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### Bovine herpes virus -1 (BoHV-1) in cattle- a review with emphasis on epidemiological parameters influencing the prevalence of bovine herpes virus -1 in cattle in India

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#### Abstract

This review article summarizes information on description of BoHV -1 virus, BoHV -1 viral genome and its proteins and the latency of BoHV -1 virus. There are different sero-survey, antigen and antibody detection tests available to identify the risk factors and the sero-positivity for BHV-1 infection but only some of them are well defined. The prevalence of Bovine Herpes Virus -1 infection by molecular detection of antigen by Polymerase Chain Reaction and molecular detection of antibody by Enzyme Linked Immuno Assay among infected cattle, which are asymptomatic but continue to circulate the virus between the environment and susceptible animals, the Age wise prevalence, Species wise prevalence and Breed wise prevalence for BoHV -1 virus infection in Cattle can determines and play a significant role in the development of the disease.

Keywords: Epidemiology bovine herpes virus-1 polymerase chain reaction Elisa

#### Introduction

Bovine Herpes Virus -1 (BHV-1) is an agent responsible for the development of a severe respiratory form of infection known as Infectious Bovine Rhinotracheitis (IBR) in high producing cattle and Infectious Pustular Vulvo-Vaginitis (IPV) and Infectious Pustular Balanoposthitis (IPB) in cows and bulls respectively. Bovine herpes virus -1 infection has a worldwide distribution and depicting significant variation in the incidence and prevalence at the regional level.

The disease poses various clinical and pathological problems in affected cattle and causes a considerable economical loss to the livestock industry attributable to the decreased milk production, reduced feed efficiency and reproductive abnormalities. All age groups of cattle are susceptible to the infection although young calves following weaning are found to be highly susceptible that may be associated with a reduction in colostral immunity. Mehrotra *et al.* (1976) <sup>[35]</sup> first reported IBR in India. Kiran *et al.* (2005) <sup>[25]</sup> described this disease as one of the most prevalent respiratory and reproductive viral disease of cattle in India. Sinha *et al.* (2003) and Malmarugan *et al.* (2004) <sup>[30]</sup> reported the IBR prevalence of 2.75 per cent and 81.0 per cent in buffaloes respectively in India. Renukaradhya *et al.* (1996) <sup>[52]</sup> reported the sero-prevalence of 50.9 per cent and 52.5 per cent in cattle and buffaloes respectively.

There are different sero-survey, antigen and antibody detection tests available to identify the risk factors and the sero-positivity for BHV-1 infection but only some of them are well defined. Testing of bulk milk by gB specific ELISA gives a clue to the prior spread of infection in the herd (Frankena *et al.*, 1997)<sup>[12]</sup>. The gE specific ELISA is suitable only when more than 10-15 per cent of the herd is infected. Wellenberg *et al.* (1998)<sup>[28]</sup> reported the bulk milk screening may not be able to describe the herd freedom from the BHV-1 infection and further it necessitates screening of individual serum sample which were negative by milk test.

Currently, PCR is becoming an inevitable molecular technique used in the diagnosis of various diseases because as it is more sensitive and more rapid than virus isolation technique Moore *et al.* (2000) <sup>[41]</sup>. Despite the presence of colostral immunity, the virus maintains latency in trigeminal ganglion of the affected cattle and as and when the cattle are stressed out due to various reasons, they shed the virus in the environment and become the source for infecting the

other susceptible cattle. It could be due to immune evasion mechanism and reactivation of virus following stress. Various intrinsic and extrinsic factors also influence the prevalence of infection among cattle population.

#### **Historical Perspective**

In US, in 1950s, IBR was noted as an emerging disease in feed lot and dairy cattle of Colarado and California. Based on the symptoms observed in infected cattle, the disease was described by several names, red nose, dust pneumonia, necrotic rhinotracheitis and necrotic rhinitis. The US Sanitary Association, in 1955, confirmed the name of the disease as infectious bovine rhinotracheitis. McKercher *et al.* (1955) <sup>[34]</sup> and Kendrick *et al.* (1958) <sup>[24]</sup> suggested the name infectious pustular vulvovaginitis. The close antigenic relationship that exists between IBR and IPV was studied by Gillespie *et al.* (1959) <sup>[17]</sup>.

In Europe, BHV-1 infection has been known for more than a century. In 1960s, only 10 per cent of cattle in Great Britain was found serologically positive for IBR conversely, in between mid and the late 1970s the incidence was found to have increased. In 1986, almost 35 per cent of cattle and 48 per cent of herds in Europe evidenced the antibody to IBR. (Cited by Ganguly *et al.* 2008) <sup>[13]</sup>

Mehrotra *et al.* (1976) <sup>[35]</sup> reported IBR for the first time in India, from naturally infected cross bred calves in Uttar Pradesh. Many research workers (Sulochana *et al.*, 1982; Singh *et al.*, 1983; Manickam and Mohan, 1987; Satyanarayan and Babu, 1987; Mohan Kumar *et al.*, 1994 and Ganguly *et al.*, 2008) <sup>[13]</sup> have reported the widespread nature of IBR in Kerala, Gujarat, Tamil Nadu, Andhra Pradesh, and Karnataka respectively.

During the 19<sup>th</sup> century, Buchner and Tommdorf identified BHV-1 as a probable cause for the venereal form of infection that inflicted cattle (Muylkens *et al.*, 2007)<sup>[43]</sup> in Germany.

#### **Description of BHV-1**

Bovine Herpes Virus-1 is a member of the family Herpesviridae, subfamily Alphaherpesvirinae and found in the genus Varicellovirus (Fenner et al., 1987)<sup>[10]</sup>. Bovine Herpes Virus-1 is the only single serotype identified so far, nevertheless, it consists of three subtypes i.e. BHV - 1.1 (respiratory subtype), BHV-1.2a (genital subtype) and BHV -1.2b (encephalitic subtype) had been recognized on the basis of endonuclease cleavage patterns and DNA technology. Bovine Herpes Virus-1.1 develops a severe respiratory disease and abortion where as BHV-1.2b strains are a less virulent in nature. Subtypes 1.1 and 1.2a has been identified in North America and parts of Europe. Meningo-encephalitis caused by BHV-1 in calves was previously included into BHV-1.3 subtypes and at present which isreclassified into BHV- 5 type (OIE, 2010) [47]. Goat herpes virus - 6 (GHV-6) serotype has close antigenic relationship with BHV-1 (Engels et al., 1983)<sup>[9]</sup> 20094. All the strains studied so far as based on the phylogenetic analysis in India have been the predominant BHV-1.1 subtypes. (Rahman et al., 2011)<sup>[49]</sup>.

#### **BHV-1** Genome and Its Proteins

BHV-1 genome belongs to a D group in the classification, consists of a long double stranded DNA molecule, encodes for a total of 70 proteins, of which 33 are structural and 15 are non-structural proteins (Ganguly *et al.*, 2011 and OIE, 2010) <sup>[14, 69]</sup>. The total molecular size of the genome is 135-140 Kb. Proteins of subfamily Alphaherpesvirinae are highly essential

for entry, pathogenesis and immunity development in the hosts. The viral genome consists of 12 enveloped glycoproteins namely; gB, gC, gD, gE, gG, gH, gI, gL, gM, gK, gN and Us9, of these, ten former proteins are glycosylated and two later are non-glycosylated. The gB, gC and gD proteins are considered as major proteins (Jones and Chowdhury, 2008)<sup>[23]</sup>.

#### Latency of BHV-1

Cattle infected with BHV-1 could excrete the virus for a prolonged period of time and develop latency following recovery from the infection. Some cattle those developed bronchopneumonia during this period become permanent carriers. Bovine Herpes Virus-1 persists in the peripheral sensory ganglia such as trigeminal, sacral, lumbar or thoracic and shed the virus in response to various stresses and such cattle were found spreading the infection to immunocompromised cattle (OIE, 2004) <sup>[46]</sup>. During an acute stage, the viral and sub-viral particles, besides, cell to cell spread (Winkler *et al.*, 1999) <sup>[14]</sup> also enter through oral, nasal or ocular route and sets up an infection in the sensory neuron of trigeminal ganglion. Jones and Chowdhury (2008) [23] described that the abundant transcription of latency related (LR) gene and g E gene coding for glycoprotein E was responsible for the latency of BHV-1. Latently infected cattle become a carrier for their life.

#### Prevalence of bhv-1 infection Global scenario

Genital form of BHV-1 infection was found more predominant in Europe than the respiratory form of infection, while the digestive disorders were found more common in calves especially in Belgium (Straub, 1991)<sup>[62]</sup>.

In Sudan, Elhassan and Co-workers (2006) identified the highest rate (73 per cent) of sero-positivity on the basis of serum neutralization test (SNT) and also found the SNT was the best and widely used technique among all the tests that were used in their study.

In Australia, for the first time, IBR was reported in 1962 from an outbreak of vaginitis and rhinitis in cattle as based on virus isolation technique. The prevalence of antibody reported in mature breeding cattle in Australia was 25 per cent to 40 per cent. Out of 80 per cent beef feedlot cattle which were negative at the time of entry into the farm in Australia atleast, 60 per cent were found as sero-converters against BHV-1 while at slaughter (Gu and Kirkland, 2008)<sup>[19]</sup>.

The prevalence of subtypes BHV-1.1 and BHV1 -1.2a were reported from North America and Parts of Europe (OIE, 2010)<sup>[47]</sup>. Mahomoud *et al.* (2009) in an investigational study in Egypt, found a higher incidence (80 per cent) of BHV-1 in apparently healthy cattle that were raised in closed farms, the lower incidence (62.5 per cent) in cattle in open farms and very less percentage positivity in buffaloes.

Though many countries around the world have reported the IBR, some countries namely, Austria, Denmark, Finland, Sweden, Italy, Switzerland, Norway, and parts of Germany have got the virtual freedom from BHV-1-IBR (OIE, 2010)<sup>[14]</sup> but the programme for control for BHV-1-IBR has been in progress in some other countries like, Australia, Belgium, Canada, India, Poland, Turkey and USA (Nandi *et al.*, 2009)<sup>[44]</sup>.

#### Indian Scenario

Mehrotra and his co-workers identified IBR for the first time

in India in 1976. Since then it has been reported from all states in India and becoming an endemic disease Cumulative sero-surveillance study carried out in 57,009 serum samples in between 1995 and 2010, identified 36 per cent of serum samples as positive for IBR antibody by AB-ELISA (Rahman, 2011)<sup>[49]</sup> in India.

Indirect hemagglutination test was found as quite a sensitive and inexpensive test for screening of the prevalence of economically important BHV-1-IBR (Kirby *et al.*, 1974) <sup>[26]</sup>. Samal *et al.* (1981) <sup>[54]</sup> also reported 56.5 per cent cattle as positive by hemagglutination inhibition test for IBR antibody, In an attempt to detect the antibodies against IBR in both vaccinated and experimentally infected animals, among the five tests conducted, correlation was found within the results obtained in three tests (PHA, VNT and ELISA) (Edwards *et al.*, 1986) <sup>[7]</sup>.

In Maharashtra, Chinchkar *et al.* (2002) <sup>[4]</sup> reported that cattle tested positive for IBR was higher (33.91 per cent) compared to buffaloes (31.0 per cent). Sontakke *et al.* (2002) <sup>[61]</sup> detected antibody to IBR in 54.28 per cent and 46.42 per cent of cattle and buffaloes with clinical signs respectively. Also they reported cattle and buffaloes with conjunctivitis had higher antibody percentage (62.5 per cent) than those animals with rhinitis (57.1 per cent) and other clinical signs.

Afonso *et al.* (2007) <sup>[1]</sup> described the Random Amplified Polymorphic DNA (RAPD) as an effective technique and that could be used to carry out the phylogenetic analysis and to study the relationship that exists between the samples isolated from bovine with different clinical symptoms.

Ganguly *et al.* (2008) <sup>[13]</sup> identified 85.29 per cent seropositive in cattle population using virus neutralisation test in Nadia district in West Bengal in contrast to the lower prevalence of 20.72 per cent in Jalpaiguri district.

Trangadia *et al.* (2010) <sup>[69]</sup> worked out the overall seroprevalence 60.84 per cent from organized cattle farms in India, surprisingly, the researchers could not achieve the isolation of BHV-1 virus through cultivation of genital and nasal swabs in Madin-Darby Bovine Kidney (MDBK) cell lines, The prevalence of IBR antibody in cattle and buffalo in Gujarat and Andhra Pradesh were reported to be 23.94 per cent and 26.49 per cent respectively Trangadia *et al.* (2012) <sup>[70]</sup>.

The overall sero-positivity in India was reported to be higher in Tamil Nadu (67 per cent) and lower (34 per cent) in Meghalaya. Individual regional based prevalence was observed as 17 per cent in Eastern India, 24 per cent in western India, 37 per cent in northern India, 39 per cent in north eastern and 25 per cent in central India respectively (Rahman *et al.*, 2011)<sup>[49]</sup>.

#### **Species Wise Prevalence**

BHV-1 affects wide range of animal species, which includes cattle, sheep, goats, water buffaloes and other wild species such as antelope, wild beast, hippopotamus, caribou and species of Mustelidae family and man (Radostits *et al.*, 2007) <sup>[48]</sup>. The prevalence of IBR in buffaloes would be lesser than it is observed in cattle (Suresh *et al.*, 1999 and Sharma *et al.*, 2009) <sup>[58]</sup>. Infectious Bovine Rhinotracheitis sero-prevalence in buffaloes with a history of reproductive disorders has been found higher (40.30 per cent) than in buffaloes with respiratory infection (29.1 per cent) in Tamil Nadu (Suresh *et al.*, 1992) <sup>[65]</sup>. In an antibody prevalence study carried out to IBR in Mithun cattle from Arunachal Pradesh, Mizoram and Nagaland in India. Rajkhowa *et al.* (2004) <sup>[51]</sup> found 38.46 per

cent, 18.8 per cent and 15.5 per cent respectively as positive and zero per cent for Mithun in Manipur. Bovine Herpes Virus-1 antibody was recorded in 60.1 per cent Yaks (Peophagus grunniens) in the National Research Centre for Yak in India (Nandi and Kumar, 2010)<sup>[10]</sup>. The Per cent positivity in male Yaks, Yak cows and Yak heifers was found to be 67.7 per cent 62.6 per cent and 50.0 per cent respectively.

#### Age Wise Prevalence

All age groups of animals are found to be susceptible for IBR; nevertheless, the disease occurs most commonly in animals over six months of age (Radostits *et al.*, 2007) <sup>[48]</sup>. There has been a report that an adult age group of cattle to be highly susceptible compared to animals in younger age group that was attributed to higher exposure to BHV-1 and the development of carrier status in the former age group of cattle (Dhand *et al.*, 2002) <sup>[6]</sup>. Lower prevalence in cattle in younger age groups might have been attributed to the presence of maternal immunity (Fenner *et al.*, 1987) <sup>[10]</sup>. An unvaccinated herd of breeding or beef lot cattle are highly prone for epidemics of respiratory form of IBR and abortion. It has been reported that the prevalence in cattle 9 years and above has been found to be more while comparing the prevalence in other age group (Sharma *et al.*, 2006) <sup>[58]</sup>.

#### **Breed Wise Prevalence**

The prevalence of infection in cross bred cattle outnumbered the prevalence in non-descript cattle (Koppad *et al.*, 2007)<sup>[27]</sup>. No significant difference was found between the occurrence of disease in cross bred and non-descript buffaloes (Suresh *et al.*, 1992). Occurrence in female cattle could be higher than in male (Sharma *et al.*, 2006)<sup>[58]</sup>.

Significantly higher rate of sero reactors has been found in cattle reared in both organized and unorganized farms than in buffaloes (Dhand *et al.*, 2002) <sup>[6]</sup> whereas, cattle maintained in the closed farms found with higher percentage of antibody to IBR (Suresh *et al.*, 1999) <sup>[67]</sup>. Cattle in the organised farms have been reported to be affected more (Ganguly *et al.*, 2008) <sup>[14]</sup>, in contrast to some cattle and buffaloes maintained in the organized dairy farms that were found to be equally susceptible (Trangadia *et al.*, 2010) <sup>[69]</sup>.

#### Epidemiology

#### Transmission

Nose to nose contact is the main mode of transmission occurs between the infected to susceptible cattle (Muylkens et al., 2007)<sup>[43]</sup>. Aerosols contaminated from the exhaled, sneezed, and coughed up materials shed by the infected animals (Mars et al., 1999). Transmission of IBR depends mainly on the rich viral sources of the infected materials. Nasal exudates, coughed out droplets, genital secretions, semen, foetal fluids and tissues are considered as potential viral materials for the transmission. Bovine Herpes Virus-1 is able to survive for up to 1 year in semen frozen in liquid nitrogen (Nandi et al., 2009) <sup>[44]</sup>. The virus in semen can be transmitted through natural service and artificial insemination. Venereal transmission becomes the method of spread for genital diseases. Bovine Herpes Virus-1 can also be spread from inanimate objects. Ticks (Ornithodorus coriaceous) can influence the mechanical transmission of BHV-1 in cattle (Straub, 1990). Direct contact, dense cattle population was found to increase the risk for BHV-1 infection (Van Schaik et al., 2002 and Vonk Noordegraaf, 2004) <sup>[71, 73]</sup>.

Virus excreted from vaginal and preputial secretion are less likely to transmit IBR to other animals. Latently infected cattle serve as carriers for other susceptible cattle (Thiry *et al.*, 1987)<sup>[67]</sup> and make the control program very difficult. Sheep may unable to transmit BHV-1 infection to cattle (Hage, 1997)<sup>[15]</sup>. Experimental infection of rabbit via intraconjunctival or the intranasal route may be possible (Meyer *et al.*, 1996)<sup>[36]</sup>.

#### **Development of the Disease**

Through nasal inhalation the virus able to make entry into the mucous membrane of the upper respiratory tract and tonsils, where in the virus undergoes multiplication in high titres. From there on distribution of the virus occurs to conjunctivae and finally reaches the trigeminal ganglion by neuro-axonal transport. Sero-nasal discharge followed by mucopurulent, salivation, fever, inappetance, and depression are the most important clinical signs exhibited by the infected cattle after 2-4 days of incubation period (OIE, 2008) <sup>[45]</sup>. Following respiratory infection the viral shedding occurs for 10 to14 days with 10<sup>8</sup> to 10<sup>10</sup> TCID<sub>50</sub>. Nasal, ocular and genital swabs are the samples can be collected for the diagnosis of BHV-1 infection.

Affected animals can clinically be identified by the development of ocular, respiratory, reproductive, alimentary and central nervous system problems and there may be a generalized new borne infection in young calves (Gibbs and Rweyemam, 1977)<sup>[16]</sup>. Acute BHV-1 respiratory infections may predispose cattle to potentially fatal bacterial pneumonia which is a major cause of death and economic losses to the beef lot cattle industry (Yates, 1982)<sup>[76]</sup>. Introduction of animals into a farm often leads to an outbreak of IBR. In reproductive tract infection the virus multiplies in mucous membranes of the vagina, prepuce and become latent in the sacral ganglia and remains in the neuron of the ganglia probably for the life time (OIE, 2008)<sup>[45]</sup>.

#### **Diagnostic tests**

## Detection of antibody against bhv-1 by enzyme linked sorbent assay (Elisa)

Though the concentration of immunoglobulin in milk is lower than those in serum, the gE ELISA is highly sensitive for the detection of antibody against BHV-1 in milk (Mach and Pahud, 1971)<sup>[29]</sup>. Similarly gE Milk ELISA was identified as a highly sensitive and specific test than serum gE ELISA (Wellenberg, 1998)<sup>[28]</sup>.

According to Shome *et al.* (1997)<sup>[59]</sup> AB-ELISA detected 89 per cent out of 203 samples as positive for antibody to IBR in cattle. Also Suresh *et al.* (1999)<sup>[64]</sup> reported 38.01 per cent positive out of 3,428 cattle screened for the presence of IBR antibody and declared AB-ELISA as the best technique among the five techniques performed.

In Maharastra, Chinchkar *et al.* (2002) <sup>[4]</sup> using Dot ELISA found 58.13 per cent in cross bred cattle with IBR antibody and suggested that could be due to exposure of animals to the virus. Regardless of breed, age, parity, health status and management practices Rajesh *et al.* (2003) <sup>[50]</sup> described the sero-prevalence in 28 to 110 cattle in Kerala.

The gB specific ELISAs are more sensitive for the detection of antibody in serum samples. Indirect ELISAs and gB blocking ELISAs had a highly comparable sensitivity and specificity (Beer *et al.*, 2003) <sup>[2]</sup>.

Enzyme Linked immuno Sorbent Assay technique has gradually replaced Viral Neutralisation test. Several ELISAs

are utilised for the detection of antibody in serum samples, however, Kramps *et al.* (2004) <sup>[28]</sup> detected antibody to BHV1-IBR in milk, AB- antibody ELISA revealed 45.01 per cent serum antibody to BHV-1 infection in bulls in Punjab State and on comparison with the results of other three different techniques performed, PCR was considered as more sensitive technique than virus isolation in bulls (Deka *et al.*, 2005) <sup>[5]</sup>.

Infectious Bovine Rhinotracheitis monoclonal antibody-based blocking ELISA showed 30 per cent bulls as overall positive for BHV-1 infection while gB gene PCR showed 42 per cent as positive (Jain *et al.*, 2009) <sup>[21]</sup>.

Since some of the samples which were positive by ELISA were negative by PCR and vice versa, Jain *et al.* (2009) <sup>[21]</sup> suggested the use of both serological and PCR diagnostic tests, they also observed 15 of 50 breeding bulls as positive by antibody-based blocking ELISA. In an investigational study, Mahmoud *et al.* (2009) <sup>[32]</sup> reported ELISA was the most rapid, reliable, inexpensive and simplest test of all the tests and could be the most suitable technique for screening of large animals in herds.

Indirect ELISAs are the most sensitive tests used in the detection of BHV-1 antibodies in milk (OIE, 2010)<sup>[47]</sup>.

In a study, out of 595 cattle and buffalo screened 362 were described to be positive by ELISA and the highest prevalence was observed in central region of India followed by southern, western, and northern region (Trangadia *et al.*, 2010) <sup>[69]</sup> this study also reported the overall prevalence rate of 60.84 per cent but failed to isolate BHV-1 from nasal or genital samples even after repeated passage in Madin Darby Kidney (MDBK) cell lines.

#### Molecular Detection of Bhv-1 Dna By Polymerase Chain Reaction (PCR)

Vilcek *et al.* (1994) <sup>[72]</sup> were successful in the detection of herpes virus DNA from samples of reindeer, red deer and goats by PCR assay and they also succeeded in the detection of BHV-1 from semen and serum samples. On comparison of PCR and the virus isolation test with experimentally inoculated bulls with BHV-1 virus, at different days of post inoculation, the analysis showed that virus isolation in egg yolk extended semen samples 24, virus isolation on fresh semen 51 and by PCR assay 118 as positive out of 162 semen samples tested. Through their study Frank *et al.* (1995) <sup>[11]</sup> understood intra-preputially infected bulls could excrete the BHV-1 virus for a longer period of time than any other route of infection.

In BHV-1 endemic countries PCR screening could be very cost effective, which can be used to reduce the spread of BHV-1 virus through semen by early and quick diagnosis (Gee *et al.*, 1996) <sup>[15]</sup>. In a molecular differential diagnosis, between the wild type Bovine Herpesvirus -1 and gE negative strain, made by PCR assay and the specificity was confirmed by restriction enzyme analysis and DNA sequencing of the amplicons (Schynts *et al.*, 1999) <sup>[42]</sup>. Based on the results obtained, the study suggested that PCR could be a useful tool for monitoring the spread of live marker vaccine and gE genotype of viral field isolates.

Tiwari *et al*, (2000) <sup>[68]</sup> found the simple boiling water method itself was sufficient for PCR amplification and moreover, they suggested that simultaneous extraction of purified DNA was not essential for comparison of PCR products.

Moakhar *et al.* (2003) <sup>[37]</sup> suggested that PCR has a great applicability in the screening of BHV-1 infected aborted

fetuses of cattle and early detection of BHV-1 in tissue culture viral isolates, comparably PCR was time saving assay than the virus isolation and the Neutralization test. Deka *et al.* (2005) <sup>[5]</sup> observed 14 of 24 bull semen samples were positive for 468bp gI gene fragment by PCR and 11 samples by virus isolation technique.

Bovine Herpes Virus-1,gB and gE gene from semen samples of naturally infected bulls was detected by PCR assay and the sensitivity of this assay was found to be comparable with virus isolation test (Grom *et al.*, 2006) <sup>[18]</sup>. Jhala *et al.* (2007) <sup>[22]</sup> suggested that PCR based assay is highly rapid and sensitive method for screening of bulls in semen collection centers.

Jain *et al.* (2009) <sup>[21]</sup> found 46.53 per cent and 42.57 per cent semen samples from bulls in Gujarat as positive for BHV-1 infection by gB gene and gC gene based PCR. Open reading frame of the gB gene from BHV-1 genomic DNA was amplified and used in PCR cloning by Momtaz and Abbasian (2009) <sup>[40]</sup>.

Polymerase Chain Reaction amplification of gB gene from semen indicated the incidence rate of 42 per cent for BHV-1 infection in bulls in Gujarat state. The incidence was found to be comparatively higher in cattle (50 per cent) than the incidence that was recorded in buffaloes (34.61 per cent) (Jain *et al.*, 2009) <sup>[21]</sup>.

Rodriguez Medina *et al.* (2009) <sup>[53]</sup> reported that even in an optimal thermal cyclic condition, a thymidine kinase (tk) based PCR method that was developed to detect Bovine Herpesvirus -1 appears to have failed in detecting the gene from either heterologous or any other bovine viruses, however, the same PCR method succeeded on the other hand in the amplification of BHV-1 fragment size of 202bp and so why the research workers considered this PCR as specific method. Real Time PCR was found successful in the detection of buffaloes experimentally inoculated with field cattle strain of BHV-1 (Teresa Scicluna *et al.*, 2010) <sup>[66]</sup>.

Chandranaik *et al.* (2010) <sup>[6]</sup> carried out a large level screening work on semen samples collected from four states of southern India and identified four samples with cytopathic changes in cell lines and also confirmed that by real time PCR technique. The per cent IBR positive in cattle bull (40.81 per cent) was comparatively higher than in the buffalo bulls (38.46 per cent). Breeding bull semen was found 39.60 per cent positive by gC gene PCR than buffalo bulls semen.

#### Conclusions

The prevalence of BHV-1 Infection is likely to increase with the advancement of age in older animals than the younger animals, higher prevalence may be found in older female cattle and pluriparous animal due to heavy milk production stress factors, likewise more prevalence of BoHV-1 infection can be found in intensive farming due to the maintenance of close contact of animals with parturition and milk production attributing the other factors. In younger animals the prevalence of BoHV-1 is low, which could be attributed due to the fact of presence of maternal antibody or immunity, the prevalence of BoHV-1 infection among breeds showed no significant difference, but the prevalence of BoHV-1 infection is recorded among Mithun and Yak species in India. Although most of the infected cattle are asymptomatic and they continue to circulate the virus between the environment and susceptible animals the virus maintains latency in the trigeminal ganglion of the affected cattle and as and when the cattle are stressed out due to various factors the virus is shed

in the environment which becomes a source of infection to other susceptible cattle, which could be attributed due to the factor that the immune evasions mechanism and reactivation of the virus following stress, the prevalence of BoHV -1 Infection can also be influence by various intrinsic and extrinsic factor. No one individual diagnostic technique has the ability to detect both antigen and antibody at a time and sometimes samples which is positive by one test may be negative by another test, ELISA is a rapid, inexpensive and highly specific test for detecting antibody titre in sera and milk of animals, that is why it is of paramount significance to detect latent virus carriers in control programme, in International trade act, sero epidemiological studies, sero surveillance during eradication programme and to evaluate antibody response during vaccination studies, PCR is becoming an imminent molecular technique used for the diagnosis of various disease since it is more rapid and sensitive technique tool. Molecular tool technique is useful for the detection, genetic characterization and the presence of genetic variation that might exists among the circulating field strains of BoHV-1.

#### **Competing Interests**

The author declare that they have no competing Interests

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