CATTLE INFECTION WITH HERPES VIRUS TYPE-1:
Etiopathogenesis, diagnostics, possibilities of control, eradication and legislative regulations

INFEKCIJA GOVEDA HERPESVIRUSOM TIP -1:
Etiopatogeneza, dijagnostika, mogucnosti suzbijanja, iskorenjivanja i zakonska regulativa

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Summary: Bovine herpesvirus type -1 (BHV-1) causes several diseases, but the most prevalent are infective bovine rhinotracheitis and infective pustular vulvovaginitis (IBR/IPV). In this paper we presented the latest discoveries about etiopathogenesis and diagnosing of BHV-1 infection based on the recent knowledge on the molecular structure and functional importance of some virus glycoproteins in the process of infection. We presented some eradication experiences in several countries as well as some legislative regulations on distinct measures for improved prevention, control and eradication of BHV-1 infection in breeding cattle herds.

Key words: BHV-1, etiopathogenesis, diagnostic, suppression, legislative regulation

Kratak sadržaj: Herpesvirus goveda tip-1 (BHV-1) izaziva vise oboljenja, a najcešće infektivni bovini rinotraheitis i infektivni pustularni vulvovaginitis (IBR/IPV). Tokom poslednje dekade dvadesetog veka utvrđena je molekulama struktura virusa i funkcionalni znacaj njegovog genoma. Utvrđivanjem funkcionalnih karakteristika pojedinih gena i njihovih produkata znacajno je rasvetljena patogeneza oboljenja, koje izaziva BHV-1, a posebno latencije, kao oblika infekcije ovog virusa. Zahvaljujuci ovim saznanjima znacajno su unapredeni postupci dijagnostikovanja i sprovoaenja imunoprofilakse, što je doprinelo efikasnijem suzbijanju i iskorenjivanju BHV-1 infekcije u mnogim zapatima goveda. U radu su opisana novija saznanja o etiopatogenezi i dijagnostikovanju BHV-1 infekcije, koja su zasnovana na najnovijim saznanjima o molekulama geni i funkcionalnom znacaju pojedinih glikoproteina virusa u procesu infekcije. Opisana su iskustva u postupku iskorenjivanja infekcije u više zemalja i deo zakonske regulative koji propisuju mere radi sprečavanja širenja infekcije i unapredjenja postupaka suzbijanja i iskorenjivanja BHV-1 infekcije u maticnim zapatima goveda.

Ključne reci: BHV-1, etiopatogeneza, dijagnostika, suzbijanje, zakonska regulativa
Introduction

Infection of cattle with herpes virus type-1 is one of the most widespread infections nowadays. Bovine herpes virus-1 (BHV-1) can cause significant health problems and economical losses. Respiratory syndrome, conjunctivitis, balanopsthitis, vulvovaginitis, endometritis, miscarriage, infertility and other diseases can develop as manifestations of BHV-1 infection. Development of the disease is influenced by age, herd management and particularly by virulence of the agent itself. However, infectious rhinotracheitis in young animals and infectious pustular vulvovaginitis in older animals are the most common manifestations. In the past, these diseases were known as: red nose, dusty fever, infectious necrotic rhinotracheitis, coital exanthema, vesicular venereal diseases etc. but taking into account the nature of the agent and the site of its isolation all these diseases were designated as infectious bovine rhinotracheitis and infectious pustular vulvovaginitis (IBR/IPV).

Significant improvement in research of molecular and biological characteristics of BHV-1 was noted by the end of 20th century and at the beginning of 21st century. The structure and function of the surface glycoproteins were clarified and major part of the genome was sequenced. These accomplishments resulted in elucidating of crucial issues on virus pathogenesis, particularly latency as an important manifestation of the infection. Development of novel effective immunoprophylactic and diagnostic instruments that are currently widely used in the control and eradication of infections caused by this virus are also the result of molecular and biological research. Better understanding of biological characteristics of BHV-1 and its pathogenesis contributed also to the designing the control and eradication programs. A number of European Union member-countries approached eradication of BHV-1 infection by adopting special regulations i.e. directives, which are mandatory for all producers of breeding cattle.

Biological characteristics of the virus

Shape, physical, chemical and biological features of the virus were the criterion for classification of BHV-1 in the family Herpesviridae, subfamily Alphaherpesvirinae and genus Varicellavirus (Roizman et al., 1982). Genome of BHV-1 consists of 135 to 140 kb double stranded linear DNA encoding synthesis of 70 proteins. Genome is completely sequenced, so
Ackremann, 1996). The genome organization allows US sequence to invert in relation to UL, which gives isomeric form (Mayerfield et al., 1983). Virus genome is surrounded with 162 capsomeres. Each capsomere is 12 nm long and 11.5 wide. Genome and capsomeres together constitute virus nucleocapsid of icosahedral symmetry. Diameter of the nucleocapsid is 95 to 110 nm. Nucleocapsid is surrounded by the tegument and the whole virion is surrounded by the double-layered lipid envelope. The envelope contains 10 different glycoproteins, which are the prerequisite for the virion-cell fusion, for entry of the virion into the cell and it's spreading from cell to cell. Some of these proteins are highly immunogenic, and are used for vaccine production. Since they localize on the surface of the virus or of the infected cells they present the target of the host's immune response. Most of these glycoproteins are, by their function and structure, homologous to glycoproteins of human \textit{Herpes simplex virus-1} (HSV-1) and therefore the following designation of the BHV-1 and HSV-1 envelope glycoproteins is accepted: gB, gC, gE, gl, gH, gL, gG, gK and gM.

Similar to other Alphaherpesviruses, replication of the BHV-1 genome can be divided in three phases: immediate early (\(\alpha\) genes), early (\(\beta\) genes) and late (\(\gamma\) genes) stage. According to the gene analysis and features of virus peptides, isolates of BHV-1 from various clinical materials are subdivided in various subtypes (Studdert, 1994). Subtype 1 causes infectious bovine rhinotracheitis, subtype 2 causes infectious pustular vulvovaginitis and in male animals infectious pustular balanopostitis. However, it is to be emphasized that all BHV-1 isolates are genetically and antigenically similar. They have preserved their antigenic structure even after multiple passages \textit{in vivo} and \textit{in vitro}. As concerning the subtypes it is to be emphasized that in 1992 strains of bovine herpes viruses that cause encephalitis in calves have been reclassified into the subtype BHV-5, based on genome characteristics of viruses isolated from clinical materials (Roizmann, 1992).

Pathogenesis

BHV-1, as well as other viruses, can multiply only in adequate (susceptible) cells. Outside the cell virus exists as a virion or as a viral particle (i.e. corpuscle). Virion is an inert form of the virus, which does not exhibit metabolic activity and does not produce molecules. It becomes infective only in contact with susceptible cells. Interaction of all Alphaherpesviruses, including BHV-1, with the cell develops in three phases. The first phase includes attachment of the virion on the cell surface; the second phase is the immediate interaction of viral glycoproteins from the envelope with cell receptors, while the terminal phase includes fusion of viral envelope and cell membrane and release of nucleocapsid in the cytoplasm of the cell. Virion-cell attachment is initiated when gC and/or gB counteract the heparan sulfate proteoglycans receptors on the surface of the cell. When attached to the cell, virion interacts with various cell receptors. It is believed that gD has the dominant role in this stadium, which interacts with receptors of immunoglobulin superfamily, such as tumor necrosis factor, Nectin-1 and 2 and CD155. Pathogenesis of this virus in cattle is highly determined by the 3-O sulphate heparan sulphate receptor, which is abundantly present in various tissue cells in cattle. This interaction initiates fusion process with the cell membrane. Most likely, gH, gL and gB as well as some other molecules from the cell membrane are involved in this process. In the course of the fusion process "crossing" of phospholipid part of the envelope and inner part of cell membrane occurs resulting in the incorporation of virus envelope into the cell membrane. After fusion some tegument proteins remain in the cell membrane and nucleocapsid is transported to the nuclear pores via the microtubules. Viral DNA is released at
the site where nuclear pore enters the nucleus. The capsid and the rest of the tegument proteins remain in the microtubules. They are thought to play the crucial role in "preparing the surrounding" for DNA virus replication. After the virus DNA enters the nucleus, process of infection is terminated, and replication of viral DNA and virus multiplication is initiated. It is well known that living cells are essential for virus multiplication. Therefore, BHV-1 "strategy" is to maintain the host cell alive as long as possible. However, cells productively infected with BHV-1 do not survive; they die as a result of the virus multiplication and of the cellular immune response related to the infection. Number of events in the infected cells is identified, e.g. the nucleus is enlarged, chromatin is destructed into the fragments and intranuclear bodies appear. Newly synthesized proteins are incorporated into the cell membrane, which results in structural and functional changes of the cell membrane. In the cytoplasm Golgy vesicles are scattered and recomposition of microtubular network occurs. These changes result in cell damage that is manifested as the lesions of different intensity and shape at the infection site, mostly the mucous of upper respiratory tract, eyes and reproductive organs. This phase presents the beginning of the clinical course of the infection, which may be manifested as infectious rhinotracheitis or infectious vulvovaginitis, i.e. balanospotitis, depending on the virus transmission route. Respiratory transmission most likely requires at least 10 to $10^4$ of infectious virions, which are transmitted by a direct contact with the infected animal, aerogenically or by droplets from nasal secretions. The virus shedding is the most pronounced at the third and sixth day after infection. At that time in one ml of nasal secret $10^7$ virion particles can be found (Babiuk et al., 1987). Virus shedding terminates after 12 to 14 days after infection (Lupton and Reed, 1980). Many factors influence the intensity of respiratory form of BHV-1 infection. Immune status of the animal and number of virions in the infectious material are considered to play dominant role in disease development. Identification of clinical symptoms is determined by the period of clinical observation of the animal and the extent of secondary infection. Lesions of the respiratory tract are mostly manifested as necrotic rhinitis, pharingitis and tracheitis. This process is highly progressive, resulting in high amounts of detritus containing exudates detected in the lumen of respiratory tract, which provides favorable environment for the growth and multiplication of bacterial microflora. Further complication of this process is manifested as hyperemia of the entire respiratory tract, infiltration and accumulation of the neutrophile granulocytes in the submucous finally resulting in an edema. Such condition enables bacterial colonization of the respiratory tract, especially by organisms Pasteurellae spp, which colonize the nose and pharynx of cattle as conditional pathogens. Superinfection with Pasteurellae significantly increases morbidity and mortality rates. Severe forms of serofibrinous bronchopneumonia, particularly in ventral parts of the lungs are developed. Lacking of prompt and adequate chemotherapy results in hypoxia and toxemia, and finally in death of the affected animals. Respiratory syndrome is often accompanied by conjunctivitis, which may well be the only symptom of BHV-1 infection. Whether conjunctivitis is the consequence of the respiratory tract infection and virus spreading to the conjunctiva or is it result of direct infection of conjunctiva still remains unclear. Though it is well known that the virus spreads in the organism via the monocytes, viremia is difficult to establish. Systemic infections occur in fetuses and in newborn calves, being quite rare in older animals. In these animals virus infects liver, kidneys and digestive tract that results in functional impairments of the affected organs and in the fatal outcome. Infection of the fetus often ends up with abortion that can occur even 90 days after infection. Fetuses are sensitive in every stage of intrauterine development, but most of the abortions take place after the fifth month of gravidity. Symptoms of genital infection occur very quickly, one to three days after infection. Vesicles can be observed on the mucous of reproductive tract, progressing to pustules and ulcers. First clinical signs indicating
genital tract infection are frequent urination, tail lifting and poor vaginal discharge. Examination of the vulva reveals edema and hyperemia. In uncomplicated infections the process terminates within 10 to 14 days, and the mucous heals leaving distinct unpigmented areas. In case of bacterial superinfection endometritis with purulent vaginal discharge and transient infertility may develop in several weeks. Slight edema of the prepuce are mostly the only symptoms in bulls, thus the disease often remains unnoticed. Penis examination can reveal presence of pustule. Bulls in this stage of infection refuse mating, because it is painful; however, if mating continues severe damage of the mucous and penis distortion occur. The described symptoms are characteristic for the acute infection, presenting the consequence of unlimited virus replication that is often designated "lytic growth" since cells are destroyed and the virus permanently replicates, attacking new cells.

Contrary to the lytic growth as a form of BHV-1 infection with the clinical outcome, the course of BHV-1 infection often lacks destruction of infected cells and subsequent symptoms. Such course of the disease with specific antibodies against BHV-1 occurring as only symptoms of the infection is called latent infection. Following the acute stage, if the infected animal survives, BHV-1 infection becomes latent. Latent infection develops in three phases: establishment of the latency, maintaining of the latency and reactivation of the latency. Latent infection is mostly established in trigeminal ganglia, though it can be detected also in the lymphoid follicles of tonsils (Winkler et al., 2000). Mechanisms of development and maintenance of latent herpesvirus infections in animals have poorly been understood until recently; however, some novel research revealed that programmed cell death, i.e. apoptosis is prevented in animals latently infected with BHV-1 (Lovato et al., 2003).

During the latent infection antigen exposition at the surface of the cell is lacking, thus they remain unrecognized by the immune system mechanisms. The viral genome is present in the nucleus of the infected cell as an extrachromosomal material. Its circular shape determines the sustained expression of the viral genes during latent infection. There is transcription of only one viral gene that is responsible for blocking apoptosis of the infected cells. Viral gene responsible for latent infection (LR gene related-latency) encodes the LR protein. This protein with the cell cyclin dependent kinase 2 inhibits productive infection and prevents the programmed cell death. Stress conditions or the corticosteroid treatment of latently infected animals results in the drop of immune status of the animals, remodeling of mRNA LR gene processing and activation of other viral genes, which finally leads to production of virus proteins and new virions. Therefore, by reactivation of the viral genome lytic cycle in cells and shedding of the virus is reestablished. Latently infected and clinically sick animals periodically excrete the virus in amounts ranging from 1000 to 100 000 virions per one ml of exudates (Siebert et al., 1995). Reactivation of latent infection under the stress conditions (transport, poor zoohygienic conditions, poor feeding etc.) or treatment of animals with corticosteroids can induce viremia and distribution of the virus in cells of parenchimatos organs (i.e. in respiratory tract, urogenital tract etc.) (Lazic et al., 2003). In that respect, latently infected animals are potential source of infection, through which infection persists in the herd for a long period of time.

Diagnostic

Diagnostic of BHV-1 infection is based on determining characteristic clinical symptoms and detection of the virus. Besides the described symptoms, the acute phase of the infection is characterized by the increased body temperature (41 to 42°C) and appetite loss. Development of the severe symptoms of bronchopneumonia is accompanied by all symptoms of the
respiratory syndrome. The most characteristic symptoms are the abundant nasal discharge, which is strongly marked in the morning before feeding, and intensive hyperemia (red nose) accompanied by lesions on the nasal mucous. Furthermore, keratoconjunctivitis on both sides is often present. Massive miscarriage in pregnant animals can also indicate BHV-1 infection. Characteristic changes observed in dead and slaughtered animals may suggest the rhinotracheitis and bronchopneumonia. However, the accurate diagnosis of latent infection is possible only on laboratory examination of blood samples in order to confirm presence of the specific antibodies that are the only symptom of infection or by examining other suspect material to prove presence of the virus and/or some of its components.

In the past decades much effort was made with an aim of developing and improving the laboratory diagnostic of BHV-1. Nowadays, laboratory diagnostic of this virus is based on two approaches. One is detection the virus or its components and the second is directed towards the detection of specific antibodies (Babiuk, 1996). Detection of the virus or its components involves application of several laboratory tests or techniques, but virus isolation in the susceptible cell cultures is considered the basic laboratory diagnostic method for BHV-1 (OIE Manual of Diagnostic Test and Vaccines for Terrestrial Animals, 2004). However, novel laboratory techniques, such as immunofluorescence, immunoperoxidase, ELISA and especially methods of molecular biology in virus gene detection (Polymerase chain reaction-PCR) are to be emphasized, which enable fast and precise diagnosis and which are preferred as the method of choice in the contemporary laboratory practice. The advantage of these methods, especially ELISA and PCR technique, is the possibility of examining the whole herd through the bulk milk sample. ELISA technique enables examination on the specific antibodies against BHV-1 in bulk milk samples collected from up to 50 cows, while PCR technique enables detection of the viral genome in pulled milk samples of even more than 100 cows. Detection of specific antibodies against BHV-1 or serology diagnostic is frequently used; especially when the suspect material is not available or when latent infection is suspected. Serology diagnostic is based on virus neutralization and various immunoenzyme techniques, which are applicable for blood sera as well as for milk samples (Perrin et al., 1993).

Control and eradication

Infections of cattle with Bovine herpesvirus-1 are of highest concern in the view of animal health status and considerable economical losses in cattle production. A drop of milk production amounting 0.92 kg during 10 weeks of lactation was established in latently infected cows (Straub, 2001). Furthermore, the service period is significantly extended as well as the calving interval resulting in a yearly loss of 150 DM per one cow (Krage Von E. et al., 1989). Besides this and many other direct loses, latent infection with BHV-1 causes other indirect loses that often cannot be presented in figures. Ban on trading breeding animals, sperm and embryos originating from infected herds, as well as from affected regions are examples of indirect loses. Such bans can induce much higher losses than epidemic outbreaks of IBR. Analyses in many countries suggest that infection with Bovine herpesvirus-1 cause losses that can be quantified as millions of national currency.

Vaccination is considered the most effective method to reduce these health and economic losses. Many authors recommend it as an effective measure to prevent shedding of BHV-1.
Nowadays, besides the conventional, attenuated and inactivated vaccines, vaccines produced by genetic engineering are commercially available. These vaccines contain the "virus mutant" lacking the gene for production of one single surface glycoprotein. These vaccines proved highly efficient in eradication of BHV-1 infection. Namely, after application of this vaccine and subsequent serologic examination, it is possible to determine whether seroconversion is the consequence of vaccination or infection. National authorities of many countries in Europe, North America, Near East and others established principles and measures for control and eradication of IBR. Herds in Switzerland, Austria, Belgium and almost in all Scandinavian countries are free of BHV-1 infection (Bommeli and Kihm, 1982). In these countries control and eradication programs started in the second half of the 20th century. Eradication was based on the principle "examine and remove" (Oirschot 1997) and was fully justified from the health and economic point of view.

Countries like Holland, Germany, Hungary, France, Slovenia and other, developed and introduced control and eradication programs with an aim of gaining status of BHV-1 negative country. Program design includes frequent and planned examination of blood and milk samples on presence of antibodies against BHV-1, mostly by the use of ELISA technique. If the infection is established in the herd, seropositive animals are removed or vaccination program is introduced.

Significant efforts to eradicate BHV-1 infection are evident in Holland. Livestock resources in Holland include ca 33 000 milking cows and 30 000 beef cattle. Prevalence of BHV-1 infection in milking cows and breeding herds is high. Some calculations revealed that some 40% of these animals are seropositive on BHV-1. Losses due to subclinical and clinical IBR, reduced milk production in expositive animals and particularly losses due to banning on export of young breeding animals, sperm and embryos into the countries of the European Union have induced development of control and eradication program in Holland that was adopted as a legislative regulation. Its application is mandatory for all farmers. According to this program cattle herd is considered BHV-1 free if infected animals are not present, in spite of vaccination regime. The program foresees rigorous control of the animals introduced in the herd. Such animals are subjected to multiple examinations and they must be BHV-1 free and must originate from BHV-1 free herds. After obtaining status of BHV-1 free herd vaccination is terminated and the herd is subjected to continuous monitoring. Breeding herds are released from trade and market banning. Eradication expenses are regulated by legislative provisions, being partly covered by a farmer himself, while the utmost part is covered by the Holland Fund For Protection of Animal Diseases (Franken, 1999, Wit de J et al., 1998).

So far Serbia and Montenegro haven't established any legislative base for eradication of BHV-1. It is carried out on voluntary basis or, more precisely, on the request of cattle breeder. Researchers from the Scientific Veterinary Institute "Novi Sad" have developed few programs for eradication of this infection, which proved effective in eradication of the infection caused by herpesvirus-1 in four industrial herds. These programs were developed according to the number of infected animals in herds and according to available capacities. The main principles of its content and application are as following: 1. "TEST AND REMOVE" 2. HERD TRIAGE and 3. VACCINATION OF HERDS.

Legislative regulations
In order to detect, control and eradicate BHV-1 infections development and introduction of standards mandatory for all cattle breeders is of essential importance. With an aim of protecting their cattle most countries of the European Union facing the problems of such infections and especially countries that are free from BHV-1, have developed control and eradication programs, which are regulated by the law. Such regulations contain detailed descriptions of the conditions of trade of breeding animals, diagnostic procedures, control and eradication of the infection and immunoprophylactic measures. It is important to emphasize that these regulations are often updated and harmonized with recent knowledge on the virus, pathogenesis, diagnosis and immunoprophylaxis. For example, a revised decree on BHV-1 was in force in Germany (from 2001 to 2003) and a decree on the ban on international trade of cattle over the territory of this country is still in force in Austria.

In Serbia and Montenegro a Regulation on the control and eradication of infectious bovine rhinotracheitis and infectious pustular vulvovaginitis was established in 1989. Of course, this Regulation presented a significant document in the moment of its adoption; however, it mostly regulated procedures for disease control. Nowadays, considering the nature and characteristics of BHV-1 infections criterions are much higher. The farmers demand novel control and eradication strategies, which will be constitutive part of the legislative regulations. The researchers from the Scientific Veterinary Institute "Novi Sad", Novi Sad and Faculty of Veterinary Medicine from Belgrade developed a proposal of the amendments to the existing Regulation based on recent knowledge on BHV-1 infections, on the requirements of the International Office of Epizootic (O.I.E.) and pursuant to the status of BHV-1 infections in cattle industry of Serbia and Montenegro. Proposal of the new Regulation is submitted for consideration and adoption to the Veterinary Department of the Ministry of Agriculture and Environmental Protection.

References