Розділ 2. Ветеринарна вірусологія

CURRENT ISSUES OF INFECTION WITH RETROVIRUSES OF CATTLE

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Retroviruses are recognized as important pathogens in humans and many animal species. Under natural conditions cattle can be infected by three retroviruses: bovine leukemia virus (BLV), bovine immunodeficiency virus (BIV) and bovine foamy virus (BFV). In general, infections with these pathogens are a major concern to the livestock industry due to:

1) the potential for disease, impairment of the immune system and economic and trade effects.

2) even if no risk of transmission to humans is evident, the retroviral nature of these viruses may evoke concern to public health if they are present in the human food chain. Also for several biotechnological processes, based on bovine tissues as source for biomaterials, it might become necessary to assess that animals are free from retroviruses.

3) one of specific patterns of retroviral infection is capacity of these viruses to cross species barrier leading sometimes to the emergence of new diseases in the new host animal species.

4) retroviruses harbour a great genetic variability, therefore the isolation and characterization of a new field isolates and the data on genetic variability are essential for investigation of the prevalence and to assess the likely impact of these forms on diagnosis.

1. Prevalence retroviral infections in cattle. Serological surveys of BLV infection revealed that the infection is widely disseminated throughout the world with high prevalence in North America compared to the Europe. A high geographic variability in rates of infection was observed and in general with higher rates of BLV prevalence in dairy cattle compared to beef cattle. Most of western European countries have already eradicated BLV infection however, it does exist as a enzootic form in some central and eastern European countries, especially in the Baltic area.

Serological evidence for BIV infection has been reported in many countries. In Europe, several countries have carried out testing for antibodies to BIV (the Netherlands, Germany, France, Poland and Italy). Even if there is some variations in the results depending of the methods used, seroprevalence in cattle was 0.5 to 10 %. However, the weak positive reactions frequently obtained with European serum samples may be due to the bias that all tests currently used in Europe are based on the antigen available from the original american R29 BIV isolate. Some emerging data suggest that variants of BIV, that are antigenically distinct from R29 and demonstrated immunologic cross-reactivity with the major capsid protein are present in Europe.

Foamy viruses are ubiquitous retroviruses of non-human primates, cats, cows and horses which prevalence is generally high and varies widely depending on the species. In the field study when 1408 serum samples from cattle from 45 dairy herds were tested BFV seroprevalnce was found in 30 % samples, using ELISA. BFV DNA was also found in 40 milk samples out of 80 tested by PCR. Quite different results showing 7 % seropositivity were noted when cattle from Germany was tested. The source of this discrepancy is unknown.

2. Transmission. In BLV and BIV-infected animals the virus replication is rarely concomitant with cell-free virus in vivo, so natural trasmission is by transfer of infected lymphocytes in blood or perhaps secretions. Horizontal transmission is the most important form of BLV propagation both within a herd or between herds. Experimentally, BIV like to BLV can be transmitted by intravenous inoculation of infectious material. Field observation, in the US and Japan, evidenced the high frequency of co-infection by BLV and BIV, which suggest a similar mechanism of transmission. In addition, the prevalence of both BIV and BLV infection is higher in dairy than in beef cattle. This may be partialy due to standard practices in the dairy industry. Newborn calves are frequently fed with pooled colostrum and milk, that may favor the viral spread. Because BIV DNA was detected in the seminal leucocytes of 82 % randomly selected semen samples from a continental US stud semen repository, thus may also contribute to the distribution of the virus to dairy herds. The route of transmission of BFV in field animals is unknown.

3. Pathogenicity/pathology. BLV induces a persistent and chronic infection that affects essentially the B lymphocyte population. The expansion of the lymphocyte population results from the polyclonal proliferation of B lymphocytes, mainly CD5+ cells. Although B cells are the natural target for BLV, the virus was found in CD8+ T lymphocytes, monocytes/macrophages and granulocytes.

Unlike BLV it is still unknown whether BIV induces a specific syndrome or whether it renders animals more susceptible to other infections. It has been shown that BIV, unlike classical immunodeficiency viruses such as HIV and FIV, does not cause remarkable depletion of CD4+ cells and induces a transient B-cell proliferation with an expansion of the CD5+ B cell population, as was reported in cows infected with BLV. BIV is not associated with specific disease in cattle however, different data argue for the pathogenic role of BIV in bovine. Observation on calves experimentally inoculated with tissue culture-adopted or moleculary cloned BIV R29 evidenced a transient lymphocytosis and a persistent lymphoadenopathy. In a long-term survey of a Louisiana dairy herd with a 80% frequency of BIV infection, the animals had various signs of disease, including skin lesion, lymphoadenopathy and lymphosarcoma. Many also exhibited neuronal lesions on histopathologic examination. The various disease observed resulted in high mortality and cull rates. Some observation confirmed an association between BIV seropositivity and the bovine paraplegic syndrome. Two new BIV isolates have been recovered in Florida from the cattle with clinical manifestation of „poor doers". When examined in experimental inoculation, some clinical signs have been noted including a transient mononuclear cell increase and lymphoproliferative response composed of a mild folliculat hyperplasia.

One report suggests that co-infection with BIV could enhance the pathogenicity of BLV in experimentally inoculated sheep and may accelerate BLV-induced leukosis. Our study confirmed that antibody response to BLV and BLV-proviral load in sheep infected with BLV/BIV were higher than those from sheep infected with BLV alone. Under natural condition co-infected cows showed lymphocytosis, weight lose, wasting and enlarged lymph nodes

Infections with BFV have not been associated to specific disease or any clinical signs. But even though BFV may not be agents of bovine disease directly, it may be significant cofactor for other viral diseases. Additionally, it was demonstrated that transgenic mice expressing human foamy virus genes were shown to develop progressive encephalopathy and myopathic disorders, suggesting a pathogenic potential of these viruses.

Although BFV is highly prevalent world-wide, valid experimental data of BFV biology in the respective host as well as its interaction with other bovine retroviruses are very limited. It has been suggested that foamy viruses, which are leucotropic, may contribute to disease caused by retroviruses of men, like HIV. There are only some data suggesting an association of BFV and BLV infections, a finding that is also supported by own studies demonstrating a higher rate of BFV infections in BLV positive herds compared to BLV free herds. Beside this, our assumption on possible interaction between BFV and BLV is supported by out recent studies on the distribution of BFV infectivity and DNA in naturally and experimentally infected cattle: lymphoid organs including peripheral blood leukocytes are consistently positive for infectious virus and BFV DNA. It can be also postulated that BLV and BFV share a tropism to several subpopulations of leucocytes, especially monocytes/macrophages, CD8+ T lymphocytes and B cells, what most probably results in *in vivo* interaction between both viruses at the molecular level. Since both viruses were found in seropositive individuals, it has been suggested that they play a role as cofactors in the virus activation. Both BFV and BLV contain accessory genes encoding the BFV *Tas* and BLV *Tax* proteins which act as *trans* activators of gene expression. Additionally, Tax not only activates BLV genes but also induces cellular gene expression thus contributing to oncogenesis Thus, we postulate that in dually infected animals both proteins can cross-transactivate BFV and BLV what might account for the increased virus replication and enhanced pathogenicity.

4. Capacity to cross species barrier and to emergence of new diseases in the new host. The natural host range of BLV and BIV is thought to be restricted to cattle, however, goats and sheep are susceptible to experimental infections. Recently the presence of BIV specific antibodies in naturally infected sheep was found, therefore, the risk that BIV can cause cross species infection in ruminants resulting in the emergence of new pathogens is to be seriously considered.

In contrast to this, foamy viruses are able to induce interspecies transmission including zoonotic transmission, as was demonstrated for people in Africa and Asia, exposed to contact with monkeys infected with simian foamy virus (SFV). In the previous study we demonstrated that all five cell lines of human origin (293T, HeLa, SiHa, CasKi, HCT116) can be easy infected *in vitro* with BFV through cell-to-cell contact, as was showed by continuous detection of viral DNA and Gag protein. To investigate the exposure of humans to BFV we collected the following specimens: 79 plasma and PBL samples from vets and dairy cattle caretakers being in close contact with seropositive animals, 176 plasma samples from farmers and people living in rural areas having permanent contact with cows and 102 plasma samples from control population, with no obvious contact with cows. All these samples were tested by BFV ELISA. Specific antibodies were found in 11 %, 6.3 % and 1.9 % in respective risk groups. Additionally, when 260 plasma samples from wild-living ruminants were tested specific antibodies were found in 4.6 %. We were also able to successfully detect the foamy virus DNA in peripheral blood leucocytes, using newly developed nested-PCR method. All together, these results showed that humans with high risk of exposure to BFV-infected cows are carriers of BFV specific antibodies, probably as a result of zoonotic transmission of BFV. Our attempt to identify a new reservoirs of foamy viruses clearly showed that wild-living ruminants can be considered as hosts for a new foamy virus, genetically slightly distant to already known foamy viruses. These data can open a possibility to explore FV infection events in other species in terms of their prevalence, interspecies transmission and possible links to other viral infections.

5. Genetic variability. BLV sequences are highly conserved among isolates from different parts of the world. Proviral sequences of three isolates from Belgium, Japan and Australia differ by 3 %, mainly in point mutations scattered throughout the entire genome. These mutations confer some differences in restriction enzyme maps and are good markers to differentiate BLV variants. Envelope genes of a larger number of isolates show little variation; substitutions present at 6 % of the nucleotides alter 4.3 % of the predicted amino acids. This minimal genetic diversity can be related to low mutation rate of BLV. The *in vivo* mutation rate was 0.009 and 0.034 % nucleotide changes/year in the *env* gene and in the LTR, respectively. Nevertheless, some data demonstrated that small nucleotide changes can lead to dramatic consequences for the biological properties of BLV variants and influence BLV antigenicity. Different variants of BLV can lack one or even two of the gp51 epitopes. Moreover, nucleotide deletion in the *prt* frame of the Japanese BLV isolate may cause the inability of this variant to express gag and env proteins and the mutation in the BLV tax protein can abrogate the LTR-directed transactivating activity.

In contrast to BLV, BIV is characterized by high genetic diversity. Similar to other lentiviruses BIV appears to mutate very rapidly. Two infectious molecular clones derived from R29 exhibit a genomic variability of 2 % with 75 % of the substitution within surface envelope (SU) gene. Sequence analysis of the env gene of nine different isolates from cattle in the US showed that the size variation between isolates can be as large as 200 bp, mostly occurring in the second hypervariable (V2) gene region of the SU gene. Analogically, pol gene segment of Japanese BIV isolate showed 99 % homology to that of R29. Jembrana Disease Virus (JDV) is considered as a genetic variant of BIV. This virus is associated with a specific and unique lethal disease syndrome in Bali cattle. Despite the significant genomic differences, both BIV and JDV show extensive regions with a high degree of nucleotide and amino acid sequence identity that would be associated with strains of the same virus.

6. Diagnosis of retroviral infection. The diagnosis of bovine retrovirus infection is based mainly on serum antibody determination of virus-exposed animals. Recently recombinant GAG protein, expressed by E.coli were developed and used as antigen in ELISA tests. Although serological tests can detect virus exposure, they may lack sensitivity or specificity, particularly in light of concomitant infection with these retroviruses. Because of its higher sensitivity and versatility, the detection of proviaral DNA or viral RNA by real-time polymerase chain reaction (RT-PCR) has become gold standard for a number of retroviral infection. This technique allows higher throughputs, decreasing turnaround times and quantitation of the viruses. Thus the development and practical application of RT-PCR to identify BLV, BIV and BFV offers a number of significant advantages over conventional PCR and leads itself well to implementation within a routine viral diagnostics

СУЧАСНІ ПРОБЛЕМИ ІНФІКУВАННЯ РЕТРОВІРУСАМИ ВЕЛИКОЇ РОГАТОЇ ХУДОБИ

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У статті розглянуті найбільш актуальні проблеми ретровірусних інфекцій великої рогатої худоби. Ретровіруси є важливими патогенами людини та багатьох видів тварин. В сучасних умовах велика рогата худоба може бути інфікована трьома ретровірусами: вірусом лейкозу ВРХ, вірусом імунодефіциту ВРХ та пінящим вірусом ВРХ. Інфекції, що спричинені цими патогенами є найбільш важливою проблемою скотарства.