Bovine herpes virus infections in cattle

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Abstract
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 main sources of infection are the nasal exudates and the respiratory droplets, genital secretions, semen, fetal fluids and tissues. The BHV-1 virus can become latent following a primary infection with a field isolate or vaccination with an attenuated strain. 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. BHV-1 infections can be diagnosed by detection of virus or virus components and antibody by serological tests or by detection of genomic DNA by polymerase chain reaction (PCR), nucleic acid hybridization and sequencing. Inactivated vaccines and modified live virus vaccines are used for prevention of BHV-1 infections in cattle; subunit vaccines and marker vaccines are under investigation.

Keywords: infectious bovine rhinotracheitis, bovine herpes virus 1, vulvovaginitis, balanoposthitis, abortion

Introduction
Infectious bovine rhinotracheitis (IBR), caused by bovine herpes virus 1 (BHV-1), is a disease of domestic and wild cattle. BHV-1 is a member of the genus Varicellovirus in the subfamily Alphabervirinae, which belongs to the Herpesviridae family. The viral genome consists of double-stranded DNA that codes for about 70 proteins, of which 33 are known to be structural and up to 15 are non-structural proteins (Muyldemans et al., 2007). The viral glycoproteins are located in the envelope on the surface of the virion and play an important role in pathogenesis and immunity. BHV-1 can be differentiated into subtypes 1.1, 1.2a, 1.2b and 1.3 (Muyldemans et al., 2007). BHV-1.3, which is a neuropathogenic agent, has been re-classified as BHV-5 (Edwards et al., 1990). The BHV-1.2 subtypes may be less virulent than subtype 1.1. BHV-1 is a cause of several infectious disease syndromes in cattle and buffaloes and occurs throughout the world, including India.

BHV-1 is primarily associated with three major clinical syndromes namely, IBR, infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IPB). The virus also causes a wide variety of other clinical syndromes such as abortion, infertility, conjunctivitis and encephalitis. BHV-1 is also one of the most important pathogens involved in the development of the respiratory disease syndrome called shipping fever (Yates, 1982; Jones and Chowdhury, 2007). BHV-1 is the cause of a notifiable list B OIE disease that includes transmissible diseases that are considered to be of socio-economic importance within the countries and that are significant in the international trade of animals and animal products (Turin and Russo, 2003). The disease is endemic in India and during the period 1986 to 2006, out of 7313 serum samples tested, 3152 were positive for BHV-1 (Anon, 2007) by indirect and competition ELISA (c-ELISA) or micro-serum neutralization test (m-SNT) and during the period 2000 to 2008, 26 of 953 semen samples were positive by polymerase chain reaction (PCR) or isolation in cell culture (Nandi et al., 2004, 2007, 2008a, b; Deka et al., 2005).

The Government of India has arranged to screen all the breeding bulls for the infection before using the semen...
for in vitro fertilization (IVF) or natural or artificial insemination (AI) purposes (Anon, 2007). The BHV-1 virus infections in cattle and buffaloes are mostly mild and non-life threatening. However, the introduction of IBR into a cattle farming can cause severe economic losses due to weight loss, decrease in milk production and restrictions in the international livestock trade. Cattle that recover from an acute IBR infection can prove very harmful to disease-free herds because they are silent carriers of BHV-1. These animals remain carriers of BHV-1 for the rest of their lives until immuno-suppressive treatments or other conditions reactivate virus replication, leading to the spread of the infection to the rest of the herd (Van Oirschot, 1995; Preston and Nicholl, 2008). It is therefore important to control the disease in the cattle population of a country by imposing regulations to ensure BHV-1 negativity for trade in livestock and their derivatives such as frozen embryo, semen straws and fresh semen.

Etiology

The BHV-1 is a lipid enveloped, hollow polygonal structure and the nucleocapsid contains the double-stranded DNA genome of approximately 140 kilobase pairs. The nucleocapsid contains 162 hollow capsomers: 150 hexamers and 12 pentamers. The capsomers are polygonal in cross section; each capsomer is approximately 12 nm long, 11.5 nm wide with an axial hole of 3.5 nm. The DNA is wrapped around a fibrous spoon-like core, whose fibers are anchored to the inner side of the surrounding capsid (Murphy et al., 1999). The virion is pleomorphic, with a diameter that varies from 120 to 200 nm. The virion contains about 70 proteins of which 11 are envelope glycoproteins. One of the glycoprotein peplomers gI and gE possess the Fc receptor and binds with the IgG molecule (Schwyzer and Ackermann, 1996).

There is only one antigenic type of BHV-1, irrespective of whether the isolate is derived from cases of IBR or IPV. On the basis of restriction enzyme digestion patterns, three genotypes of BHV-1 can be distinguished: subtype 1 is a respiratory subtype; subtype 2 is a genital subtype, which is subdivided into BHV-1.2a and BHV-1.2b; and subtype 1.5 (5), is an encephalitic subtype (Edwards et al., 1990, 1991). BHV-1 subtypes 1 and 2a mainly cause the respiratory form of the disease (IBR), with fever, drop in milk production and abortion; infections with these subtypes have a mild outcome (Edwards et al., 1990; Miller et al., 1991). Isolates of BHV-1.2a cause abortion, whereas BHV-1.2b isolates are not abortifacient. Isolates of BHV-1.1 are more virulent than are isolates of BHV-1.2b. BHV-1.3 or BHV 5 has been isolated from calves that died of encephalitis and from aborted fetus (Schudel et al., 1986).

Physiochemical properties of the virus

The virus BHV-1 is resistant to environmental influences. Inactivation of the virus in the environment depends on factors such as temperature, pH, light, humidity and kind of medium harboring the virus (Gibbs and Rweyemamu, 1977). At 4°C, the virus is stable for 1 month. BHV-1 is inactivated at 56°C within 21 min, at 37°C within 10 days and at 22°C within 50 days (Gibbs and Rweyemamu, 1977). The virus may survive for more than 30 days in feeds. As the virus is enveloped, it is sensitive to organic solvents such as chloroform, ether and acetone. The virus is sensitive to many disinfectants and is readily inactivated by 0.5% NaOH, 0.01% HgCl2, 1% chlorinated lime, 1% phenolic derivatives, 1% quaternary ammonium bases and 10% Lugol’s iodine. Formalin (5%) inactivates BHV-1 within 1 min (Straub, 1990).

Host range

BHV-1 is an alphaherpesvirus. These viruses have restricted cattle and buffalo host range and do not commonly or stably cross species barriers (Brake and Studdert, 1985). The host range of the virus is determined by both host and viral factors. The circ, UL0.5, UL3.5 and US15 genes are peculiar to BHV-1. The repertoire of BHV-1 proteins consists of the major glycoproteins (glycoprotein B (gB), gC and gD), additional glycoproteins (gE, gI, gH, gL, gG, gK and gM), thymidine kinase (TK) (UL23), a number of enzymes such as UL2, UL12, UL40 (ribonucleotide reductase), UL42 (DNA polymerase), UL50 (dUTPase), UL52 (helicase), US3 (protein kinase) and a group of regulatory proteins (BICP0, BICP4, BICP22 and BICP27, α TIF). Investigations into the functions of these proteins and recombinant viruses containing deletions or replacements of individual genes were aimed at vaccine development and insights into pathogenesis, latency, neurotropism and interference with host functions (Engels and Ackermann, 1996; Schwyzer and Ackermann, 1996). Successful viral infection depends upon attachment and penetration of the virus into a host cell, followed by replication and viral export. Viral glycoproteins of herpes viruses are involved in several steps of the viral cycle, such as the attachment, penetration, maturation and egress of the virus.

Host response to BHV-1 infection can be divided into a specific response mediated by B- and T-cells and a non-specific response mediated by polymorphonuclear neutrophils (PMNs), macrophages, natural killer (NK) cells, interferon, complement and other factors that can limit virus attachment at the respiratory epithelium (Denis et al., 1993). Interferon α and β molecules are present within 5 h post-infection, reach peak levels in the nasal secretions and blood by 36–72 h post-infection, and remain elevated until virus replication ceases.
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Russo, 2003; Boelaert et al. 1990, 1991; Noordegraaf et al. 1996. According to OIE data, BHV-1 is distributed worldwide, but has been eradicated from Austria, Denmark, Finland, Sweden, Italy (Province of Bolzano), Switzerland and Norway, and control programmes are in place in some other countries including Australia, Belgium, Canada, India, Poland, Turkey and USA (Edwards et al., 1976; Suresh et al., 1999; Chinchkar et al., 2002; Sharma et al., 2004; Kiran et al., 2005; Jain et al., 2006). Sero-surveillance studies indicate that about 48% of serum samples are positive for antibodies to BHV-1 detected by screening with the m-SNT and up to 54% by testing the samples by indirect and c-ELISA (Pharande et al., 2004; Nandi et al., 2004, 2007, 2008a, b). It was observed that cattle were more susceptible to IBR than buffaloes and crossbred cattle were more susceptible than indigenous cattle. Animals 3–6 years of age were more prone to infection than were younger animals (Sarumathi et al., 2002). The seroprevalence of BHV-1 ranges from 14 to 60% in Africa and from 36 to 48% in Central and South America (Straub, 1990). According to OIE data, BHV-1 is distributed worldwide, but has been eradicated from Austria, Denmark, Finland, Sweden, Italy (Province of Bolzano), Switzerland and Norway, and control programmes are in place in some other countries including Australia, Belgium, Canada, India, Poland, Turkey and USA (Edwards et al., 1990, 1991; Noordegraaf et al., 2000; Salwa et al., 2000; Galiero et al., 2001; Madinelli et al., 2001; Turin and Russo, 2003; Boelaert et al., 2005).

Distribution

The disease is widely distributed around the world. In India, the disease is endemic and outbreaks have been reported from almost all the states (Mehrotra et al., 1976; Suresh et al., 1999; Chinchkar et al., 2002; Sharma et al., 2004; Kiran et al., 2005; Jain et al., 2006). Sero-surveillance studies indicate that about 48% of serum samples are positive for antibodies to BHV-1 detected by screening with the m-SNT and up to 54% by testing the samples by indirect and c-ELISA (Pharande et al., 2004; Nandi et al., 2004, 2007, 2008a, b). It was observed that cattle were more susceptible to IBR than buffaloes and crossbred cattle were more susceptible than indigenous cattle. Animals 3–6 years of age were more prone to infection than were younger animals (Sarumathi et al., 2002). The seroprevalence of BHV-1 ranges from 14 to 60% in Africa and from 36 to 48% in Central and South America (Straub, 1990). According to OIE data, BHV-1 is distributed worldwide, but has been eradicated from Austria, Denmark, Finland, Sweden, Italy (Province of Bolzano), Switzerland and Norway, and control programmes are in place in some other countries including Australia, Belgium, Canada, India, Poland, Turkey and USA (Edwards et al., 1990, 1991; Noordegraaf et al., 2000; Salwa et al., 2000; Galiero et al., 2001; Madinelli et al., 2001; Turin and Russo, 2003; Boelaert et al., 2005).

Clinical signs

The incubation period varies from 10 to 20 days under natural conditions. The clinical signs may vary widely and have been grouped as: respiratory form, genital form, ocular form and encephalomyelitis form.

Respiratory form of the disease (IBR)

IBR occurs as a subclinical, mild or severe disease. Morbidity approaches 100% and mortality may reach 10%. In mild cases, clinical signs may be limited to a serous nasal and ocular discharge. Classical IBR is characterized by pyrexia (40.5–42°C), inappetence, increased respiratory rate, dyspnea, persistent harsh cough, depression and severe drop in milk production in milking cows. There is bilateral nasal discharge that is initially serous and later muco-purulent. The nasal mucosa is hyperemic and lesions progress from putustular necrosis to large hemorrhagic and ulcerated areas covered by a cream colored diphtheritic membrane (Murphy et al., 1999). If the encrustations are removed, the underlying tissues are hyperemic hence the name ‘red nose’. Foul breath, mouth breathing, salivation and a deep bronchial cough are common. Secondary bacterial or viral infection may lead to an increase in the low mortality rate. Animals may show signs of bronchitis and pneumonitis. Acute uncomplicated cases last 5–10 days and the animals recover rapidly but remain as carriers to shed the virus for a considerable period (Tikoo et al., 1995). On auscultation, tracheitis is evident but lung sounds are normal. About 10% of the affected animals lose body condition and pneumonia is a sequel (Gibbs and Rweyemamu, 1977). Abortion is a consequence of a respiratory BHV-1 infection of a seronegative cow. Following a viremia, BHV-1 crosses the maternal–fetal barrier to produce lethal infection of the fetus (Owen et al., 1964). The route of BHV-1 from the placenta to the fetus is unknown but since viral lesions are consistently observed in the fetal liver, hematogenous spread occurs most likely via the umbilical vein. Although the lesions are observed in the placenta and in several fetal organs, it was suggested that placental degeneration would be secondary to the fetal death induced by BHV-1 (Molello et al., 1966). BHV-1 is one of pathogens involved in the bovine respiratory disease (BRD) complex or shipping fever. Other viruses and bacteria that are involved are bovine virus diarrhea virus, bovine parainfluenzavirus-3, bovine respiratory syncytial virus (BRSV), Mannheimia haemolytica, Pasteurella multocida and Histophilus somnis (Yates, 1982).

Conjunctival form of the disease (IBR)

Cattle with typical IBR show conjunctivitis, which is either unilateral or bilateral and associated with profuse lacrimation. The affected animals show photophobia. Epiphora is the most characteristic symptom and the hair beneath the eye becomes heavily soiled. Eversion of the eyelids may occur. Secondary bacterial infection is common and pus may be seen in the lacrimal discharge. The cornea is usually unaffected but where there is secondary bacterial infection, keratitis and corneal ulceration occur with permanent scarring of cornea (Murphy et al., 1999; Turin and Russo, 2003). In uncomplicated cases, the symptoms regress within 5–10 days. This form may appear along with the respiratory form.
**Genital form of the disease (IPV and IPB)**

Acute IPV usually develops 1–3 days after mating and frequent micturition and tail swishing are characteristic signs noticed initially. Affected animals develop fever, depression and anorexia; they seek to avoid contact of the tail with the vulva. The vulva is swollen and hyperemic with small pustules (1–2 mm in diameter). The pustules usually coalesce to form yellowish white fibrinous membranes that gradually detach to form ulcers. Secondary bacterial infection is common and varying amounts of pus are discharged. The lesions usually heal 10–14 days after the onset of the disease but in some animals purulent vaginal discharge persists for several weeks (Turin and Russo, 2003). IPB also develops after an incubation period of 1–3 days. Lesions similar to those of IPV develop on the mucosa of the penis and prepuce. Secondary bacterial infection is common. If mating is continued, scar tissue may form. The sequelae of this condition include extensive adhesions, annular constrictions and penile distortions. Healing occurs in uncomplicated cases within 10–14 days but some animals may lose libido, have painful erection and ejaculation and require several weeks to resume regular mating. Mild or subclinical forms of IPV/IPB are also common (Pastoret et al., 1982; Tikoo et al., 1995).

Semen contaminated with BHV-1 can cause IPV, cervicitis with copious mucopurulent discharge and endometritis in cows and epididymitis in bulls. BHV-1 is also associated with poor semen quality. Abortions due to BHV-1 have been reported in the USA, India, Argentina, Italy, Japan, Belgium, China and Canada (Edwards et al., 1990, 1991; Galiero et al., 2001; Nandi et al., 2008a, b; Yan et al., 2008). Abortions usually occur within 4–7 months of gestation after natural infection or vaccination. The placenta is often retained and traction may be required to remove it. The cotyledons are usually blanched and degenerate without any gross lesions on the aborted fetus. The abortion is due to the death of the fetus. Endometritis, poor conception rate and a short estrus can occur after insemination with infected semen (Parsonson and Snowdon, 1975; Miller and Vander, 1984; Miller et al., 1991).

**Encephalitic form of the disease**

Affected cattle show incoordination initially, which progresses to ataxia. There is depression followed by excitement characterized by inordinate running, tremor, cycling and terminating in stumbling and falling. In the fallen position they develop clonic spasms of the legs, neck and lumbar muscles and show opisthotonus. Coma and death usually occur within 4 days from the onset of the neurological disorders. Some animals recover but are blind (Gibbs and Rweyemamu, 1977; Schudel et al., 1986). Outbreaks of encephalitis caused by neuro-invasive variant BHV-5 have been reported from Australia, Canada, Argentina and Hungary (Straub, 1990) and the free movement of animals between European states has greatly increased the risk of BHV-5 transmission.

**Transmission**

The main sources of infection are nasal exudates and cough droplets, genital secretions, semen, fetal fluids and tissues. The virus may survive up to 1 year in semen frozen at −196°C. All the animals of a herd inside a building can become infected within a short time by aerosol transmission. Animals that excrete virus from the vagina or prepuce (IPV or IPB) transmit the virus less efficiently and only a limited number of animals become infected (Gibbs and Rweyemamu, 1977; Edwards et al., 1991). Virus can be transmitted by semen during natural service or AI and cattle infected by AI can transmit the virus to cattle in contact (Van Oirschot, 1995). The virus survives well enough in the atmosphere for airborne transmission to occur. Survival is optimal at low temperature and a high relative humidity. The virus is also transmitted through inanimate objects. In the USA, BHV-1 has been isolated from ticks (Ornithodorus coriaceus) that fed on animals positive for BHV-1. Ticks are mechanical transmitters of the virus, although multiplication of the virus in the tick might also occur. Transmission by ticks occurs when they feed on animals during the first stage of the disease when the virus is present in the macrophages and monocytes (Straub, 1990).

During acute primary infection, BHV-1 is excreted in the nasal fluids at concentrations up to 10^10 50% TCID50 ml⁻¹. The virus is excreted over a period of 10–17 days with a peak at 4–6 days post infection (p.i.) (Straub, 1990). Cattle infected with BHV-1 subtype 1 excrete much higher titers of virus (10–100 times) in nasal fluids than do cattle infected with BHV-1.2b and are therefore more likely to transmit infection to animals than are cattle infected with BHV-1.2b (Edwards et al., 1991). Bulls can start shedding BHV-1 from the prepuce between 2 and 7 days after primary preputial infection. The pattern of virus shedding can vary considerably and shedding may last for several days to several weeks (Snowdon, 1965). Bulls can excrete high concentrations of the virus up to 10^8 TCID50 ml⁻¹ in semen. After the primary phase of infection, intermittent virus shedding has been observed over years and latently infected bulls can reexcrete the virus after natural or artificial stress (Bitsch, 1973; Pastoret et al., 1982). The amount of virus excreted is considerably lower than during the primary phase of infection.

Semen is probably contaminated with BHV-1 that replicates in the mucosa of the prepuce and the penis, rather than with virus produced in the testis, epididymis or accessory glands (Snowdon, 1965). The virus excretion
profile in semen varies from bull to bull and depends on the route of infection, viral and host factors such as virulence, tissue affinity, infecting dose, age and genetic make up of the animal. Cows infected with BHV-1 intravaginally can excrete virus at concentrations of $10^{11}$ TCID$_{50}$ ml$^{-1}$ in vaginal secretions (Straub, 1990). In acute IPV, the vaginal fluid contains virus titers of the order of $8.0 \log_{10}$ TCID$_{50}$ ml$^{-1}$ and virus is excreted 4–5 days after onset of symptoms. In acute IPB, bulls excrete the virus over a long period at a titer of about $4.0 \log_{10}$ TCID$_{50}$ ml$^{-1}$ in preputial washing. The titer of the virus in the tissues of the aborted fetus ranges from 3.5 to $6.0 \log_{10}$ TCID$_{50}$ g$^{-1}$. The villi of the cotyledons may contain up to $7.5 \log_{10}$ TCID$_{50}$ g$^{-1}$ of tissue (Wentink et al., 1993). Latently infected animals excrete BHV-1 in nasal or vaginal secretions during the time of recrudescence up to $10^6$ TCID$_{50}$ ml$^{-1}$. Vaccinated cattle can also excrete the BHV-1 virus after exposure to infection but at a lower level and for a shorter period of time than do unvaccinated animals (Frerichs et al., 1982; Wentink et al., 1990).

**Latency**

The BHV-1 virus can become latent following a primary infection with a field isolate or vaccination with an attenuated strain. Latency is believed to develop in almost all animals that are infected with high or low doses of attenuated or virulent BHV-1 (Pastoret et al., 1982). The viral genomic DNA is usually detectable in the sensory ganglia of the trigeminal nerve in IBR (Homan and Easterday, 1980) and in sacral spinal ganglia in IPV/IPB cases. Latency may also occur in tonsillar lymphoid cells and peripheral blood lymphocytes (Ackermann and Wyler, 1984). Transportation of cattle with latent infection can reactivate the virus, resulting in re-excretion of virus and a rise in neutralizing antibodies. 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 (Jones et al., 2000). Vaccination in latently infected animals also does not prevent re-excretion of a wild strain. The vaccine virus and the field isolates can be excreted after live virus vaccination and subsequent challenge with field strains. Colostral anti-BHV-1 antibodies do not prevent initial virus replication in calves and latency can persist after the decline in colostral immunity and the calves are seronegative (Homan and Easterday, 1983). Latent virus produced only latency related proteins that protect latently infected cells from apoptosis. Infectious virus is not produced during latent infection (Rock, 1994; Engels and Ackermann, 1996).

After clinical or subclinical infection of the respiratory tract, BHV-1 spreads along the nerves to the trigeminal ganglia where the viral DNA may remain latent. In herpesvirus infection, the site of latency is the local sensory ganglion. However, virus can be distributed throughout the body following intranasal (IN) infection and can establish latency in more distant ganglia (Winkler et al., 2000). After intravenous infection of calves with BHV-1, virus could be isolated from the prepuce 3–4 months later following dexamethasone treatment (Grom et al., 2006). Varying virus titers ranging from $10^3$ to $10^6$ TCID$_{50}$ ml$^{-1}$ during reactivation and re-excretion have been reported after stress (Straub, 1990; Pastoret et al., 1982), transport (Thiry et al., 1987), superinfection with parainfluenza-3 (PI-3) virus (Mensik et al., 1976) or *Dictyocaulus viviparous* (Msolla et al., 1983), parturition, treatment with dexamethasone and adreno cortico tropic hormone (ACTH), and uptake of three methylindole. Spontaneous reactivation without clinical signs has also been observed at irregular interval (Schultz et al., 1977; Grom et al., 2006).

Intravaginal infection also resulted in latency of BHV-1 in the sacral ganglia (Ackermann and Wyler, 1984) and shedding of BHV-1 from vaginal and nasal fluids after stress or corticosteroid treatment. Bulls once infected are considered as lifelong potential shedders of BHV-1 (Goffaux et al., 1976). Spontaneous re-excretion from the prepuce, vagina and nasal mucosa of cattle has been reported up to 578 days after experimental intrapreputial, intravaginal and IN infections (Rock, 1994; Schyns et al., 2003). Latently infected animals have low levels of circulating antibodies for BHV-1 after infection with an IPV strain (Gibbs and Rweyemamu, 1977; Straub, 1990) or after vaccination with a temperature sensitive (ts) mutant of BHV-1 (Pastoret et al., 1982).

Instead of serological tests, delayed type hypersensitivity is a better method of detection of latently infected animals especially in calves with maternal antibodies. The lymphocyte stimulation test has not been reliable for the detection of latently infected cells (Thiry et al., 1987). Viral DNA can be shown by *in situ* hybridization in the nuclei of the local sensory ganglia (Ackermann and Wyler, 1984). Additional sites of latency in the lymph nodes and nasal mucosa have been determined for BHV-1 by PCR (Van Engelenburg et al., 1994). In the process of reactivation, latent viral genomes enter a new lytic cycle and productive viral gene expression is readily detected in sensory neurons; viral enzymes such as TK, may supply metabolic functions normally down-regulated in neurons (Rock, 1994), open reading frame-E (ORF-E) and latency related (LR) gene expression decrease dramatically and infectious virus is secreted from nasal or ocular swabs (Jones et al., 2000, 2006).

**Pathogenesis**

Under natural condition, bulls are infected via the IN or genital route. There is circumstantial evidence that, after natural respiratory infection, BHV-1 is also excreted in semen. After experimental IN and genital infection of
bulls, virus has been isolated from preputial washing for 2–10 days from the distal urethra but not from the proximal urethra, accessory glands, epididymis or testicles (Engels and Ackermann, 1996). The virus multiplies in the respiratory tract and causes inflammatory changes such as rhinitis, laryngitis and tracheitis leading to destruction of the tracheal microvilli. Infection of cattle with BHV-1 impairs resistance to secondary bacterial infection such as *M. haemolytica*, *P. multocida* and *H. somnis*, leading to fatality and depression of cell-mediated immunity (CMI; Yates, 1982; Leite et al., 2002).

Lesions may extend from the nasal tract to the eyes through the nasolacrimal duct and may give rise to conjunctivitis and nasal discharge. The virus may enter the brain tissues from the nasal mucosa via the trigeminal nerves causing meningo-encephalitis. The virus causes changes in the placenta and fetus resulting in abortion. The virus may occasionally cause a systemic form of disease with high mortality rate in young calves. Following IN inoculation of calves with BHV-1, the virus was isolated from the prepucce but there were no signs of balanoposthitis (Brake and Studdert, 1985; Turin and Russo, 2003; Ackermann and Engels, 2005).

Viremia in BHV-1 infection can barely be demonstrated. Nyaga and McKercher (1980) have shown by *in vitro* examinations that BHV-1 can infect blood monocytes, where a limited virus replication and release is possible. In addition, BHV-1 is able to adsorb to lymphocytes which also may serve as vehicles, at least as long as no neutralizing antibodies are present. In alphaherpesvirus infections, systemic spread is achieved by invasion of lymph nodes and lymph vessels, followed by a lymphocyte-associated virematia. During the initial replication at the portal of entry the herpes viruses may enter the axons of local nerve cells. Then, by intra-axonal transport, the viruses reach the neuron bodies in the regional ganglia, where latency can be established.

Viral entry into cells is a multi-step process involving several glycoproteins and at least two cellular receptors (Mettenleiter, 1994). Glycoprotein gC of alphaherpesviruses initiates these steps by binding to heparan sulfate proteoglycans on the cell surface. These receptor molecules are present on many cells, thus allowing adsorption of herpesviruses to a variety of different cell types. The binding of gC to heparan sulfate moieties leads to a loose attachment that is followed by fixed binding of gD to the putative second cellular receptor. Binding of gD is necessary for initiation of viral entry (Karger et al., 1995) and for steps between virus binding and membrane fusion by interacting with other cellular or viral components. Viral entry into the cell is finally mediated by fusion of the viral envelope with the cell membrane, due to interactions of gB, gH and gL (Liang et al., 1995). The BHV-5 gE is involved in neural spread and neurovirulence within the central nervous system and cannot be substituted by BHV-1 gE. However, BHV-5 gE is not essential for the initial entry into the olfactory pathway (Chowdhury et al., 2000). After recovery, BHV-1 establishes a state of latency primarily in the trigeminal ganglionic neurons and in some cases in the germinal center of the pharyngeal tonsils. Reactivation from latency occurs through a complex and largely unknown mechanism initiated by natural or artificial stress factors (Engels and Ackermann, 1996; Winkler et al., 2000).

### Histopathology

The gross pathology of IBR in a typical but uncomplicated case is characterized by serous rhinitis with hyperemia and edema of the mucosa. The lesions extend to the trachea and sinuses if complicated with secondary bacterial infections. The nasal catarrh is copious and the nasolabium becomes excoriated. The mucosa in the sinuses may develop cattahal purulent exudates and in some case croupous pseudomembrane in the nasal cavity that subsequently detaches to expose an ulcerated area. A purulent inflammation of the larynx and trachea can occur and is associated with edema of the larynx and hemorrhages of the trachea. Pneumonia is a complication and not part of the primary disease.

Histological changes in uncomplicated cases include acute catarrhal inflammation. The mucosa is edematous containing emigrating neutrophils while the submucosa is infiltrated with lymphocytes, macrophages and plasma cells. Intranuclear Cowdry type A inclusion may be present in the epithelial cells during the first few day of infection (Gibbs and Rweyemamu, 1977; Murphy et al., 1999). The gross pathology in IPV includes hyperemia of the vulval and vaginal mucosa with focal hemorrhages over the lymphocytic follicles of the submucosa. There is edema of the vulva with copious mucopurulent discharge. Small (2–3 mm) yellow colored pock-like lesions replace the focal hemorrhages over the lymphoid follicles. The epithelium over the lesions is lost and an ulcer is revealed. Microscopically, there is a ballooning degeneration of the epithelium and Cowdry type A intranuclear inclusion bodies are seen (Engels and Ackermann, 1996). The epithelial disruption and ulceration are due to infiltration of neutrophils. The lamina propria is acutely inflamed and infiltrated with numerous plasma cells. The lesions regress within 8 days.

The pathology of IPB is similar to that for IPV. There is widespread necrosis with little inflammatory reaction in the aborted fetus. There are hemorrhages in brain, kidney, adrenal cortex and lymph nodes. Intranuclear inclusion bodies are found in affected cells (Murphy et al., 1999). There is usually little gross pathology associated with encephalitis caused by BHV-1. Histologically, intranuclear inclusion bodies in the astrocytes and neurons and perivascular cuffing in the cerebrum are found throughout the brain. Diffuse areas of degeneration of the cerebral cortex with vacuolation around the neurons are also evident.
Immunosuppression

BHV-1 causes broad immuosuppression in infected cattle, leading to susceptibility to secondary viral and bacterial infections. There is impairment of function of macrophages, PMNs and lymphocytes (Tikoo et al., 1995), decreased expression of interleukin-2 (IL-2) receptor, decreased mitogenic stimulation of peripheral blood mononuclear cells (PBMCs) and a reduced number of circulating T cells (Winkler et al., 1999). There is impaired phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC) and T cell stimulation due to infection of monocytes and macrophages. The effect of immunosuppression is partly mediated by the BHV-1 gG, a broad-spectrum chemokine binding protein that blocks chemokine binding and activity. The virus also infects CD4+ T cells inducing a loss of CD4 expression followed by apoptosis of these cells (Babiuk et al., 1996; Winkler et al., 1999). BHV-1 is known to down-regulate the expression of MHC class I molecules on the surface of infected cells (Hariharan et al., 1993). It probably interferes with the protective function of CD8+ cytotoxic T lymphocytes (CTLs), which are involved in lysis of BHV-1-infected cells (Denis et al., 1993). Other effector mechanisms are likely to play a role: CD4+-mediated CTL activity has been proposed as a strategy aimed at providing cellular protection in BHV-1 infection. Stimulation of PBMCs of cattle immunized with attenuated live BHV-1 has shown that cytolytic effectors, primarily CD4+ stimulated CD8+ T lymphocytes, are able to lyse macrophages infected with BHV-1 (Hariharan et al., 1993).

Laboratory diagnosis

BHV-1 infections can be diagnosed on the basis of serologic tests that detect virus or virus components, or antibody and by nucleic acid-based tests that detect genomic DNA by PCR, nucleic acid hybridization and sequencing.

Isolation of the virus in cell culture

The 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) cells. However, primary cell culture is more sensitive. The virus can be isolated from nasal swabs, conjunctival swabs, vaginal swabs, preputial washing, placental cotyledons of aborted fetus, fetal liver, lung, spleen, kidney, lymph node, mucous membrane of respiratory tract, tonsils and lungs collected in virus transport medium (Homan and Easterday, 1980). Raw or frozen semen with preservatives may also be collected for virus isolation (Snowdon, 1965). The swabs and other clinical materials are processed and passaged in cell culture.

The presence of virus in specimens is detected by a cytopathic effect (CPE). The CPE of BHV-1 is characteristic and usually appears within 3 days after inoculation. There are grape-like clusters of rounded cells present around a microplaque in cell culture. 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 is passaged at least 3 times before the sample is considered negative (Straub, 1990; Turin and Russo, 2003). Only one infective particle of BHV-1 is necessary to produce one plaque. IFN-γ (interferon-γ) plays an important role in the generation of non-MHC-restricted cytotoxic responses of cattle to BHV-1, which represents the most apparent cellular-mediated immune response during BHV-1 infection (Campos et al., 1989). The IFN-γ, by enhancing T cell-mediated clearance mechanisms, might be a critical mediator of immunity to BHV-1. IFN-γ should be considered in terms of the major and compensatory mechanisms available to the body to affect immunity to viral infection. α- and β-IFN inhibit the induction of TK enzyme activity in vitro (Turin et al., 1999).

Histopathology

Intranuclear viral inclusions of Cowdry type A can occasionally be identified in the epithelial cells of vaginal biopsy tissues collected in the early stage of IPV but not in
cells collected in nasal discharge of cattle with IBR. These inclusions are also present in the brain from cases of encephalitis and in tissues of aborted fetuses. As these inclusions are transitory, the use of histological examination for diagnosis is of limited value (Turin and Russo, 2003).

**Electron microscopy**

The use of electron microscopy to identify virus particles in clinical material has been a rapid method for the diagnosis of BHV-1. It can be used to identify the virus in the early stage of the disease but it must be considered provisional as all herpesviruses are indistinguishable by electron microscopy. It cannot be used routinely due to non-availability of an electron microscope in the small laboratory. There is therefore greater reliance on serological or other tests (Gibbs and Rweyemamu, 1977).

**PCR**

PCR is more sensitive than virus isolation and is a practical alternative for rapid detection of the virus. The result is available within 12 h compared to virus isolation which requires 7 days. Virus can be detected in nasal swabs up to 14 days after experimental infection. PCR can also detect virus in bovine fetal serum and semen samples and has equivalent sensitivity to dot-blot hybridization (Fuchs et al., 1999). The PCR technique detects positive semen samples at a frequency 5 times that of virus isolation in cell culture (Xia et al., 1995). However, extreme precautions should be taken to avoid false positive and false negative results (Rocha et al., 1998; Tiwari et al., 2000; Schyns et al., 2001; Nandi et al., 2008a, b). The primer set (forward 5'-CAC GGA CCT GGT GGA CAA GAA G-3' and reverse 5'-CTA CCG TCA CGT GAG TGG TAC G-3') used are based on the sequences of the gI glycoprotein of BHV-1 and produce a PCR product of 468 bp (Nandi et al., 2008a, b). Real-time PCR provides satisfactory reproducibility as well as high specificity and sensitivity, in combination with significant reduction of time for detecting amplified products, making it a valuable alternative to the time and labor consuming virus isolation for detection of BHV-1 in extended semen (Jianning et al., 2008).

**Serology**

A battery of serological tests is available for detection of antibody and a rise in titer between the acute and convalescent phases of infection. The immune response to primary BHV-1 infection in experimentally inoculated cattle is characterized by the development of specific IgM and IgG antibodies at 7 days post inoculation. Secondary immune responses are characterized by development of IgG 2 antibody (Turin et al., 1999). Paired serum samples will indicate recent infection as it usually takes 2–3 weeks to develop antibody response; this is extremely useful in diagnosing the disease. A variety of ELISAs namely indirect ELISA, c-ELISA and avidin–biotin ELISA have been employed to screen serum samples of cattle and buffaloes in India (Nandi et al., 2004, 2007). The indirect ELISA kit uses density gradient purified virus and detects antibodies to envelope protein and capsid protein whereas the c-ELISA is based on the monoclonal antibodies to gB of BHV-1. A combination of different immunogenic surface glycoproteins is targeted in the indirect ELISA, whereas a single protein is targeted in the c-ELISA.

Both virus neutralization test (VNT) and ELISA have been used for the detection of antibodies against BHV-1 infection. The ELISA is a specific, sensitive and practical test for the detection of antibody and has advantages over the VNT (Kaashoek et al., 1995; Van Oirschot et al., 1997; Nandi et al., 2008a, b). The IgM ELISA is useful for diagnosis of recently infected calves. The detection of latent BHV-1 infection in cattle is important in control programs and in international trade activities. So, the ELISA test must be highly sensitive (100% sensitivity) to detect the low level of antibodies in the serum. Both c-ELISA and indirect ELISA have been carried out and provided qualitative not quantitative results. The primary infection stimulates a strong humoral and cell-mediated immune response in the host. In the BHV-1 infection, the cell-mediated immune response is first detected at about 5 days p.i. and peaks at approximately 8–10 p.i. Neutralizing antibodies, mainly of the IgG followed by the IgG class, are usually detected around 7 days after infection (Turin and Russo, 2003). A commercial anti-BHV-1 blocking ELISA kit is available to differentiate between vaccinated and naturally infected cattle but the sensitivity is only 74% (Turin and Russo, 2003). Bulk tank milk testing for BHV-1 antibodies may be useful in eradication and monitoring programs (Yan et al., 2008). If BHV-1 is detected in the bulk milk there is a high probability that more than one animal in a herd is infected and that the infection has spread.

**Vaccines**

There are four kinds of vaccines namely modified live virus (MLV) vaccines, inactivated vaccines, subunit vaccines and marker vaccines that are available to be used in cattle against BHV-1 infections.

**MLV vaccines**

There are three MLV vaccines that are available. One is intended for parenteral use and is of bovine fetal kidney
tissue culture origin; one is an IN vaccine of rabbit tissue culture origin; and the third is of bovine tissue culture origin containing a ts mutant of BHV-1 that is administered by the IN route. The live vaccine strains can be differentiated from the field strains by digestion with restriction endonucleases. The MLV vaccines induce a rapid immune response, long lasting immunity, and result in local and mucosal immunity (Whetstone et al., 1986). However, MLV vaccines are potentially abortigenic and cannot be used in non-immune pregnant cattle and may develop the latent state that may lead to shedding of the vaccine virus.

Following IN or intramuscular (IM) vaccination with MLV vaccines, animals have been protected from infection and disease within 40–96 h due to induction of IFN locally (Endsley et al., 2002). Besides, IN vaccination also induces secretory IgA and CMI (Frerichs et al., 1982; Castrucci et al., 2004). The parenteral and IN vaccines stimulate the production of humoral antibody but the parenteral vaccine of bovine tissue culture origin is abortigenic especially in non-immune cows, whereas the IN vaccine is safe for use in pregnant cows and is highly effective for the prevention of abortion due to the virus. The IN vaccine also induces protection following challenge against respiratory disease 72 h after vaccination and can be used in the face of an outbreak where all in-contact animals are vaccinated in an attempt to reduce the number of new cases. The ts mutant cannot replicate at the body temperature of the animal.

**Inactivated vaccines**

Inactivated vaccines have been developed because of the disadvantages of MLV vaccines. They do not cause immunosuppression, abortion or latency although they do not prevent the development of latency following exposure to field virus. They are safe in pregnant animals, stable in storage and do not cause the shedding of virus. However, inactivated vaccines are not as efficacious as MLV vaccines because of potential destruction of some of the protective antigen during the inactivation process by alkylating agents and there is a need to add an adjuvant (Johannes et al., 2004). Usually two doses are administered at an interval of 10–14 days and protection is observed 7–10 days following the second dose (Patel, 2005). Due to potential disadvantages of MLV vaccines and inactivated vaccines, genetically engineered attenuated vaccines as well as subunit vaccines have been developed employing the advantages of molecular biology and protein purification techniques (Frerichs et al., 1982; Castrucci et al., 2002).

**Subunit vaccines**

A subunit vaccine contains one or more of the antigens of the virus necessary to evoke protective immunity and lacks nucleic acid and other components that might cause unwanted side effects (Brun et al., 1988). In BHV-1, gB, gC and gD, glycoproteins are immunogenic and are separated from the virus-infected cells or the peptide is synthesized. Animals immunized with these proteins develop high levels of antibody and are protected from experimental challenge. The level of immunity is based on serum antibody titers and protection against experimental challenge is greater with the individual glycoproteins than with commercially available inactivated vaccines (Babiuk et al., 1987).

An experimental subunit vaccine containing truncated BHV-1 glycoprotein protected calves vaccinated at 3 and 7 or 6 and 7 months of age from experimental aerosol infection with BHV-1, 12 days after the second vaccination. A low level of maternal antibody did not interfere with the antibody response of the calves. Another subunit BHV-1 vaccine containing only gD glycoprotein along with recombinant M. haemolytica vaccine was reported to be superior to MLV BHV-1 vaccine in reducing mortality due to respiratory disease. To produce a glycoprotein-based subunit vaccine, a truncated secreted version of glycoprotein (gB, gD and gE) can be constructed and expressed in MDBK cells under the regulation of bovine heat shock 70A (hsp70) gene promoter (van Drunen Littel-van den Hurk, 2006). The subunit vaccines primarily elicit Th2 response, while DNA immunization with plasmids encoding single glycoprotein stimulates Th1 responses (Cox et al., 1993).

**Marker vaccines**

A marker vaccine is based on changes in one or more microbial proteins, which allow the differentiation of vaccinated animals from infected animals. The test for infected cattle detects antibodies against a glycoprotein that is lacking in the vaccine. Mutants of BHV-1 have been developed by deleting one or more of the non-essential glycoproteins. A TK negative gC deletion mutant protected calves against disease and reduced shedding of the virus following challenge. A double vaccination with a killed gE-negative vaccine also gave similar results. IN vaccination of animals with a live gE-negative vaccine and double vaccination with subunit gD BHV-1 reduced the challenge virus replication and excretion (Kaashoek et al., 1995; Van Oirschot et al., 1997). Calves immunized with a full length gD or a truncated secreted form of gD (tgD) developed higher neutralizing antibodies in serum and nasal mucosa than animals vaccinated with an inactivated or an MLV vaccine (Lehmann et al., 2002). There was minimum weight loss and reduced virus shedding in the vaccinated animals. Vaccination of animals with a live or killed gE-deleted marker vaccine induced both humoral and cell-mediated immune responses against BHV-1 and prevented the spread of BHV-1 (Kaashoek et al., 1995). The live vaccine is safe in breeding cows, bulls and
pregnant cows. It is also efficacious in the presence of maternal antibody. However, the inactivated gE-negative BHV-1 vaccine causes slight decrease in milk production after double vaccination (Kaashoek et al., 1995).

**Combined or multivalent vaccines**

In countries such as the USA, Australia and the European countries, multivalent vaccines containing parainfluenza-3 (PI-3) virus, BRSV and bovine viral diarrhea (BVD) virus (Castrucci et al., 2002) are most frequently used for control of diseases caused by BHV-1. Antibody responses to BHV-1 are higher in calves vaccinated with MLV vaccines than in those vaccinated with inactivated vaccines. Following single vaccination, there are no differences in the seroconversion rates and titers to BHV-1 between IN and IM MLV vaccines. However, both seroconversion rates and changes in titers to the virus are higher in calves vaccinated with the IM compared to those vaccinated with the IN vaccine (Frerichs et al., 1982). Some genetically modified BHV-1 strains containing genes coding for biologically active cytokines such as IFN-γ or ILs display immunomodulating properties (Campos et al., 1989). These engineered strains have not been more effective and protective than negative marker vaccines. Further, negative marker vaccines do not impair the ability of the virus to establish latency and undergo reactivation. Vaccine strains can be excreted both in naïve and passively immunized calves (Reddy et al., 1993; Babiuk et al., 1996).

Recombinant BHV-1 expressing BVD virus E2 protein or BRSV G protein or PPRV B and C glycoprotein or gB, gC, gD and gl in combination induce a protective immune response in cattle (Thiry et al., 2006). Due to the poor antigenicity displayed by the subunit vaccines or epitope-based vaccines; a carrier molecule is required to stimulate adequately the immune response. Bovine heat shock protein gp96, conjugated in vitro with BHV-1 epitopes elicited cytotoxic T cells and antibody response in a mouse model (Reddy et al., 1993; Gerds et al., 2002). Human adenoviruses type 3 and 5 expressing the BHV-1 gC or gD induce high levels of neutralizing antibody in a rabbit model after IN or IM administration and in cattle after IN/intratracheal or subcutaneous inoculation. These vaccines display a high degree of biosafety due to the use of replication incompetent adenoviruses. Adenoviruses are also excellent vectors because of the absence of pre-existing anti-adenovirus neutralizing antibody in cattle; and these can be administered by the IN route mimicking the natural antigen presentation in the airway mucosal as happens in BHV-1 infection (Gupta et al., 2001).

**Conclusion**

BHV-1 is a successful herpesvirus as it adapts to its hosts and follows a strategy of infection, a mild disease course and life long reservoirs of virus in latent infections. It is an example of intricate virus–host interaction and the cause of a major disease affecting livestock. Although the infection is infrequently life threatening, the introduction of BHV-1 into a cattle farm can cause severe economic losses due to production losses and restrictions in the international trade of livestock. In India, the disease is highly endemic and cases are detected on the basis of sero-surveillance or virus isolation. The picture of BHV-1 infection in cattle has been further complicated due to the establishment of latency. During latency, virus is secured from the immune response by the host animal. Studies are needed to elucidate the mechanism of establishment of latency by BHV-1 virus and the interaction between the virus protein and host protein; information from such studies may enhance management of latent infections.

In India, AI is largely practiced to improve the indigenous germplasm with exotic ones; however, the virus can be transmitted to cattle at distant locations through semen. Recently, the Government of India has taken several steps to screen all the semen samples of bulls for the presence of organisms causing sexually transmitted diseases including BHV-1 before permitting its use in AI. Although many vaccines are available to control BHV-1 infection in cattle, each has disadvantages. It is difficult to differentiate the animals vaccinated with conventional vaccines and animals with infection. So, the marker vaccines and subunit vaccines offer the ability
to monitor the herd and are critical for trade purposes. Marker vaccines can be genetically engineered gene deleted, subunit or vectored vaccine and the marker protein needs to be present in all wild virus strain and to induce a long lasting immune response. New research on vaccines may be the means to control the disease more effectively.

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References


