ATLANTIS PRESS

Advances in Intelligent Systems Research, volume 167

International Scientific and Practical Conference "Digitization of Agriculture - Development Strategy" (ISPC 2019)

Application of omix technologies in studying of BLV biological diversity by gag gene

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***Abstract*—This paper is the part of a large-scale project concerning studying of bovine leukemia virus biological diversity and of the influence of BLV structural characteristics on virulence and hematolytic blood parameters of infected animals in Western Siberia. This part is devoted to the study of the influence of haplotypes which are determined by mutations in gag gene on several blood parameters and on the number of viral particles per 1000 healthy cells. Emphasis is made on the importance of bioinformatics analysis for processing large data arrays during the multivariant analysis of BLV carriers' parameters. It was established that about ¾ of infected animals were infected with II haplotype virus, whereas the remaining part was infected with BLV of I haplotype. The number of leukocytes, including lymphocytes, erythrocytes, and platelets in the blood of animals infected with II haplotype virus, was generally higher than that of the carriers of I haplotype. Viral load in carriers of II haplotype was 800-1000 viral particles per 1000 healthy cells, whereas the number of viral particles per 1000 healthy cells in the blood of individuals with I haplotype ranged from 600 to 900. It is worth noting that among carriers of I haplotype there were no animals with clinical signs of leukemia, and viral load in healthy animals exceeded that in suspicious individuals. In general, it was shown that gag gene, along with LTR region, is a rather significant factor in maintaining of "viral load – immune response" correlation.**

Keywords— cattle, BLV, leukemia, gag gene, PCR analysis, omix technologies.

I. INTRODUCTION

Today, leukemia is the most common infectious disease of cattle in Russia and several foreign countries [1]. According to 2015 data, leukemia is the cause of more than half of the reported cases of pathologies caused by infectious diseases [2]. One of the generally accepted preventive measures to prevent the spread of leukemia is to isolate sick animals from healthy ones or to slaughter infected animals [3]. However, the implementation of both methods requires accurate identification of carriers [4]. The majority (70–90%) of animals infected with BLV have a prolonged asymptomatic stage [5] during which it is almost impossible to identify an infected animal without using highly sensitive techniques. The current methods of BLV identifying, such as RID and ELISA, have rather a low accuracy and sensitivity [7]. In addition, false-negative results are possible in the course of the diagnostics of mutant strains [8, 9]. Thus, development and implementation of more accurate methods for cattle diagnostics for BLV carrier state is a very interesting, important and relevant scientific and practical issue.

A number of specialists consider PCR analysis as the most promising method for identifying viruses, not only BLV [7, 9, 10]. One of the advantages of this method is not only determining the presence or absence of a virus in a sample, but also the possibility of quantitative analysis and identification of specific strains [11].

Gag gene, along with env and pol genes, is a structural gene of bovine leukemia virus. There are indications [12, 13] that the expression of viral gag gene makes some changes in the metabolic processes of host cells at the molecular level what may well explain the level of BLV recognition by immune agents.

The aim of this work is to implement omix technologies in big data processing, including multifactorial, bioinformatic analysis, as tools for monitoring the occurrence of separate BLV haplotypes determined by gag gene polymorphism, as well as the connection of mutations with hematological parameters of cattle blood in Western Siberia.

II. MATERIALS AND METHODS

We used total DNA samples recovered from whole blood of 780 cows of black-and-white Holsteinized breed. Blood samples were taken in 2015-2016 from the tail vein using sterile catheters and EDTA as an anticoagulant. DNA was recovered using "DNA-Sorb-B" kit (Federal State Research Institution "Central Research Institute of Epidemiology", Russia). Cytofluorometric and morphological blood parameters were determined using PCE-90 Vet automated veterinary hematology analyzer. A bioinformatics analysis (Ugene) of genome-wide BLV nucleotide sequences recorded in the NCBI database was performed. Taking into account the mutational status of isolate sequences, oligonucleotide primers were designed.

The annealing temperature of primers (62.03°C for direct and 53.83°C for inverse) was selected taking into account their nucleotide composition. The direct PCR test was carried out according to the author's previously published method [14] using real-time computer analysis of amplicon. Sample was colored with SuberGreen. Temperature profile: 2 min - 94 ° C; (30 s - 95°C; 30 s - 68°C; 30 s - 72°C) - 40 cycles; 3 min - 72°C. Restriction scheme is given in Table 1.

The work is performed on the means of Finance on the instructions of the Ministry of agriculture of Russian Federation (No. AAAA-A18-118101190004-2).

TABLE I. MAKING OF BLV SAMPLE HAPLOTYPES AS A RESULT OF RFLP ANALYSIS CARRIED OUT USING RESTRICTION ENZYMES HAEIII AND FAEI

	Restriction enzyme	
Haplotype	***HaeIII***	***FaeI***
I	563, 487	160, 205, 300, 385
II	563, 487	15, 385, 650

III. RESULTS AND DISCUSSION

According to the results of PCR-RFPL analysis, two BLV haplotypes by gag gene were found. The first haplotype of leukemia provirus occurred in 26% of livestock, the second one was found in the remaining 74% of livestock (Fig. 1). The results of the hematological analysis are shown in the Table.

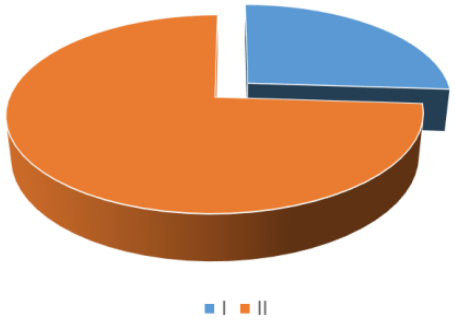

Fig. 1. Distribution of BLV haplotypes by gag gene in cattle of Novosibirsk region

Comparison of hematological parameters of cattle blood using Wilks's lambda distribution showed a fairly high similarity (0.940) between the carriers of different BLV haplotypes. It is interesting to note that despite the seemingly insignificant difference in the absolute numbers of leukocytes as a whole (WBC), as well as their different types, in particular, lymphocytes (LYM), a comparison using Student's criterion shows high reliability of differences in absolute parameters (Table 2).

TABLE II. HEMATOLYTIC STATUS OF BLV CARRIERS OF DIFFERENT HAPLOTYPES BY GAG GENE

Parameter	**Haplotype I**				**Haplotype II**				t_f
	$\bar{X}$	$S_{\bar{x}}$	$D[(X)]$	***99 %***	$\bar{X}$	$S_{\bar{x}}$	$D[(X)]$	***99 %***	
WBC, 10^9/L	9.37	0.23	10.36	0.59	10.98	0.17	16.94	0.44	***
LYM, 10^9/L	4.23	0.13	3.38	0.34	5.14	0.12	8.09	0.31	***
MON, 10^9/L	0.80	0.03	0.19	0.08	0.89	0.01	0.11	0.04	***
GRA, 10^9/L	4.35	0.12	3.12	0.32	4.85	0.06	2.44	0.17	***
LYM, %	44.53	0.75	111.00	1.95	45.75	0.44	113.70	1.15	n/a
MON, %	8.72	0.24	11.07	0.61	8.38	0.08	3.38	0.20	n/a
GRA, %	46.76	0.72	103.30	1.88	45.81	0.44	109.50	1.13	n/a
RBC, 10^{12}/L	4.98	0.09	1.58	0.23	5.27	0.03	0.66	0.09	***
HGB, g/L	89.59	1.62	526.10	4.22	91.74	0.65	246.02	1.69	n/a
HCT, %	25.89	0.44	39.39	1.15	27.17	0.17	16.19	0.43	***
MCV, fL	51.80	0.29	17.10	0.76	52.15	0.20	23.52	0.52	n/a
MCH, pg	17.74	0.22	9.81	0.58	17.70	0.14	11.26	0.36	n/a
MCHC, g/L	344.10	3.86	2987.60	10.05	339.13	2.35	3176.73	6.06	n/a
RDW, %	15.69	0.09	1.55	0.23	15.56	0.05	1.25	0.12	n/a
PLT, 10^9/L	212.34	6.25	7808.70	16.25	236.00	4.00	100.00	11.00	***
MPV, fL	6.92	0.05	0.42	0.12	6.87	0.03	0.48	0.07	n/a
PDW	16.90	0.04	0.29	0.10	16.95	0.02	0.30	0.06	n/a
PCT, %	0.14	0.01	0.01	0.01	1.15	0.58	192.70	1.49	n/a

Moreover, the number of leukocytes in animals with II haplotype found exceeded the upper limit of the norm and, thus, was a pathology.

It should be noted that the absolute content of lymphocytes in the blood of carriers of the abovementioned haplotype was 5.14×10 9/L, although this parameter exceeded the norm, but did not reach the lower limit for diagnosing leukemia. This situation can be explained by the fact that the majority (69%) of diagnosed animals had a viral infection at the time of blood sampling, and it did not give rise to leukemia development. It was also observed that in the blood of the carriers of BLV II haplotype there were 0.29 × 1012/L ($p<0.001$) more red blood cells (RDW) and 23.66×109/L ($p<0.001$) more platelets (PLT). Hematocrit

(HCT) associated with the number of erythrocytes, was higher by 1.28%.

The percentage of formed elements and different hematological parameters: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW) and thrombocrit (PCT) showed no differences between the compared samples (Table 2).

Viral load in carriers of the more common II haplotype (800-1000 virus particles per 1000 healthy cells) was generally higher than in animals with I haplotype (Fig. 2). The clear distinction comes under notice, between healthy and (800-900 viral particles per 1000 healthy cells) and suspicious individuals (600-700 viral particles per 1000 healthy cells) with I virus haplotype identified. It is worth noting that among carriers of I haplotype there were no animals with clinical signs of leukemia, and the viral load in healthy animals exceeded that of suspicious individuals (Fig. 2).

Since the content of leukocytes in the blood of cattle with II haplotype was also somewhat higher, it can be concluded that gag gene, along with LTR region, is a rather significant factor in maintaining of "viral load – immune response" correlation. The high occurrence and virulence of haplotype II are well consistent with the concept of the evolutionary advantage of virulent strains [15]. Moreover, the number of haplotypes and the occurrence of each one correlate with the data of LTR-region studies [15] what suggests the linkage of mutations in LTR-region and gag gene. In this case, the question of the contribution of each mutation to the overall rate of replication of viral particles and, as a consequence, to the viral load of infected animals seems quite interesting. Variant when a single mutation was a cause and other concomitant mutations spread due to the linkage, cannot be excluded. However, more specific conclusions on the questions defined will be possible only after conducting additional research.

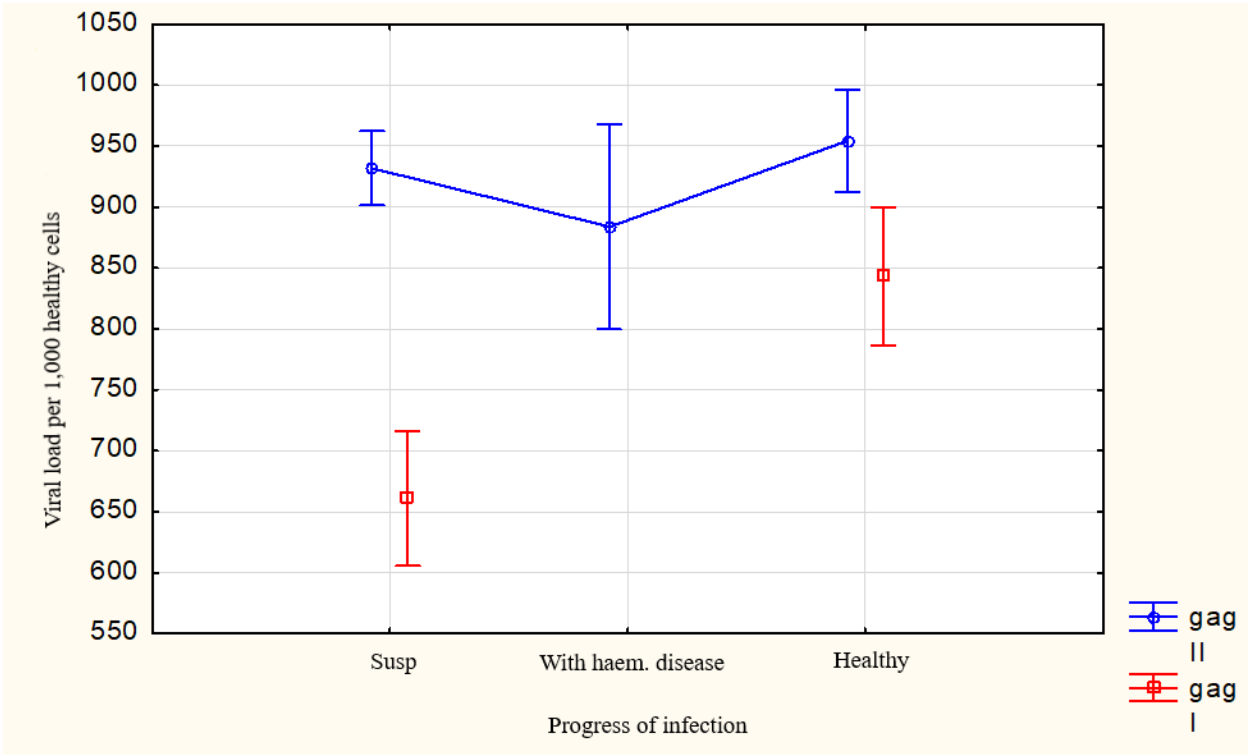

Fig. 2. Viral load in carriers

IV. Conclusions

1. The prevalence of carriers of the first haplotype was 26%, of the second one – 74%.

2. According to the content of formed elements, a significant difference was observed between the carriers of I and II BLV haplotypes by gag gene.

3. The difference should be noticed in viral load between animals with I and II BLV haplotypes: from 300 to 900 and from 800 to 1000 virus particles per 1000 healthy cells, respectively.

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