SIGNIFICANCE OF LATENT BOVINE INFECTION DUE TO IBR VIRUS AND ITS REACTIVATION BY CORTICOSTEROIDS

S. Lazic, T. Petrovic, Diana Lupulovic, M. Jovicin

Abstract: Mechanism of origin and maintenance of latent bovine infection by IBR virus have not been discovered, but the consequences of this disease are very well known. It is known that latently infected animals are seropositive on bovine herpesvirus (BHV; IBR), that they occasionally shed the virus, don't show signs of the disease, but due to stress, infection may have all the characteristics of an acute infection. It is also known that production of latently infected animals is reduced. It has been discovered that latently infected cows have milk yield reduced for 0.94 kg daily, conception is lower and multiple insemination is needed.

Five seropositive-latent cows, infected by bovine herpes virus-1, were 5 days treated by corticosteroids with an attempt to reactivate the infection. After sacrificing of 4 cows, an isolation of IBR virus was performed and determined its distribution on organs.

Key words: BHV-1, corticosteroids, latent infection, pathogenesis, reaction on infection.

Introduction

Infection by bovine herpesvirus-1 (BHV-1) or IBR virus is widely spread throughout the world and especially in the countries with developed livestock production and intensive cattle breeding in big agglomerations. Diseases generated by the infection with this virus are various. They are influenced by the age of animal, way of keeping cattle and by causative agent virulence. However, the most often disease caused by this virus is infective bovine rhinotracheitis (IBR) in younger cattle, while in older categories it is infective pustular vulvovaginitis (IPV). IBR and IPV are often used as synonyms for the infection by bovine herpesvirus-1 and not only they define pathological process but indicate the illness of known etiology with all clinical manifestations and consequences. Mentioned diseases are mostly of a systematic character, meaning that more organs are seized by the pathological process, but it is not rare that infection by bovine herpesvirus-1 is manifested by illness of only one organ, such as endometritis, oophoritis, ovarian cysts in females and by orchitis, balanopsthitis in males, often resulting in infertility.

Clinical manifestation of keratoconjunctivitis and meningitis in calves or mastitis in cows is possible when there is infection by bovine herpesvirus-1, either in general or local manifestation of illness. Besides mentioned clinical manifestations, the consequence of infection can also be the occurrence of latent infection, when clinical

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The origin and development of infection of cells of host animal by bovine herpesvirus-1 is widely known, while establishing and managing of latent infection by this virus still represents insufficiently studied problem. Virus through its surface glycoproteins gP and gC is being adsorbed to receptors (heparan sulfate-Glycosaminoglycan) on the cell membrane, and afterwards through glycoproteins gD, gB i gH penetrates into receptive host cell. In the cytoplasm of infected cell there is a process of disintegration of nucleocapsid of virus and penetration of its DNA into the cell nucleus where the virus replication is underway. It is a known fact that herpesviruses, therefore also BHV-1, in the cell, inhibit the process of translation of cell mRNA. Ribosomes become liberated, they do not synthesize cell proteins but join in the synthesis of virus proteins. In that way the virus secure undisturbed production of new virions. After multiplication of virus DNA and synthesis of capsomere they start to unite with one another and form nucleocapsides which by passing through nucleus membrane obtain cover, and afterwards as a ripen virions they leave the cell by exocytosis. The cell in which the replication of virus has been performed most often ends in deterioration.

Besides acute infection, when infected cell ends in lysis, BHV-1 causes also latent infection, but without deterioration of infected cell. For a long time we believed that BHV-1 causes only latent infection of sensory neurons. However, by the research of Winkler et al. (2000), the presence of virus DNA was observed also in tonsils lymph follicles of latently infected calves. The mechanisms of creation and maintenance of latent infection by herpesviruses in animals have been unknown for a long time but due to recent research we learned that in latently infected cells by bovine herpesvirus-1 the programmed death of cells - apoptosis has been prevented (Asanin Ružica et al, 2002). In the course of latent infection there is no exposition of antigen virus on the surface of infected cells, therefore they are unrecognizable for the mechanism of the immune system of the organism. Virus genome is present in the nucleus of infected cell in the form of extrachromosomal material, of circular shape, what is a reason for the limited expression of virus genes. The transcription of only one gene of virus responsible for blocking the mechanisms of apoptosis of infected cells has been performed. The gene of virus responsible for latent infection (LR gene-latency related) is coding LR protein. This protein with cell cyclin-dependant kinase 2 inhibits the productive infection and prevent the programmed death of cells. Under the influence of stress or treatment of latently infected animals by corticosteroids there occurs the fall in the animal immune status, different processing of mRNA LR gene as well as the activation of other virus genes leading to the production of virus proteins and new virions. Therefore, by the reactivation of virus genome the lytic cycle in cells is established again together with its secretion from the organism. Latently infected animal secrete virus periodically, as well as the clinically diseased one, having from 1000 to 100.000 virus particles in one millilitre of exudates (Siebert S. et al., 1995). The reactivation of latent infection due to the stress (transport, poor zoo-hygienic conditions of keeping, inadequate nutrition and so on) or the treatment of animals by corticosteroids leads to "remia and distribution of viruses into the cells of parenchimatosal organs such as the organs of respiratory tract, urogenital tract and other organs. However, in the cases of cell infection by apathogenic and vaccine strains of viruses the certain "tube structures" have been discovered which probably have some role in the process of intracellular transport of some viral antigens (Tyshehenko I.P. 1991).
Infection by bovine herpesvirus-1 from the aspect of medicine and economy represents a special problem, and the damages ensued are substantial and various. It was established that latently infected cow in the course of the first 10 weeks of lactation has a reduced milk yield by 0.92 kg \(0.92\) (Straub O.C. 2001). Also the service period is significantly prolonged as well as the calving interval, and because of that about 150 DM is lost yearly per cow (Krage Von E. et al, 1989). Besides these and many other direct losses, latent infection by bovine herpesvirus-1 brings also some other indirect losses which often cannot be shown by numerical values. The ban regarding the transfer of breeding cattle, sperm and embryos from infected breeding stocks, and even regions are the examples of indirect losses. These bans can bring even greater losses than the occurrence of the epidemic by IBR in some regions. The analysts from many countries have established that infection by bovine herpesvirus-1 brings great damages which are measured by huge money losses.

**Material and method**

**Trial and treatment of animals**

In the breeding stock of race Black Holstein it was observed that 72% of animals is infected by bovine herpesvirus-1. The group of 5 cows, inseminated 5 or more times with the calving interval in all cows being more than 150 days was separated. In the observed period the pregnancy was detected in neither cow. All 5 cows were seropositive to BHV-1. Antibody titre, established by the method of microserum neutralization ranged from 1:32 to 1:128. Examined cows did not show clinical signs of infection, and due to problems in conception they were meant for culling. The cows received daily in the period of 5 days Hostacorin H ("Hoechst-Roussel", Germany; 1 ml contains 10 mg prednisolone acetate) in the dose of 10 ml. 10 days after the last application of this drug 4 cows were sent to slaughter, because one cow was found pregnant before sending to slaughter. The cows were in the course of treatment and after the treatment by Hostakortin H, and before being sent to slaughter clinically monitored every day.

**Inspection material for virus isolation**

During the treatment of cows by Hostacorin H on the third and fifth day and two days after the treatment the nasal swabs were taken. On the slaughter line from all cows were taken sections of parenchymatous organs (lungs, spleen, kidney and liver), ovaries, small sections of endometrium, nasal mucous membrane and parts of mandibular and mediastinal lymph node. The material for inspection was transported to laboratory in the medium for multiplying cell cultures.

**Virus isolation**

The isolation of virus was performed on the culture MDBK (Madin Darbi Bovine Kidney) cells with previous treatment of the material for inspection to virusological examination. Treated material was cultivated by three-day passages of 7 days each. For the confirmation of virus isolation, the cells of third passage were examined also by the method of direct immunofluorescence, that is by the conjugate of polyclonal antibodies for BHV-1 manufactured by the American Bio Research (USA).
Results and discussion

Clinical manifestation

Out of 5 cows treated by Hostakortin $H$ in the cow tattoo no. 3267, on the third day of treatment seromucous nasal secretion and hyperaemia of the mucous membrane of the nasal cavity were observed. Other signs of disturbed health state were not observed. The presence of nasal secretion as well as the hyperaemia of the mucous membrane of the nasal cavity in this cow was observed in next two days. The nasal secretion was best observed in the morning before food intake. In other cows no signs of disturbed health condition were observed. All cows consumed predicted daily quantity of food, and the milk production was not reduced in relation to period before the treatment by Hostakortin $H$.

The isolation of BHV-1:

From nasal swabs the BHV-1 was isolated only in the swabs of cow tattoo no. 3267 taken on the third and fifth day of the treatment by Hostacortin $H$, while in other nasal swabs the virus was not isolated. The isolation of virus from the parts taken from parenchymatous organs, ovaries, lymph nodes and sections of nasal mucous membrane and endometrium is shown in the Table 1.

<table>
<thead>
<tr>
<th>Ispitujući material</th>
<th>Material for inspection</th>
<th>Tetovir broj krava / Tattoo no. of cows</th>
<th>Ukupno / Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slezina / Spleen</td>
<td>+</td>
<td>3267, 323, M01, 0468</td>
<td>1</td>
</tr>
<tr>
<td>Bubreg / Kidney</td>
<td>+</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Jeta / Liver</td>
<td>+</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Jajnici / Ovaries</td>
<td>+</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mandibulami limfni cvor</td>
<td>+</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Mandibular lymph node</td>
<td>+</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Medijastinal limfni cvor</td>
<td>+</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mediastinal lymph node</td>
<td>-</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Sluzokoža nosa Nasal mucous membrane</td>
<td>+</td>
<td>12/36</td>
<td></td>
</tr>
<tr>
<td>Endometrijum / Endometrium</td>
<td>-</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

According to the results shown in Table 1, it can be seen that BHV-1 was isolated in all investigated material of the cow tattoo no. 3267. In this cow, as already said, the virus was isolated both from nasal swabs taken on the third and fifth day of the treatment by Hostacortin $H$. Therefore, the results obtained show that in this cow the viremia was observed as well as distribution of virus in almost all organs. The works of numerous researchers indicate that latent infection by bovine herpesvirus-1 can reactivate under the stress influence or by treatment by corticosteroids. Miler J.H and Van der Maaten M.J (1987) reisolated BHV-1 on the fifth day of the treatment by dexamethasone, 3-4 months.
after conducted artificial infection. The virus was reisolated from nasal and vaginal secretions in 5 from 13 observed heifers and in one heifer from ovaries, ovarian infundibulum and uterus. Winkler M. T. et al (2000) have by RT-PCR technique only 6 and 24 hours post-treatment by corticosteroids proved the presence of DNA of bovine herpesvirus-1 in trigeminal neurons and lymphoid follicles of tonsils, but 48 hours post-treatment the researchers did not succeed to prove the presence of virus in these tissue by the same technique. In the inspection material of the third cow, tattoo no. M01, the virus was not isolated, while in other cow, tattoo no. 323, the virus was isolated only in the mandibular lymph node, while in the material of the fourth cow, tattoo no. 0468, BHV-1 was isolated in mandibular lymph node and in the cut section of nasal mucous membrane. 

It is a known fact that the reactivation of the infection and isolation of BHV-1 in latently infected animals even besides the effect of stress is influenced by many factors. It is certain that decisive role in the process of reactivation of infection has immune state of the animals and virus virulence. Therefore, non-isolation of virus in the materials of the third cow and in majority of materials of the second and fourth cow can be explained by the mentioned facts, but also by the methodological procedure of isolation. The isolation of virus by PCR technique is much more sensitive than the technique by the cell cultures. By the multiplication of viral DNA in PCR technique even minimal quantities of virus can be detected.

By the use of the techniques of molecular biology during a few previous years the researchers have succeeded to explain partly the origin, maintenance and reactivation of latent infection in bovine herpes virus-1. However, some elements of biological cycle of this virus which can be of importance in genesis of latent infection still remain unexplained. Probably in the next period the research in that direction will be intensified, not only in order to explain and understand the factors of latency but in the first place because of preventing the infection of cattle by herpesvirus-1.
Literature