## **BRIEF REPORT**

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# Bovine leukemia virus *tax* gene/Tax protein polymorphism and its relation to Enzootic Bovine Leukosis

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## ABSTRACT

Bovine leukemia virus (BLV) is an oncogenic retrovirus of the *Deltaretrovirus* genus, which causes persistent infection in its natural hosts – cattle, zebu, and water buffalo with diverse clinical manifestations through the defeat of B-cells. The BLV proviral genome, along with structural genes (*gag, pro, pol,* and *env*), includes nonstructural ones (*R3, G4, tax, rex, AS, pre-miRs* (for miRNAs). We have shown in our previous data the association of some *pre-miRs-B'* (for BLV miRNA) alleles with leukocyte (WBC – white blood cell) number in BLV-infected cows. Multifunctional properties of Tax protein have led us to an assumption that *tax* gene/Tax protein could have too population variations related to WBC counts. Here we report about several *tax* alleles/Tax protein variants, which have a highly significant association with an increase or a decrease of WBC number in BLV-infected cows. We have provided evidence that Tax A, H variants (*tax b, c, d, f, e* alleles) are correlated with reduced WBC counts at the level of BLV-negative groups of animals and thus could be the feature of the aleukemic (AL) form of BLV infection. We suggest this finding could be used in BLV testing for the presence of Tax A, H in the proviral DNA consider such strains of BLV as AL ones, and because of this, minimize the clinical losses due to BLV infection in cattle.

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Bovine leukemia virus (BLV) with other three species: Primate T-lymphotropic virus 1, 2, 3 (PTLV-1, PTLV-2, PTLV-3, respectively), belongs to the *Deltaretrovirus* genus (*Orthoretrovirinae* subfamily, *Retroviridae* family, order *Ortervirales*) (the ICTV taxonomy database website: http://ictv.global/virus Taxonomy.asp).

BLV causes a persistent infection of B-cells in natural hosts – cattle, yak, zebu, and water buffalo resulting in the disease called Enzootic Bovine Leukosis (EBL). Two types of the disease manifestations can be distinguished: Persistent Lymphocytosis (PL) (about 30% infected animals) and B-cell leukemia/lymphoma (lymphosarcoma) or other types of tumors (< 5% ones). The remaining 70% of infected animals are asymptomatic and this is referred to as the aleukemic (AL) stage [1–5].

PL is a subclinical form of the BLV infection. It is a usually stable benign form of the disease with a nonmalignant polyclonal expansion of immature B-cells, which has been resulting in the increase of absolute WBC count (leukocytosis) in the peripheral blood [1,2]. Cattle with PL may suffer from disturbances of the immune system, and because of this could be susceptible to other infectious diseases (e.g. mastitis) [2,6]. The onset of PL usually occurs before the age of 5 years, and after that, PL may develop into the tumor stage (B-cell leukemia/lymphoma) with a peak of incidences between 5 and 8 years of cattle age, or may be not. As well the tumor stage could develop with or without prior PL [1,2,5].

So, it is difficult to say: is the PL type (form) or stage of the BLV infection disease? Some authors consider that only B-cell leukemia/lymphoma (lymphosarcoma) in the case of the BLV infection should be termed EBL [1]. Clinical symptoms of tumors may manifest in digestive disturbances, weight loss, weakness. Superficial lymph nodes may be enlarged. A wide range of tissues and lymph nodes are found to be infiltrated by neoplastic cells, which can be detected at necropsy. Organs such as the abomasum, heart, spleen, intestine, liver, kidney, omasum, lung, and uterus are most commonly affected [5]. Experimentally, BLV can also infect rabbits, rats, chickens, pigs, guinea-pigs, cats, dogs, rhesus monkeys, chimpanzees, antelopes, goats, and sheep, but the only sheep can develop leukemia [5].

The BLV proviral genome has 8.7 kbp in length and includes structural genes (*gag, pro, pol,* and *env*) and non-structural ones (*R3, G4, tax, rex, AS, pre-miRs* (for miRNAs) [1,3,7–9].

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In our previous data, we have shown an association of some pre-miRs-B' alleles with WBC counts in 2-5 yeas old cows [9]. Also, we have noted that a direct Tax-1 (of PTLV-1) and Drosha interaction has been found [10]. Drosha is one of the proteins participating in the biogenesis of host miRNAs [11], but it is not in the biogenesis of BLV miRNAs (miRs-B), which are processed without Drosha [12]. Tax 1 and Drosha interaction leads to the regular host miRNA production decrease [10]. The consequence of this is an increase of viral miRNA production. BLV Tax and host Drosha interaction has not been found, but it has been shown for miRs-B that their production increased so much, that BLV proviral DNA has been called "a prodigious producer of viral microRNAs" in host cells [7]. So, we have been suggesting that BLV Tax has interaction with Drosha too, and by this, it influences miRs-B' production. Overall, there are much data that Tax (BLV Tax except its primary function and Tax-1), as a transactivator of proviral gene transcription, has essential roles in pathogenicity/oncogenicity. Tax involved in the transcriptional and posttranscriptional regulation of a wide variety of cellular genes. So, it takes part in many host cell processes: signal transduction, growth/proliferation/cycle, apoptosis, cell stress response, immunere sponse, DNA repair, and others [1,13–17]. So, having such multifunctional properties, the assumption about BLV Tax interaction with host cell Drosha is not illusive. Thereby, our suggestion about the effect of BLV Tax on miRs-B' production could lead to tax gene/Tax protein polymorphism, and its association with WBC counts as it has been found for pre-miRs-B' genes [9].

In this study we have been investigating *tax* gene and Tax protein polymorphism, and its association with WBC counts. For this purpose, we have been used DNA from 42 peripheral blood samples of 2 to 5 years old female Holstein cattle (farming in Moscow region, Russia), all of which are the same as in previous works [9,18]. WBCs of the samples have been counted on an Abacus Junior Vet 5 Automatic Hematology Analyzer (Diatron, Austria).

All samples have been checked for BLV proviral DNA by high sensitive PCR test system based on two primers to *gag* and *pol* genes [19], and at the same time, all of them have been tested for antibodies to BLV envelope glycoprotein gp51 antigen by AGID (agar gel immunodiffusion).

The envelope glycoprotein gp51 is the main target for neutralizing antibodies in the host animal and is necessary for the virus to enter B-cells [5,8]. BLV antibodies can be detected by AGID, ELISA (enzyme-linked immunosorbent assay), RIA (radioimmunoassay), and immunoblot. These antibody detection methods, PCR detection of BLV proviral DNA, and BLV virus isolation are recommended to apply on the same clinical samples [5,8]. However, it should be noted, all of those methods reflect only the fact of a contact of an individual animal with the virus, but they do not allow predicting the spread of BLV infection to other susceptible cattle or development of tumors [18].

All of 42 samples have been divided into two groups: 16 BLV-seropositive and proviral BLVpositive ones (further: BLV-positive), and 26 BLVseronegative and proviral BLV-negative (further: BLV-negative) ones and WBC count reference intervals (i.e. mean  $\pm$  2 SD) have been ranged as 4,710--29,322 and 5,906-16,609 WBCs/µL, respectively [9]. Further, 16 BLV-positive samples have been used to study tax gene/Tax protein polymorphism by PCR tax fragment cloning and sequencing. For this, two primers TBLV-F and TBLV-R (5'-GCAAGTGTTGTTGGTTGGGGGGCC-3' and 5'-CCCTCAAAAAGGCGGGGAGAGCC-3', respectively) have been designed based on the alignment of BLV complete genomic sequences retrieved from GeneBank used in our previous work [9]. (Primers had been synthesized by Evrogen JSC, Moscow, Russia.) Using these primers, ~ 900 bp PCR fragments of the second big exon of tax gene have been cloned with help of CloneJET PCR Cloning Kit (Thermo Scientific TM), and, further, the total of 83 clones (5-6 clones for each of 16 BLV-positive samples) have been sequenced by Sanger's sequencing method (by Evrogen JSC, Moscow, Russia). The obtained sequences (GeneBank number from MN072344 to MN072355) have been aligned and analyzed with the GeneRunner program. We should note that the *tax* gene is presented in the provirus as composed of two exons, the first of which consists only of 4 bp. We have not found any substitutions in this short region after the alignment of complete genomic sequences of BLV from GeneBank. Because of this, we use only the second exon of tax gene, and further, when we are speaking about tax alleles and Tax protein variants, we consider only II-nd tax exon and Tax protein without Met and Ala, respectively, although we put them for comparisons with others from GeneBank.

As a result, we have detected twelve different tax alleles (a - l), which have many silent mutations. So, all tax alleles can represent eight variants of Tax

Table	1.	The	occurrence	e of	tax	prot	ein	varia	nts	in	each	I SI	ub-
group	of	cow	s: BLV+ I, I	BLV.	+ 1/11	and	BL	V+ II	are	BL	V- po	osit	ive
subar	oup	os of	animals.										

Tax alleles		The occurrence of Tax protein variants in each cow (in absolute numbers of 5–6 clones investigated) by subgroups										(in				
and Tax protein			BL	_V+	I				BL	V+	I/II			В	LV-	- II
variants		2	3	4	14	15	16	13	5	6	9	10	11	8	7	12
tax    Tax      b, c, d, f    A      e    H      h    D      i    C      g    E      k    F      a, I    B      j    G	6	5	5	4	6	5	5	6	5	5	5	5	4	4	5	5

protein (A – H). Moreover, all samples have been divided into three subgroups (I, I/II, II) with different representations of *tax* alleles/Tax protein variants (Table 1). I-st BLV-positive (BLV+ I) includes 6 cows (#1-4,14,15) with *tax b, c, d, f* alleles, which represent Tax A protein, and *tax e* allele/Tax H protein. I/ II-nd BLV-positive (BLV+ I/II) includes 7 cows (#5,6,9–11,13,16) with *tax a, l* alleles, which represent Tax B protein, and *tax j* allele/Tax G protein. II-nd BLVpositive (BLV+ II) includes 3 cows (#7,8,12) with *tax h* allele/Tax D protein, *tax i* allele/Tax C protein, *tax g* allele/Tax E protein, and *tax k* allele/Tax F protein (Table 1). Fisher's exact test (http://mathworld.wolfram.com/FishersExactTest.html) has shown the highly significant (P < 0.01) association

of such division of BLV-positive cows into three subgroups with different representations of Tax variants (and tax alleles). Namely, ones more, Tax A, H (tax b, c, d, f, e alleles) are present in the I-st BLVpositive subgroup, Tax B, G (tax a, l, j alleles) are present in the I/II-nd BLV-positive subgroup, and Tax D, C, E, F (tax h, i, g, k alleles) are present in the II-nd BLV-positive subgroup (Table 1). WBC count reference intervals for BLV+ I, BLV+ I/II, and BLV+ II subgroups have been ranged as 8,009-16,191, 7,097-30,540, and 10,140-35,146 WBCs/µL, respectively. We can see from ANOVA test (GraphPad Prism V.7.04, (1992-2017 GraphPad Software, Inc.) (Figure 1) and unpaired t-test (Table 2 (http://www. graphpad.com/quickcalcs/), that the difference between BLV+ I/II and BLV+ II subgroups has not been significant, so, it can be considered as one subgroup (Figure 1, Table 2). The opposite situation is with the BLV+ I subgroup. There is the significant difference in WBC counts of this subgroup with BLV+ I/II and BLV+ II subgroups. Moreover, the BLV+ I subgroup has not difference with the BLV-negative group. It means that the BLV+ I subgroup of cows remains AL. The same (with some exceptions) situation we had been found in our previous study for some of pre-miRs-B' alleles [9]. So, we can say that Tax A, H (tax b, c, d, f, e alleles) have represented in the BLV+ I subgroup could associate with the AL form (stage) of BLV infection, and these Tax (tax alleles) could be



**Figure 1.** ANOVA test results for BLV-negative (BLV-), BLV-positive (BLV+) groups, I-st BLV-positive (BLV+ I), I/II-nd BLV-positive (BLV+ I/II) and II-nd BLV-positive (BLV+ II) subgroups of cows. The number of WBCs ( $y \times 10^3$  cells/µL) is on the *y*-axis. BLV- and BLV+ status of these groups was determined by both serology (AGID) for BLV antibody, and PCR detection of proviral BLV DNA.

**Table 2.** Unpaired *t-test* results for groups and subgroups of cows. The two-tailed *P* values with a 95% confidence interval. WBC RI (the WBC reference interval) has been measured in WBC/ $\mu$ L. BLV -, BLV +, BLV + I, BLV + I/II, and BLV + II areas in Table 1 and Figure 1. BLV- and BLV + status has been determined by both serology (AGID) for BLV antibody, and PCR detection of proviral BLV DNA.

BLV - (N = 26)				
WBC RI is				
5,906 — 16,609				
$P \leq 0.0001$	BLV + (N = 16)			
This difference is considered to be	WBC RI is			
extremely statistically significant.	4,710 — 29,322			
	P = 0.1650	II BLV + $(N = 3)$		
	This difference is	WBC RI is		
	considered to be not	10,140 — 35,146		
	statistically significant.			
	P = 0.5195	<i>P</i> = 0.3797	I/II BLV + (N = 7)	
	This difference is	This difference is	WBC RI is	
	considered to be not	considered to be not	7,097 — 30,540	
	statistically significant.	statistically significant.		
P = 0.4768	P = 0.0730	P = 0.0054	P = 0.0223	I BLV + (N = 6)
This difference is considered to be not	This difference is	This difference is	This difference is considered	WBC RI is
statistically significant.	considered to be not quite	considered to be very	to be statistically significant.	8,009 — 16,191
	statistically significant.	statistically significant.		

considered as AL ones. The rest Tax B, C, D, E, F, G (*tax a, g, h, i, j, k, l* alleles) have represented in the BLV+ I/II and BLV+ II subgroups could associate with the non-AL (PL or tumor) form (stage) of BLV infection.

Furthermore, we have performed multiple alignments (Figure 2) and phylogenetic analysis (Figure 3) of Tax protein sequences obtained in this work and ones from GeneBank with known disease manifestations (Table 3) [4,8,20–25] using MEGA 10 program [26]. The evolutionary history had been inferred by using the Maximum Likelihood method and the JTT matrix-based model [27].

We can see (Figure 3) that the Tax A, H (from the BLV+ I subgroup, AL ones) have clustered in the separate group from others. So, the AL form of the Tax has been separated in the phylogenetic tree from othes, which supposed to be non-AL (presented in BLV+ I/II and BLV+ II subgroups). A similar situation has been found for genotypes G1 and G2. The classification of BLV into ten genotypes (G1 - G10) has been made based on phylogenetic studies of env genes and proved by complete proviral genomic sequences [28]. In our phylogenetic analysis, based on Tax protein, the G1 and G2 phylogenetic groups (Figure 3) include BLV strains with AL and PL (for G2), and tumor (for G1) form (stage) of BLVinfection disease. We can, therefore, say that the AL or non-AL (PL or tumor) variants of Tax protein may be a refinement of BLV genotypes based on phylogenetic studies of env genes and complete proviral genomic sequences.

Farthemore, we can see (Figure 2) that the main difference between Tax A, H from Tax D, C is a mutation E51G in the putative zinc finger motif (ZNF) (Figure 2), one of the structural domains has been found in the Tax protein [14]. It is known that ZNF proteins participate in the regulation of a wide variety of genes and take part in diverse cell processes, such as DNA recognition, RNA packaging, transcriptional activation, apoptosis, cancer progression, and others [29]. Those properties of ZNF proteins remind the functions of the Tax proteins noted above. So, since the mutation E51G is located in the zinc finger (ZNF), it may be a key to different functionality of the Tax A, H (Tax E51) and Tax D, C (Tax G51) variants, which occur in different BLV+ subgroups (I and II, respectively) (Figure 1-3, Table 1), and I-st of which is considered as AL subgroup, and IInd does as non-AL one of the BLV+ animals (see above and Table 2).

In this study, we have discovered the existence of the *tax* gene/Tax protein polymorphism, which is related to the different WBC count in BLV-infected cows. We have provided evidence that Tax A, H variants (*tax b, c, d, f, e* alleles) are correlated with reduced WBC counts at the level of BLVnegative groups of animals and thus could be the feature of the AL form of BLV infection. So, we can suggest that BLV with Tax A, H variants may be considered as the AL strains of BLV. Moreover, this finding could be used in BLV testing for the presence of Tax A, H in their proviral DNA consider them as AL strains of BLV, and because of this, minimize the clinical losses due to BLV infection in cattle.

	Zn finger domen							
EBLBAX04241.1	MASVIGWGPH	SLHACPALVL	/- SNDVTIDAWC	PLCGPHERLQ	FERIDTTLTC	ETHRVNWTAD	GRPCGLNGTL	FPRLHVSETR
TCCJ67619.1 EBLBAX04259.1	V v	• • • • • • • • • • • •	• • • • • • • • • • • •	• • • • • • • • • • • •	•••••	I	•••••	• • • • • • • • • • • •
T#EBL*,AL-AXL95014.1	v					I		
ALBAX04169.1	v					I		Q
ALBAX04142.1 ALBAX04205.1	V					I		
EBLBAX04277.1 EBLBAX04178.1	V v	•••••	•••••	•••••	•••••	I	•••••	•••••
TBAU59290.1	v					I	M.	
EBLAXL95008.1 ALBAX04133.1	v					I	M.	
ALAUV64408.1 ALACR15161.1	v		D	R		HIT	FA.	
EBLAAF97920.1	v				H	IT	FA.	RDP
H = BLV+ I H = BLV+ I	v					IT	FA.	
D - BLV+ II C - BLV+ II	v					GIT GIT	FA.	
E - BLV+ II F - BLV+ II	V v	•••••	•••••	•••••	•••••	.IIT	F F	•••••
B - BLV+ I/II	V					IT	F	
G - BLV+ I/II ALAUV64420.1	V		D			HIT	F	
ALAUV64414.1 ALAUV64426.1	v		D		s	HIT	FM	
ALAUV64438.1	V					IT	F	
ALAUV64432.1	v		•••••		•••••	IT	<b>F</b>	
DDT DDV04041 1	DOGDDDIWIN		+	appopyogor	PARAGRAGET			/
TCCJ67619.1	PQGPKKLWIN	CPLPAVRAQP	GPVSLSPFER		PSASSDGCPI	IGHGLLPWNN	K.	
EBLBAX04259.1 T#EBL*,AL-AXL95014.1							K.	
TBAP46819.1			W	• • • • • • • • • • • •			К. к	
ALBAX04103.1						т	K.	
EBLBAX04205.1			•••••		•••••	•••••	K.	•••••
EBIBAX04277 1							к	v
EBLBAX04178.1							к.	•••••
TBAU59290.1 EBLAXL95008.1		v						
ALBAX04133.1 ALAUV64408.1		v.	vo				K.	к.
ALACR15161.1					V		K.	
A - BLV+ I	APRA				V		K.	
H - BLV+ I D - BLV+ II					F		K.	
C - BLV+ II F - BLV+ II					F		K.	
F - BLV+ II		T	Q				.AK.	
G - BLV+ 1/11 G - BLV+ 1/11		S	Q	н.		s	K.	
ALAUV64420.1 ALAUV64414.1	G	I	AQ	S	S		K.	н
ALAUV64426.1		<b>T</b>	L.Q			K	.EK.	.T
ALAUV64432_1	s							
			·····				.EK.	.T
	lausing si	ah activation de	b.Q			к	.EK.	
	leucine-ri	ch activation do	omein	\		k	.EK.	.т/
EBLBAX04241.1 TCCJ67619.1	leucine-ri	ch activation do	omein DTRGAIRYLS	\ TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	.T/ LPLIQTPGLS
EBLBAX04241.1 TBAX04241.1 EBLBAX04259.1 #EBL*-BAX195014_1	leucine-ri	ch activation do	DTRGAIRYLS	TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/LPLIQTPGLS
EBLBAX04241.1 TCCJ67619.1 EBLBAX04259.1 T#EBL*,AL-AX195014.1 TBAY46819.1	leucine-ri	ch activation do	DTRGAIRYLS	\ TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/LPLIQTPGLS
EBLBAX04241.1 TBAX04259.1 T#EBLBAX04259.1 T#EBL*,AL-AXL95014.1 TBAY04169.1 ALBAX04169.1 ALBAX04142.1	leucine-ri	ch activation do	DTRGAIRYLS	TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS
EBLBAX04241.1 TBAX04259.1 T#EBLBAX04259.1 T#EBLBAX04259.1 ALBAY04169.1 ALBAX04122.1 EBLBAX04205.1 EBLBAX0427.1	leucine-ri	ch activation do	DTRGAIRYLS	TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS
EBLBAX04241.1 TCUJ67619.1 EBLBAX04259.1 T*EBL*A.AX195014.1 TBAX04163.1 ALBAX04163.1 ALBAX04163.1 BELBAX04176.1 EBLBAX04176.1	LLPSFDTLLV	ch activation do	DTRGAIRYLS	TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS
EBLBAX04241.1      TCCJ67619.1      EBLBAX04259.1      T*EEDL*A.AX1595014.1      TBAX04169.1      ALBAX04169.1      ALBAX04120.5      EBLBAX04277.1      EBLBAX04178.1      TBAX04178.1      EBLBAX04178.1      EBLBAX04178.1      EBLBAX04178.1      EBLBAX04178.1      TBAX05008.1	leucine-ri	ch activation do	DTRGAIRYLS	TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS 
EBLBAX04241.1 TCUJ67619.1 EBLBAX04259.1 TFEEDL*A.AX195014.1 TBAX04169.1 ALBAX04169.1 ALBAX04277.1 EBLBAX04277.1 EBLBAX04277.1 EBLBAX04178.1 TBAX04178.1 TBAX04133.1 ALBAX0433.1	leucine-ri	ch activation dd	DTRGAIRYLS	\ TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS 
EBLBAX04241.1      TCCJ67619.1      EBLBAX04259.1      T*EBL*AAX195014.1      TBAX04169.1      ALBAX04169.1      ALBAX04270.1      EBLBAX04270.1      EBLBAX04270.1      EBLBAX04277.1      EBLBAX04178.1      TBAX04133.1      ALAX195008.1      ALAX195008.1      ALAX195008.1      ALAX195008.1      ALAX195008.1      ALAAP16408.1      ALAAP16408.1      ALAAP16408.1      ALAAP17920.1	LLPSFDTLLV	ch activation dd	DTRGAIRYLS	TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS 
EELBAX04241.1      TCCJ67619.1      EELBAX04259.1      TFEEL*J.A.XIA59014.1      TBAX04169.1      ALBAX04169.1      ALBAX04270.1      EELBAX04270.1      EELBAX04270.1      EELBAX04127.1      TBAX04178.1      TBAX04133.1      AL	LLPSFDTLLV	ch activation do	T	TILITICPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS 
EBLBAX04241.1    TCJG7619.1    EBLBAX04259.1    TBAX04163.1    ALBAX04163.1    ALBAX04163.1    BAX0427.1    EBLBAX0427.1    EBLBAX0427.1    EBLBAX0427.1    EBLBAX0427.1    EBLBAX04230.1    EBLBAX04178.1    ALBAX04217.1    EBLBAX0413.1    AL	LLPSFDTLLV	ch activation dc	T.	\ TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS 
EBLBAX04241.1    TCCJ67619.1    EBLBAX04259.1    TBAX04259.1    TBAX04169.1    ALBAX04169.1    ALBAX04205.1    EBLBAX04205.1    EBLBAX04205.1    EBLBAX04130.1    ALARJ55200.1    FLARJ55200.1    EBL	leucineri LLPSPDTLLV	ch activation do	T. T. J. C. Smein	TILTICPATC	ILPLGEPFSP	NVPICRFPRD	EK. SNEPPLSEFE	/ LPLIQTPGLS 
EBLBAX04241.1      TCCJ67619.1      EBLBAX04259.1      F#EDL*,ALX155014.1      TBAX04169.1      ALBAX04169.1      ALBAX04277.1      EBLBAX04277.1      EBLBAX04178.1      TBAX04178.1      TBAX04178.1      ALAUS9008.1      ALAUV6408.1      ALAVV6408.1      ALBAV920.1      A =      BL-V+I      D = BLV+I      D = BLV+I      D = BLV+I      B = BLV+II      F = BLV+II      F = BLV+II      B = BLV+II	leucine-ri	ch activation dd	. T T	\ TILITICPATC	ILPLGEPFSP	NVPICRFFRD	SNEPPLSEFE	/ / LPLIQTPGLS
EBLBAX04241.1      TCCJ67619.1      EBLBAX04259.1      TTBAX04259.1      TBAX04169.1      ALBAX04169.1      ALBAX04169.1      ALBAX04127.1      EBLBAX04178.1      TBAX04178.1      TBAX04178.1      TBAX04178.1      BLBAX04133.1      ALALV95008.1      ALALV95008.1      ALALV95008.1      ALALV95008.1      ALALV95008.1      ALALV95008.1      ALALV95008.1      AL	LUCENTIAL	ch activation dd	mein DTRGAIRVLS 	TLUTICEPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	LPLIQTPGLS 
EBLBAX04241.1      TCUJ67619.1      EBLBAX04259.1      TTBAX04259.1      TTBAX04169.1      ALBAX04169.1      ALBAX04169.1      ALBAX04127.1      EBLBAX04127.1      EBLBAX04127.1      EBLBAX04178.1      TBAX04133.1      ALAVV6408.1      ALAVV6408.1      ALAVV6408.1      BLV+ I      D - BLV+ II      C - BLV+ II      F - BLV+ II      B - BLV+ I/II      A - BLV+ I/II      A - BLV+ I/II	leucine:ri      LLESPTILU	ch activation dc	mein DTRAIRYLS TT. TT. TT. .K.	\ TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	.E	/ LPLIQTPGLS 
EBLBAX04241.1      TCCJG7619.1      EBLBAX04259.1      TFEDL*ALAX155014.1      TBAX04169.1      ALBAX04169.1      ALBAX04169.1      ALBAX04169.1      BLBAX04120.5      EBLBAX04127.1      EBLBAX04178.1      TBAX04178.1      AL      BLV	Isosoft      I.I.SPEPTILI      I.I.SPEPTILI      P.      P.	ch activation dc		TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	.E	/ LPLIQTPGLS
EBLBAX04241.1      TCUJ67619.1      EBLBAX04259.1      TBAX04259.1      TBAX04163.1      ALBAX04163.1      ALBAX04163.1      ALBAX04178.1      EBLBAX04207.1      EBLBAX04207.1      EBLBAX04178.1      ALBAX04178.1      ALBAX04178.1      ALBAX04178.1      BALARCH5161.1      EBLARCH5161.1      EBLARV54200.1      F = BLV4 II      Ø = BLV4 VI      Ø = BLV4 VI      Ø = BLV4 VI      Ø = BLV4 I/II      ALAUV64426.1      ALAUV64432.1	Ieucine-ri      LD-BEPOTLIV	ch activation dd	mein DTRGATRYLS T. T. T. T. T. T. T. T. T. T. T. T. T.	TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD	.EA. SNEPPLSEFE 	/ LPLIQTPGLS 
EBLBAX04241.1      TCCJ67619.1      EBLBAX04259.1      TBAX04259.1      TBAX04169.1      ALBAX04169.1      ALBAX04169.1      ALBAX04169.1      BLBAX04169.1      BLBAX04169.1      BLBAX04133.1      ALAXU59008.1      ALAXU59008.1      ALAXU59108.1      EBLAXU59108.1      EBLV+ I      D = BLV+4      D = BLV+4      D = BLV+4      B = BLV+4      D = BLV+4      B = BLV+4      J = BLV+4 <td< th=""><th>P. P. P</th><th>ch activation dd</th><th></th><th>\ TLUTLCPATC</th><th>ILPLGEPFSP</th><th>X.</th><th>SNEPPLSEFE</th><th>/ LPLIQTPGLS</th></td<>	P. P	ch activation dd		\ TLUTLCPATC	ILPLGEPFSP	X.	SNEPPLSEFE	/ LPLIQTPGLS
EBL BAX04241.1    TCJG7619.1    EBL BAX04259.1    TT	LUCEPTILL LLEPETILL P. D.	ch activation dd			ILPLGEPFSP	NVPICRFFRD	SNEPPLSEFE	/ LPLIQTPGLS
EBLBAX04241.1      TCCJ67619.1      EBLBAX04259.1      TTBAX04259.1      TTBAX04259.1      TT	leucine-ri LLESPTOTILU 	ch activation dd		TLITICPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS
EBLBAX04241.1    TCCJ67619.1    EBLBAX04259.1    TTBAX04259.1    TBAX04169.1    ALBAX04125.1    EBLBAX04127.1    EBLBAX04178.1    TBAX04178.1    TBAX04178.1    TBAX04178.1    EBLBAX04178.1    LELBAX04178.1    LELAVV6408.1    ALAVV6408.1    ALAVV6408.1    ALAVV6408.1    ALAVV6408.1    AAVV6408.1    AAVV6408.1    A	leucine-ri	ch activation de PPLRLSVPAP		TILITICPATC	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS
EBLBAX04241.1      TCJG7619.1      EBLCJG7619.1      EBLBAX04259.1      TBAX04163.1      ALBAX04163.1      ALBAX04163.1      ALBAX04163.1      ALBAX0427.1      EBLBAX0427.1      EBLBAX04178.1      TBAX04178.1      ALAXL95008.1      BLAXL95018.1      EBLAXL940420.1      TAXVF64432.1      B	Ieucine-ri      LDSBYDTLV	ch activation de	T. T	TLLTLCPATC	ILPLGEPFSP	NVPICRFPRD		/ LPLIQTPGLS
EBLBAX04241.1      TCCJG7619.1      EBLBAX04259.1      TBAX04259.1      TBAX04163.1      ALBAX04163.1      ALBAX04163.1      ALBAX04163.1      BLBAX04178.1      BELBAX04133.1      ALBAX04133.1      ALAX195008.1      BL	P. P	ch activation dd		\ TLUTLCPATC	ILPLGEPFSP	NVFICRPPRD	E	/ LPLIQTPGLS
EBLBAX04241.1      TCCJG7619.1      EBLBAX04259.1      TBAX04259.1      TBAX04169.1      ALBAX04169.1      ALBAX04169.1      ALBAX04169.1      BLBAX04169.1      BLBAX04177.1      EBLBAX04177.1      EBLBAX04177.1      EBL	P. P	ch activation dd	T THE TRAINING STRATEGYLS	RELEIDTLIT	ILPLGEPFSP	NVPICRFFRD	E	/ LPLIQTPGLS
EBL BAX04241.1    TCCJ67619.1    EBL BAX04259.1    TBAX04259.1    T	P. P	ch activation dd		RELEDETIT	ILPLGEPFSP	NVPICRPFRD	SNEPPLSEFE	/ LPLIQTPGLS
EBL    BAX04241.1      T    CCJ67619.1      EBL    BAX04259.1      T    BAX04259.1      T    BAX04259.1      T    BAX04169.1      AL    BAX04169.1      AL    BAX04169.1      AL    BAX04169.1      AL    BAX04127.1      EBL    BAX04277.1      EBL    BAX04178.1      T    T      BAX04178.1    T      C    BAX04178.1      AL    ACM5161.1      EBL    BAX04120.1      AL    ACM5161.1      EBL    BAX0420.1      AL    ACM5161.1      EBL    BAYN 1      B    BIX+1      F    BIX+1      F    BIX+11      F    BIX+11      F    BIX+1/11      G    BIX+1/11      G    BIX+1/11      G    BIX+1/11      B    BIX+1/11      B    BIX+1/11      C </th <th>leucine-ri</th> <th>ch activation dd</th> <th></th> <th>TLUTICEATC</th> <th>JLPLGEPFSP</th> <th>NVPICRFPRD</th> <th>SNEPPLSEFE</th> <th>/ LPLIQTEGLS</th>	leucine-ri	ch activation dd		TLUTICEATC	JLPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTEGLS
EBL    BAX04241.1      TCJG7619.1      EBL    BAX04259.1      TBAX04259.1      TBAX04259.1      TBAX04163.1      AL      BAX04163.1      AL      BAX04205.1      EBLBAX04163.1      AL      BAX04205.1      EBLBAX04178.1      T	Leucine-ri	ch activation dd		RELEDPTITW	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTPGLS
EBL    BAX04241.1      T    CCJ67619.1      EBL    BAX04289.1      T    BAX04289.1      T    BAX04289.1      T    BAX04183.1      AL    BAX04163.1      AL    BAX04163.1      AL    BAX04163.1      AL    BAX04178.1      T    BAX04178.1      T    BAX04133.1      AL    ACR5161.1      EBL    BAX04133.1      AL    ACR515161.1      EBL    AAF97920.1      M    BEV+ I      D<    BEV+I      D    BEV+I      D    BEV+I      D    BEV+I      D    BEV+I      J    BEV+I      J    BEV+I      J    BEV/HIT      F    BEV+I      J    BEV/HIT      B    BEV+I      J    BEV      AL    AUV64438.1      AL    AUV64432.1      EBL    B	Leucine-ri LD-BEPOTILI 	ch activation dd		RELHDPTLTW	ILPLGEPFSP	NVFICRPPRO	E	/ LPLIQTPGLS
EBL  BAX04241.1    TCCJG7619.1    EBLBAX04259.1    TBAX04259.1    TBAX04189.1    AL    BAX04189.1    AL    BAX04189.1    AL    BAX04189.1    AL    BAX04189.1    AL    BAX04189.1    AL    BAX04120.1    EBL    BAX04130.1    AL    BAX04133.1    AL    ARP3920.1    F    BUV+ I    D    BUV+ II    G    BUV+ II    G    BUV+ II    G    BUV+ II    G    BUV+ I/II    G    BUV+ I/II    B    BUV+ I/II    BEL    BL    BL    BL    BL    BUV+ I/II    B    BL    BL    BL	leucine-ri	ch activation dd		RFLHDPTLTW	ILPLGEPFSP	NVPICRPPRD	SNEPPLSEFE	/ LPLIQTPGLS
EBL    BAX04241.1      TCCJG7619.1      EBL    BAX04259.1      TBAX04259.1      TBAX04169.1      AL    BAX04169.1      AL    BAX04169.1      AL    BAX04169.1      AL    BAX04169.1      AL    BAX04169.1      AL    BAX04127.1      EBL    BAX04128.1      TBAX050133.1    AL      AL    ACR15161.1      EBL    ACR15161.1      EBL    ACR15161.1      EBL    BIV+1      D<= BIV+1    D      D = BIV+1    T      B = BIV+1    T      C = BIV+4    T      B = BIV+1    T      AL=BAV04240.1    AL=AUV6425.1      T=BAV0425.1    T <th>Levine-ri LLPPTTLU</th> <th>ch activation dd</th> <th>THE THE THE THE THE THE THE THE THE THE</th> <th>RELHDERT</th> <th>ILPLGEPFSP</th> <th>NVPICRFFRD</th> <th>SNEPPLSEFE</th> <th>/ LPLIQTPGLS</th>	Levine-ri LLPPTTLU	ch activation dd	THE	RELHDERT	ILPLGEPFSP	NVPICRFFRD	SNEPPLSEFE	/ LPLIQTPGLS
EBL CCJG7619.1    EBL CCJG7619.1    EBL	leucine-ri	ch activation dd		RFLEDPTLTW	ILPLGEPFSP	NVPICRFFRD	SNEPPLSEFE	/ LPLIQTEGLS
EBL    —      EBL    —      CCJG7619.1      EBL    —      EBL    —      BAX04259.1      T    —      BAX04259.1      T    —      BAX04259.1      T    —      BAX04189.1      AL    —      BAX04120.1      EBL    —      BAX04277.1      EBL    —      BAX04127.1      EBL    —      BAX04200.1      AL    —      BAX04133.1      AL    —      BBL    —      BL    —      AL    —      BBL    —      BL    —      BL    —	LUCINE TI LLESETOTIAL P. P. N. P.	ch activation dd	T. T	RELHOPTLTW	LLPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTEGLS
EBL    BAX04241.1      TCCJG7619.1      EBL    BAX04259.1      TBAX04259.1      TBAX04163.1      AL    BAX04163.1      AL    BAX04163.1      AL    BAX04163.1      AL    BAX04163.1      AL    BAX04163.1      AL    BAX04163.1      AL    BAX04178.1      TBAX04178.1      EBL    BAX04178.1      AL    CAR15161.1      EBL    BAX04133.1      AL    AAF37920.1      A = BUX+1    BUX      B = BUX+1    II      B = BUX+1    II      B = BUX+1    II      J = BUX+1    II      J = BUX+1    II      J = BUX+1    II      AL    AUV64420.1      AL    BUX+4      AL    BUX+4      AL    BUX+4      AL    AUV64432.1      EBL    BUX+4      AL    AUV64432.1      EBL    <	Leucine-ri	ch activation dd		RELEOPTIT	ILPLGEPFSP	NVPICRFPRD	SNEPPLSEFE	/ LPLIQTEGLS
EBL    BAX04241.1      T    CCJ67619.1      EBL    BAX04289.1      T    BAX04289.1      T    BAX04289.1      T    BAX04183.1      AL    BAX04142.1      BEL    BAX04163.1      AL    BAX04163.1      AL    BAX04163.1      AL    BAX04178.1      F    BAX04178.1      T    CSM05008.1      AL    ACM55008.1      AL    SAX04303.1      AL    ACR15161.1      EBL    BAX041408.1      AL    SAX04241.1      F    BUX+1 II      G    BUX+1 II      G    BUX+1 II      G    BUX+1 III      AL    SAX04241.1      T    CCJ67619.1      EBL    BUX+41/11      AL    SAX04241.1      T    SAX04242.1      T    SAX04242.1      T    SAX04242.1      T    SAX04229.1      T    SAX04229.1<	Leucine-ri	ch activation dd	T. T	RELEDELTA	LIPIGEPFSP	NVFICRPPRO	E	/ LPLIQTPGLS
EBL    BAX04241.1      TCCJG7619.1      EBL    BAX04259.1      TBAX04259.1      TBAX04259.1      TBAX04189.1      AL    BAX04189.1      AL    BAX04189.1      AL    BAX04189.1      AL    BAX04189.1      AL    BAX04129.1      EBLBAX04205.1    EBLBAX04205.1      EBL	Levine-ri	ch activation dd PPLRLSVFAP Dalladoren TGPPSPCDRL		RFLHDPTLTW	ILPLGEPFSP	NVPICRPPRD	SNEPPLSEFE	/ LPLIQTPGLS

**Figure 2.** Alignment of Tax amino acid sequences./ – \— structural domains, + — phosphorylation sites. BLV+ I, BLV+ I/II and BLV+ II areas in Figure 1. PL — Persistent Lymphocytosis, T — tumor (B-cell leukemia/lymphoma), AL — aleukemic (asymptomatic, healthy or non-EBL), EBL\* — the disease has developed during the observation period (AL  $\rightarrow$  EBL), EBL — Enzootic Bovine Leukosis (PL or T are unknown) (Table 3).



**Figure 3.** Tax primary protein sequence evolutionary analysis by Maximum Likelihood method. The percentage of trees in which the associated sequences clustered together is shown next to the branches. BLV+ I, BLV+ I/II, BLV+ II, PL, T, AL, EBL\*, EBL areas in Figure 1, 2. G1, G2, G6, and G10 — BLV genotypes, based on *env* genes and complete proviral genomic sequences, for which references are given in Table 3. Square brackets indicate the sequence numbers that represent groups of identical ones (Table 3).

GeneBank numb	ers of Tax sequences			
different	the same	Genotypes	Manifestations of the disease	References
BAX04241.1		G1	EBL	[20]
CCJ67619.1		G1	Т	[21]
BAX04259.1		G1	EBL	[20]
BAR47041.1	BAR47041.1	G?	Т	[21]
	CCJ67631.1	G?	Т	[21]
	CCJ67625.1	G?	Т	[21]
	BAX04286.1	G1	EBL	[20]
	BAX04268.1	G1	EBL	[20]
	BAX04223.1	G1	EBL	[20]
	BAX04160.1	G1	AL	[20]
	BAX04151.1	G1	AL	[20]
	BAX04124.1	G1	AL	[20]
	BAX04115.1	G1	AL	[20]
	BAX04106.1	G1	AL	[20]
	BAX04088.1	G1	EBL*	[20]
	BAX04061.1	G1	AL	[20]
	BAX04052.1	G1	EBL*	[20]
	BAX47046.1	G?	Т	[30]
BAP46819.1		G?	Т	
BAX04169.1	BAX04169.1	G1	AL	[20]
	BAX04079.1	G1	AL	[20]
BAX04142.1		G1	AL	[20]
BAX04205.1	BAX04205.1	G1	EBL	[20]
	BAX04196.1	G1	EBL	[20]
	BAX04187.1	G1	EBL	[20]
BAX04277.1		G1	EBL	[20]
BAX04178.1		G1	EBL	[20]
BAU59290.1		G?	Т	[22]
BAX04250.1	BAX04250.1	G1	EBL	[20]
	BAX04232.1	G1	EBL	[20]
	BAX04214.1	G1	EBL	[20]
BAX04133.1	BAX04133.1	G1	AL	[20]
	BAX04097.1	G1	AL	[20]
	BAX04070.1	G1	AL	[20]
AUV64408.1		G10	AL	[4]
ACR15161.1		G2	AL	[7,23]
AAF97920.1		G2	PL	[24,25]
AUV64420.1		G6	AL	[4]
AUV64414.1		G10	AL	[4]
AUV64426.1		G6	AL	[4]
AUV64438.1		G10	AL	[4]
AUV64432.1		G10	AL	[4]

Table 3. Tax protein sequences have been used for the alignment (Figure 2) and the phylogenetic analysis (Figure 3). PL, T, AL, EBL\*, EBL areas in Figure 2,3.

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# **Disclosure statement**

No potential conflict of interest was reported by the authors.

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