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Detection and immunobiological characterization of bovine leukemia vírus in Russian Federation territory in dependence on geographical variations

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Abstract

BLV is a cancerous lymphoproliferative disease of cattle widely spread all over the world, including the Russian Federation. The genetic characterization of BLV is an important task in scientific research in many countries of the world. According to the phylogenetic analysis of routinely sequenced env gene region, BLV isolates are allocated in different geographical locations of the world, up to 10 different genetic groups of the virus were identified and classified. However, at the moment there are no detailed data on the immunobiological characteristics of BLV genotypes from Russia.

We selected groups of infected animals (n = 54) in Tyumen region by ELISA method. Immunological evaluation of animals in all test groups is given. A nested-PCR study was performed, which resulted in was received a fragment of the env 444 bp gene in the studied samples. RFLP analysis of this fragment allowed to establish that in 94% of the samples there was a «Belgian genotype» of the leukemia virus, in 4% of samples – «Australian» and in 2% - a «mixed type». In this region of Russia, the «Belgian genotype» eventually prevailed. Samples were sent for sequencing.

Аннотация

Вирус лейкоза – злокачественное лимфопролиферативное заболевание крупного рогатого скота, которое широко распространено во всем мире, включая Российскую Федерацию. Генетическая характеристика ВЛ КРС является важной задачей научно-исследовательских работ во многих странах мира. Согласно филогенетическому анализу секвенированных участков гена – env изолятов ВЛ КРС, выделенных в разных географических точках мира установлено и классифицировано до 10 различных генетических групп вируса. Однако на данный момент нет подробных данных об иммунобиологических характеристиках генотипов ВЛ КРС из Российской Федерации.

Нами были подобраны группы инфицированных животных (n=54) в Тюменской области методом ИФА. Дана иммунологическая оценка животных во всех опытных группах. Было выполнено nested-ПЦР исследование, в результате которого в исследуемых образцах был получен участок гена env 444 п.н. RFLP анализ этого фрагмента позволил установить в 94% образцов «Бельгийский генотип» вируса лейкоза, в 4% образцов - «Австралийский» и в 2% - «смешанный генотип». В исследуемом регионе Российской Федерации превалировал «Бельгийский генотип». Образцы были отправлены на секвенирование. Таким образом, исследованием фрагментов env области генома ВЛ КРС, выделенных на территории Тюменской области Российской Федерации, методом рестрикционного полиморфизма (RFLP) показано изменение генетического ландшафта и доминантного генотипа ВЛ КРС.

Keywords: Bovine leukemia virus in Russian Federation, nested PCR, RFLP analysis, several genetic groups

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1. Introduction

Bovine leucosis is a cancerous lymphoproliferative disease, the etiologic factor of which is the bovine leukemia virus belonging to the family Retroviridae, the genus Deltaretrovirus.

The bovine leukemia virus is a huge problem for animal husbandry in many countries of the world, including the Russian Federation. In addition to the fact that the disease is widespread and causes billions of dollars in annual economic damage to the industry, there are also risks to public health [7]. The only effective method to eradicate the disease in cattle is the introduction of comprehensive recovery programs, including diagnostic studies of the entire population for the presence of antibodies to the capsid proteins of the pathogen, followed by the replacement of infected cattle. However, despite the relatively high efficiency of modern methods of diagnosing the disease, there is a possibility of incomplete identification of animals infected with leukemia virus in the healthy herds, which significantly affects the timing of the implementation of recovery programs. Earlier it was noted that this could be due, among other things, to the effect of genetic variability of individual virus isolates. The study of genetic diversity and regional phylogenetics of the virus is an important task in many countries around the world.

Molecular characterization of bovine leukemia virus

Like other retroviruses, the BLV gene contains the structural and regulatory genes: gag, pol and env. The env gene encodes the transmembrane glycoproteins gp51 and gp30 of the virus capsid, which cause the infectivity of the virus. In connection with this, the phylogenetic analysis of BLV genotypes was mainly based on the env gene [8, 10].

Characterization of the genetic diversity of BLV is an important task in scientific research in many countries of the world. According to the sequenced gene site – env BLV isolates are allocated in different geographical locations of the world, up to 10 different genetic groups of the virus were identified and classified [8].

The isolates allocated in Russia and published in NCBI Gene Bank are classified in 4, 7 and 8 genetic groups. Analysis of the amino acid sequences of isolated strains revealed that the main changes were localized in the C-part of the CD4+ epitope, the zinc binding peptide region, CD8+ T cell epitope and overlapping linear epitope E, and the greatest number of changes were noted in G4 («Belgian genotype») [3, 11].

However, there are no detailed data on the immunobiological characteristics of individual genetic groups, no difference in their effect on the animal's organism and no data on the detectability of the approved system tests (serological, molecular-genetic tests), and the recombinant interaction of several genetic groups.

Purpose of exploration: To study the antigenic landscape of bovine leukemia virus pathogens on the territory of the Tyumen region of the Russian Federation using PCR, nested PCR and polymorphism methods for following immunobiological and molecular genetic characteristics.

2. Material and Methods

As part of the research, we monitored the epizootic situation of the bovine leukemia virus in Russia. Groups of animals Holstein-Frisian (imported breed) and Russian Black Pied (local breed) breed (n = 54) were selected, belonging to agricultural organizations of the Tyumen region. Serological (ELISA, AGID) methods of screening the cattle population were used to identify infected animals. Studies by ELISA were performed using an IDEXX Leukosis Serum Screening test (IDEXX Montpellier SAS, France). For the AGID (agar gel immunodiffusion) - diagnostics used kit by the Kursk Biofactory - the "BIOK" company according to kit instructions.

Primary PCR study was performed using a standard commercial test system. Proviral DNA extraction was performed using the “Diatom DNA Prep 200” (IsoGen, Moscow) according to the manufacturer’s protocol.

For carrying out nested PCR, a BioMaster HS-Tag PCR kit (2x) by «Biolabmix» (Novosibirsk) was used. The concentrations of the solutions and the temperature regimes of the amplification reaction were established experimentally, a research protocol was formed. As a control, DNA isolated from cell culture FLK-BLV was used.

The env gene fragment was amplified using Nested PCR using the following primers: env 5032 TCT-GTG-CCA-AGT -CTC-CCA-GAT-A, env 5608 AAC-AAC-AAC-CTC-TGG-GAA-GGG-T, env 5099 CCC-ACA-AGG-GCG-GCG-CCG-GTT-T, env 5521 GCG-AGG-CCG-GGT-CCA-GAG-CTG-G [1], synthesized by «Syntol» (Moscow).

The DNA concentrations were determined on the MaxLife H100 Mod.2 kit of MVM Diagnostics (Barnaul). Amplification was performed using an Applied Biosystems 2720 thermal cycler (Singapore) with the following cycle parameters: 2 minutes at 94 ° C (1 cycle), 30 seconds at 95 ° C, 30 seconds at 62 ° C (external primers) or 30 seconds 70 ° C (internal primers), 60 seconds 72 ° C (40 cycles), 4 minutes 72°C. PCR was performed in a 50 μl volume of the reaction mixture per sample (25 μl BioMaster set HS-Tag PCR (2x), 1 μl of each primer (1 pkm/μl), 1 μl MgCl2 (50 μM), 500 ng of genomic DNA, diluted bidistilled water.

The product was visualized on a 1.5% agarose gel in the presence of ethidium bromide.

At the first stage of the Nested PCR electrophoretic mobility amplicons corresponded to a fragment length of 600 bp. At the second stage of Nested PCR, positive amplicons correspond to a length of 444 bp.

To conduct genotyping of the env region (444bp-gp51 region), a polymorphism reaction (RFLP) was used, BamHI, BclI, PvuII were used as restriction enzymes. Amplification was performed with the following cycle parameters: BamHI, PvuII - 37°C 2 hours; BclI - 55°C 2 hours. PCR was performed in a volume of the reaction mixture of 20 μl per

sample (5 µl of PCR product, 1 µl of the enzyme, 2 µl of buffer, 12 µl of bidistilled water) [1].

3. Results

In As a result of the study, we obtained amplification products of the BLV env 444 bp gene region in 48 samples from the Tyumen region. In the process of research, there were animals that react negatively when examined with a standard commercial kit, however, during the Nested PCR reaction, the desired fragment of the virus gene site was found (Table 1).

RFLP analysis of this fragment allowed to establish that in 94% of the samples there was a «Belgian genotype» of the leukemia virus, in 4% of samples – «Australian genotype» and in 2% - a «mixed type» (Figure 1).

It is established that in Tyumen area of Russia the «Belgian genotype» of BLV dominates the «Australian». Mainly, this type was found in Holstein animals, the disease was characterized by a high rate of development of the acute form and rapid spread. The presence of negative samples in the study of the standard commercial test system could be associated with a low proviral load in the studied animals, as well as with the variability of the gene region in the primer sites. A complete picture characterizing the phylogenetic analysis and the nature of amino acid substitutions in the targeted part of the gene will be obtained from the results of DNA sequencing. The results of immunological studies of animals may allow to establish reliable correlations and to give immunobiological characteristics of individual isolates of the pathogen.

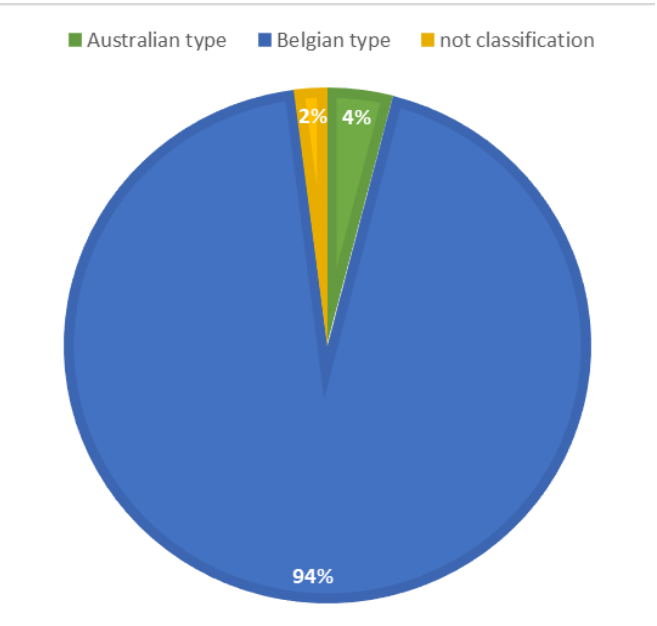

Figure 1 | Characterization of the genetic groups of the BLV in cattle of Tyumen area by RFLP method (n=48). Характеристика генетических групп вируса лейкоза крупного рогатого скота районов Тюменской области методом RFLP (ПДРФ), n=48.

4. Concluding Remarks

Summarizing the preliminary results, we can conclude that the application of the methodological approaches described in the article can serve to determine the geographical origin of leukemia virus isolates. So, we found that in the studied samples obtained from the Tyumen region mainly "Belgian genotype" of bovine leukemia virus is determined. The alleged source of infection could be local and imported cattle (previously imported in 2011) from America. It is also possible that there were false-negative serological tests in a batch of cattle from the Eastern Europe.

Earlier studies have determined that the territories of the Tyumen, Kurgan, and Sverdlovsk regions were dominated by the “Australian genotype”. We recorded the “Belgian genotype” in a smaller percentage in these areas [3, 11]. This genotype mainly dominated on the territory of the Chelyabinsk region, where the spread of the disease is the highest, and the course is characterized by severity and a rapid transition to the terminal stage (hematological). The high detection and dominance of the “Belgian genotype” on the territory of the Tyumen Region may be due to its more aggressive effect on the immune system of susceptible animals. In such animals, an increased proviral load is possible, which leads to a more rapid spread of the pathogen among susceptible herds. This is to be determined by the results of DNA sequencing and the results of immunological expertise of the studied cattle population.

Заключение

Установлено, что применение описанных в статье методологических подходов может служить для определения и установления географического происхождения изолятов вируса лейкоза. Так, нами было установлено, что в исследованных нами образцах, полученных из Тюменской области преимущественно, определялся «Бельгийский генотип» вируса лейкоза крупного рогатого скота.

Предполагаемым источником заражения мог являться местный и импортированный крупный рогатый скот (ранее импортированный в 2011 году) из Америки. Также возможно, что серологические тесты были ложноотрицательными в партии крупного рогатого скота из Восточной Европы.

Проведенными ранее исследованиями было определено, что на территориях Тюменской, Курганской, Свердловской областей циркулировал в доминирующем количестве (Австралийский генотип. Бельгийский генотип) нами регистрировался в меньшем процентном соотношении в этих областях [3,11]. Данный генотип в основном доминировал на территории Челябинской области, где распространение заболевания наиболее высоко, а течение характеризовано тяжестью и быстрым переходом в терминальную стадию (гематологическую). Высокое

Table 1 | List of samples and research results. Результаты исследований, выделенных образцов.
*Studies were conducted in the veterinary laboratory of the Tyumen region. Исследования проводились в районной ветеринарной лаборатории Тюменской области.

#	Origin	AGID*	ELISA	PCR	Nested PCR (second stage)	RFLP
1	Tyumen region, farm 1	+	no data	-	+	belgian type
2	Tyumen region, farm 1	+	no data	+	+	belgian type
3	Tyumen region, farm 1	+	no data	+	+	belgian type
4	Tyumen region, farm 1	+	no data	+	+	belgian type
5	Tyumen region, farm 1	+	no data	-	+	belgian type
6	Tyumen region, farm 1	+	no data	+	+	belgian type
7	Tyumen region, farm 1	+	no data	+	+	belgian type
8	Tyumen region, farm 1	+	no data	+	+	australian type
9	Tyumen region, farm 1	+	no data	-	+	belgian type
10	Tyumen region, farm 1	+	no data	+	+	belgian type
11	Tyumen region, farm 1	+	no data	-	-	-
12	Tyumen region, farm 1	+	no data	+	+	belgian type
13	Tyumen region, farm 1	+	no data	+	+	belgian type
14	Tyumen region, farm 1	+	no data	-	-	-
15	Tyumen region, farm 1	+	no data	+	+	belgian type
16	Tyumen region, farm 1	+	no data	-	-	-
17	Tyumen region, farm 1	+	no data	+	+	belgian type
18	Tyumen region, farm 1	+	no data	+	+	belgian type
19	Tyumen region, farm 1	+	no data	+	+	belgian type
20	Tyumen region, farm 1	+	no data	-	-	-
21	Tyumen region, farm 1	+	no data	+	+	belgian type
22	Tyumen region, farm 1	+	no data	-	-	-
23	Tyumen region, farm 1	+	no data	+	+	mixed type
24	Tyumen region, farm 1	+	no data	+	+	belgian type
25	Tyumen region, farm 1	+	no data	+	+	belgian type
26	Tyumen region, farm 1	+	no data	+	+	belgian type
27	Tyumen region, farm 1	+	no data	+	+	belgian type
28	Tyumen region, farm 1	+	no data	+	+	belgian type
29	Tyumen region, farm 1	+	no data	-	-	-
30	Tyumen region, farm 1	+	no data	+	+	belgian type
31	Tyumen region, farm 2	+	+	+	+	belgian type
32	Tyumen region, farm 2	+	+	+	+	belgian type
33	Tyumen region, farm 2	+	+	+	+	belgian type
34	Tyumen region, farm 2	+	+	+	+	belgian type
35	Tyumen region, farm 2	+	+	+	+	belgian type
36	Tyumen region, farm 2	+	+	+	+	belgian type
37	Tyumen region, farm 2	+	+	+	+	belgian type
38	Tyumen region, farm 2	+	+	+	+	belgian type
39	Tyumen region, farm 2	+	+	+	+	belgian type
40	Tyumen region, farm 2	+	+	+	+	belgian type
41	Tyumen region, farm 2	+	+	+	+	belgian type
42	Tyumen region, farm 2	+	+	+	+	belgian type
43	Tyumen region, farm 2	+	+	+	+	belgian type
44	Tyumen region, farm 2	+	+	+	+	belgian type
45	Tyumen region, farm 2	+	+	+	+	belgian type
46	Tyumen region, farm 2	+	+	+	+	belgian type
47	Tyumen region, farm 2	+	+	+	+	belgian type
48	Tyumen region, farm 2	+	+	+	+	belgian type
49	Tyumen region, farm 2	+	+	+	+	australian type
50	Tyumen region, farm 2	+	+	+	+	belgian type
51	Tyumen region, farm 2	+	+	+	+	belgian type
52	Tyumen region, farm 2	+	+	+	+	belgian type
53	Tyumen region, farm 2	+	+	+	+	belgian type
54	Tyumen region, farm 2	+	+	+	+	belgian type

обнаружение «Бельгийского генотипа» на территории Тюменской области может быть связано с его более агрессивным воздействием на иммунную систему восприимчивых животных. У таких животных возможна повышенная провирусная нагрузка, что приводит к более быстрому распространению возбудителя среди восприимчивого поголовья. Этот тезис предстоит установить по результатам секвенирования ДНК и результатам иммунологической экспертизы исследуемой популяции крупного рогатого скота.

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