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Bovine Alphaherpesvirus-1 (BoHV-1) Infection in Cattle: An Overview of Epidemiology, Role of Envelope Proteins in Disease and Control

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ABSTRACT

In the past decade, substantial progress has been made towards the evaluation of various antigenic proteins from Bovine alphaherpesvirus-1 (BoHV-1) that can likely be targeted for designing of a suitable control strategy. Growing knowledge on the immunology of the host systems has been highly useful in the assessment of antigenic epitopes from the virus and its receptors on the host. BoHV-1 are enveloped double stranded DNA viruses studded with a dozen (gB, gC, gD, gE, gG, gI, gH, gK, gL, gM, UL49.5 and US9) of protein molecules on their surface where, ten are glycosylated and two are non-glycosylated. Each of these proteins are known to play a role in various functions such as cell attachment, entry, viral multiplication, cell to cell movement, packaging, egress of cell, retrograde and anterograde movement of virus. Knowledge about the properties and function of envelope proteins helps to explore the right protein for in-house vaccine or diagnostic test development. Overall, the review provides a comprehensive overview of the various aspects of Bovine alphaherpes infection including the epidemiology, envelope proteins involved in enforcement of infection, vaccines and methods of detection.

Key words: BoHV-1, Envelope proteins, gB, gC, gD, gE, Negative mutants, Prevalence.

Bovine alphaherpes virus-1 (BoHV-1) is a highly contagious virus that affects cattle worldwide. BoHV-1 is classified under order *Herpesvirales,* family *Herpesviridae,* subfamily *Alpha herpesvirinae* and genus Varicello virus. Three known subtypes of virus are BoHV-1.1, BoHV-1.2a and BoHV-1.2b (Biswas *et al*., 2013). BoHV-1.1 is responsible for a wide variety of clinical diseases, including conjunctivitis of one or both the eye, upper respiratory tract infection known as infectious bovine rhinotracheitis (IBR), reproductive tract lesions in cows, infectious pustular vulvovaginitis (IPV) and bulls infectious balanoposthitis (IBP) and new-born systemic infection (Jones and Chowdhury 2007; Tikoo, 1995). Latent infections and bovine respiratory disease (BRD) complex are the known sequelae of infection. Although economic loss is not well documented in India, these infections are known to lead economic downward spiral with losses up to over 3 billion dollars annually in the global livestock industry (Chen *et al*., 2018).

History

The oldest recorded case of BoHV-1 producing illness was described in Germany, in 1841 as a venereal disease in bulls and the cows that were in contact with them. This condition was described as Blaschenausschlag in German literature (Rychner *et al*., 1841). Reisinger and Reimann established the disease's viral origin in 1928. Schroeder and Moy's published the first report on respiratory IBR in 1954; they described a respiratory form of the disease in California milching cattle that was characterised by pyrexia, agalactia and respiratory symptoms. A disease that first manifested in a Colorado feedlot in 1950 and has subsequently spread throughout the state was reported in the following year by Miller (1955). Madin *et al*. (1956) originally isolated the etiological agent for IBR on cell culture

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and the initial live attenuated vaccine was produced around the same time by Kendrick *et al.* (1958). In India Ocular form of IBR was reported for the first time from exotic or crossbred cattle from Uttar Pradesh (Mehrotra *et al*., 1976), since then a substantial number of studies on seroprevalence are being conducted in different parts of country (Nandi *et al*., 2011; Kollannur *et al*., 2014).

Virion morphology

Herpesviruses are large, enveloped, double-stranded DNA viruses. Herpesvirus core contains a linear 136 kbp, double-stranded DNA genome protected by 162 capsomers in an icosahedral symmetry (Engels *et al*., 1987). The diameter of nucleocapsid is 100-110 nm consisting of 150 hexamers and 12 pentamers. A tegument layer made of globular proteins separates outer envelope and the core nucleocapsid. The outer envelope is a lipid bilayer derived from host cell, contain a large number of viral encoded glycoproteins. The size of the mature virion particle varies from 120 to 300 nm in diameter (Thiry *et al*., 2006).

The genes on BoHV-1 is arranged as one unique long unit (UL) and one unique short unit (US) flanked by two inverted repeat sequences, referred as internal repeat and terminal repeat (Fig 1). The UL segment has six glycoproteins, while the US segment contains four (Thiry *et al*., 2006). Three subtype variants of BoHV-1, designated as BoHV-1.1a, BoHV-1.2a and BoHV-1.2b, are described based on genomic DNA restriction endonuclease profiles (Metzler *et al*., 1985). The most prevalent BoHV subtype circulating in India is BoHV-1.1 and all isolated strains have homology to Cooper stain (Chandranaik *et al*., 2010), except in one study only one subtype of BoHV-1.2 from nasal swab was isolated from the aborted animal (Saha *et al*., 2010). Subtype 1 virus isolates are associated with IBR and abortion and are commonly found in North America, South America and Europe. Subtype 2a in Brazil are linked to IBR, IPV IPB and abortions. Respiratory form/IPV/IPB excluding abortion are linked to subtype 2b strains found in Australia or Europe (Righi *et al*., 2023). Los Angeles (LA) and Cooper are the most prevalent BoHV-1 strains in United States and Europe respectively.

Disease transmission and clinical signs

Direct transmission from infected animals can happen by aerosols or contact with their respiratory, ocular, or reproductive tract secretions, which aids in the spread of the disease in cattle herds. Indirect virus transmission can occur through contaminated individuals, objects, semen (Coitus or artificial insemination) and even embryo transfer (Van Engelenburg *et al*., 1995). Infected animals remain carriers for the remainder of their lives. When calves with latent BoHV-1 infection are exposed to natural stresses like transportation, harsh weather, overcrowding, or immunosuppressive medications (such as dexamethasone), the latent virus reactivates and spreads to hosts which are vulnerable to it (Chase *et al*., 2017). IBR is mainly a disease of cattle and buffaloes. Virus is also known to circulate in sheep, goat, horse, pigs, hippopotamus and mithun (Singh *et al*., 2017), yak and camel (Selim *et al*., 2022). Though mortality is less, morbidity is 100%. Anorexia, pyrexia, depression and a copious nasal discharge that is initially serous but later becomes mucopurulent are among the earliest symptoms. The nasal mucosa is hyperaemic and lesions inside the nasal cavity advance from focal necrosis with accompanying purulent inflammation to significant areas of hemorrhagic, congestion, ulcerated mucosa covered by a cream-colored diphtheritic membrane. These lesions may be difficult to see. The breath could smell foul. Deep bronchial coughs, mouth breathing, salivation and dyspnea are frequent symptoms. Acute, uncomplicated forms may last for 5-10 days.

Unilateral or bilateral conjunctivitis, along with profuse lacrimation, could be an exclusive clinical sign in cattle herd with IBR (Mehrotra *et al*.,1976). Gastroenteritis is prominent finding in the generalized disease of neonatal calves, also in adult cattle and could be fatal. Abortion may occur at 4 to 7 months gestation, with no much gross lesions in the foetus. Virus has also been reported to cause mastitis or agalactia. Infectious pustular vulvovaginitis infections are associated with dairy cows. Many a times subclinical cases go unnoticed. Affected cows develop fever, depression, anorexia and stand apart, lifts the tail away from contact with the vulva; micturition is frequent and painful. The vulval

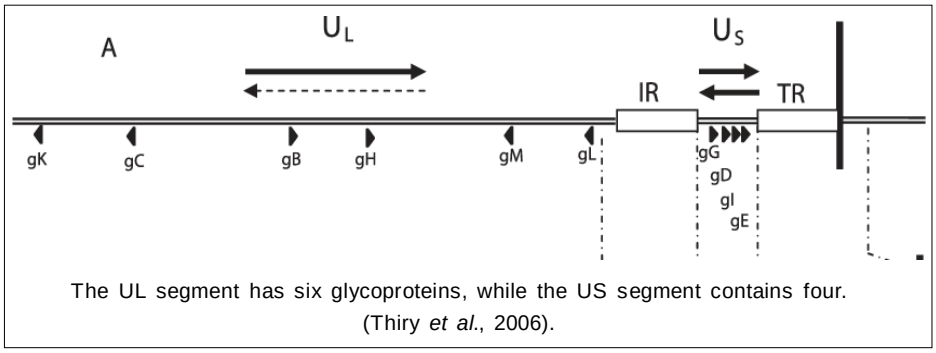

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Fig 1: The genes on BoHV-1 is arranged as one long unique unit (UL) and one short unique unit (US) flanked by two inverted repeat sequences, named internal repeat (IR) and terminal repeat (TR).

labia, vestibular mucosa is reddened, swollen, with many tiny pustules and discharge. Nearby pustules coalesce to form a fibrinous pseudomembrane that covers an ulcerated mucosa (Turin *et al*., 2003). Uncomplicated lesions usually heal 10-14 days.

Infectious Balanoposthitis develops after 1-3 days of infection (Huck *et al*., 1971). Initially Penis and prepuce are reddened with coalescing pustules and discharges. Gradually adhesion, annular constriction and penile distortions may develop. In mild cases healing occurs within 10-14 days, but some animal may lose libido, have painful arousal and ejaculation and difficult to mate with female partner. BoHV-1 reduces the semen quality (Majumder *et al*., 2015). Recovered bull semen may be contaminated with virus because of periodic shedding. However, contaminated semen may not affect pregnancy in cattle by natural service or artificial insemination but animal in later stage develop IPV. Pregnant cows that acquire infection rarely abort. At a given point of time presence of both genital form and respiratory form in a herd is rare event.

Epidemiology

BoHV-1 is a successful pathogen distributed worldwide except few European countries Finland, Switzerland, Austria, Denmark, parts of Italy and Czech Republic (Iscaro *et al*., 2021). The incidence and prevalence of IBR are extremely variable under different geographical location and systems of rearing (Nandi *et al*., 2009). Prevalence of IBR is low in Slovenia 0.1% a due to ongoing eradication programmes (Hostnik *et al*., 2021) and in Spain region highest 99.92% (Dias *et al*., 2013). Pooled seroprevalence of IBR in China as per meta-analysis is 40.00% (Chen *et al*., 2018), prevalence in Colombia is 57.5% (Ortiz *et al*., 2022), Brazil, 71.30 (Abad *et al*., 2016) Mexico 76.32% (Posado *et al*., 2013) and Ethiopia, 25.6 (Tadeg *et al*., 2021).

A number of seroprevalence studies in different parts of India is given in Table 1. Prevalence is ranging from as low as 10.39% in Uttaranchal (Jain *et al*., 2006) to 89.00% in Andaman and Nicobar Islands (Patil *et al*., 2021b). According to prevalence studies, older female cattle were reported be more susceptible than males due to the stress factors associated with parturition and substantial milk production. Many authors have described correlation between age and disease, older animals aged between 24 months to nine years of age are much more susceptible than younger animals due to maternal immunity (Palmer *et al*., 1990; Nyaga and McKercher, 1980). In certain studies, male animals were more susceptible than females. Disease was found to affect exotic and crossbred cattle in India during inception than native breeds. In Slovenia and Colombia HF cow breed (Hostnik *et al*., 2021) and in Nepal's Jersey breed of cattle have shown higher prevalence than other breeds (Sanjay *et al*., 2022). One frequent risk factor reported by many authors is herd size, where the disease was more prevalent in intensive rearing or feed lots or organised farms or farms with more than 10 animals compared to free-range

Table 1: Seroprevalence of BoHV-1 in India.

Author, year	Test used	Sample type	Prevalence	Place
Renukaradhya *et al*., 1996	ELISA	Bovine serum	50.90%	Andra Pradesh, Tamil Naadu,
		Buffalo serum	52.50%	Karnataka
Vaid *et al*., 1991	Passive HA	Bovine serum	64.47%	Himachal
Suresh *et al*., 1992	Passive HA	Bovine serum	33.97%	Tamil Naadu
Pandita and Srivastav, 1995	Dot ELISA	Bovine serum	51.90%	Haryana
Shome *et al*., 1997	AB-ELISA	Bovine serum	89.00%	Andaman and Nicobar Islands
Suresh *et al*., 1999	AB-ELISA	Bovine Serum	38.01%	18 states India
Dhand *et al*., 2002	AB-ELISA	Buffalo serum	28.76%	Punjab
Rajesh *et al*., 2003	AB-ELISA.	Bovine Serum	14.88%	Kerala
Singh *et al*., 2004	ELISA	Bovine Serum	11.82%	Punjab
Deka *et al*., 2005	PCR	Breeding bulls semen	45.09%	India
Jain *et al*., 2006	AB-ELISA	Bovine Serum	10.39%	Uttaranchal
Sharma *et al*., 2006	AB ELISA	Bovine serum	50.00%	Himachal Pradesh
Koppad *et al*., 2007	ELISA	Bovine serum	19.20%	Karnataka
Singh and Yadav, 2010	ELISA	Bovine serum	32.31%.	Uttar Pradesh
Verma *et al*., 2014	ELISA	Bovine serum	46.51%	Uttar Pradesh
		Buffalo serum	35.28%	
Samrat *et al*., 2016	ELISA	Bovine serum	34.69%	Chhattisgarh
Patil *et al*., 2017	AB ELISA	Bovine serum	61.60%.	India
Thakur *et al*., 2017	AB ELISA	Bovine serum	29.03%.	Uttrakhand
Kathiriya *et al*., 2018	ELISA	Bovine serum	36.31%	Gujarat
Patil *et al*., 2021 (a)	ELISA	Bovine serum	29.50%	North East
Patil *et al*., 2021 (b)	ELISA	Bovine serum	25.60%	Andaman and Nicobar
Dharmeshkumar *et al*., 2023	Indirect ELISA	Bovine serum	29.73%	South Gujrat

animals or the unorganised sector (Rajkhowa *et al*., 2004). Studies have also evaluated the possibility of cross-species infections. In this regard, caprine, sheep, yak, mithun, horse, pigs, hippo and mustelid were suggested to be involved in this (Biswas *et al*., 2013).

Viral glycoproteins

Viral glycoproteins are key molecules that play an important role in the interactions between viruses and their host-cells. They are involved in various steps of the viral replication cycle, such as the attachment, penetration, maturation and egress of the virus. Therefore, they constitute an important target for the host immune response. Some glycoproteins have greater immunogenic properties, allowing their use as a component of vaccines and also diagnostic tests. An overview of all the Glycoproteins and their properties are listed in Table 2. The unique long segment of the genome code for six glycoproteins gB (UL27), gC (UL44), gH (UL22), gL (UL1), gK (UL53) and gM (UL10) and one non glycosylated protein UL49.5 while four genes corresponding to gG (US4), gD (US6), gI (US6), gE (US8) and a non-glycosylated protein US9 are grouped in a tandem organisation in the US segment (Barber *et al*., 2017). Although 6 glycoproteins (gC, gB, gD, gH, gK and gL) are involved in viral attachment and entry to host cell four glycoprotein (gB, gD, gH and gL) interactions are must for a virus to gain entry. Based on deletion mutant virus construction studies gC, gI, gE, gG and gM glycoproteins are found to be non-essential. On the contrary, gB or gD are essential for in vitro replication (Kamiyoshi *et al*., 2008). UL 49.5 in Pseudorabies virus is designated as glycoprotein N (gN) due to presence of glycosylation and known to inhibit the transporter associated with antigen processing driven peptide import into the endoplasmic reticulum. The same UL 49.5 is non glycosylated in BoHV-1 and has the same immune evasion function. A modified diagram of BoHV-1 from Barber *et al.* (2017) showing all envelope proteins are depicted in Fig 2.

Table 2: Properties of BoHV-1 glycoproteins.

Envelope protein type	Coding gene	Size (Amino acid)	Mol wt. (K Da)	Functions	Interactions
gD	US 6	417	71	Cell attachment	
gC	UL 44	508	91	Cell attachment	
gB	UL 27	932	130	Cellentry/cell-to-cell spread	
gE	US 8	575	92	Cell-to-cell spread	gI us9
gI	US 7	380	40	Cell-to-cell spread	gE
gK	UL 53	332	35	Infectious virus production and spread	UL20
gH	UL 22	842	108	Cellentry/cell-to-cell spread	gH
gL	UL 1	156	17	Cell entry/cell-to cell spread	gL
gM	UL 10	411	43	Virion morphogenesis/membrane fusion	UL49.5, VP8
gG	US 4	444	47	Cell-to-cell spread	

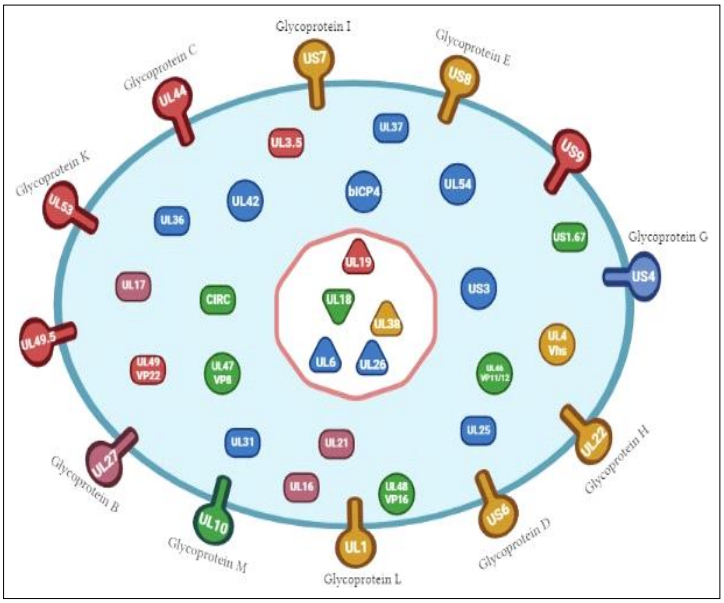

Fig 2: Envelope proteins of BoHV-1.

Glycoprotein D (gD)

This protein is a significant component of BoHV-1 and plays a crucial role in the fusion of the viral envelope and plasma membrane of the host cell. Along with gB and the gH-gL complex, it performs fusion process. gD has been considered as the primary vaccine candidate due to its ability to induce a robust cellular immune response and the highest neutralizing titres of antibodies against it (Dubuisson *et al*., 1992). Glycoprotein D can also be used to distinguish between BoHV-1 and BoHV-5, with 79.9% amino acid identity between the two (Delhon *et al*., 2003). Structurally gD is a type I membrane glycoprotein, coded by US 6 gene with a molecular weight of 71 kDa. In BoHV-1 gD, 28 amino acids of cytoplasmic tail and a hydrophobic transmembrane anchor sequence make up the carboxy-terminus and extracellular domain forms the amino terminus.

Researchers have developed various subunit and viral vector vaccines using gD that could elicit cell-mediated immune response. Subunit gD vaccines have proven to be effective in controlling spread of both BoHV-1 and BoHV-5 infections but are expensive. Recombinant gD expressed in a prokaryotic system had the ability to induce high antibody response but low virus neutralization capacity (Parker *et al*., 1993). Subsequently eukaryotic expression systems such as yeast, mammalian, plant and insect cells, are also tested to develop subunit vaccine incorporating adjuvants like oil based adjuvant, avridine and CpG to lower the cost (Zhu *et al*., 1997; Rankin *et al*., 2002). DNA vaccine and Viral vectored vaccine (Human adeno virus/Bovine adeno virus) encoding gD though induce better cell mediate immune response and mucosal response respectively needs further improvement in eliciting balanced immune response (Dummer *et al*., 2014). PCR targeting conserved region of gD gene (Smits *et al*., 2000; Wiedmann *et al*., 1993) and indirect ELISA based on gD recombinant protein (Ratta *et al*., 2020) could be used to diagnose BoHV -1 infections.

Glycoprotein B

Glycoprotein B (gB) is one of the immunodominant antigens present on the viral envelope and of plasma membrane of infected cells. This protein plays a vital role in the virus attachment to host cell, entry, cell-to-cell movement and fusion (Raaperi *et al*., 2012). Immunity to glycoprotein B develops rapidly after infection and lasts a long time. It also induces a strong neutralizing antibody response (Clinton and Chowdhury, 2010) and is recognized by CD4+ helper T lymphocytes (Hutchings *et al*., 1990). This makes glycoprotein B a potential target for subunit vaccines and diagnostics based on recombinant proteins. The glycoprotein B gene UL27 codes for a polypeptide consisting of 932 amino acids with an unusually long signal sequence, transmembrane sequence and five potential sites for the addition of N-linked oligosaccharides (Misra *et al*., 1988). This gene has sufficient variability to produce high-resolution phylogenetic trees and helps to classify the virus into different groups, providing more insight into its transmission and distribution (Surendra *et al*., 2015).

Amplification and sequencing of the gB gene by employing PCR to diagnose and also to understand the molecular epidemiology has been followed in India as well as worldwide (Patil *et al*., 2006; Patil *et al*., 2012; Chandranaik *et al*., 2014; Sobhy *et al*., 2014; Surendra *et al*., 2015; Chandranaik *et al*., 2016). The phylogenetic analysis of the gB gene sequences of BoHV-1 isolates by Patil *et al.* (2016), revealed the dominance of subtype 1.1 from India. The gB specific ELISAs are more sensitive for the detection of serum antibodies. Indirect ELISAs and gB blocking ELISAs have a highly comparable sensitivity and specificity (Beer *et al*., 2003).

Glycoprotein C (gC)

One of the glycoproteins involved in virus entry is glycoprotein C along with other 5 glycoproteins (gB, gD, gH, gK and gL). The gC binds to the host cell's surface ligand, heparin sulphate, aiding in attachment to the host cell and influencing the host cell type tropism, (Liang *et al*., 1993; Okazaki *et al*., 1999). On the other hand, research on gC null mutants demonstrated that virus entrance may be independent of gC binding, though gC binding could increase the infectiousness of the virus.

BoHV-1, gC is coded by UL44 and size is 521 amino acid. It is a high immunogenic protein present on the plasma membrane of infected host cells as well as in the virus's envelope. Both CD4 and CD8 T lymphocytes can detect it (Anonymous, 2005). It is a member of the immunoglobulin superfamily and a type I transmembrane glycoprotein (Fitzpatrick *et al*., 1989). There are four potential sites for addition of N linked oligosaccharide and a serine /threonine rich region (amino acid 32-92) for addition of O linked oligosaccharide. In addition the BoHV-1 gC gene has a region with strong homology to the constant domain of MHC class II antigens, but its functional significance is unknown (Fitzpatrick *et al*., 1989). Differences in the amino acid residue 76 between the BoHV-1.1 (valine) and BoHV-1.2 (glycine) strains helps to differentiate the two subtypes (Rijsewijk *et al*., 1999, Ranganath *et al*., 2013). A DNA vaccine expressing glycoprotein C (gC) of BoHV-1 was prepared and evaluated by Gupta *et al*. (2001). However it failed to induce protective immunity in bovines. In order to distinguish BoHV-1 from BoHV-5, Brower *et al.* (2008) targeted and sequenced the gC gene for the identification of BoHV-1 in the brain tissues of aborted foetuses (an unusual manifestation of BoHV-1). In India, gC gene amplification and phylogenetic analysis study from keratoconjunctivitis cases by Chintu Ravishankar *et al.* (2012) and from semen of cattle by Chandranaik *et al.* (2010) and buffalo Supriya *et al.* (2014) helped to group the isolate as a 1.1 subtype, with a 100% nucleotide sequence homology to the USA (Cooper) strain.

Glycoprotein (gE) and glycoprotein (gI)

The open reading frame US8 gene codes for gE, which is a key factor of the virus's virulence that has been investigated in vaccine development efforts against the virus. Although

the gE is not required for viral replication, it can interact with other proteins to boost the virus's neurovirulence and cell-to-cell transmission as well as the secondary envelope coating of its virions. The US7 gene-encoded gI forms a heterodimer with gE always and together they participate in a variety of viral activities. The gE/gI heterodimer can also associate with the immunoglobulin G (IgG) Fc fragment, which makes it easier for the virus to evade the immune system following infection (Jones, 2007). Overall, gE exhibits most of the characteristics suitable for the development of deletion mutant vaccines.

The extracellular domain (ETD), transmembrane domain (TMD) and cytoplasmic domain (CTD) make up the structure of glycoprotein E. The formation of the gE/gI heterodimer is mainly through interaction with conserved gE ETD, which is crucial for cell-to-cell spreading and immune evasion (Ning *et al*., 2022). The gE/gI complex's attachment to the IgG Fc region prevents the traditional complement pathway from being activated and shields virus-infected cells from antibody-dependent cellular phagocytosis and cytotoxicity in almost all alpha herpes viruses except bovine alphaherpes virus (Jenks *et al*., 2019). The interaction of gE with nearby proteins present on unique long sequences, such as UL 11, UL 16, UL 7 and UL 51, enhances cell fusion and cell-to-cell dissemination. The association of the unique short sequence protein US 9 with glycoprotein E aids in the formation of mature virions, axonal sorting and reverse axonal transport of viruses in neurons (Miranda *et al*., 2016).

Vaccination is the economical, safe and best method of reducing viral circulation. Traditional vaccination strategy involves use of killed or attenuated virus that elicits an immune response indistinguishable from the naturally infected animal. To overcome this problem and to eradicate the disease based on segregation of infected animals from vaccinated ones, researchers came up with new kind of vaccines called marker vaccines. Marker vaccines or DIVA strategy involves addition of novel gene or deletion of non-essential gene from the pathogen that serves as a marker to differentiate vaccinated and infected animals. This concept came up by the end of 20th century and the first licensed vaccine developed of this type was for Porcine Herpes virus (Van Oirschot *et al*., 1990), subsequently extended to other diseases such as Newcastle Disease, Infectious Laryngo Tracheitis, Infectious Bursal Disease, Avian influenza, Classical Swine Fever *etc* (Erdem *et al*., 2022).

Number of gE deletion mutant vaccine constructs of alpha herpesvirus has demonstrated appreciable safety and immunogenicity, with a significant reduction in the replication and spread of the virus in the central nervous system. Examples include gE-deficient form of Equine Herpes Virus EHV-1 vaccine equine abortion (Ning *et al*., 2022), gE-deficient marker vaccine prepared by replacing the gE gene with β-galactosidase, BoHV-1ΔgEβgal by Romera *et al*. (2014). In some studies along with gE other virulent genes has been considered for deletion. EGFP-labelled gE/gI-free Porcine herpes virus (Yin *et al*., 2017), gE, gI and TK triple deletion mutants of Porcine herpes virus (Hu *et al*., 2015) and recombinant virus without gE/gI/TK genes, rPRVTJ-delgE/ gI/TK-E2 in addition expressing E2 protein of classical swine fever virus (CSFV), mutants have been developed to protect against pseudorabies in pigs (Lei *et al*., 2016). Protection against challenge with wildtype BoHV-1 was found to be lower with Triple (gG-/tk-/gE-) mutant vaccine compare to double mutant vaccine (gG-/tk) (Zhang *et al*., 2011; Marawan *et al*., 2021). The reason for lower protection could be the reduced replication efficiency of triple mutants *in vivo* (Kaashoek *et al*., 1995). Another triple gene deleted mutant vaccine (gE-/US9/UL-49.5-) administered intranasally notably cleared the challenge virus three days earlier than the BoHV-1 gE-deleted virus vaccinated group (Chowdhury *et al*., 2014). Both gE- killed, gE-live vaccines are marketed and used by European countries in IBR control programmes. Details of commercially available gE negative mutant vaccines are given in the Table 3. Inactivated gE-marker vaccines were found to be efficient in inhibiting virus secretion after reactivation (Bosch *et al*., 1997) and live gE- marker vaccines administered intranasally were found to induce early immune response than gE killed vaccines (Kaashoek and Van Oirschot., 1996). In addition to gE- deletion tk- deleted double mutants are known to reduce neurovirulence and latency are also presently marketed against IBR (Petrini *et al*., 2019). Recently, novel gene editing technology CRISPER/CAS9 has been tested to knock out gE gene and also gE and US9 gene at the same time, proveing that the CRISPR/Cas9 system is a powerful tool for rapidly generating mutant viruses (Liu *et al*., 2022).

Recombinant glycoprotein E (ectodomain/cytoplasmic domain/truncated protein) has been cloned and expressed in prokaryote, yeast cell, Baculovirus and in human embryonic kidney cells to develop ELISA, a companion test to differentiate infected and gE deletion mutant vaccinated animal (Ning *et al*., 2022). Indirect gE ELISA are more specific compared to gE blocking ELISA (Bertolotti *et al*., 2015). Glycoprotein E Milk ELISA was identified as easy and cost-effective method for surveillance programs than serum gE ELISA (Righi *et al*., 2022). Glycoprotein E gene amplification by PCR has also been used to differentiate

Table 3: List of commercially available gE deleted marker vaccines against IBR.

Name of the (Company)	Active substance	Dose	Route
Hiprabovis IBR marker live(Hipra)	Live gE-, TK- Double gene deleted	Single 2 ml	i.m.
Cattle marker IBR inactivated (Zoetis)	gE- Inactivated	Single 2 ml	s.c.
Bayovac IBR marker vivum (Bayer)	gE- Modified live	Single 2 ml	i.m.
Bovalto ibraxion inactivated IBR (Merial)	gE- Inactivated	Single 2 ml	s.c.

infected from vaccinated animals in wild and domesticated animals (Grom *et al*., 2006, Wernike, 2012).

Glycoprotein (gK)

For the generation and spread of infectious viruses, BoHV-1 gK is necessary. Like Herpes Simplex Virus 1, BoHV-1 gK physically interacts with and binds with UL20 in infected cells and induces cell fusion, virus entrance and infectious virus propagation. Deletion of gK gene conducted by Muzammel *et al.* (2016) resulted in, failure of these viruses to form viral plaques and inability to spread infection beyond a single infected cells.

The BoHV-1 gK is coded by UL53 gene, consisting of 338 amino acids with the apparent molecular mass of 36 kDa, with four transmembrane domains, a 29 aa cleavable signal sequence and two N-glycosylation sites at asparagine positions 46 and 67, glycoprotein K is believed to be a hydrophobic protein (Khadr *et al*., 1996). Conserved domains on structures of gK of other members of alphaherpeviruses suggest, domains may serve similar functions. Amino acid sequence homologies of 46%, 44%, 53%, 43% and 46% with the gK counterparts of the herpes simplex viruses 1 and 2, equine herpesvirus 1, Marek's disease virus and varicella zoster virus respectively, were found when the gK proteins of other members of herpesviruses were compared. Glycoprotein K deletion mutants vaccines are also promising in control of IBR (Muzammel *et al*., 2016).

Glycoprotein G (gG)

Glycoprotein G is Coded by US4 gene with 444amino acids and size approximately 47kDa. BoHV-1 gG is required for movement of virion form one cell to another in epithelial bovine kidney (MDBK) cells (Nakamichi *et al*., 2000) and also to maintain Cell-to-Cell Junctional Adherence among viral infected Cells (Nakamichi *et al*., 2002). The structure of gG is considered to be unique compared to other viral envelope proteins due to its ability to exist in different isoforms. A full-length membrane-bound form, a smaller membrane-bound form and a secreted form are three isoforms of gG. Glycoprotein G is known to interfere with the innate immune response by altering the chemokines binding, migratory directions and positioning of immune cells (Griffith *et al*., 2014).

Glycoprotein M (gM)

A highly conserved gM forms complex with gN in all herpesviruses *via* disulphide bonds. The gM/gN complex has been shown to be crucial in the packaging of different proteins during the maturation of BoHV-1 (Li *et al*., 2021). Along with morphogenesis it is also involved in viral movement in cells. Only gM interacts with tegument protein VP8 and plays a role in translocation of VP8 to the Golgi apparatus and packaging into the virions (Sucharita *et al*., 2022). In gM-deleted virus (ΔgM BoHV-1) study, VP8 failed to move from cytoplasm to the golgi apparatus resulting in reduced VP8 concentration in mature virion. PCR targeting gM gene to study genetic variability of BoHV 1.1 showed the high similarity of genes (98-100%) to BoHV-1.2 (Hidayati *et al*., 2018). Glycoprotein M is coded by UL 10 gene, with 411 aa, Mol wt 43kDa.

Glycoprotein H (gH)

Essential for infectious entry and spread from cell-to-cell of BoHV-1 (Christina and Gunther, 1999). Glycoprotein H is one of the highly conserved glycoproteins among envelope proteins of herpesviruses, coded by UL 22 gene, with 842 aa having molecular weight of 88.3kDa.

Glycoprotein L (gL)

Glycoprotein L is a 17 kDa protein, comprising of 156 amino acids. It is coded by UL 1 gene. It is a conserved glycoprotein helps in folding of gH, entry as well as transport of virus (Jones, 2019).

UL49.5

BoHV-1 non glycosylated protein UL49.5 is named as glycoprotein N (gN), in other members of alphaherpes viruses. UL 49.5 downregulates host CMI response by proteolytic degradation of the TAP complex, (Koppers *et al*., 2005) which is known to block the transporter-associated antigen processing (TAP)-mediated transport of cytosolic peptides into the endoplasmic reticulum affecting the endocytic pathway of antigen processing. Peptide transport by TAP is pre-requisite for loading of antigen on to MHC class I and activation of $CD8^+$ T cells. Experimental Infection of calves with a UL49.5 free BoHV-1 mutant lead to enhanced virus neutralizing antibody and cellular immune responses when compared to the parental wild-type virus (Jones, 2019).

US9

Interaction of US9 with other nearby envelope proteins such as gE and gI helps in axonal anterograde transit of viruses in neurons, axonal sorting and formation of mature virions (Butchi *et al*., 2007, DuRaine and Johnson, 2021). US9 belong to type II membrane polypeptide with no extracellular domain. Cytoplasmic domains of gE, gI and US9 are loaded with the intracellular sorting motifs (di-leucine, tyrosine motifs and acidic and basic clusters) of gE, gI and US9 play vital roles in movement of the proteins to sites of virus assembly and then beyond to the cell surface and into axons (Tirabassi and Enquist, 2000). Deletion mutant study specific to cytoplasmic domain of US9 aminoacid 83 to 90 has resulted in inhibition of reactivation of virus in the trigeminal ganglion and anterograde axonal transport from trigeminal ganglion to nose and eye (Chowdhury *et al*., 2011).

Diagnosis and control

Virus culture on MDBK cell lines, Immune peroxidase or Immune florescent test are the direct methods of detecting virus. Virus infected MDBK cell line shows syncytia, grape like clustering and characteristic eosinophilic intranuclear inclusion bodies. Virus nucleic acid detection can be performed by targeting specific genes of BoHV-1 gB, gE,

gC or gD genes by PCR technique (OIE, 2018). The serological tests used for the detection of IBR viral antibodies are Serum Neutralization, Indirect Enzyme Linked Immunosorbent Assay, Monoclonal antibody-based blocking ELISA, indirect haemagglutination test, dot enzyme linked immunosorbent assay, fluorescent antibody technique, complement fixation test, agar gel immunodiffusion test and counter immunoelectrophoresis *etc*. (Chatterjee *et al*., 2016). Detection of Antibodies in milk or serum by ELISA are regularly conducted in surveillance programmes (Righi *et al*., 2022).

Control measures includes following strict hygienic measures at the farm premises, Vaccinating the animals at the right time, detection and removal of infected animals, Quarantine for the period of 4 weeks before introducing new animal to the herd, avoiding natural mating, artificial insemination using semen from BoHV-1 negative bulls and restriction on import of animal semen or embryo.

CONCLUSION

BoHV-1 is an important pathogen that affects milk yield, body condition and birth of young ones incurring financial loss to farmers. Except in some parts of European countries virus is reported from throughout the world. Government of India has implemented only screening of semen samples for BoHV-1 in breeding mother bulls but various epidemiological studies carried out in India indicate the need of implementation of vaccination policy and eradication programmes. Among dozens of envelope proteins exploring right glycoproteins is important in developing cost effective, in-house vaccines or diagnostic tools. Glycoprotein D is significantly immunogenic and subunit vaccine candidate confer protection against BoVH-1 and also BoVH-5. Glycoprotein C gene analysis by polymerase chain reaction helps to differentiate BoVH-1.1 and BoVH-1.2. A gE- live or killed marker vaccine has proven to be promising in inducing protective immune response and in such animals gE companion ELISA are used to differentiate infected animals from vaccinated. Analysis of genes of gB/ gC/ gD and gE by PCR or use of expressed and purified proteins in ELISA plays an important role in diagnosis and cost effective epidemiological studies. The gene US9 deletion inhibits the reactivation of virus in the trigeminal ganglion and anterograde axonal transport. This could be exploited with tk and gE deletion mutants to resolve the problems associated with latent infections. Further, novel CRISPER/CAS9 targeted gene editing system could be employed to produce deletion mutants and also as an antiviral tool to cure infected cells.

Conflict of Interest

The authors declare that they have no conflict of interest.

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