Infectious Bovine Rhinotracheitis - Major Pathogen of Ruminants

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Infectious diseases have serious economic implications in terms of productivity. Inspite of stupendous growth in milk production in India, the major factors that contribute to low productivity of animals is losses due to infectious diseases. There are number of diseases which frequently affect bovine production systems. Among the viral diseases of cattle, infectious bovine rhinotracheitis (IBR) / infectious balanoposthitis (IPV) occupies a key position with pandemic distribution. Infectious bovine rhinotracheitis is endemic to India but often neglected disease, and thus causes great economic loss to the industry (Deka *et al.*, 2005, Nandi, 2005). Considering the range, seriousness and rate of spreading of the infection and social aspects, the disease is classified as one of the List B diseases by Office Internationale des Epizootics (OIE) (OIE, 2000).

Infectious bovine rhinotracheitis / infectious pustular vulvovaginitis (IBR/IPV), caused by bovine herpesvirus 1 (BHV-1), is a major pathogen of cattle. Primary infection is accompanied by various clinical manifestations, and systemic infection in neonates. When animals survive, a life-long latent infection is established in nervous sensory ganglia. Several reactivation stimuli can lead to viral re-excretion, which is responsible for the maintenance of BHV-1 within a cattle herd (Muylkens *et al.*, 2007).

Bovine herpesvirus 1 infections are easily transmitted from one animal to another because, large quantities of the virus are shed in the secretions of respiratory, ocular and reproductive tract of infected cattle. Since, semen is a potential source of BHV-1, the virus can be transmitted by natural service or artificial insemination. Bulls may shed virus in semen during both clinical and subclinical infections. The spontaneous intermittent virus shedding has been frequently observed. In artificial insemination centres, preserved semen is stored at -196°C and sent to other places when necessary. Unfortunately, the antimicrobial agents added during the preservation of semen to avoid contamination with microorganisms, do not have any effect on viruses in the semen. Because of this reason, bulls used for insemination should be periodically tested for viruses in suitable laboratories, particularly to prevent from risk of outbreak of viruses-induced diseases. The infectivity of BHV-1 remains stable during storage of semen in liquid nitrogen. Bovine herpesvirus 1 in extended semen was well preserved at 4°C for 7 days and

at ambient temperature for 5 days (Lata Jain, 2006).

Several research works have highlighted the need for research into the molecular epidemiology and vaccinology of IBR around the world. As per the Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS) report (2004) there is an increase in the seroprevalence of IBR (85.9 per cent) in Tamil Nadu in recent years. But, vaccination for IBR is never carried out in Tamil Nadu. It is absolute necessity to know the status and molecular epidemiology of IBR in cattle and buffaloes, because it often occurs as sub-clinical infection due to latent virus establishment in the nerve ganglia of the infected but clinically normal animals after primary infection despite the development of neutralizing antibody. So, this preliminary work on seroprevalence using avidin-biotin enzyme linked immunosorbent assay (AB-ELISA) combined with isolation of BHV-1 from suspected cases using *Madin-Darby bovine kidney cell line*, identification and molecular characterization by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), and screening of frozen semen for the detection of BHV-1 by PCR has been carried out, which would start further research work on IBR in Tamil Nadu.

Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV), caused by bovine herpesvirus 1 (BHV-1), is a disease of domestic and wild cattle. *The disease is characterised by clinical signs of the upper respiratory tract, such as (muco) purulent nasal discharge and conjunctivitis. Signs of general illness are fever, depression, inappetance, abortions and reduced milk yield. The virus can also infect the genital tract and cause pustular vulvovaginitis and balanoposthitis. Post-mortem examinations reveal rhinitis, laryngitis and tracheitis. Mortality is low. Many infections run a subclinical course. Secondary bacterial infections can lead to more severe respiratory disease* (*OIE, 2008;* Kamaraj *et al.,* 2008) and encephalitis in bovine species. The main sources of infection are the nasal exudates and the respiratory droplets, genital secretions, semen, foetal fluids and tissues (Nandi *et al.,* 2009). Infectious bovine rhinotracheitis is the most common BHV-1 infection, which causes significant economic losses to the livestock industry worldwide.

Despite a pronounced immune response, the virus is never eliminated from an infected host, but establishes life-long latency and may be reactivated at intervals (Ackermann and Engels, 2006). Several reactivation stimuli can lead to viral re-excretion, which is responsible for the maintenance of BHV-1 within a cattle herd (Muylkens *et al.*, 2007).

Infectious bovine rhinotracheitis was described in Germany during the 19th century as "*Bläschenausschlag*" (coïtal vesicular exanthema), a cattle disease probably caused by BHV-1. The viral etiology was demonstrated in 1928 and this venereal disease was transmitted by a filterable agent. The manifestations of BHV-1 infections known as "infectious

pustular vulvovaginitis" in cows and "infectious pustular balanoposthitis" (IPB) in bulls were confined to the genital organs until the early nineteen-fifties. At this time, a respiratory form arose in North American feedlots. This type of severe disease due to BHV-1 infection was called "infectious bovine rhinotracheitis" (Muylkens *et al.,* 2007).

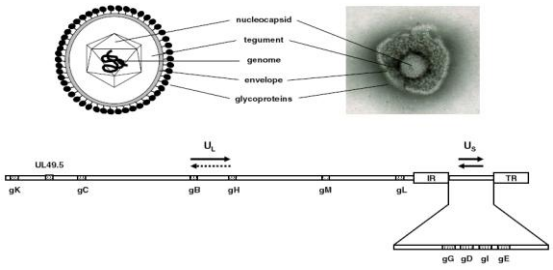
Historically in 1955, IBR was described as a distinct disease in feed-lot cattle in Western USA. Soon after that, the etiologic virus was isolated. However, clinical IPV has been known in Europe for years before the discovery of IBR in the USA. The virus is distributed worldwide, although it has been eradicated in some countries (Elzein *et al.,* 2008).

Infectious bovine rhinotracheitis was originally recognized as a respiratory disease of feeder cattle in the western United States during the early 1950s. The first published report on respiratory form of IBR came in 1954 and an apparently new upper respiratory disease of dairy cattle that occurred in California in 1953 was described. It appeared suddenly and was characterized by high fever and agalactia in addition to respiratory signs. The cause was undetermined at that time, but in 1954 it was reported that the disease could be transmitted through tissues and exudates from natural cases. In the next year, in 955 the disease was first seen in a Colorado feedlot in the fall of 1950, which was 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. It was known by names such as "red nose", "dust pneumonia". In the same year, the accepted name for the disease became "infectious bovine rhinotracheitis". After the respiratory form of IBR had been characterized in the USA in the mid 1950's, greater attention was paid to the genital form of infection as seen in female cattle. In 1958, this form of infection was referred to as "infectious pustular vulvovaginitis" to replace the various terms used previously (vesicular venereal disease, vesicular vaginitis, coital vesicular exanthema, blaschenausschlag, *etc.*). The origin of the word "herpes" was reviewed in 1973 and that it has been a medical term for at least 25 centuries, although its meaning has varied during that time. Hippocrates used it to mean "shingles" (now known as herpes zoster infection) whereas subsequent medical writers used it more broadly to include several skin conditions, from the true herpes lesions to ringworm. The original word meant "the creep", probably a reflection of the belief that herpes lesions resulted from acrid wastes being excreted onto the skin, creeping forth from a focus to give the characteristic appearance (Yates, 1982).

Herpesviruses are large, enveloped, double-stranded DNA viruses. The inner part of the herpesvirus virion consists of a core containing linear 136 kilobasepairs (kbp) double-stranded DNA genome protected by an icosahedral nucleocapsid of 100 to 110 nm in diameter made up of 150 hexamers and 12 pentamers. This structure is surrounded by a proteinaceous layer, defined as the tegument. The latter is surrounded by a lipid bilayer, the envelope, containing a large number of

viral glycoproteins, among which glycoproteins gB, gC and gD are the most abundant. The mature virion particle ranges from 120 to 300 nm in diameter (Thiry *et al.,* 2006) (Figure 1).

Figure 1 Morphology and Genomic Organisation of Ruminant Alphaherpesvirus



The genome consists of a double-stranded linear DNA. It contains an arrangement of one long unique unit (U_L) and one short unique unit (U_S) flanked by two inverted repeat sequences, named internal repeat (IR) and terminal repeat (TR). The genome includes ten genes encoding glycoproteins: six are located in the U_L segment and four in the U_S segment. The U_S segment can have two possible orientations (depicted by the arrows). The U_L segment is predominantly present in one orientation. The broken arrow illustrates that about 5% of the genomes show the U_L segment in this inverted orientation (Thiry *et al.,* 2006).

Enzyme linked immunosorbent assay could be efficiently applied to the diagnosis of IBR virus infections because of its greater sensitivity and specificity. Furthermore, the test is less expensive and time consuming and can be used to titrate rapidly a large number of sera (Payment *et al.,* 1979; Riegel *et al.,* 1987). Detection of cattle latently infected with BHV-1 is of importance in control programmes and in international trade activities (Kramps *et al.,* 1996). Therefore, tests to detect specific antibodies in serum must be highly sensitive. Blocking ELISA, which is highly sensitive, has also been developed for serodiagnosis of BHV-1 infection (Kramps *et al.,* 1994; van Wuijckuise *et al.,* 1998).

Satyanarayana and Suri Babu (1987b) compared ELISA with indirect-HA and found that ELISA to be rapid, reliable and more sensitive than indirect-HA test in the detection of antibodies to BHV-1.

Avidin Biotin - Enzyme Linked Immunosorbent Assay (AB-ELISA)

The serum based IBR avidin-biotin ELISA kit was developed and commercially available at Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS), Bangalore, India. This kit is widely used in India for the detection of antibodies against BHV-1 and to assess the seroprevalence of IBR among cattle population of Tamil Nadu (Narsimha Reddy, 1993), Andaman and Nicobar group of islands (Shome *et al.,* 1997), Kerala (Rajesh *et al.,* 2003), Mithun breeds of cattle in Arunachal Pradesh, Mizoram, Nagaland and Manipur (Rajkhowa *et al.,* 2004), cattle and buffaloes of Tamil Nadu (Malmarugan *et al.,* 2004), Andhra Pradesh (Sarumathi *et al.,* 2004), Himachal Pradesh (Sharma *et al.,* 2006), Uttaranchal (Sweta Raghuvanshi *et al.,* 2006), Karnataka

(Koppad *et al.,* 2007) and Arunachal Pradesh (Rahman *et al.,* 2007).

Suresh *et al.* (1999) studied the seroprevalence of IBR in different states using AB-ELISA and found it to be economical, very sensitive and specific.

Infectious bovine rhinotracheitis was first reported in India by Mehrotra *et al.* (1976), who isolated IBR virus from cases of keratoconjunctivitis amongst crossbred calves at an organized cattle herd in Uttar Pradesh. Subsequently, Mehrotra *et al.* (1979) reported the isolation and characterization of IBR/IPV virus from a case of abortion in cow.

Suri Babu and Mallick (1983) collected vaginal and nasal swab materials during an outbreak of disease amongst Jersey exotic cattle (Central Cattle Breeding Farm, Sunabeda, Koraput, Orissa), and confirmed the etiological agent as BHV-1 / IBRV strains on the basis of physico-chemical, biological and neutralization tests and concluded that IBR viral agents were responsible for abortions in cattle.

Singh *et al.* (1989) isolated IBRV in Madin-Darby bovine kidney (MDBK) cell line from nasal swabs and buffy-coat of 6 affected Holstein Friesian cattle during two outbreaks of respiratory tract infections, and the virus was identified by its characteristic cytopathic effect (CPE) with typical intranuclear inclusions, physicochemical properties and morphological characteristics of the viral isolates.

Narsimha Reddy (1993) reported that IBR viral isolates could be well adapted to MDBK cell line. Infectious bovine rhinotracheitis virus was isolated from cattle in Erode and Coimbatore in Tamil Nadu. The cytopathogenicity of the IBR viral isolates was characterized by rounding, grouping and formation of intranuclear inclusion bodies. He concluded that the field isolates were not very different from the two known referral viruses with regard to their growth characters in cell culture, physico-chemical properties and protein fractionation studies. However, he reported that further studies on restriction endonuclease analysis, immunological and biochemical characterization are warranted.

Mehrotra *et al.* (1994) isolated BHV-1 from cervico-vaginal swabs and organs collected from a cow during a storm of abortion in an organized farm and the virus was identified by its characteristic CPE with typical inclusion bodies, physico-chemical and neutralization with reference IBR antiserum.

Mohan Kumar *et al.* (1994) isolated IBR virus from nasal and conjunctival swabs collected from crossbred cows in Hassan during two suspected outbreaks of IBR infection in Hassan and Mulbagal areas of Karnataka and the isolate was designated as the Hassan strain of IBRV. This is perhaps the first record of isolation of IBRV in Karnataka. The IBRV was identified on the basis of cytopathology in the infected MDBK cells, demonstration of virus specific immune fluorescence, neutralization tests and the characteristic herpesvirus morphology in the electron

microscopy. The virus was recovered after five serial passages in MDBK cells. Initially this Hassan isolate produced CPE with rounding and ballooning of the affected cells, followed by clumping and giving the appearance of bunches of grapes. Inclusion bodies were not demonstrable in the haemotoxylin and eosin stained coverslip culture.

Bulut and Yavru (2004) isolated BHV-1 from semen and nasal swab samples inoculated into foetal bovine kidney (FBK) and MDBK cell cultures and the CPE was determined, then ELISA/Ag and polymerase chain reaction (PCR) methods were applied for detection of BHV-1 in semen and nasal swab samples.

Elhassan *et al.* (2005) collected samples from cases suffering from infertility, nasal discharges, vaginal discharges, lacrimation and abortion from different regions of Sudan and isolated BHV-1.

Rajesh *et al.* (2007) isolated two isolates from clinical cases and both showed characteristic CPE on MDBK cell line consisting of rounding, grape like bunch appearance and cell detachment. Intranuclear inclusion bodies were observed in the stained MDBK infected cell preparations. The isolates were further confirmed by SNT. The isolation and identification of IBR on MDBK cell line along with molecular analysis was found to be highly specific and reliable technique for the confirmation of the diagnosis.

Trangadia *et al.* (2009) collected genital and nasal swab samples and reported that none of the sample was found to be positive for presence of BHV-1 upon repeated passage in MDBK cell lines.

Van Engelenburg *et al.* (1993) developed a PCR assay to detect BHV-1 in bovine semen using a purification method that eliminated interfering components. This was acheived by separating the semen into a seminal fluid, a non sperm cell and a sperm head fraction and preparing seminal lysates from these fractions using sodium-N-laurylsarcosine, proteinase K and sheared salmon sperm DNA. Seminal lysates were centrifuged at 12000 x *g* and equal volume of 6M NaCI was added to the supernatant and incubated for 5 minutes at room temperature. The DNA was extracted from this mixture using chloroform:isoamyl alcohol and precipitated with isopropanol.

Wiedmann *et al.* (1993) used chelating resin Chelex 100 to prepare viral DNA from artificially inoculated samples of extended and raw semen for use in the PCR assay.

The basic principle of PCR is amplification of the template (target) DNA. This is achieved by the use of synthetic oligonucleotides that correspond to sequences within the target and use of a thermostable DNA polymerase. The exponential increase in target is achieved by subsequent rounds of denaturation, primer annealing and extension by DNA polymerase. After the amplification of DNA, the reaction is assayed for the specific product in agarose gel with ethidium bromide staining, by hybridization with a cloned probe or oligonucleotide probe, or by the digestion with a restriction enzyme. The widespread

success of PCR as a technique comes from the fact that it is rapid, automated, efficient, sensitive and specific (Ramadass, 2008). The PCR method has been applied for the direct detection of BHV-1 in clinical samples, cell culture supernatant and raw and extended semen.

Kibenge *et al.* (1994) and Vilcek *et al.* (1994) described the detection of BHV-1 gB, gC, gD and thymidinekinase (*tk*) DNA by PCR.

Kopp *et al.* (1994) reported that the glycoprotein B homologs represent the most highly conserved group of herpesvirus glycoproteins. They exist in oligomeric forms based on a dimeric structure.

Vilcek *et al.* (1994) used PCR assay based on primers from the viral gI glycoprotein gene for rapid detection of BHV-1. The PCR assay detected 3 fg pure BHV-1 DNA, 0.1 to 1.0 $TCID_{50}$ or a single infected cell. No amplification was observed with DNA from BHV-2, BHV-3, BHV-4, OHV-1 or OHV-2. However, a fragment of the correct size was amplified using DNA from herpesviruses isolated from reindeer, red deer and goats. They concluded that the PCR assay is as sensitive as virus isolation and is a practical alternative for the rapid detection of BHV-1. The PCR technique may become the first choice for the routine diagnosis of BHV-1.

Vilcek *et al.* (1995) developed a PCR assay with primers selected from the gI gene and flanking a 468 bp DNA fragment. Out of 27 samples (nasal swabs, lung, lymph nodes, and tracheal mucosa) collected from 16 different outbreaks in Scotland, 18 were found positive by PCR and 13 by virus isolation. Some samples of isolated DNA had to be diluted by a factor 50 to 100.

Rai *et al.* (2002) isolated DNA from Indian isolate of BHV-1 and amplified 520 bp sequence of glycoprotein C gene by PCR.

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 BHV-1 in clinical specimens because of their rapidity, sensitivity and specificity (Jhala *et al.*, 2007).

Dhami *et al.* (2008) opined that PCR is more sensitive for detection of BHV-1 in aborted placental tissue.

Kapgate *et al.* (2008) performed gI specific PCR for the amplification of BHV-1 from vaginal swabs, nasal swabs and aborted foetus from cow.

Lata *et al.* (2009) used two pairs of primers, gB1 F (5’-TAC GAC TCG TTC GCG CTC TC-3’), gB2 R (5’-GGT ACG TCT CCA AGC TGC CC-3’) and gC1 F (5'-CTG CTG TTC GTA GCC CAC AAC G-3'), gC2 R (5'-TGT GAC TTG GTG CCC ATG TCG C-3') for PCR amplification. The gB1/gB2 primer was selected according to the DNA sequence published for glycoproteins gB (BHV-1.1 Cooper; accession no. M21474) by Fuchs *et al*. (1999), and was predicted to produce a PCR product of 478 base pairs (bp). The gC1/gC2 primer sequences are based on the sequence of the BHV-1 glycoprotein C (gC) gene as used by van Engelenburg *et al*. (1993).

Nandi *et al.* (2009) reported that 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 PCR, nucleic acid hybridization and sequencing.

Bruce *et al.* (1985) studied the isolates of BHV-1 to determine if a single form of BHV-I was responsible for the different virus-associated diseases or whether subpopulations of various isolates produced different clinical symptoms. Studies were initiated to examine the DNA restriction enzyme patterns and nucleic acid homology between virus isolates from respiratory infections and other clinical syndromes. Differences between the genomes of several virus isolates were detected using DNA restriction enzyme analyses.

Brake and Studdert (1985) carried out restriction endonuclease DNA fingerprints for molecular epidemiology of herpesviruses.

Whetstone *et al.* (1989) reported that REA of virus isolates from animals during acute infection, after reactivation of virus from latency, and after reactivation followed by superinfection showed changes in the BHV-1 genome after one passage in the host animal.

Whitbeck *et al.* (1988) carried out the complete nucleotide sequence of the BHV-1 gI gene and predicted 932 amino-acid sequence of the gI primary translation product.

Schwyzer *et al.* (1996) reported that the nucleotide sequence of a 31-kb segment at the left genome end of BHV-1 comprises of 19 different open reading frames (ORFs). All 19 ORFs exhibited strong amino acid sequence homology to the gene products of other alphaherpesviruses. The BHV-1 ORFs were arranged colinearly with the prototype sequence of HSV-1 in the range of the UL54 to UL37 genes. No BHV-1 homologs of the HSV-1 UL56, UL55, and UL45 genes were identified.

Nadin-Davis *et al.* (1996) performed a polymerase chain reaction targeted to the central portion of the BHV-1 genome. The PCR products generated from cell cultures infected with BHV-I.1 were consistently smaller than the corresponding products from cells infected with BHV-1.2. The nature of the sequence differences between these isolates within the target region was found to be a consequence of variable numbers of small GC rich repeats, particularly the sequence 5'-G(A/T)CC-3', present in the region downstream of the gI coding region.

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