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Immunoreactivity of VLCRS-infected cows

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Abstract. The article describes the results of the ELISA and PCR-RDF blood serum analysis of the env BLV gene in order to study the features of immunoreactivity of infected cattle leukemia virus. The ELISA results showed that 63.8% of the studied blood serum samples from HCV infected cows reacted positively to mycobacterial antigens. The results of a comparative study of PCR-RDF data from DNA analysis of isolates and ELISA indicated that antibodies against antigens of the 4 genotype of VLCRS are more likely to cross-react with antigens of M.Avium, and antibodies against 7 genotype – with antigens of M.Bovis. The presented research results allow us to conclude both about the possible determinant commonality of VLCRS and mycobacteria antigens, and about changes in the immunoreactivity of cows infected with VLCRS. The reason for this heterogeneity and cross-reactivity is, of course, the constant change in titers and spectra of antibodies to HCV in infected animals with the development of the infectious process. And also, the fact that infection of an organism with HCV leads to disruption of the host immune system regulation processes at both the cellular and humoral levels.

1 Introduction

The associative course of leukemic infection with viral and bacterial infections, including tuberculosis in cattle, is a serious problem during diagnostic measures. Questions about the possibility of reactions to tuberculin in cattle infected with VLCRS and about a positive reaction in RID in cows infected with mycobacteria remain open in the scientific literature [1].

Despite the fact that the most modern highly effective techniques and technologies are offered to combat these infections, the pathogenesis and genetic determinants of pathogens are well studied, this problem remains unresolved and there are statements of a diverse nature. While some identify a direct relationship between the level of infection with HCV and the response of animals to tuberculin, others argue that cattle diagnosed with leukemia according to hematological parameters, as well as infected with HCV, usually do not respond to tuberculin [2, 3].

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Cow leukemia, also known as infectious brucellosis or leukemic viral infectious leukemia (Bovine Leukemia Virus, BLV), is a serious problem in animal husbandry, having a significant impact on the productivity and health of the herd. This viral disease caused by the leukemic cow virus is transmitted through blood, milk and other biological fluids, which makes its spread especially relevant in conditions of intensive care [4].

The main problem associated with leukemia is its asymptomatic course in the early stages. Animals can be carriers of the virus for a long time without showing clinical signs. This makes it difficult to diagnose and control the disease, which in turn leads to its spread in the herd. Leukemia can cause various pathological changes in the cow's body, including the development of tumors, anemia and immunodeficiency, which significantly reduces productivity and increases the risk of other diseases [5].

One of the main methods of diagnosing leukemia is serological testing, which allows detecting antibodies to the virus in the blood of animals. Early detection of infected individuals and their isolation are key measures to control the spread of infection. It is important to note that vaccination against leukemia has not been developed to date, which underscores the need for an integrated approach to disease prevention and control [6].

To reduce the risk of leukemia infection in the herd, strict biological measures must be implemented, including regular checks for the presence of the virus, isolation of new animals before entering the herd, and monitoring the use of common equipment. Attention should also be paid to the conditions of detention and feeding, since stress factors can contribute to the activation of the virus in carriers [7].

An important aspect of the fight against leukemia is educational work among farmers and veterinarians. Training in the diagnosis, prevention and management of the disease can significantly increase awareness and help in making effective decisions. In addition, it is necessary to develop scientific research in the field of leukemia in order to better understand the mechanisms of its pathogenesis and develop new methods of diagnosis and treatment [8].

Scott D. Fitzgerald et al. (2009) prove that coinfections of cattle with both BLV and M. bovis can occur only in geographical areas where both diseases are endemic, and the claim that leukemia as a background infection increases the predisposition of animals to infection with M.bovis cannot be definitively confirmed [9].

Claudia M. Lutzelschwab et al. (2016), having studied the effect of Mycobacterium bovis infection on some immunological parameters of the host in BLV infection, concluded that an associative course of leukemia infection and tuberculosis in cattle carrying a genetic marker of resistance against BLV is impossible [10].

The reasons for such statements are undoubtedly the complex antigenic structure of mycobacteria, which have many antigens common to all groups of mycobacteria, as well as heterogeneity, cross-reactivity, constant changes in antibody titers and spectra, and the ratio between free and bound antibodies to HCV in infected animals with the development of the infectious process [11, 12].

The purpose of this work was to study the immunoreactivity of VLCRS infected cows based on PCR-RDRF analysis of the env BLV gene.

2 Materials and methods

Blood serum samples from cattle infected with leukemia virus were examined. Enzyme immunoassay was performed according to the standard method in a non-competitive version of the verdophase. The lipopolysaccharide antigens M. bovis, M. avium, and M. sctochromogenes obtained by extraction with organic solvents were used to detect anti-tuberculosis antibodies. Antigens obtained in this way are widely used in laboratory practice.

Enzyme immunoassay for the detection of anti-leukemia antibodies was performed using a "Kit for the detection of antibodies to bovine leukemia virus (BLV)" manufactured by NPO Narvak.

The PCR was performed using nest primers according to the procedure described by Fechner Hetal. DNA was isolated from the sediment after centrifugation using the Ribot-prep kit manufactured by Interlabservice according to the manufacturer's instructions.

PCR testing for leukemia was performed according to the following protocol. The preparation of PCR mixtures (per 1 sample) was carried out according to the following recipe: 2 µl of 10-fold PCR buffer, 2 µl of 2.5 mM nucleoside triphosphate solution, 2 µl of 25 mM magnesium chloride solution, 1 µl of 10 pM each direct (5’-ggcaccgggtctcgcaagtatg-3'), reverse (5'-cggttaggctggtcatgtggcc-3') primers and a TaqMan type oligonucleotide probe (ROX-aaactacgacttgcaatcttacaggccgac-RTQ2), 1 µl Taq polymerase (5 units/ml), 10 µl of the DNA sample under study.

To identify M. bovis by PCR-RV, primers IS1081_Forward – ggctgctcgacgttcatc, IS1081_Reverse - cgctgattggaccgctcat and the oligonucleotide probe IS1081_Probe – FAM-ctgaagccgacgccctgtgc-MGBNFQ) developed by Duffy SC et al [6] were used.

The following restrictases were used for restriction analysis: PvuII, SspI, BstDEI, BamHI. The results were detected by horizontal electrophoresis in 2.5% agarose gel.

3 Results and discussion

In total, more than 500 samples of cow blood serum were examined by ELISA, which reacted positively in RID. 95.6% (478) of the samples studied showed positive results. All ELISA positive samples for bovine leukemia were tested for the detection of mycobacterial antibodies using the antigens M.bovis, M. avium and M. sctochromogenes. As a result, 63.8% (212) of the samples revealed specific antibodies to mycobacterial antigens. Including, in 29 samples — to all three types of mycobacterial antigens, in 123 - to two types of antigens and in 60 – to only one type of antigen. Of the last group, 37 samples reacted only to M.Avium, 17 to M.Bovis, and 6 to M.Scot.

All 212 samples were further examined by PCR for leukemia and tuberculosis. Previously, blood serum samples were deposited at 13,000 rpm for 10 minutes. DNA was isolated from the sediment after centrifugation using the DNA Sorb B kit (Interlabservice) according to the manufacturer's instructions.

PCR tests for leukemia and tuberculosis were performed according to the above protocols. It should be noted that all samples were obtained from tuberculosis-safe farms and the results of PCR for the detection of mycobacterium genetic material were negative in all samples. PCR results on VLCRS were negative in three samples.

To further study the immunoreactivity, identify possible pathogenetic relationships, and perform PCR-RDF analysis of the envBLV gene, 15 samples were randomly selected, with the first 5 reacting to M.Avium, the next 5 reacting to M.Bovis and M.Scot.

The results were detected by horizontal electrophoresis in 2.5% agarose gel (Figures 1 and 2). This method allows you to visualize the reaction results and assess the presence or absence of target DNA molecules in the samples. Agarose gel electrophoresis is a key step in molecular biology for analyzing the size and number of DNA molecules.

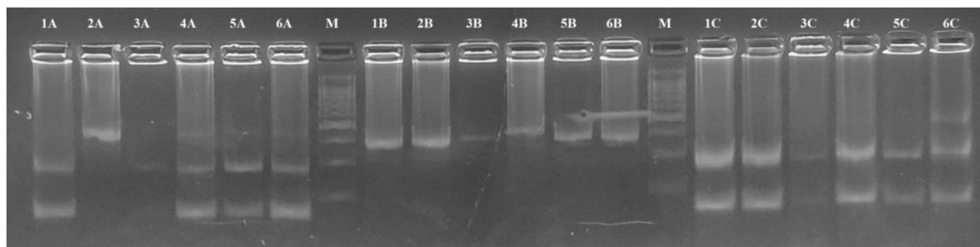

Fig. 1. Results of PCR-RDF of the env BLV gene.

Designations: M is a marker of the molecular weight of DNA from 100 to 1000 pairs of nucleotides (bp); 1A – 6A are DNA samples treated with PvuII restrictase; 1B – 6B are DNA samples treated with SspI restrictase; 1C – 6C are DNA samples treated with BstDEI restrictase.

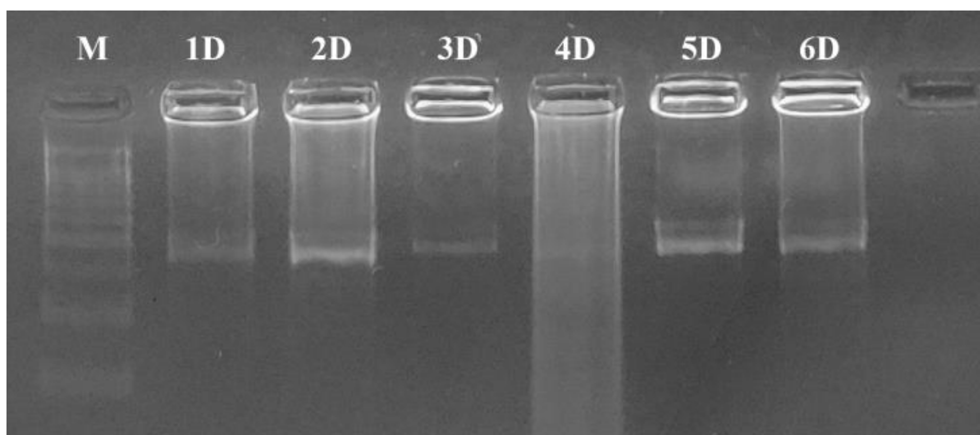

Fig. 2. Results of PCR-RDF of the env BLV gene.

Designations: M is a marker of the molecular weight of DNA from 100 to 1000 pairs of nucleotides (bp); 1D – 6D are DNA samples treated with BamHI restrictase. PCR-RDF analysis of DNA from isolates 1-5 using PvuI restrictase identified two patterns of 164 and 280 nm in size, which, according to the phylogenetic classification, the presented isolates may be related to the fourth genotype. And in the DNA of isolates 6-15, a single pattern of 444 bp was found, therefore, this isolate is related to the seventh or eighth genotype.

When using SspI restrictase, a single pattern ranging from 395 to 444 bp is found in the DNA of all isolates, which makes it impossible to accurately determine whether they belong to a particular genotype. Additional research needs to be done. The use of BstDEI restrictase, which is an isoisomer of DdeI endonuclease, allowed us to determine the presence of two patterns of 168 and 276 bp in all the isolates studied, which most likely refers them to the fourth or seventh or eighth genotypes. Using BamHI restrictase, it was found that only a 444 bp pattern was detected in the first 5 analyzed DNA samples, therefore, these isolates probably belong to the fourth genotype.

Thus, the restriction analysis allows us to conclude that isolates No. 1-5 belong to the fourth genotype, and 6-10 to the seventh. However, it is desirable to confirm the results obtained by additional studies, for example, DNA sequencing.

Genetic analysis of cows for resistance to the leukemia Virus (Bovine Leukemia Virus, BLV) is becoming increasingly important in modern animal husbandry, especially in the context of the fight against infectious diseases that have a negative impact on the

productivity and health of the herd. Cow leukemia is a viral disease that is transmitted through biological fluids and can occur in an asymptomatic form, making it difficult to diagnose and control.

Current research shows that the genetic predisposition of animals to BLV resistance can vary significantly depending on their genetic background. Some cow lines show a higher level of resistance to the virus, which is due to the presence of certain genetic markers. These markers may be associated with the immune response, as well as with other physiological mechanisms that protect the body from infectious agents.

The use of genetic analysis makes it possible to identify cows with high resistance to the leukemia virus in the early stages of their life. This, in turn, enables breeders to carry out targeted work to improve the health of the herd. Selection based on BLV resistance can lead to the creation of more viable and productive lines capable of withstanding viral loads and minimizing the risk of infection spread.

In addition, genetic analysis can help in developing effective herd management strategies. For example, the introduction of cows with high rates of leukemia resistance into breeding can reduce the incidence and, as a result, the cost of treatment and prevention. It also helps to increase the overall productivity and economic efficiency of animal husbandry.

Scientific research in the field of genetics and molecular biology opens up new horizons for understanding the mechanisms of resistance to BLV. Genomic association studies (GWAS) can identify specific alleles and genetic variations that are responsible for the body's defense responses. These data can be used to develop molecular markers, which will speed up the breeding process and increase its accuracy.

4 Conclusion

The presented research results allow us to conclude both about the possible determinant commonality of VLCRS and mycobacteria antigens, and about changes in the immunoreactivity of cows infected with VLCRS. The results of a comparative study of PCR-RDF analysis and ELISA data from DNA isolates indicate that antibodies against antigens of the VLCRS genotype 4 are more likely to cross-react with M.Avium antigens, and antibodies against genotype 7 with M.Bovis antigens. However, it should be remembered that more than 100 of the blood serum samples studied showed positive results in ELISA simultaneously against several types of mycobacterial antigens. The reason for this heterogeneity and cross-reactivity is, of course, the constant change in titers and spectra of antibodies to HCV in infected animals with the development of the infectious process. Undoubtedly, it should also be remembered that infection of an organism with HCV leads to disruption of the host's immune system regulation processes both at the cellular level (damage to B-lymphocytes, parts of T-lymphocytes, monocytes) and at the humoral level (IgM, IgA deficiency).

Of course, more in-depth research is required for further claims, however, it is indisputable that when carrying out diagnostic measures for leukemia and tuberculosis in cattle, the epizootic situation for these infections must be taken into account.

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