UDC 619: 578: 616.98: 578.828.11

Baschenko M.,
Academician of the National Academy of Sciences of Ukraine, Doctor of Agricultural Sciences, National Academy of Agrarian Sciences of Ukraine
Stegniy B.,
Academician of the NAAS, Doctor of Veterinary Sciences,
Gherilovich A.,
Doctor of Veterinary Sciences
Lymanska O.,
Doctor of Biological Sciences
Hema I., Gorbatenko S.,
Candidate of Veterinary Science National Research Center "Institute of Experimental and Clinical Veterinary Medicine"

Molecular-genetic and phylogenetic researches of virus of leukosis of cattle circulating in Ukraine

The purpose. To compare the effectiveness of the application of molecular genetic methods and RID in the diagnosis of bovine leukemia and to investigate the phylogenetic relationships between isolates of the leukemia virus (VL) of cattle. Methods. Detection of the pro-viral DNA of VL of cattle was carried out using the domestic PCR test system "BLV-provirus OL / L-hesg", developed by the NSC "IEKVM". Results. The molecular genetic features and phylogenetic connections in the population of HL circulating in different regions of Ukraine are researched. The higher specificity of PCR analysis in comparison with RID for detecting HL of the cattle is confirmed. Conclusions. It has been established that the results of phylogenetic research can be used to identify and study possible subgroups (or genotypes), to create a basis for the search for genes that determine the high biological activity of viruses.

Key words: virus, leukemia of cattle, molecular-genetic methods, phylogenetic analysis.

Viruses, the genome of which is represented by RNA, are characterized by the high rate of mutations of the nucleotide sequences and the associated significant lability of the structure of the genetic material [2, 6, 12-15]. Genomes retro viruses, like other RNA viruses enclos are vysokovariabelnymy vidsut¬nist through the mechanism of correcting errors when copying vy¬nykayut matrix during replication, and possible genetic recombination. At the same time, the nucleotide modifications can lead to a change in the amino acid composition of the synthesized proteins. The consequence of genetic variability is the differences in the biological properties of isolates of retrovirus - cellular tropism, cytopathic action, surface antigenic characteristics. In view of this, the study of the genetic variability of infectious agents is one of the major implications of biological monitoring, the ultimate goal of which is not so much in the study as in explaining this phenomenon [10]. One of the representatives of retroviruses is the leukemia virus of cattle (VL of cattle) - an etiologic agent of cattle leukemia. Ley¬kozu bovine virus structurally and genetically related viruses funktsi¬onalno T kpitynnoho leukemia person STI _ \ / - 1 and STI _ \ / - 2 development of diseases caused by these viruses has much in common [3, 17] . That is why VL of cattle is a very convenient model for studying leukemia. An important aspect of such studies is the problem of genetic variability of HL CVA [4, 9, 11, 18]. M.ysyhei colleagues using methods lan¬tsyuhovoyi polymerase reaction (PCR) restriction fragment length polymorphism (RFLP) and sekvenu- tion compared the 42-zraz¬kiv provirus overhead cattle received in Japan and Argentina, with options provirus VL from different geographic regions (France, Belgium, Italy, North America, Australia) and showed that the variability of genomic material of VL of cattle reaches 3.5% [8]. MR SatahdoB and colleagues conducted a comparative analysis of gene sequence epu 3 strains overhead cattle allocated in 3 different regions in Brazil, with a 7-relevant sequences strains overhead cattle from different countries and found that Brazilian isolates have a much greater rate of mutation [1]. Knowledge of the features of the structure of the HL genome of the cattle, on the one hand, and the spatio-temporal interaction of the virus with the animal's organism, on the other hand, is a prerequisite for the successful establishment of control over the spread of leukemia. The purpose of the research is to compare the efficiency of the application of molecular genetic methods and RID for the detection of HL of the cattle and the study of phylogeny-non-specific relationships between isolates of HL of cattle. Materials and methods of research. Proviral DNA was detected using overhead tion of cattle in clinical zraz¬kah performed using domestic test kits for the detection of proviral DNA overhead cattle by standard PCR "BI _ \ / - rhouihyz OMA Test" design NSC "IECVM" according to social class-producer. To prevent coagulation of blood as an anti-coagulant, a solution of 0.056 M sodium citrate, 0.166 M glucose in a ratio of 1: 5 was used. An analysis of the products of amplification was carried out by electrophoresis in a 1.5% agarose gel stained with bromide ethidium. For the construction of phylogenetic trees, the MESA program, version 4.1 was used. Research results. As a clinical material for PCR, peripheral blood of RID-positive and RID negative animals was used from farms of Kharkiv, Rivne, Poltava regions of Ukraine, Crimea. Figure 1 shows a fragment of electrophoretic analysis of amplicons formed as a result of PCR with the use of the test system "BI_ \ / - pOуигиз OMA-test". It should be noted that in rezul¬tatamy PCR analysis of clinical material obtained from two Reed-positive animals were found genetic material overhead cattle in clinical material obtained from one ROD-negative animals, on the contrary, it has been established. The identified discrepancies are related not only to the lack of specificity of RID, but also to the low sensitivity of this reaction. This is confirmed, for example, ^ I. Koiaga eh ai. [7], which detected pro-viral DNA of VL of cattle by means of PCR

Fig. 1. Elektroforehrama PCR products detektsyiyi gene fragment em overhead cattle, with the help of my test systems "VI.] / - rhouihyv ОЫА test" after dyeing 1.5% agarose gel th ethidium bromide. 1, 13 - molecular weight marker; 2-10 - clinical material from cattle; 11 - negative control; 12 - culture of cells of PC, infected with VL of cattle. The length of the received amplicon is 241 n.

Fig. 2. Dendrohrama constructed from fragments of the gene ales / proviral DNA isolates overhead cattle from different geographical regions of the world

in BI _ \ / - infected animals at 5 weeks rani¬she than antibodies overhead by RID and KE Tough and so on. [16], which, on a large sample of cattle from 363 different breeds, demonstrated that the specificity of RID compared to PCR analysis was 79.7%. The efficacy of PCR for detecting HBV infected animals (especially in the absence of anti-24 antibodies) was confirmed by M, A. ^ Iiahepa ee: ah. [5] during the control, at intervals of 6 months, the study of several herds from different provinces of Argentina by means of serological, hematologic methods and PCR. The results of our moleku¬lyarno genetic studies indicate bez¬perechnu need at least selective tes¬tuvannya RID-positive animals by PCR availability of proviral DNA overhead cattle at an early stage and gender-negative animals at the final stage of rehabilitation of various shapes hospodarstv Property of leukemia. This, we believe, will contribute to the successful implementation of the national program for the improvement of livestock farms from leukemia. Samples of the pro-viral DNA of VL of cattle were sequentially sequenced in order to determine the sequence of the ethyl activator gene and to conduct a phylogenetic analysis. To study the phylogenetic vzayemovidnosyn between organisms and clarifying the time of their divergence using methods for determining evolutionary distances based on nucleotide sequences po¬rivnyanni homolo¬hichnyh genes or amino acid sequences of the corresponding proteins. In order to establish phylogenetic zv'yaz¬kiv between overhead cattle isolates circulating in Ukraine and their phylogenetic relationships with tso¬ho isolates of the virus isolated in other regions of the world (Europe, Asia, North and South America), was constructed phylogenetic Deleu on the basis of sequenced sequences of the env gene of the pro-viral DNA of VL of cattle (Fig. 2). The constructed dendrogram illustrates the proximity of the isolates of HL of cattle circulating in Ukraine to the isolates of the European and Asian subgroups (1, Fig. 2). This isolates overhead cattle proviral DNA which was ekstra¬hovano from peripheral blood of animals hospodarstv Rivne, Poltava and Kharkiv regions, closer to the European sub¬hrupy (isolate Austria), and leukemia virus that the farms tsyr¬kulyuye The Autonomous Republic of Crimea, is close to the VL of the cattle of the Asian subgroup (isolates Zanjan, Tehran). Isolates of HL of cattle, isolated in the countries of the American continent, form a separate, American, subgroup (see Figure 2).

Conclusions

The higher specificity of the PPI-analysis compared to the RHD for the detection of HL of the cattle has been confirmed, and it has been demonstrated that the results of phylogenetic research can be used to identify and study possible subgroups (or genotypes), to create a basis for the search for genes that determine the highest Biological activity of viruses.

Bibliography

1. Camargos M.F. Partial sequencing of env gene from bovine leukemia virus from Brazilian samples and phylogenetic analysis / M.F. Camargos, D. Stancek, M.A. Rocha // J Vet Med. B. infect Dis Vet Public Health. - 2002. - V. 49, No. 7. - P. 325-331.
2. Dariix J. High spontaneous mutation rate of Rous sarcoma virus demonstrated by direct sequencing of the RNA genome / J. Dariix, P.F. Spahrf / Nucleic Acids Res. - 1983. -V. 11. - P. 5953-5967.
3. Dube S. Degenerate and Specific PCR Assays for the detection of bovine leukemia virus and primate T cell leukemia / lymphoma virus. Pol DNA and RNA: phylogenetic comparisons of amplified sequences from cattle and primates from around the world / S. Dube, S. Bachman, T. Spicer // J. Of General Virology. - 1997. - V. 78. - P. 1389-1398.
4. Hemmatzadeh F. Sequencing and phylogenetic analysis of gp51 gene in bovine leukemia virus in Iranian isolates / F. Hemmatzadeh / A / et. Res Commun - 2007. - No. 6. - P. 783-789.
5. Juiiarena M.A. Determination of Proviral Load in Bovine Leukemia Virus Infected Cattle with and without Lymphocytosis / M.A. Juiiarena, S.E. Gutierrez, C. Ceriani // Am. J. Vet Res. - 2007 -V. 68, No. 11. - P. 1220-1225.
6. Katz R. Generation of diversity in retroviruses / R. Katz, A. Skalka // Annu. Rev. Genet - 1990. - Vol. 24. - P. 409-415.
7. Kohara J. Experimental transfer of Bovine Leukemia virus in the cattle through rectal palpation / J. Kohara, S. Konna, M. Onuma // Jpn. J. Vet. Res - 2006. - V. 54, No. 1. - P. 25-30.
8. Licars M. Provirus variant of bovine leukemia virus in naturally infected cattle from Argentina and Japan / M. Licursi, Y. Inoshima. D.Wu // Vet. Microbiol - 2003. - V. 96, No. 1. - P. 17-23.
9. Mamoun R. Sequence variability of the bovine leukemia virus env gene and its relevance to the structure and antigenicity of the glycoproteins / R. Mamoun, M. Morisson, N. Rebeyrotte // J. Virol. - 1990. - V. 64. - P. 4180-4188.
10. Manini P. Exposure assessment at the workplace: implications of biological variability / P. Manini, G. De Palma, A. Mutti // Toxicol. Lett - 2007. - Vol. 168, No. 3. - P. 210-218.
11. McGirr K.M. Tax and rex Sequences of bovine leukemia virus from globally diverse isolates: rex amino acid sequence more variable than tax / K. M. McGirr, G. C. Buehring // J. Vet Med. B Infect Dis Vet Public Health. -2005 -V 52, No. 1. - P. 8-16.
12. Meyerhens A. Temporal fluctuations in HIV quasipecies in vivo are not reflected by sequential HIV isolates / A. Meyerhens, R. Cheynier, J. Albert // Cell. - 1989.-V. 58.- P. 901-910.
13. Parvin J. Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1 / J. Parvin, A. Moscona, W. Pan // J. Virol. - 1985. - V. 59. - P. 377-383.
14. Steinhauer D. Extreme geterogeneity in a population of vesicular stomatitis virus / D. Steinhauer, J. de la Torre, E. Meier // J. Virol. - 1989. - V. 63. - P. 2072-2080.
15. Steinhauer D. Rapid evolution of RNA viruses / D. Steinhauer, J.J. Holland // Annu. Rev. Microbiol - 1987, -V. 41, -P. 409-433.
16. Trono K.G. Seroprevalence of bovine leukemia virus in dairy cattle in Argentina: comparison of sensitivity and specificity of different detection methods / K.G. Tron // Vet. Microbiol - 2001. - V. 83, No. 3. - P. 235-248.
17. Willems L. Bovine Leukemia Virus, an animal model for the study of intrastrain variability / L. Willems, E. Thienpont, P. Kerkhops // J. Of virology - 1993. - V. 67, № 2 - P. 1086-1089.
18. Zhao X. Sequence polymorphisms in the long terminal repeat of Bovine Leukemia virus: Evidence for the selection of pressures in regulatory sequences / X. Zhao, C. Jimenez, H. Sentsi // Virus Res. - 2007. - V. 124, No. 1-2. - P. 113-124.

Posted 03.03.2015.