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Bovine herpes Virus-1: Viral latency and reactivation induced infections in bovines

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Abstract
Bovine reproductive disease attributable to bovine herpes virus-1 (BoHV-1) was first described in Germany in the 19th century, being recognised primarily as the cause of infectious vulvovaginitis and balanoposthitis until the mid-1950s when a more virulent strain of the virus (BoHV-1.1) associated with respiratory disease (infectious bovine rhinotracheitis; IBR) emerged in the western United States. Subsequently, IBR emerged as a clinical condition in Europe, from the 1970s onward. Acute infection of cattle leads to abundant expression of lytic cycle viral genes, high levels of virus shedding, and clinical symptoms. Following acute infection, lifelong latency is established in sensory neurons. While the ability of BoHV-1 to produce respiratory disease is now well recognised, the potential negative outcomes of infection on fertility and reproduction are less frequently considered. Latency and reactivation of the virus is the main cause of infections in cattle. In this review viral latency and reactivation induced infections in cattle is discussed.

Keywords: BoHV-1, IBR, latency, LRT, MDBK, ORF-E, reactivation, TG

1. Introduction
Infectious bovine rhinotracheitis is a disease caused by Bovine Herpes Virus (BoHV-1) in domestic and wild cattle. BoHV-1 is a member of the genus vericellovirus in the subfamily Alphaherpesvirinae, which belongs to the herpesviridae family order herpesvirales. Three subtypes of BoHV-1 are recognized worldwide: BoHV-1.1, BoHV-1.2a, BoHV-1.2b. Viruses from the BoHV-1.1 subtype cause severe respiratory disease and can be associated with abortion. Subtype 1 isolates are frequently detected in aborted foetuses suggesting that infection caused the abortion. Abortions due to herpes virus have been reported worldwide. Abortions usually occur between four to seven months of gestation after natural infection or vaccination. The bohv1 becomes latent following primary infection with field isolate or vaccination with an attenuated strain [1, 2]. The viral DNA is detected in sensory ganglia of the trigeminal nerve. Latency may also occur in tonsillar lymphoid cells and peripheral blood lymphocytes. Latent virus may be reactivated by several stress factors such as transportation and climatic changes. Attenuated virus strain remains latent in the dam and vaccination does not provide protection against latent infection with field isolate and reappearance of virus. This virus causes late-term abortions and conception failure due to venereal diseases.
BoHV-1 typically infects the trigeminal nerve endings, through which it establishes a latent infection in ganglionic neurons. Even after the animal stops exhibiting symptoms of infection, the latent infection can be reactivated later in life, at which point the animal begins shedding the virus. After genital infection, BoHV-1 replicates in the mucous membranes of the vagina or prepuce, and becomes latent in the sacral ganglia [3]. The viral DNA remains in the neurons of the ganglia, probably for the entire life of the host (status of latency). Stress, such as transport and parturition, but also the application of corticosteroids can induce reactivation of the latent infection leading to standard viral lytic cycle which can lead to abortions.
BoHV-1, by means of viral latency mechanism, is able to maintain the viral genome in the nucleus of infected cells, as provirus. Under this viral condition, the infected animal may remain without clinical signs of infection for a long period [4]. Eventually, the viral latency is broken and the infected animal may present clinical manifestations accompanied by viral re-excretion, perpetuating the infection in the herds [5]. If Initial exposure to the virus, reactivation of the virus from latency, or vaccination using live virus occurs during pregnancy, it can cause abortion [6, 7, 8].

2. Virion structure
BoHV-1 contains an icosahedral nucleocapsid of 95-110 nm in diameter consisting of 162 capsomeres, each being 12 nm long by 11.5 nm wide, with an axial hole of 3.5 nm [9, 10]. The nucleocapsid is surrounded by an electron-dense zone called the tegument and a lipid bilayer envelope, forming pleomorphic virions 150-200 nm in diameter.

3. Transmission
Natural portal of entry of BoHV-1 is the mucous membrane of either upper respiratory or genital tracts. Transmission of infection can also occur through the inoculation of conjunctival epithelium. Preferential way of BoHV-1 transmission is direct nose to nose contact. However, airborne transmissions by the aerosol route were demonstrated on short distances (120). Animals that excrete virus from the vagina or prepuce transmit the virus less efficiently and only limited number of animals becomes infected [11, 12]. Genital transmission occurs through semen contaminated with virus [13]. The virus may survive up to one year in semen frozen at - 196C. For this reason bulls used for artificial insemination are required to be seronegative for BoHV-1.

4. Pathogenesis

Following initial infection, 2-3 days of post exposure animals develop a fever and virus multiplies in the respiratory tract and causes inflammatory changes such as rhinitis, laryngitis and tracheitis leading to destruction of the tracheal microvilli. In dairy cattle there will be drop in milk production. Focal necrosis of the nasal mucosa is evident, often leading to serous nasal/ ocular discharge and conjunctivitis. Infection of cattle with BHV-1 debilitates resistance to secondary bacterial infection such as M. haemolytica, P. multocida and H. somnis, leading to fatality and depression of cellmediated immunity [14, 15]. The severity of clinical disease appears to be related to the virus strain, the immunological status of the animal, environmental stress and the age of the animal. α-Herpesvirus glycoproteins are required to bind viral particles and penetrate lenient cells. A subset of glycoproteins is necessary for viral release, cell fusion and cell to cell spread. Ten envelope proteins such as gB, gC, gD, gE, gG, gH, g1, gL, gM and gK are glycosylated and two (gN and Us9) are non-glycosylated [16, 17]. For growth in cultured cells five envelope proteins gB, gD, gH, gL and gK are essential. Whereas gC, gE, gG, gI, gM, gN and Us9 are not essential for growth in cultured cells [18, 19, 20].

5. Latency

All herpes virus are able to establish latent infections. Viral latency is the ability of a pathogenic virus to lay dormant (latent) within a cell, denoted as the lysogenic part of the viral life cycle. A latent viral infection usually does not cause any noticeable symptoms and can last a long period of time before becoming active and causing infections. Long living, non-replicating and highly differentiated cells such as neurons, or lymphoid cells are ideal for virus to harbour during latency. The viral genomic DNA is usually detectable in the sensory ganglia of the trigeminal nerve in IBR and in sacral spinal ganglia in IPV/ IPB cases. Additional sites of latency in the lymph nodes and nasal mucosa, have been determined for BHV- 1 by PCR [21, 22]. Relatively high levels of viral gene expression or infectious virus can be detected in trigeminal ganglia within 1–6 days after infection. Latency may also occur in tonsillar lymphoid cells and peripheral blood lymphocytes.

After replication in mucosal epithelium BoHV-1 establishes lifelong latency in sensory neurons of the peripheral nervous system. BoHV-1 penetrates the terminus of the sensitive nerves distributed in the infected epithelium. It is then transported along the microtubules of the axons to reach the neuron body in the nervous ganglion. In acute infections all viral genes are expressed abundantly while the latency-related (LR) gene is the only viral transcript expressed abundantly in sensory neurons within trigeminal ganglia (TG) [23]. An elevated level of transcription occurs from the latency-related (LR) gene and open reading frame-E (ORF-E). ORF-E is at the downstream of the gene, encoding the major viral transcriptional activator bICP0. The LR gene encodes at least two micro-RNAs and more than one protein. These proteins and micro-RNAs are detected in a subset of latently infected neurons implying that they regulate certain aspects of the latency-reactivation cycle. BoHV-1 region containing the latency- related transcript (LRT) is expressed leading to the inhibition of the lytic virus cycle and induction of the antiapoptotic state of the infected cells in latently infected cells. In latently infected neurons, reduced levels of viral DNA were detected but higher levels of apoptosis occurred in trigeminal ganglion at the end of the acute infection. Latency develops in almost all animals that are infected with high or low doses of attenuated or virulent BoHV-1 [24]. Attenuated vaccine strains can remain in a latent state in the body and vaccination does not provide protection against establishment of a latent infection with a wild strain [25]. Inoculation with live vaccine strains of BVH-1 can also lead to latent infection [26]. Vaccination in latently infected animals does not prevent re-excretion of a wild strain.

6. Reactivation from latency

The latent virus serves as a reservoir in an immune host which becomes persistent upon reactivation. Certain factors such as stress, immune suppression, transport, experimental corticosteroid treatment, parturition can reactivate the virus. Reactivation of latent virus promotes perpetuation and virus transmission to the cattle population. New virus is synthesized at the site of latency and re-excreted. In BoHV-1 the reactivated virus is transported intra axonally to the original portal of entry where it can persist and transmitted to the herd. Viral DNA persists in the tonsil and can be reactivated. During reactivation from latency, three significant events occur: (i) productive viral gene expression is readily detected in sensory neurons, (ii) ORF-E and LR gene expression decrease dramatically and (iii) infectious virus is secreted from nasal or ocular swabs [27, 28, 29]. Administration of dexamethasone to cattle latently infected with BoHV-1 leads to activation of viral gene expression and reactivation of latent virus.

Furthermore, it has to be stressed at this point that not only the virus but also the host contributes significantly to the efficiency of reactivation. It was, for example, easy to reactivate BoHV-1 from latently infected calves by the administration of corticosteroids.

Table 1: Steps of latency and reactivation

Steps	Mechanism
Establishment of latency	Entry of virus into ganglionic neurons, viral gene expression and replication, LRT expression.
Maintenance of latency	Expression of LRT, lytic viral gene expression and replication not detected.
Reactivation from latency	External stimulus such as stress, transport, glucocorticoid treatment, extensive viral gene expression and replication, LRT expression.

7. Diagnosis

Diagnosis of viral infection may be performed on the basis of serologic tests that detect the virus or antibodies, and through nucleic acid based tests that detect the DNA via PCR and nucleic acid hybridization and sequencing[30]. Isolation of BoHV-1 can be carried out using cell cultures of bovine kidney cell lines such as MDBK (Madin-Darby Bovine Kidney).

7.1 PCR amplification: The PCR assay is sensitive and practical alternative for the rapid detection of virus. The PCR assay is considered equivalent to that of standard virus isolation and dot blot hybridization [31]. The PCR assay with southern blot hybridization is considered to be highly sensitive and can detect the virus in aborted tissue [32].

7.2 Real-time PCR: Real-time PCR provides satisfactory reproducibility as well as high specificity and sensitivity in combination with significant reduction in time for detecting

amplified products. BoHV-1 DNA was detected by real-time PCR in 10 (2.5%) of the 400 tissue pools from abortion cases. The original diagnostic procedures had identified just 2 of these cases as BoHV-1 related, 4 were considered likely to have a bacterial cause, 1 was due to a non-infectious cause, and no diagnosis was reached for the remaining 3 cases. In one case, clinical signs of IBR had been observed in the dam 1 month before the sample was submitted. Another case had received an IBR vaccination.

7.3 Electron microscopy: The use of electron microscopy to identify virus particle is a rapid method for the diagnosis of BHV-1 but it should be used in the early stage of the disease. Electron microscopy may also be used to identify the virus particles in foetal samples.

7.4 Serology: Several serological tests are available for the detection of antibody and a rise in titre between the acute and convalescent phase of infection. The primary immune response to BHV-1 experimental inoculation of cattle is characterized by the formation of both IgM and IgG antibodies. Secondary immune responses are characterized primarily by the formation of IgG2 antibody.

7.4.1 Enzyme –linked immunosorbent assay: Enzyme – linked immunosorbent assay for viruses are qualitative enable to determine the presence or absence of virus.

The ELISA is a specific, sensitive, and more practical test for detection of BHV-1 antibodies. A variety of ELISAs, namely indirect ELISA, c-ELISA, and AB-ELISA have been employed to screen serum samples of cattle and buffaloes in India.

7.4.2 micro-ELISA: is being used for the control program of BHV-1 infection in Switzerland. The test is simple, rapid, and convenient compared to the serum neutralization test, which requires cell culture facilities and is time-consuming.

7.5 Virus neutralization test: The VNT has been widely used and is the gold standard by which other techniques have been evaluated [33], based on the titration it is possible to establish the magnitude of antibody titres present in the blood serum.

7.6 Histopathology and immunohistochemistry: Tissue samples, where Bohv-1 DNA was detected by real time-PCR were analyzed using histopathological methods. Samples were fixed in 10% formalin for at least 24 hr and then embedded in paraffin wax blocks. Five micron sections were cut using a microtome, placed on slides, and stained with hematoxylin and eosin (HE) by standard methods.

7.7 Virus identification: Serum neutralization tests were done using MDBK cells and monospecific antisera (obtained from experimentally infected natural hosts) directed against BoHV-1.

7.8 Indirect immunofluorescence assays: Indirect immunofluorescence assays were done on infected coverslip cultures following fixation with acetone using monospecific antisera.

7.9. Virus isolation: BHV-1 can be readily isolated in cell culture of primary or secondary bovine kidney, lungs, testis, turbinate, or trachea and established cell lines such as Madin–Darby Bovine Kidney (MDBK) or CRIB cells [34]. The virus can be isolated from nasal swabs, conjunctival swabs, vaginal swabs, preputial washing, placental cotyledons of aborted foetus, fetal liver, lung, spleen, kidney, lymph node, mucous membrane of the respiratory tract, tonsils, and lungs collected in virus transport medium. The presence of virus in specimens is detected by a cytopathic effect (CPE). The CPE of BoHV-1 is characteristic and usually appears within 3 days of inoculation. There are grape-like clusters of circular cells present around a microplaque in cell culture. Giant cells or syncytia are also observed. Giant cells or syncytia are also observed. The virus is cytolytic if the cells are incubated for a prolonged time and there is total sloughing of the rounded cells from the plastic/glass surface of the container. The cell cultures inoculated with specimens are observed for 7 days. The cell culture should be passaged at least three times before the sample is considered negative [35, 36].

8. Conclusion

Bovine herpes virus 1 (BHV-1) is primarily associated with clinical syndromes such as rhinotracheitis, pustular vulvovaginitis and balanoposthitis, abortion, infertility, conjunctivitis and encephalitis in bovine species. The BHV-1 virus can become latent following a primary infection with a field isolate or vaccination with an attenuated strain. Latency can be interrupted periodically, leading to reactivation from latency and spread of infectious virus. Latent infections can be reactivated by wide variety of stimuli such as transport, stress, glucocorticoid treatment and parturition. The viral genomic DNA has been demonstrated in the sensory ganglia of the trigeminal nerve in infectious bovine rhinotracheitis (IBR) and in sacral spinal ganglia in pustular vulvovaginitis and balanoposthitis cases. Attenuated vaccine strains can remain in a latent state in the body and vaccination does not provide protection against establishment of a latent infection with a wild strain. Vaccination in latently infected animals does not prevent re-excretion of a wild strain.

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