-terminal region of gp51 (SU). An extra potential glycosylation site may affect protein folding and stability, which correlates with antibody interaction.

The R121H substitution is typical of all BLV G4 samples. However, we registered its reverse variant, H121R, in two BLV G4 isolates from the Krasnodar Region (kk1, kk7). H121R is typical of G7 (in this study as well). As with A119T, this phenomenon indicates cross-recombination between the G4/G7 genotypes of BLV. Arginine has a positively charged guanidine group while histidine has a variable charge, i.e., it can be neutral or positive, depending on the environment pH. This substitution can significantly alter the electrostatic properties of this glycoprotein region, thus disrupting salt bridges or hydrogen bonds with other amino acids. Changes in the electrostatic potential affect the binding to cellular receptors and recognition by neutralizing antibodies due to the changes in epitope conformation.

Epitope H is a crucial neutralizing epitope. We identified the amino acid substitution substitution of S56F in the G4 samples from the Tyumen Region (TO3, TO11, TO14, TO15, TO19, TO24, TO26, TO32, TO33, TO36, TO38, TO41). It was previously reported in Novosibirsk, Moldova, Kazakhstan, and France. A small polar amino acid (serine) is replaced with a large hydrophobic aromatic amino acid (phenylalanine), which can cause steric obstacles and significant changes in protein conformation. The S56F substitution must alter the antigenic architecture of this epitope, which allows the virus to evade recognition by existing antibodies. Mamoun *et al.* [37] proved that the amino acid substitution of S56F in the H epitope altered the recognition of the monoclonal antibody to this immunogenic region by replacing a small serine residue with a large hydrophobic phenylalanine residue [25].

The N-terminal region of gp51 (SU) contains conformational epitopes F, G, and H, which are critical for recognition by neutralizing antibodies [38]. It contains (1–173 amino acids) the receptor-binding domain (RBD), which mediates binding to the cellular receptor (CAT1/SLC7A1) and triggers membrane fusion [9]. Johnston *et al.* [8] reported systematic single amino acid substitutions in 23 BLV *env* gene variants. The substitutions in the N-terminal region (SU) often retained the

ability to induce syncytia. The substitutions sometimes exhibited signs of misfolding, which reduced the binding of monoclonal antibodies to conformational epitopes.

Callebaut *et al.* [10] built a structural model of the N-terminal region of BLV gp51SU 1 based on alignment with the RBD of the ecotropic murine leukemia virus (Friend, F-MuLV). Most of the residues with substitutions that determine the antigenic properties of the leukemia virus (including region 73) were exposed on the surface of the molecule. This fact means that they were accessible for direct contact with antibodies and immune cell receptors. Therefore, a substitution at one of these positions, e.g., A73P, can directly alter the efficiency of immune recognition of the virus.

The substitutions of D81N and N84D (T-cell epitope N5) were random: we detected them in one sample from the Krasnodar Region (kk1) and one from the Tyumen Region (to31). The D81N polymorphism from negatively charged aspartic acid to neutral asparagine can eliminate the negative charge at position 81. This result potentially disrupts the electrostatic interactions with positively charged regions of the T-cell receptor (TCR), e.g., in CDR3. N84D is the reverse substitution: it adds a negative charge, which can lead to a new electrostatic repulsion or attraction with the TCR.

The D81N and N84D substitutions in antigenic BLV peptides may represent a mechanism for T-cell evasion. This mechanism is due to the modification of the charged residues that are critical for electrostatic interaction with the TCR. This effect alters recognition of the peptide-MHC-I complex, which is consistent with the mechanisms of immune evasion reported for other viruses, e.g., HIV-1. In these viruses, such substitutions directly reduce the TCR binding affinity and the efficiency of T-cell activation [39]. However, in the case of BLV, this mechanism remains hypothetical and requires experimental confirmation.

Substitutions in the zinc-binding motif (137–156 amino acids) of gp51 (SU) belonged to ND2 and $CD8^+$ epitope (N11, N12). Mutations of I144T were found in most isolates from the Tyumen Region (to1, to3-to9, to11, to14-to17, to19, to24, to26, to28, to29, to31-to34, to36, to38). This amino acid substitution was previously identified in some historical samples from Chelyabinsk (G4), Tyumen (G4), Novosibirsk (G4), Krasnodar (G4) (2010, 2020), Moldova (G4), Kazakhstan (G4), Mongolia (G4), France (G4), South Africa (G4), Zambia (G4), Iran (G4), China (G11), Thailand (G10), Myanmar (G10), Brazil (G6), and Bolivia (G9). Substitutions of L149V (13kk) and K150R (kk1-kk4, kk6-12kk, 14kk, 16kk, 18kk-20kk) were previously reported for isolates from Bashkortostan and Kazakhstan. Substitutions of L155I (kk2, kk4, kk14) were found in the Krasnodar Region.

The zinc-binding region is critical for protein stability and structure. Substitution of the hydrophobic isoleucine with the polar, hydrophilic threonine at position 144 may disrupt the hydrophobic core. Altered hydrophobicity may compromise the stability of the zinc-binding motif or alter the conformation of the ND2 and $CD8^+$ epitopes, potentially reducing antibody recognition. Leucine at position 149 has a larger side chain (isobutyl group). Its substitution with valine (isopropyl group) may lead to minor changes in the compaction of the hydrophobic core and flexibility of the protein chain. Arginine at position 150 has a longer and more rigid guanidine group in the side chain than lysine (amino group). This substitution maintains a positive charge. This fact is important for interactions with negatively charged cellular receptors or other molecules but may alter steric parameters and hydrogen bonding. Leucine and isoleucine at position 155 are stereoisomers with very similar sizes and hydrophobic properties. The only difference is the position of the methyl group: it is branched in leucine, and it is linear in isoleucine. This substitution is conservative (neutral) and has a slight effect on the local compaction of the gp51 structure (SU).

A substitution in the $CD8^+$ epitope occurs in the GYPD motif region (D166G) [11]. The GYPD motif is conserved and typical of other retroviruses. It is presumably involved in the formation of a hinge in the structure of the gp51 glycoprotein (SU) [40]. The D166G substitution may disrupt this hinge, altering the conformation of the region.

As described by de Brogniez *et al.* [15], gp51 SU (137–156 amino acids) contains residues (H150, D153) that coordinate a zinc ion (Zn^{2+}) and forms part of a conformational epitope. Zn^{2+} binding is crucial for stabilizing the tertiary structure of the protein, which, in turn, is necessary to develop a neutralizing epitope [15]. The functional importance of this region is confirmed by monoclonal antibody-based assays, e.g., mAbs 3C8 or 1H1. Binding to this epitope entirely depends on the presence of a Zn^{2+} and the native conformation of the protein [41].

Biophysical studies of ion mobility and mass spectrometry demonstrate that amino acid substitutions in zinc-binding motifs can significantly affect zinc affinity and structural conformations of peptides [42]. Extrapolating these data to the current study, we may conclude that amino acid substitutions or combinations in ND2 (I144T, L149V, K150R, L155I) can disrupt Zn^{2+} coordination and destroy the zinc-binding site. It partially or completely destabilizes the conformational epitope, making it unrecognizable to neutralizing antibodies. This mechanism is crucial for the formation of BLV resistance to neutralization, as confirmed by other studies.

Furthermore, substitutions in $CD8^+$ T-cell epitopes (N11, N12) can disrupt the processing and presentation of peptides by histocompatibility complex class I (MHC I) molecules. This, in turn, inhibits the activity of virus-specific cytotoxic T-lymphocytes (CTLs). This means weak cytotoxic T-cell response and a weak key mechanism for the elimination of virus-infected cells [12].

CONCLUSION

Amino acid substitutions in glycoprotein gp51 of bovine leukemia virus (BLV) proved to concentrate in the key functional domains that interact with the host

immune system. Being a mechanism of immune evasion for the virus, these substitutions are often associated with high pathogenicity.

Genotype G4 possesses a selective advantage over G7 due to the cumulative effect of specific amino acid substitutions (in epitopes G, H, zinc motif of ND2, etc.) and their combinations in crucial neutralizing epitopes of the glycoprotein gp51 (SU).

We identified sequences with up to five simultaneous different amino acid substitutions, e.g., in the G4 isolates from the Tyumen Region. For example, isolate to26 from this region had six substitutions, i.e., T47A, A73P, R121H (G epitope); S56F (H epitope); I144T (ND2), and D166G ($CD8^+$ T-cell epitope). In the same region, we identified a shift in the genetic landscape from G7 to G4.

Substitutions in the N-terminal region of gp51(SU) lead to antigenic drift, and the combined effects of weakening both the cellular (CTL) and humoral (antibody) immune responses allow the virus to evade immune control. The poor immune recognition ultimately leads to viral persistence in the host. The combined effect of high viral infectivity may also be achieved through improved receptor binding and accelerated membrane fusion, as indicated by substitutions in the receptor binding domain polypeptide region. Mutations are markers of virulence. Mechanisms and their cascades associated with impaired immune control allow the virus to replicate freely. They correlate with the development rate of clinical symptoms, persistent lymphocytosis, and the development of B-cell lymphomas in susceptible animals. Enzootic bovine leukemia induced by G4 BLV in cattle with an average age of 3.5 years was most often described in the subleukemic and leukemic stages [43]. The rate of pathological development is an important indicator in leukemia. An increase in the proviral load mediates the spread of the pathogen in the population [44]. This thesis is confirmed by compared results of epizootic, molecular-genetic, and clinical immunological analysis across Russia. The lowest infection rate belonged to the G7 genotype while medium infection could be caused by both G7 and G4, and high infection rate was associated with G4.

Changes in the antigenic structure of the glycoprotein may reduce the effectiveness of enzyme-linked immunosorbent assay and polymerase chain reaction diagnostics, thus leading to false-negative results.

Monitoring the antigenic landscape of the leukemia pathogen proved to be a relevant research issue, as did the studies of the epizootiology and immunobiology of its genetic variants in various Russian regions. They may contribute to more effective diagnostic strategies and disease control measures. New genotype-specific diagnostic tests and innovative vaccines should take into account the antigenic characteristics of the particular strain.

Further scientific research requires whole genome sequencing to monitor the genetic variability of BLV. It will help predict risks using molecular modeling, immunochemical analysis, and interreceptor interactions.

CONTRIBUTION

M.V. Petropavlovsky and I.M. Donnik developed the research concept and supervised the project; A.S. Krivonogova and A.G. Isaeva were responsible for the validation; M.V. Petropavlovsky and N.A. Martynov provided formal analysis, methodology, and research; A.G. Isaeva and A.S. Badretdinova performed the data curation and proofread the article. The authors have reviewed and approved of the final version of manuscript.

CONFLICT OF INTEREST

The authors declared to conflict of interest regarding the publication of this article.

REFERENCES

1. Brujeni GN, Houshmand P, Soufizadeh P. Bovine leukemia virus: A perspective insight into the infection and immunity. Iranian Journal of Veterinary Research. 2023;24(4):290–300. https://doi.org/10.22099/IJVR.2023.48236.7023
2. Kuczewski A, Mason S, Orsel K, van der Meer F. Pilot implementation of a newly developed bovine leukemia virus control program on 11 Alberta dairy farms. Journal of Dairy Science. 2021;104(4):4549–4560. https://doi.org/10.3168/jds.2020-19251
3. Lv G, Wang J, Lian S, Wang H, Wu R. The global epidemiology of bovine leukemia virus: Current trends and future implications. Animals. 2024;14(2):297. https://doi.org/10.3390/ani14020297
4. Pereira JG, Silva CA, Silva LD, Lima CAA, do Rosário CJRM, *et al*. Diagnosis and phylogenetic analysis of bovine leukemia virus in dairy cattle in northeastern Brazil. Frontiers in Veterinary Science. 2022;9:1080994. https://doi.org/10.3389/fvets.2022.1080994
5. Zhao Y, Wang J, Chen J, Chen Y, Huet C, *et al*. Bovine leukemia virus: Origin, prevalence, phylogenetic diversity, risk factors, and strategies for control. Animals. 2025;15(9):1344. https://doi.org/10.3390/ani15091344
6. Poryvaeva AP, Pechura EV, Petrova OG, Bezborodova NA, Lysova YaYu, *et al*. The effectiveness of scientifically based monitoring programs and therapeutic and preventive measures for controlled infectious diseases of animals. International Journal of Veterinary Medicine. 2023;(4):96–110. (In Russ.) https://doi.org/10.52419/issn2072-2419.2023.4.96
7. Shrestha S, Orsel K, Barkema HW, Martins L, Shrestha S, *et al*. Effects of bovine leukemia virus seropositivity and proviral load on milk, fat, and protein production of dairy cows. Journal of Dairy Science. 2024;107(1):530–539. https://doi.org/10.3168/jds.2023-23695

8. Johnston ER, Albritton LM, Radke K. Envelope proteins containing single amino acid substitutions support a structural model of the receptor-binding domain of bovine leukemia virus surface protein. Journal of Virology. 2002;76(21):10861–10872. https://doi.org/10.1128/jvi.76.21.10861-10872.2002
9. Tomé-Poderti L, Olivero-Deibe N, Carrión F, Portela MM, Obal G, *et al*. Characterization and application of recombinant bovine leukemia virus *env* protein. International Journal of Scientific Reports. 2024;14:12190. https://doi.org/10.1038/s41598-024-62811-8
10. Callebaut I, Voneche V, Mager A, Fumière O, Krchnak V, *et al*. Mapping of B-neutralizing and T-helper cell epitopes on the bovine leukemia virus external glycoprotein gp51. Journal of Virology. 1993;67(9):5321–5327. https://doi.org/10.1128/JVI.67.9.5321-5327.1993
11. Zhao X, Buehring GC. Natural genetic variations in bovine leukemia virus *envelope* gene: Possible effects of selection and escape. Virology. 2007;366(1):150–165. https://doi.org/10.1016/j.virol.2007.03.058
12. Bai L, Takeshima S, Isogai E, Kohara J, Aida Y. Novel $CD8^+$ cytotoxic T cell epitopes in bovine leukemia virus with cattle. Vaccine. 2015;33(51):7194–7202. https://doi.org/10.1016/j.vaccine.2015.10.128
13. de Brogniez A, Bouzar AB, Jacques JR, Cosse JP, Gillet N, *et al*. Mutation of a single envelope N-Linked glycosylation site enhances the pathogenicity of bovine leukemia virus. Journal of Virology. 2015;89(17):8945–8956. https://doi.org/10.1128/JVI.00261-15
14. Murakami H, Uchiyama J, Suzuki C, Nikaido S, Shibuya K, *et al*. Variations in the viral genome and biological properties of bovine leukemia virus wild-type strains. Virus Research. 2018;253:103–111. https://doi.org/10.1016/j.virusres.2018.06.005
15. de Brogniez A, Mast J, Willems L. Determinants of the bovine leukemia virus envelope glycoproteins involved in infectivity, replication and pathogenesis. Viruses. 2016;8(4):88. https://doi.org/10.3390/v8040088
16. Pluta A, Rola-Łuszczak M, Hoffmann FG, Donnik I, Petropavlovskiy M, *et al*. Genetic variability of bovine leukemia virus: Evidence of dual infection, recombination and quasi-species. Pathogens. 2024;13(2):178. https://doi.org/10.3390/pathogens13020178
17. Olaya-Galán NN, Corredor-Figueroa AP, Velandia-Álvarez S, Vargas-Bermudez DS, Fonseca-Ahumada N, *et al*. Evidence of bovine leukemia virus circulating in sheep and buffaloes in Colombia: Insights into multispecies infection. Archives of Virology. 2022;167:807–817. https://doi.org/10.1007/s00705-021-05285-7
18. Blanco R, Quezada-Romegialli C, Muñoz JP. Bovine leukemia virus and human breast cancer: A review of clinical and molecular evidence. Viruses. 2025;17(3):324. https://doi.org/10.3390/v17030324
19. Khan Z, Abubakar M, Arshed MJ, Aslam R, Sattar S, *et al*. Molecular investigation of possible relationships concerning bovine leukemia virus and breast cancer. Scientific Reports. 2022;12:4161. https://doi.org/10.1038/s41598-022-08181-5
20. Polat M, Takeshima SN, Aida Y. Epidemiology and genetic diversity of bovine leukemia virus. Journal of Virology. 2017;14:209. https://doi.org/10.1186/s12985-017-0876-4
21. Bai L, Soya M, Ichikawa M, Matsuura R, Arimura Y, *et al*. Antigenicity of subregions of recombinant bovine leukemia virus (BLV) glycoprotein gp51 for antibody detection. Journal of Virological Methods. 2023;311;114644. https://doi.org/10.1016/j.jviromet.2022.114644
22. Ramalho GC, Silva MLCR, Falcão BMR, Limeira CH, Nogueira DB, *et al*. High herd-level seroprevalence and associated factors for bovine leukemia virus in the semi-arid Paraíba state, Northeast Region of Brazil. Preventive Veterinary Medicine. 2021;190:105324. https://doi.org/10.1016/j.prevetmed.2021.105324
23. Donnik IM, Gulyukin MI, Busol VA, Kovalenko LV, Kovalenko AM Bovine leukemia virus infection – diagnostics, eradication, and anthropozoonotic potential (background) (review). Agricultural Biology. 2021;56(2):230–244. (In Russ.) https://doi.org/10.15389/agrobiology.2021.2.230eng
24. Marawan MA, Alouffi A, Tokhy SE, Badawy S, Shirani I, *et al*. Bovine leukaemia virus: Current epidemiological circumstance and future prospective. Viruses. 2021;13(11):2167. https://doi.org/10.3390/v13112167
25. Suzuki A, Chapman R, Douglass N, Carulei O, van Rensburg J, et al. Phylogenetic analysis of South African bovine leukaemia virus (BLV) isolates. Viruses. 2020;12(8):898. https://doi.org/10.3390/v12080898
26. Maezawa M, Fujii Y, Akagami M, Kawakami J, Inokuma H, *et al*. Phylogenetic analysis based on whole genome sequence of bovine leukemia virus in cattle under 3 years old with enzootic bovine leukosis. PLoS One. 2023;18(1):e0279756. https://doi.org/10.1371/journal.pone.0279756
27. Pluta A, Rola-Luszczak M, Kubis P, Balov S, Moskalik R, *et al*. Molecular characterization of bovine leukemia virus from Moldovan dairy cattle. Archives of Virology. 2017;162(6):1563–1576. https://doi.org/10.1007/s00705-017-3241-4

28. Rola-Łuszczak M, Sakhawat A, Pluta A, Ryło A, Bomba A, *et al*. Molecular characterization of the *env* gene of bovine leukemia virus in cattle from Pakistan with NGS-Based evidence of virus heterogeneity. Pathogens. 2021;10(7):910. https://doi.org/10.3390/pathogens10070910

29. Baboshko DA, Gashnikova NM, Totmenin AV, Nefedova AA, Osipova IP, *et al*. Genetic diversity of BLV, common in the territory of the Kochenevsky district of the Novosibirsk region. Veterinariya i kormleniye. 2022;2:17–19. (In Russ.) https://doi.org/10.30917/ATT-VK-1814-9588-2022-2-4

30. Donnik IM, Petropavlovsky MV, Makutina VA, Gulyukin MI, Barsukov YuI. The current bovine leukemia spread situation in the Russian Federation. Veterinariya. 2024;(11):18–22. (In Russ.) https://doi.org/10.30896/0042-4846.2024.27.11.18-22

31. Sultanov A, Rola-Łuszczak M, Mamanova S, Ryło A, Osiński Z, *et al*. Molecular characterization of bovine leukemia virus with the evidence of a new genotype circulating in cattle from Kazakhstan. Pathogens. 2022;11(2):180. https://doi.org/10.3390/pathogens11020180

32. Lee E, Kim EJ, Joung HK, Kim BH, Song JY, *et al*. Sequencing and phylogenetic analysis of the gp51 gene from Korean bovine leukemia virus isolates. Journal of Virology. 2015;12:64. https://doi.org/10.1186/s12985-015-0286-4

33. Úsuga-Monroy C, Díaz FJ, González-Herrera LG, Echeverry-Zuluaga JJ, López-Herrera A. Phylogenetic analysis of the partial sequences of the *env* and *tax* BLV genes reveals the presence of genotypes 1 and 3 in dairy herds of Antioquia, Colombia. VirusDisease. 2023;34:483–497. https://doi.org/10.1007/s13337-023-00836-9

34. Yang Y, Chen L, Dong M, Huang W, Hao X, *et al*. Molecular characterization of bovine leukemia virus reveals existence of genotype 4 in Chinese dairy cattle. Journal of Virology. 2019;16(1):108. https://doi.org/10.1186/s12985-019-1207-8

35. Rola-Łuszczak M, Pluta A, Olech M, Donnik I, Petropavlovskiy M, *et al*. The molecular characterization of bovine leukaemia virus isolates from Eastern Europe and Siberia and its impact on phylogeny. PLoS One. 2013;8(3):e58705. https://doi.org/10.1371/journal.pone.0058705

36. Bruck C, Rensonnet N, Portetelle D, Cleuter Y, Mammerickx M, *et al*. Biologically active epitopes of bovine leukemia virus glycoprotein gp51: Their dependence on protein glycosylation and genetic variability. Virology. 1984;136(1):20–31. https://doi.org/10.1016/0042-6822(84)90244-7

37. Mamoun RZ, Morisson M, Rebeyrotte N, Busetta B, Couez D, *et al*. Sequence variability of bovine leukemia virus *env* gene and its relevance to the structure and antigenicity of the glycoproteins. Journal of Virology. 1990;64(9):4180–4188. https://doi.org/10.1128/jvi.64.9.4180-4188.1990

38. Rola-Łuszczak M, Grabowska A, Szewczyk B, Kuźmak J. Baculovirus expression and potential diagnostic application of the gp51 envelope glycoprotein of genetic mutants of the bovine leukaemia virus. Journal of Veterinary Research. 2019;63(1):1–6. https://doi.org/10.2478/jvetres-2019-0020

39. Wu X, Li T, Jiang R, Yang X, Guo H, *et al*. Targeting MHC-I molecules for cancer: Function, mechanism, and therapeutic prospects. Molecular Cancer. 2023;22:194. https://doi.org/10.1186/s12943-023-01899-4

40. Moratorio G, Fischer S, Bianchi S, Tomé L, Rama G, *et al*. A detailed molecular analysis of complete bovine leukemia virus genomes isolated from B-cell lymphosarcomas. Veterinary Research. 2013;44:19. https://doi.org/10.1186/1297-9716-44-19

41. Gatot JS, Callebaut I, van Lint C, Demonté D, Kerkhofs P, *et al*. Bovine leukemia virus SU protein interacts with zinc, and mutations within two interacting regions differently affect viral fusion and infectivity *in vivo*. Journal of Virology. 2002;76(16):7956–7967. https://doi.org/10.1128/jvi.76.16.7956-7967.2002

42. Adomako RA, Owusu MB, Oberdick RK, Senyah K, Asare P, *et al*. Zn (II) affinity and structural conformations of 2His-2Cys zinc finger-like motif peptide determined by ion mobility-mass spectrometry and PM6 molecular modeling. Journal of Mass Spectrometry. 2025;60(3):e5113. https://doi.org/10.1002/jms.5113

43. Donnik IM, Petropavlovsky MV. Cattle leukosis: Modern approach. Dairy cattle breeding. 2022;S2:57–59. (In Russ.) https://doi.org/10.25701/ZZR.2022.03.03.011

44. Petersen MI, Suarez Archilla G, Miretti MM, Trono KG, Carignano HA Whole-transcriptome analysis of BLV-infected cows reveals downregulation of immune response genes in high proviral loads cows. Frontiers in Veterinary Science. 2025;12:1550646. https://doi.org/10.3389/fvets.2025.1550646

Additional information about the authors

Maksim V. Petropavlovskiy ORCID 0000-0002-9892-6092; eLIBRARY SPIN 7603-9476
Irina M. Donnik ORCID 0000-0001-8349-3004; eLIBRARY SPIN 2610-5633
Anna S. Krivonogova ORCID 0000-0003-1918-3030; eLIBRARY SPIN 8427-7651
Albina G. Isaeva ORCID 0000-0001-8395-1247; eLIBRARY SPIN 7308-9644
Alisa S. Badretdinova ORCID 0000-0003-0189-2963; eLIBRARY SPIN 9488-1815
Nikolay A. Martynov ORCID 0000-0001-9251-0056; eLIBRARY SPIN 7332-1403