

## Review Article

# A Review on Current Status of Infectious Bovine Rhinotrachitis in India

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## ABSTRACT

Livestock plays an important role in welfare of rural population of India and its economy. It contributes 9% of GDP and employs 8% labor force of the country. However respiratory and reproductive disorders in dairy animals due to various etiological agents have led to significant economic losses to dairy animals. The losses are due to abortions, metritis, retention of placenta, repeat breeding, death of animals, loss of production and trade restrictions etc. IBR caused by BoHV1, is a disease of domestic and wild cattle. BoHV1 is a member of the genus Varicellovirus in the subfamily Alphaherpesvirinae, family Herpesviridae and order Herpesvirales. Based on restriction endonuclease fingerprinting, BoHV1 is classified into three subtypes, namely, BoHV1.1, BoHV1.2a and BoHV1.2b. Most BoHV1.1 strains have been isolated from respiratory tract diseases or abortion cases and BoHV-1.2 strains from lesions of genital organs. Subtypes 1.1 and 1.2a have been associated with severe diseases including infection of the foetus and abortion and BoHV1.2b strains are generally involved in mild rhinitis or vaginitis. BoHV1 infection occurs in all continents and is responsible for the significant losses resulting from disease and trade restriction.

### Keywords

Current status,  
Infectious  
Bovine  
Rhinotrachitis

## Introduction

Livestock plays an important role in welfare of rural population of India and its economy. It contributes 9% of GDP and employs 8% labor force of the country. However respiratory and reproductive disorders in dairy animals due to various etiological agents have led to significant economic losses to dairy animals. The losses are due to abortions, metritis, retention of placenta, repeat breeding, death of animals, loss of production and trade restrictions etc. IBR caused by BoHV1, is a disease of domestic

and wild cattle. The first report of disease caused by BoHV1 was made as a venereal disease in a bull and contact cows (Rychner, 1841) which was subsequently referred to as Blaschenausschlag in German literature. The viral aetiology of the disease was proved by Reisinger and Reimann (1928). The first published report on respiratory IBR came from Schroeder and Moys (1954) where they described an apparently new upper respiratory disease of dairy cattle that occurred in California in 1953. It appeared

suddenly and was characterized by high fever and agalactia in addition to respiratory signs. The following year, Miller (1955) described a disease that was first seen in a Colorado feedlot in the fall of 1950 and which has been present in that state ever since. By 1954 it was occurring in dairy cattle and all ages of beef cattle both in feedlots and occasionally in cattle on pasture. The actual cause was undetermined at that time, but the disease could be transmitted with tissues and exudates from natural cases (Schroeder and Moys, 1954). The disease was known as red nose, necrotic rhinitis or dust pneumonia. In the year 1955, at a meeting of the US livestock sanitary association, the accepted name for the disease became infectious bovine rhinotracheitis (Mckercher *et al.*, 1957). Madin *et al.*, (1956) first isolated the causative agent, and it was further characterized by Tousimis *et al.*, (1958). Armstrong *et al.*, (1961) suggested that the IBR virus (BoHV1) belongs to the herpesvirus group. The virus was first isolated from respiratory disease in the United Kingdom in the early 1960 s (Darbyshire *et al.*, 1964). Kendrick *et al.*, (1958) described its association with infectious pustular vulvovaginitis (IPV). Huck *et al.*, (1971) described its association with panoposthitis. The virus has been reported to be associated with infection of respiratory tract causing rhinotracheitis and conjunctivitis; reproductive tract causing vulvovaginitis and balanoposthitis, skin lesions as well as neonatal infection causing red nose, necrotic rhinitis, epididymitis, abortion, infertility, dermatitis and mastitis (Gibbs and Rweyemamu, 1977 and Kahrs, 1977).

### **Economic importance**

IBR is not a highly fatal disease but it can cause considerable economic losses due to

abortion, loss of body condition, milk yield, loss of new born calves, temporary failure of conception, insufficient feed conversion, secondary bacterial pneumonia and cost of treatment. Morbidity and mortality rates vary considerably and were lower in dairy herds (8% morbidity and 3% mortality) than in beef cattle, in feed lots in which the mortality rate was usually 20-30% and rarely up to 100 (Barenfus *et al.*, 1963). Wiseman *et al.*, (1979) concluded that the losses were due to market value of fatal and culled cases, feeding cost for 1-6 wk when fattening cattle did not put on weight, treatment cost and value of lost milk production. In large milking herds, the losses varying from \$ 25- \$ 55/cow were estimated (Townley, 1971). In another study, it has been shown that American livestock owners experience a loss of quarter of a billion dollar each year due to BoHV1 infection. Schroeder and Moys (1954) estimated heavy losses due to IBR in USA and 100% loss due to IBR was reported in Hungary (Bartha *et al.*, 1974). Townley, (1971) reported 30% morbidity and loss of \$ 500 in an acute outbreak of respiratory form of IBR in dairy herd. In India, no such estimates have been made till date, but the epidemiological surveys indicate a substantial loss due to the disease. Morbidity rate of 90% and mortality rate of 30% was recorded in an outbreak of nervous form of disease in Australia (Gardiner *et al.*, 1964). Conception rate fall down from 80% to 45-50% in majority of cows suffering from herpes vulvovaginitis which are artificially inseminated (Laveso *et al.*, 1984). BoHV1 is responsible for significant losses incurred by disease and trading restriction in cattle industry.

### **Disease status in India**

In India, IBR/IPV is one of the endemic diseases of cattle resulting from

crossbreeding programme. The disease was first reported by Mehrotra *et al.*, (1976) who isolated IBR virus from cases of keratoconjunctivitis amongst crossbred calves at an organized cattle herd in Uttar Pradesh. Since then the disease has been reported in most of the states of India. The disease was found to be more prevalent in exotic and crossbred cattle than in indigenous breeds. Mallick (1986) studied the seroprevalence of IBR disease in seven states and observed that 65.3% exotic, 73% crossbred and 62% indigenous cattles were seropositive. Since then, several seroprevalence studies have been carried out by different researchers and it was seen that the disease is prevalent in almost all the states of India. The disease has been recorded from states of Kerala (Sulochana *et al.*, 1982), Gujarat (Singh *et al.*, 1983), Tamil Nadu (Manickam and Mohan, 1987), Uttar Pradesh (Mehrotra, 1977), Orissa (Misra and Misra, 1987), Karnatak (Mohan Kumar *et al.*, 1994), West Bengal (Ganguly *et al.*, 2008) and Andhra Pradesh (Satyanarayana and Suri Babu, 1987). An outbreak of balanoposthitis was reported from A.I. centre in U.P. (Pandey *et al.*, 2000). An 8.56% of serum samples from Tamil Nadu and Andhra Pradesh were found to be positive for IBR (Selvaraj *et al.*, 2008). In a seroprevalence study carried out in three south Indian states by Renukaradhya *et al.*, (1996), 50.9% cattle and 52.5% buffalo were found positive to BoHV1. In Gujarat, Patel (1983) reported that 24 out of 32 paired sera of aborted buffaloes were positive for IBR antibodies. Khan (2004) from Gujarat reported 21.30% seroprevalence of IBR in cattle and buffaloes. Seroprevalence of BoHV1 is also reported in yaks (Nandi and Kumar, 2010) and mithuns (Rajkhowa *et al.*, 2004) with an overall seroprevalence of 60.1% and 19%, respectively. An 32.34% seropositivity was observed in bulls by Singh *et al.*, (1986).

Deka *et al.*, (2005) observed 45.09% seropositivity in breeding bulls and detected presence of virus in semen by isolation and PCR methods. The seroprevalence in buffalos varied from 2.75-81% (Sinha *et al.*, 2003). Pandita and Srivastava (1993) carried serological survey against IBR with 94 serum samples from aborted cows and 135 inconstant cattle serum using indirect ELISA kit and found an incidence rate of 73.40% and 37.78% respectively. In addition to that while indigenous crossbred, exotic cattle and buffaloes showed an incidence rate of 55.50%, 76.70% and 50.50%, respectively with a history of abortion, the in-contact animals showed an incidence rate of 40%, 36%, 66.70% and 30%, respectively. Pandita and Srivastava (1995) studied the efficacy of dot ELISA and plate ELISA with 239 bovine serum samples from Haryana against IBR antibody and 51.9% and 48.5% were positive by plate ELISA and dot ELISA respectively. S. S. Patil (2015) studied a total of 1000 cattle serum samples were tested for the presence of antibodies against IBR using Avidin Biotin ELISA. The results revealed that IBR antibodies were widely prevalent in all regions of the country ranging from 36.5% in Central region to 84.5% in Northern region with an overall prevalence of 61.6%. The prevalence of IBR antibodies was different between various age groups being 22.3%, 62.1%, 59.3%, 76.1% and 66.78 % in the age groups less than 1 year, 1-2 years, 2-3 years, 3-4 years and more than 4 years old respectively. Based upon the medical history of the herd, it was found that 83% abortion cases, 76% metritis cases, 83% repeat breeding cases and 65% retention of placenta cases were seropositive for IBR. Amit Kumar Verma *et al.*, (2014) studied seroprevalence of Infectious Bovine Rhinotracheitis (IBR – BHV-1) in dairy animals of western parts of Uttar Pradesh, India.

## **Virology**

The etiological agent for IBR was first isolated by Madin *et al.*, (1956) and classified subsequently as a herpesvirus and BoHV1 (Tousimis *et al.*, 1958 and Armstrong *et al.*, 1961). It belongs to family- *Herpesviridae*, sub family- *Alphaherpesvirinae* and genus- *Varicellovirus*. Bovine herpesvirus 1 (BoHV1) is the official species name of the virus. Gibbs and Rweyemamu (1977) stated that the term BoHV1 refers to all virus isolates that are serologically related to IBRV and IPVV. By DNA restriction enzyme analysis, it can be divided into BoHV1.1 and BoHV1.2. Conventional serological assays cannot distinguish between immune responses induced by BoHV1.1 from those induced by BoHV1.2. BoHV1.1 and BoHV1.2 cause IBR and IPV, respectively. BoHV1.2 may be less virulent than BoHV1.1. BoHV1.2 can be further subdivided into BoHV1.2a and BoHV1.2b. BoHV1.2a causes IPV in cows and IPB in bulls (Edwards *et al.*, 1990) whereas BoHV1.2b is less virulent compared to the earlier one (Metzler *et al.*, 1985). Sequence homology between BoHV1.1 and 1.2 is more than 95% (Engels *et al.*, 1986). BoHV1 has an icosahedral nucleocapsid of 95-110 nm in diameter (Armstrong *et al.*, 1961 and Cruickshank *et al.*, 1963) consisting of 162 capsomeres each 12 nm long and 11.5 nm wide with an axial hole of 3.6 nm. Nucleocapsid is surrounded by an electron dense zone called tegument (Valíček and Smíd, 1976) and a lipid bilayer envelope forming pleomorphic virion of 150-200 nm indiameter (Armstrong *et al.*, 1961). DNA is wrapped around a fibrous spool like core, whose fibers are anchored to the inner side of surrounding capsid. The virion possesses haemagglutinin activity which is coded by a 90 kDa glycoprotein (Trepanier *et al.*, 1985). There is only one

antigenic type irrespective of whether the isolate is derived from cases of IBR or IPV. The virus has a single linear molecule of double stranded DNA approximately 135-140 kbp (Wyler *et al.*, 1989). Buoyant density of DNA molecule is approximately 1.730 g/mL (Russell and Crawford, 1964) and the GC ratio is 72% (Plummer *et al.*, 1969). The genome can be divided into unique long (UL) and unique short (US) segment which are 102-104 kbp and 10.5-11 kbp long, respectively. The US segment is bracketed by inverted internal (IR) and terminal repeat (TR) regions of approximately 24 kbp (Muylkens, 2006). Majority of BoHV1 gene repertoire consists of ORF homologous to other alphaherpes viruses. Entire gene of BoHV1 has been sequenced by international collaboration in 1995 (Genbank accession No. AJ004801). Virus is quite resistant to environmental influence. Inactivation depends on factors such as temperature, pH, light, humidity, and kind of medium harbouring the virus (Gibbs and Rweyemamu, 1977). At 4 °C virus is stable for 1 month while gets inactivated at 56 °C within 21 minutes, at 37 °C within 10 days and at 22 °C within 50 days (Gibbs and Rweyemamu, 1977). It may survive more than 30 days in feed. Virus is sensitive to organic solvents such as chloroform ether and acetone. It gets inactivated by 0.5% NaOH, 0.01% HgCl<sub>2</sub>, 1% Chlorinated lime, 1% phenolic derivatives, 1% quaternary ammonium bases and 10% Lugols iodine. Formalin (5%) inactivates the virus within 1 min (Straub, 1990).

### **BoHV1 Genes and their encoded proteins**

BoHV1 genome is believed to encode approximately 70 proteins. Electrophoretic analysis of radiolabelled virions by SDSPAGE has revealed 33 structural proteins (Misra *et al.*, 1981), among these 13

are shown to be associated with viral envelope, 14 with nucleocapsid and 6 not classified. Other than these 16 non-structural proteins are also coded by BoHV1 genome (Misra *et al.*, 1981). BoHV1 glycoproteins are homologous in structure and function to HSV1 glycoproteins and they are involved in several steps of the viral cycle such as attachment, penetration, maturation and release of the virus. 10 genes code for glycoproteins and among them 6 are present in UL region i.e. gK (UL53), gC (UL44), gB (UL27), gH (UL22), gM (UL10) and gI (UL1) and 4 are in Us region i.e. gG (US4), gD (US6), gI (US7), gE (US8). gP, gI and gE possess the Fc receptor and binds with the IgG molecule (Schwyzer and Ackermann, 1996). UL49.5 can be considered as a false glycoprotein. Indeed the protein coded by UL49.5 is not glycosylated in BoHV1 while in other alphaherpesviruses it is glycosylated and is known as gN. It has conserved function and forms disulphide linked hetero duplex together with gM. The gC, gD, gE, gG, gI, UL49h and thymidine kinase genes are involved in viral virulence (Kit *et al.*, 1985; Kit *et al.*, 1986; Smith, 1991; Smith *et al.*, 1994).

### **Transmission**

The virus is mainly transmitted from infected animal to uninfected one by contact with mucosal droplet. Infectious virus is nasally shed for 10-14 days during acute respiratory infection (Gibbs and Rweyemamu, 1977). Airborne transmission of BoHV1.1 can occur under experimental conditions at distances of 3.85 m, although this is probably not a major route of transmission (Wentink *et al.*, 1993) and is dependent upon environmental temperature and relative humidity (Mars *et al.*, 1999). It can be mechanically transmitted between bulls in AI centres and virus may also be

spread by artificial insemination (Van Engelenburg *et al.*, 1995) and it is the main route of infection of the virus causing IPV. Semen not only spread the disease but also associated with reduced fertility and abnormal fetal development (Wyler *et al.*, 1989). As the virus causes latent infection animal infected once carry the virus silently and under some stressful conditions, shed the virus and infect the healthy animals of the herd.

### **Pathogenesis**

Virus enters through aerosol route or by direct contact with the nasal secretion in case of respiratory tract infection and by direct contact or by semen containing virus (coitus or AI) in case of genital infection. Within animal, BoHV1 is transported by monocytes and white blood cells to target organs. BoHV1 replicates in the nasal and ocular epithelia during primary infection of the respiratory tract and then, 2-3 days post exposure animal develop a fever with subsequent increase in respiratory rate and inappetance and in dairy cattle there is decrease in milk yield. Areas of focal necrosis are evident, often leading to serous nasal/ocular discharge and conjunctivitis. In both genital and respiratory form of disease there is a focal area of epithelial cell necrosis in which there is ballooning of epithelial cells. Typical herpesvirus inclusions may be present in nucleus of periphery of necrotic foci. There is intense inflammatory response within the inflamed mucosa frequent with formation of overlying accumulation of fibrin or cellular debris (pseudomembrane). Gross lesions are not frequent in aborted foetus but micro necrotic foci are present in tissues. Liver and adrenal are affected most. It may lead to secondary bacterial infection contributing to the complex syndrome called shipping fever (Bovine respiratory disease complex) and

culminates in severe pneumonia caused by *Mannheimia haemolytica*.

### **Latency**

The BoHV1 causes latent infection in immunoprivileged sites following productive viral infection (Rock *et al.*, 1987; Rock *et al.*, 1992 and OIE 2000). Latency usually occurs in the body of the trigeminal and sacral ganglia but may also occur in tonsillar lymphoid cells and peripheral blood lymphocytes (Mweene *et al.*, 1996). Latent virus only produces latency-related proteins, which protect latently infected cells from apoptosis (Schang *et al.*, 1996). Infectious virus is not present during latent infection (Engels and Ackermann, 1996). After primary infection the viral capsid together with tegument are transported retrogradely to the ophthalmic and maxillary branches of the trigeminal nerve located in the nasopharynx and eye (respiratory tract infection) or the sacral ganglia (genital tract infection) where the virus establishes a lifelong latent infection. The episomal form of viralgenomic DNA, transcripts originating from the latency-related gene (LR gene) and proteins encoded by the latency-related gene can be detected in trigeminal ganglia during latency (Rock *et al.*, 1987). The reactivation of the virus occur after stressful condition like transportation, parturition and corticosteroid treatment leading to shedding of the virus and is thought to be responsible for the perpetuation and transmission of virus in cattle population (Kutish *et al.*, 1990). In a study carried out by Thiry *et al.*, (1987) showed that the stress of transport has been shown to cause viral reactivation in latently infected cattle, leading to virus shedding from days 1-4 after the day of transport. Treatment with corticosteroid is done to eliminatethe carrier bulls from semen collection station.

### **Role of BoHV1 in viral bacterial synergy**

Pneumonia in cattle has been a liability to beef producers and consumers as well as a topic of interest and frustration for veterinary practitioners and researchers over several decades. The most important component of this complex is shipping fever, a fibrinous pneumonia associated with pathogenic microorganisms and various stressors, especially transportation. BoHV1 is one of the most important causes of respiratory disease complex called shipping fever (Yates, 1982 and Jones and Chowdhury, 2007). Other bacteria and virus that are involved are BVDV, Bovine parainfluenzavirus 3, Bovine respiratory syncytial virus, *M. haemolytica*, *P. multocida* and *Histoplasma somnis* (Martin and Bohac, 1986). As a result of virus infection of the upper respiratory tract, bronchi, lower trachea and lung two important events occur that increase the colonization of the lower lung with bacterial pathogens, leading to the development of severe pneumonia (Babiuk *et al.*, 1988).

### **Clinical signs and symptoms**

Incubation period of the disease varies from 10-12 days under natural condition. The virus mainly affects the respiratory and genital tracts. The infection of the respiratory tract is known as IBR. It is the commonest form of BoHV1 infection. It occurs as a subclinical, mild or clinical disease. It is characterized by symptoms like fever, coughing, anorexia, depression, decreased milk production, weight loss, increase respiratory rate nasal and ocular discharge which is serous at beginning and become mucopurulent later and increased salivation may also accompany these respiratory tract problems. Nasal mucosa becomes hyperaemic and lesion progress from pustular necrosis to large haemorrhagic

and ulcerated area covered by cream coloured diphtheritic membrane. A nasal discharge along with nasal congestion may develop and is referred to as red nose. Foul breath, mouth breathing, salivation and a deep bronchial cough are common. Animal may show the signs of bronchitis and pneumonia. Abortion is a consequence of a respiratory BoHV1 infection. Following viraemia BoHV1 crosses the maternal fetal barrier to produce lethal infection to the foetus. The route of BoHV1 from placenta to foetus is unknown but since viral lesions are consistently observed in fetal liver, haematogenous spread most likely occurs via umbilical vein. In typical BoHV1 infection conjunctivitis is a predominant symptom which is either unilateral or bilateral and associated with profuse lacrimation. Animal show photophobia, epiphora and the hair beneath the eye become heavily soiled. Secondary bacterial infection is common and pus may be seen in the lacrimal discharge. Cornea usually remains unaffected but if secondary bacterial infection occurs, keratitis and corneal ulceration occur with permanent scarring of cornea (Turin and Russo, 2003). In uncomplicated cases symptoms regress within 5-10 days. A second BoHV1 syndrome, IPV in the cow or IBP in bulls, causes pustular lesions of the genital tract and may lead to abortion. IPV is observed 1 to 3 days after mating and often leads to painful inflammation. The first sign of IPV are frequent micturition (urination) and followed later by small pustules (1-2 mm) on the vulva. Pustules usually coalesce to form yellowish fibrinous membrane that gradually detaches to form ulcers. Affected animal develops fever, depression and anorexia. They avoid contact of tail with vulva. Secondary bacterial infections are common and varying amount of pus is discharged. 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). Outbreaks of both the respiratory form and genital disease together are rare. IPB also develops after 1-3 days of infection. Lesions similar to that of IPV appear on the penis and prepuce. Sequelae of this condition include extensive adhesion, annular constriction and penile distortions. Healing occurs in uncomplicated cases within 10-14 days, but some animal may lose libido, have painful erection and ejaculation and require several weeks to resume normal mating. BoHV1 is also associated with poor semen quality. The respiratory tract lesions in IBR are usually acute necrotic rhinotracheitis or ne crotizing rhinitis, p haryngitis and laryngotracheo bronchitis (Allan *et al.*, 1980). There is serous rhinitis with mucosal hyperaemia oedema, mucopurulent exudate, focal areas of necrosis, and finally overlying accumulation of fibrin and cellular debris which leads to pseudomembrane formation. In the early stages, the histological picture is one of mild catarrhal inflammation with an oedematous mucosa containing migrating neutrophils, a submucosa infiltrated with lymphocytes, macrophages, and plasma cells, and hyperaemia and haemorrhages throughout (Mckercher, 1959 and Gibbs and Rweyemamu, 1977). With progression, there is a variable amount of epithelial necrosis in which there is ballooning of epithelial cells (and concomitant loss of cilia in cells so endowed) with cellular debris and exudates on the mucosal surface or within lumina of airways, and congestion, oedema, neutrophil infiltration, and nodular mononuclear cell accumulation in the lamina propria and submucosa (Mckercher *et al.*, 1957). Gross lesions are frequently not observed in aborted foetuses, but microscopic necrotic foci are present in most tissues and the liver and adrenal glands are affected most consistently. The gross pathology in IPV includes hyperaemia of the

vulval and vaginal mucosa with focal haemorrhages over the lymphocytic follicles of the submucosa. There is oedema of the vulva with copious mucopurulent discharge. Small (2-3 mm) yellow coloured pock-like lesions replace the focal haemorrhages over the lymphoid follicles. The epithelium over the lesions is lost and an ulcer is revealed. The lesions regress within 8 days in uncomplicated cases.

### **Diagnosis**

Currently, methods of BoHV1 detection used in diagnostic virology laboratories include virus isolation, examination of tissues by fluorescent antibody technique (FAT), antigen detection by enzyme-linked immunosorbent assay (ELISA) and immunoperoxidase test. Virus isolation in cell culture is a routinely used technique. However, cell lines are difficult to maintain, making the process troublesome, slow and costly. The need for the viral particle to be infective is another inconvenience of this technique because in BoHV1 abortion the foetus is usually autolysed and rarely is expelled in a fresh state. Immunoperoxidase, immunofluorescence, and enzyme immunoassays for antigen detection do not require the infectiousness of the viral particle, but results are compromised if the virus is damaged. Thus, lack of adequate sample conservation and transportation to the laboratory adversely affect the diagnosis. In recent years, molecular biology has contributed to the development of highly sensitive, new diagnostic approaches. Polymerase chain reaction (PCR) is one of the molecular techniques that has been adopted to detect BoHV1 infection in aborted foetus, calves, cows, and in semen samples (Takiuchi *et al.*, 2005). This technique has been shown to be more sensitive and specific when compared to other diagnostic methods such as virus

isolation, immunofluorescence and nucleic acid hybridization (Kataria *et al.*, 1997). Realtime PCR using primers of glycoprotein C gene 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 labour consuming virus isolation for detection of BoHV1 in extended semen (OIE, 2010). Recent attention in diagnostic virology has been directed towards the development of nucleic acid techniques for the detection of virus in clinical specimens. Nucleic acid hybridization and PCR were developed as ideal diagnostic tools for the detection of BoHV1 in clinical specimens because of their rapidity, sensitivity and specificity. Several hybridization formats such as dot blot hybridization (Vilcek *et al.*, 1993a and Vilcek *et al.*, 1993b), in situ hybridization and Southern blot hybridization (Kibenge *et al.*, 1994 and Xia *et al.*, 1995) with radioisotope (Kibenge *et al.*, 1994 and Xia *et al.*, 1995), labelled probes have been applied for the detection of BoHV1 in nasal swabs and semen. PCR with Southern blot hybridization has been developed as a diagnostic in which 0.01 TCID<sub>50</sub>/100 l of BoHV1 could be detected in 1:20 diluted bovine semen (Kibenge *et al.*, 1994 and Xia *et al.*, 1995). Various PCR assays for the detection of BoHV1 have been described using the primer of gB gene (Vilcek *et al.*, 1993a) gC gene (Van engelenburg *et al.*, 1993), gD gene (Wiedmann *et al.*, 1993) and (de Gee *et al.*, 1996) and the thymidine kinase (*Tk*) gene of BoHV1 (Kirkbride, 1992) with variable sensitivities.

### **Control**

Control measures include normal hygienic measures and maintaining 2-3 weeks of quarantine period before introducing new

stock to the herd and precludes of virus positive animals, use of live attenuated or whole virus vaccines, use of semen from positive animals. Successful eradication prompts strict import restriction on cattle, semen and embryos because the reintroduction of the virus into these immunologically naive populations is likely to have serious consequences and lead to severe economic loss.

Cattle are the primary reservoir and infection is transmitted during initial clinical disease or from reactivation of latent infection with subsequent virus shedding. As soon as a cow with a clinical BoHV1 infection is diagnosed, the whole herd may be vaccinated to protect the animals from disease, if there is a clinical outbreak of BoHV1 in the close vicinity. In such a case, cattle can be vaccinated before the infection finally reaches them. In order to eliminate BoHV1 from a herd, every infected animal must be identified and removed, because of the possibility of reactivation of latent virus. BoHV1 infected animals can be identified by the presence of BoHV1 specific antibodies in their serum.

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 BoHV1 infections (Van Drunen Littel-van den Hurk *et al.*, 1993 and Nandi *et al.*, 2009). Although, vaccines do not prevent infection, they significantly reduce the incidence and severity of disease. Importantly, breeding animals in enzootic countries, except those for export to countries free of BoHV1, should be vaccinated before coitus, to prevent the virus inducing abortion. In enzootic regions, vaccination to maintain population immunity is best done prior to stressful situations such as weaning or transport.

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