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Review Article

BOVINE HERPES VIRUS-1 AND ITS INFECTION IN INDIA - A REVIEW

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This review article summarizes recent information on the molecular biology of bovine herpesvirus 1 (BoHV-1). This may provide a better understanding of BoHV-1 latency. DNA fingerprinting of isolates allow tracing diseases and gaining a picture of the molecular epizootiology. The major immunogenic proteins, especially a 74 Kd glycoprotein which gives rise to neutralizing antibodies, have been defined as those structural components being involved in cross-reactions with the goat herpesvirus (BoHV-6) and being of importance for diagnostic assays. Besides the known clinical appearance of BoHV-1 infections, expressing them mainly as respiratory or genital infections, the immune response is discussed and its value outlined for diagnostics and epizootiology. In conclusion, recommendations are given to control or eradicate BoHV-1 infections.

Key words: Bovine herpes virus, Cattle diseases, Immunity, Latent infection, Viral diseases

Bovine herpesvirus1 (BoHV-1) causes two diseases in cattle: Infectious bovine rhinotracheitis (IBR) and Infectious pustular vulvovaginitis (IPV). These infections occur worldwide. The clinical signs are characterised by fever and involvement of the upper respiratory tract, including conjunctivitis, rhinitis and tracheitis. Secondary bacterial infections may lead to pneumonia, especially in intensively managed livestock, such as beef cattle and in feedlots. The venereal forms of the disease result in pustular lesions in the prepuce and penile epithelium of the bull and vulva and vagina of the cow. These lesions can impair reproduction. Strains of BoHV-1 that can cause abortion are found in many countries. In India, the disease is endemic. As BoHV-1 is excreted in semen and can be spread by artificial insemination, bulls entering artificial breeding centres are screened for freedom from infection. Freedom from infection is most frequently determined by serological methods, using the virus neutralization test (VNT) or enzyme-linked immunosorbent assay (ELISA). Disease is diagnosed by demonstration of seroconversion with paired sera or by virus isolation from specimens collected during the acute phase

of the disease. Semen certification is achieved by use of the real time polymerase chain reaction (rPCR) to detect viral DNA.

Aetiology :

Infectious bovine rhinotracheitis or Infectious pustular vulvovaginitis (IBR or IPV), caused by Bovine herpesvirus (BoHV-1), is a disease of domestic and wild cattle. BoHV-1 is a member of the genus Varicellovirus in the subfamily Alphaherpesvirinae, which belongs to the Herpesviridae family, order Herpesvirales. Three subtypes of BoHV-1 are recognised worldwide: BoHV-1.1, BoHV-1.2a and BoHV- 1.2b. Viruses from the BoHV-1.1 subtype cause severe respiratory disease and can be associated with abortion. Viruses isolated from buffaloes and goats and previously identified as BoHV-1 by serological tests have a different restriction enzyme profile than the subtypes of BoHV-1, are now regarded as separate viruses and have been classified as BoHV-2 and caprine herpesvirus, respectively. The bovine herpesvirus causing meningoencephalitis (previously BoHV1.3) has been classified as BoHV-5.1. BoHV-4, which was found wide spread in Israel, can cause mastitis, pneumonia, metritis, vaginitis, conjunctivitis, inter digital dermatitis and abortion in cattle.

History :

The first report of disease caused by BoHV-1 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. 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 (BoHV-1) belongs to the herpesvirus group. The virus was first isolated from respiratory disease in the United Kingdom in the early 1960s (Darbyshire *et al*., 1964). Kendrick *et al.*

(1958) described its association with Infectious pustular vulvovaginitis (IPV). Huck *et al*. (1971) described its association with Balanoposthitis. 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).

Virion structure :

BoHV-1 has a typical herpesvirus virion structure and it's double stranded DNA genome is contained within an icosahedral protein capsid. The capsid is wrapped in a protein complex called the tegument, which is made up of about 20 viral proteins The tegument connects the capsid with the outer cell-derived envelope, which contains the viral membrane proteins and glycoproteins that are essential for the successful penetration of the cell membrane, including glycoproteins gD, gB, gH, and gL.

Replication cycle :

The BoHV-1 virion penetrates the cell membrane via a three step process. First, glycoproteins gB and gC on the virion envelope interact with certain cellular structures, creating a low affinity attachment between the virus and the host cell. Second, glycoprotein gD binds to cell membrane protein nectin-1, an immunoglobulin protein. This initiates the third phase, during which the virion envelope fuses with the cell membrane, allowing the capsid and tegument to enter the cytoplasm. Upon entering the host cell, the virion begins to move towards the nucleus. As it is transported, the tegument proteins surrounding the capsid are shed into the cytoplasm. Although many of these tegument proteins are poorly understood some are known to have important functions, such as disabling host defenses or subverting the host's resources. For example, the virion host shutoff (vhs) tegument protein is responsible for halting the host's regular protein synthesis and tegument protein VP16 is required to induce expression of early BoHV-1 genes. Once it has breached the nuclear membrane, the linear viral genome end-joins and becomes a combination of viral and cellular proteins induces the expression of the "immediate early" (IE) genes. The IE gene products induce the expression of "early" genes, at which point viral DNA replication begins. "Early late" gene expression begins during viral genome replication, and then finally the "true late" genes are expressed, which code for the capsid-forming structural proteins. Meanwhile, replication of the now-circular viral genome proceeds via a "rolling circle" mechanism, which produces multiple genomes connected to each other in sequence from head to tail (concatemeric DNA). This long strand of DNA is then cleaved into individual copies of the viral genome, which are loaded into the newly-

formed virion capsids without leaving the nucleus. How the virion capsid proceeds to exit the nucleus and acquire its tegument proteins and outer envelope is a matter of debate. The leading theory suggests that the mature capsid acquires a primary envelope as it buds out of the inner nuclear membrane, and into the perinuclear space. The new primary membrane then fuses with the outer nuclear membrane, so that the naked capsid is released into the cytoplasm. The capsid then acquires its tegument coating and final envelope by budding with a trans Golgi compartment.

Classification and biology of the viruses:

The IBR /IPV viruses have been classified as BoHV-1. This is based on serology. Their identity was later proved by DNA studies. With certain restriction enzymes such as 'HpaP, however, a clear separation into IBR-like and IPV-like strains is possible, grouping all the foetal isolates, some of the brain isolates and abortigenic viruses with the IBR-like viruses. Different findings with viruses from other countries, where the classical picture of European IPV is not known or has become modified, most probably reflect rapid changes of the viruses in cattle populations. BoHV-1 does not cross-react with BoHV-2 (Bovine herpes mammillitis virus), with the African malignant catarrhal fever virus (tentatively grouped as BoHV-3) or with BoHV-4. Caprine herpesvirus (tentatively grouped as BoHV-6) is antigenically and serologically closely related to BoHV-1, but clearly differs in DNA fingerprinting. The BoHV-1 genome is similar to that of pseudorabies virus, equine herpesvirus types 1 and 3, and varicella-zoster virus, belonging to the class D herpesviruses. The BoHV-1 genome has a molecular weight of 135 Kd and is known to be composed of a unique short (Us; 13 Kd) sequence which inverts relatively to the unique long (UL; 100 Kd) sequence, leading to two isomeric forms of DNA which are flanked by internal (11 Kb) and terminal repeats (11 Kb). Recent findings show that the BoHV-1 genome has a small unique DNA "tail" on the terminal repeat sequence which might be involved in replication and could turn out to be important for the latency of the virus. Short repeats in BoHV-1 DNA terminal fragments which are responsible for the heterogeneity of the genomes appear to play a role in the circularization or concatamerization of the viral genome during DNA replication and subsequent cutting. Although BoHV-1 replication is far from being understood, genome structures of this virus have proved to be a good model of the general properties of herpesviruses, e.g. the terminase cleaving the DNA into the right genome length size (Pandey *et al.*, 2000)

Proteins and antigens :

BoHV-1 induces approximately fifty infected cell proteins, about thirty of which are structural proteins of the virus and approximately fifteen are non-structural

polypeptides. Eight to eleven are glycosylated (Misra *et al.*, 1981).The glycoproteins appear on the outer envelope of viral particles as well as on the surface of infected cells and represent the main target for the immune system, inducing a response which may neutralize free virus and destroy infected cells. BoHV-1 glycoproteins are divided into three groups, each consisting of two or three polypeptides which appear to be different forms of the same basic molecule, i.e. as a monomer or dimer or as a precursor and its final products. It emerged from different studies and was essentially pointed out for the first time by that a glycoprotein with a molecular weight between 74,000 and 82,000 represents the main immunogenic component, which induces the strongest neutralizing immune response. Therefore, the 74 Kd glycoprotein is the most suitable candidate for a possible future subunit or recombinant vaccine. Antibodies against other glycoproteins seem to neutralize BoHV-1 weakly or only in the presence of complement BoHV-1 strains of different origin and pathogenicity exhibit a high degree of uniformity in their protein composition, although strain-specific differences were observed with some minor polypeptides. It is of special interest that the goat herpes virus (BoHV-6) shares the major immunogenic glycoproteins with BoHV-1, which is the reason for strong immunological cross-reactivity and a marked one-way cross-neutralization of BoHV-6 by antibodies directed against BoHV-1.

Virus ecology and pathology :

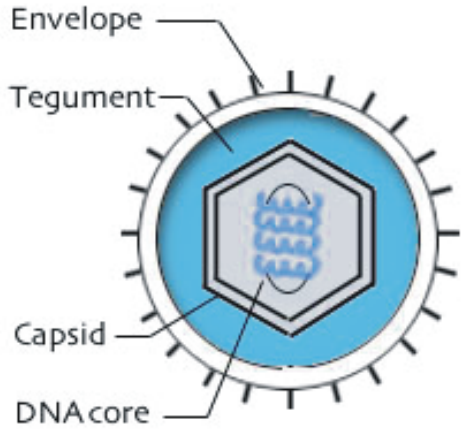
Cattle are very commonly kept in extremely crowded and unsanitary conditions which allow BoHV-I to easily spread through a herd. The most common route of infection by BoHV-1 is via the direct exchange of nasal runoff between an infected individual and an uninfected individual, although other routes of horizontal transmission include genital contact, semen transmission, and short-distance aerosol transmission (Mars *et al.*, 1999). The virus can also be transmitted vertically from mother to calf during pregnancy. The virus immediately infects the new host's epithelial cells at the contact site, where it rapidly begins its standard lytic replication cycle, spreading to surrounding cells via local dissemination. If the virus makes contact with the ordinary infection site, the nasal epithelial cells, then it can very quickly establish secondary infections via the shedding of virion-loaded nasal mucus. After spreading within the same tissue, the virus may go on to infect the host's blood, although the mechanism of this advance is poorly understood. Access to the circulatory system gives the virus access to many other types of the host's tissue, accounting for the large diversity in symptoms. In addition to this systemic spread, the virus can also make contact with nerve endings in nasal mucosal surfaces, through which the infection

spreads to the central nervous system. BoHV-1 typically infects the trigeminal nerve endings, through which it establishes a latent infection in ganglionic neurons. Even after the animal stops exhibiting symptoms of infection, the latent infection can be reactivated later in life, at which point the animal begins shedding the virus. After genital infection, BoHV-1 replicates in the mucous membranes of the vagina or prepuce, and becomes latent in the sacral ganglia (Miller, 1991). The viral DNA remains in the neurons of the ganglia, probably for the entire life of the host (status of latency). Stress, such as transport and parturition, but also the application of corticosteroids can induce reactivation of the latent infection leading to standard viral lytic cycle which can lead to pustular vulvovaginitis or balanoposthitis.

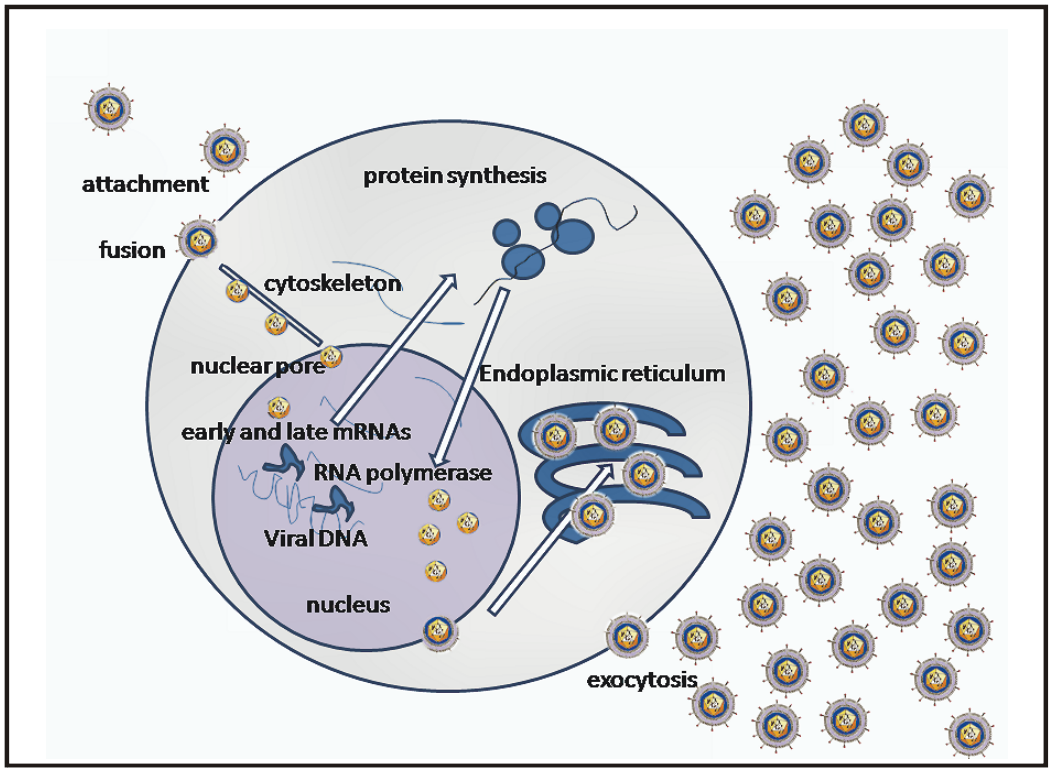
Clinical signs :

BoHV-1 infection causes an acute, contagious disease that affects either the respiratory or reproductive tracts in the following forms :

Respiratory infection - The respiratory form of the disease is most frequently observed in cattle managed under intensive conditions (for example, feedlots) and is not often noticed in cattle under grazing conditions. The clinical signs and pathological changes of BoHV-1 infection of cattle are not characteristic and could easily be confused with disease produced by a number of other pathogens. Laboratory confirmation is therefore essential. The incubation period ranges from 2-7 days. If the cases are uncomplicated, the disease may be very mild with only slight serous nasal discharge and a modest rise in body temperature over one to two days. Many cases remain unnoticed. In more severe cases, there is pronounced pyrexia of 40 - 42^{o}C, which may last for several days. Affected animals are depressed with an increased respiratory rate and show a decline in milk production. The initial serous nasal discharge often becomes muco-purulent within a few days. The mucosa of the nares becomes reddened and shallow erosions may be present. Some animals develop excessive salivation. Oral lesions, which are uncommon, consist of shallow erosions of the oral mucosa. Some animals develop unilateral or bilateral conjunctivitis and have a clear ocular discharge, which may later become muco-purulent. In feedlot or other intensively managed cattle, there can be a severe necrotising laryngotracheitis and pneumonia that is complicated by secondary bacterial infections. These infections are usually encountered within the first 3 - 4 weeks of animals entering a feedlot. From time to time, there can be outbreaks of severe pneumonia due to BoHV-1 infection at later times after entry to the feedlot. Abortion as a complication of the respiratory form of BoHV-1 infection has been frequently reported.



BoHV-1 Structure



Replication cycle of BoHV-1

Genital infection - Genital infection with BoHV-1 occurs in both sexes and is a more frequent manifestation of this herpesvirus Infection in cattle on pasture. The infection may result in the development of vesicles, pustules and erosions or ulcers in the mucosa of the vulva and vagina or on the penis and prepuce (Afshar, 1965).

Vulvovaginitis - This painful condition, which is known as Infectious pustular vulvovaginitis (IPV), may be observed within a few days of mating. Frequent micturition and raising of the tail are the first clinical signs. There may be hyperaemia or oedema of the vulva and the posterior third of the vagina. Small red to white ulcers develop into pustules (0.5 – 3 mm in diameter). There may be a thick yellow or white mucopurulent exudate, especially in cases complicated by secondary bacterial infection.

Balanoposthitis - The disease in bulls is known as Infectious pustular balanoposthitis (IPB). After a 2–3 day incubation period, pustules appear on the mucosal surface of the penis and prepuce. These pustules can progress to ulcers with a mucopurulent discharge and may prevent a bull from servicing. A proportion of infected bulls will also excrete virus in their semen. In turn, infected semen can infect susceptible females, by natural or artificial insemination(Afshar and Eaglesome, 1990).

Conjunctivitis - The conjunctival form of BoHV-1 infection, which resembles 'pink eye', is relatively uncommon. There can be occasional involvement of the cornea and a panophthalmitis. In some cases, the only sign of infection is conjunctivitis.

Occurrence and distribution :

IBR occurs worldwide. In India, the disease was first reported by Mehrotra *et al.* (1976) who isolated IBR virus from cases of keratoconjunctivitis amongst crossbred calves in an organized cattle herd in Uttar Pradesh. Since then the disease had 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. Satyanarayana and Suri Babu (1987) using indirect Haemagglutination test, reported 56.5 % BoHV-1 seroprevalence in the states of Uttar Pradesh, Haryana, West Bengal, Orissa, Andhra Pradesh, Tamil Nadu and Karnataka. Suri Babu *et al*. (1984) screened serum samples for BoHV-1 antibodies by indirect Haemagglutination test and reported a seroprevalence of 64.72 % in the states of Andhra Pradesh, Karnataka, Orissa, Tamil Nadu and West Bengal. Renukaradhya *et al.* (1996) found that 50.9 % cattle population in the states of Andhra Pradesh, Karnataka and Tamilnadu are seropositive for BoHV-1 antibodies. Suresh *et al*. (1999) standardised Avidin Biotin ELISA for detection of BoHV-1 antibodies in serum samples. They conducted the first large scale seroprevalence of BoHV-1 in India covering eighteen states and union territories. They reported 38.01 % of cattle

in India are seropostive for BoHV-1 antibodies. The state wise BoHV-1 seroprevalence what they recorded is as follows: 96.55 % in Andaman and Nicobar; 37.56 % in Andra Pradesh; 69.05 % in Arunachal Pradesh; 13.64 % in Assam; 76.74 % in Bihar; 42.50 % in Goa; 10 % in Gujarat; 9.095 in Haryana; 12.82 % in Himachal Pradesh; 95.35 % in Jammu and Kashmir;64.22 % in Karnataka; 46.67 % in Madyapradesh; 77.90 % in Maharashtra; 51.11 % in Manipur; 13.64 % in Mizoram; 100 % in Orissa; 23.66 % in Punjab; 60.16 % in Rajastan; 20.16 % in Tamilnadu and 82 % in the state of Uttarpradesh. Mallick (1986) studied the seroprevalence of IBR disease in seven states and observed that 65.3% exotic, 73% crossbred and 62% indigenous cattle 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), Gujurat (Singh *et al*., 1983), Tamil Nadu (Manickam and Mohan, 1987), Uttar Pradesh (Mehrotra, 1977), Orissa (Misra and Misra, 1987), Karnataka (Mohan *et al*., 1994), West Bengal,(De *et al*., 1989 and 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 BoHV-1 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. A 32.34% seropositivity was observed in bulls by Singh *et al.* (1985, 1986 a,b). 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 in 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. Some of the other important reports on seroprevalence of BoHV-1 in Indian states are 18.75 % in Kerala (Rajesh *et al*., 2003); 17.68 % in Andaman and Nicobar (Sunder *et al*., 2005); 10.75 % in Uttaranchal (Jain *et al*., 2006) and 19.2 % in Karnataka (Kiran *et al*., 2007). In India, the most recent reports on BoHV-1 seroprevalence are by Ravi (2009) who reported seroprevalence of BoHV-1 at 18.18 % and 12.42 %, in cows and heifers respectively in the state of Karnataka using Avidin - Biotin ELISA kits supplied by PD_ADMAS, Bangalore. Nandi *et al.* (2004) recorded that 17.5 % of breeding bulls in India are seropositive to BoHV-1 when tested by an indirect ELISA. Ganguly *et al*. (2008) adopted serum neutralisation test for detecting antibodies in serum samples and they reported 38 % of cattle in West Bengal state are seropositive for BoHV-1. Analysis of the Annual Activity Report of the I. A. H. & V. B., Kolkata, ARD Department, Government of West Bengal over a period of more than 10 years depicts that the sero prevalence of IBR in organized farms is more (60.95%) compared to rural area (35.86%) with an overall prevalence 48.98 %. Saha *et al*., 2010 reported nucleotide sequencing of part of the regions encoding gH, gB and gC from different isolates in the state of West Bengal and showed in gH sequence analysis, strain India 3 has identical sequences with the other isolates from India (strains India 4,5 and 6). Bhat *et al.*(1997) reported Serological evidence of bovine herpesviruses 1 and 2 in Asian elephants.

Epidemiology :

Infection occurs via the respiratory and genital routes. The virus is spread both within and between herds mainly by horizontal transmission such as direct and indirect contact (fomites) and aerosol droplets or from infected bulls by coitus and in infected semen either by artificial or natural insemination. Frozen semen is preserved at a condition that is optimal for virus survival. As with other herpesviruses, infection with BoHV-1 results in lifelong latent infection. This may occur in the absence of clinical signs and in the absence of detectable serum antibody. Corticosteroid treatment may induce a recrudescence of infection and excretion of virus (Rock *et al*., 1987 and Rock *et al*.,1992). Natural excretion may occur following stress but the mechanism of latency and reactivation has not yet been fully elucidated. BoHV-1 isolates vary in virulence in a manner unrelated to subtype. When introducing new animals into a closed herd or importing animals from overseas, those animals with antibody should be rejected, as they will be latently infected. Sero negative animals should be checked repeatedly for antibody and should preferably be treated with corticosteroid

and sampled for virus excretion before being allowed entry to a breeding herd.

Diagnostic tests:

A. Identification of the agent:

The virus can be detected from nasal or genital swabs from animals with respiratory signs, vulvovaginitis or balanoposthitis, taken during the acute phase of the infection and in severe cases, from various organs collected at post-mortem by antigen capture ELISA (Collins *et al.*,1988) and reverse passive Haemagglutination test (Edwards and Gitao, 1987). Following infection, BoHV-1 may persist in infected animals in a latent state in sensory neurons e.g. in the trigeminal or sacral ganglia (Ashbaugh *et al*., 1997). The virus can be reactivated and this results in virus shedding (re-excretion) without exhibition of clinical disease (Darcel and Dorward, 1975 and Grom *et al*., 2006). Therefore, antibody-positive animals have to be classified as infected with BoHV-1 (with two exceptions: serological responses induced by vaccination with an inactivated vaccine or by colostral antibodies). For virus isolation, various cell cultures of bovine origin are used, for example, secondary lung or kidney cells or the Madin–Darby bovine kidney cell line (MDBK) (Mehrotra *et al.*,1987 and Mehrotra *et al.*,1994). The virus produces a cytopathic effect in 2 – 4 days. It is identified by neutralisation or antigen detection methods using monospecific antisera or monoclonal antibodies (Madbouly *et al.*, 2008). BoHV-1 isolates can be further be subtyped by DNA restriction enzyme analysis (RFLP) (Magyar *et al.*, 1993) into subtypes 1.1 and 1.2. BoHV- 1.2 isolates can be further differentiated into 2a and 2b (Metzler *et al.*, 1985). Development of rhinotracheitis or vulvovaginitis /balanoposthitis depends more on the route of infection than on the subtype of the virus. The virus previously referred to as BoHV-1.3, a neuropathogenic agent, is now classified as BoHV-5. Viral DNA detection methods have been developed and the polymerase chain reaction technique is increasingly used in routine diagnosis including real-time polymerase chain reaction (PCR) (Engelenburg *et al.*, 1993; Wiedmann *et al.*, 1993; Engelenburg *et al.*, 1995; De Gee *et a*l., 1996; Rocha *et al.*,1998; Masri *et al.*, 1996; Santurde *et al.*,1996; Schynts *et al.*,1999; Kataria *et al.*, 1997; Fuchs *et al.*, 1999; Smits *et al.*, 2000; Tiwari *et al.*, 2000; Rola *et al.*, 2003; Gupta *et al.*, 2006; Jain *et al.*, 2009 and Rana *et al.*, 2011). Sachin *et al. (2014)* described loop-mediated isothermal amplification (LAMP) assay for rapid detection of bovine herpesvirus 1 in bovine semen. On comparison with TaqMan real-time PCR, they claimed that the LAMP assay had a diagnostic sensitivity of 97 %, specificity of 100 %, and accuracy of 99.2 % for detection of BoHV-1 in bovine semen and could be used under field condition.

B. Serological tests :

The virus neutralisation test and various enzyme-linked immunosorbent assays (ELISA; indirect or gB-blocking) are most

widely used for antibody detection (Riegel *et al.*, 1987; Kramps *et al.*, 1993; Kramps *et al.*, 1994; Deregt *et al.*, 1993; Kramps *et al*, 2004 and Reghuvanshi *et al*., 2006). With the ELISAs, antibodies can be detected in serum or plasma, and with lower sensitivity in milk or bulk milk samples. Avidin – Biotin indirect ELISA kits supplied by PD_ADMAS, Bangalore has been the most widely used tool for detection of BoHV-1 antibodies in India (Suresh *et al*., 1999 and Kiran *et al*., 2005).

Prophylaxis against BoHV-1 virus:

Inactivated and attenuated live vaccines are available(Kaashoek *et al*., 1994). The vaccines protect cattle clinically in case of infection and markedly reduce the subsequent shedding of field virus (Babiuk *et al*., 1987; Babiuk *et al*., 1988 and Bosch *et al*., 1998). Although vaccination may not prevent field virus infection of individual animals, spreading of wild-type virus in infected herds is efficiently reduced. The vaccines must not induce disease, abortion, or any local or systemic reaction, and must be genetically stable (Abdelmagid *et al*., 1998). Live gE- /tk- double gene deleted Bovine herpesvirus type 1 (BoHV-1), strain CEDDEL: $10^{6.3}$ – $10^{7.3}$ CCID50 is the only approved live marker vaccine in EEU countries (Regina, 2014). This vaccine contains a BoHV-1 (CEDDEL) strain with double deletion of the genes that encode the surface glycoprotein E (gE) and the thymidine - kinase enzyme (tk). The gE deletion increases the safety of the vaccine strain. Moreover, the gE deletion means that the vaccine does not induce gE antibodies (marker vaccine). This fact allows cattle vaccinated with HIPRABOVIS® IBR MARKER LIVE to be differentiated from those animals infected with a field strain of BoHV-1 or those vaccinated with a conventional (non-marker) BoHV-1 vaccine (hence called DIVA). The diagnostic tools designed to detect the anti gE antibodies must be acquired to do so. The tk deletion is related to a reduction of viral neuro tropism, with a lower establishment of latency and with lower viral reactivation in infected animals. The gE- /tk- double deletion makes greater vaccine safety and stability and maintains the features of the gE deleted vaccine (DIVA), which are essential for eradication of IBR (Regina, 2014).

REFERENCES

Abdelmagid OY, Mansour MM, Minocha HC and van Drunan Littel-van den Hurk S, 1998. Evaluation of baculovirus expressed bovine herpesvirus-1 (BHV-1) glycoproteins for detection and analysis of BHV-1 specific antibody responses. Vet Microbiol, 61: 249–259

Afshar A, 1965. Virus disease associated with bovine abortion and infertility. Vet Bull, 35: 736-752

Afshar A and Eaglesome MD, 1990. Viruses associated with bovine semen. Vet Bull, 60 (2): 93-109

Armstrong JA, Pereira HG and Andrewes CH, 1961. Observations on the virus of infectious bovine rhinotracheitis, and its affinity with the Herpesvirus group. Virology, 14: 276-285

Ashbaugh SE, Thompson KE, Belknap EB, Schultheiss SC and Chowdhury S *et al*., 1997. Specific detection of shedding and latency of bovine herpesvirus 1 and 5 using a nested polymerase chain reaction. J Vet Diagn Invest, 9: 387–394

Babiuk LA, L Italien J, van Drunen Littel-van den Hurk S, Zamb T and Lawman JP *et al.*, 1987. Protection of cattle from bovine herpesvirus type I (BHV-1) infection by immunization with individual viral glycoproteins. Virology, 159(1): 57-66

Babiuk LA, Lawman MJ and Ohmann HB, 1988. Viral-bacterial synergistic interaction in respiratory disease. Adv Virus Res, 35: 219-249

Bhat MN, Manickam R and Kumanan K, 1997. Serological evidence of bovine herpesviruses 1 and 2 in Asian elephants. J Wildl Dis, 33: 919-920

Bosch JC, De Jong MCM, Franken P, Frankena K and Hage JJ *et al*., 1998. An inactivated gE- egative marker vaccine and an experimental gD-subunit vaccine reduce the incidence of bovine herpesvirus 1 infections in the field. Vaccine, 16: 265–271

Collins JK, Ayers VK and Carman J, 1988. Evaluation of an antigen-capture ELISA for the detection of bovine herpesvirus type 1 shedding from feedlot cattle. Vet Microbiol, 16: 101–107

Darbyshire JH, Dawson PS, Paterson AB and Loosmore RM, 1964. Infectious Bovine Rhinotracheitis (IBR). A clinical condition of cattle occurring in United Kingdom. Vet Rec, 74: 1379-1383

Darcel CQ and Dorward WJ, 1975. Recovery of infectious bovine rhinotracheitis virus following corticosteroid treatment of vaccinated animals. Can Vet J, 16 (3) : 87-88

De BN, Chatterjee A, Sen GP and Biswas G, 1989. Investigation of outbreak of bovine abortion in a large

organized dairy farm. Indian Vet J, 66: 283–287

De Gee AL, Wagter LH and Hage JJ, 1996. The use of a polymerase chain reaction assay for the detection of bovine herpesvirus 1 in semen during a natural outbreak of infectious bovine rhinotracheitis. Vet Microbiol, 53(1-2): 163-168

Deka D, Ramneek, Maiti NK and Oberoi MS, 2005. Detection of bovine herpesvirus-1 infection in breeding bull semen by virus isolation and polymerase chain reaction. Rev Sci Tech, 24(3): 1085-1094

Deregt D, Cho HJ and Kozub GC, 1993. A comparative evaluation of two sensitive serum neutralization tests for bovine herpesvirus-1 antibodies. Can J Vet Res, 57: 56–59

Edwards S and Gitao GC, 1987. Highly sensitive antigen detection procedures for the diagnosis of infectious bovine rhinotracheitis: amplified ELISA and reverse passive haemagglutination. Vet Micorobiol, 13: 135–141

Engelenburg FAC van, Maes RK, Oirschot JT van and Rijsewijk FAM, 1993. Development of a rapid and sensitive polymerase chain reaction assay for detection of bovine herpesvirus type 1 in bovine semen. J Clin Microbiol, 31 (12): 3129-3135

Engelenburg FAC van, Schie FWV, Rijsewijk FAM and Oirschot JT van, 1995. Excretion of bovine herpesvirus 1 in semen is detected much longer by PCR than by virus isolation. J Clin Microbiol, 33 (2): 308-312

Fuchs M, Peter H, Jan D and Hanns JR, 1999. Detection of bovine herpesvirus type 1 in blood from naturally infected cattle by using a sensitive PCR that discriminates between wild-type virus and virus lacking glycoprotein. E J Clin Microbiol, 37: 2498-2507

Ganguly S, Mukhopadhya SK and Paul I, 2008. Studies on seroprevalence of infectious bovine rhinotracheitis in cattle population of West Bengal. Ind J Comp Microbiol Immunol Infect Dis, 29(1-2): 12-16

Gibbs EPJ and Rweyemamu MM, 1977. Bovine herpesviruses. Part I. Bovine herpesvirus 1. Vet Bull, 47: 317-343

Grom J, Hostnik P, Toplak I and Barlic-Maganja D, 2006. Molecular detection of BHV-1 in artificially inoculated semen and in the semen

of a latently infected bull treated with dexamethasone. Vet J, 171: 539–544

Gupta PK, Saini M and Rai A, 2006. Rapid and sensitive PCR- based test for detection of bovine herpesvirus-1 in semen. Indian J Virol, 17: 23-27

Huck RA, Millar PG, Evans DH, Stables JW and Ross A, 1971. Penoposthitis associated with infectious bovine rhinotracheitis infectious pustular vulvovaginitis (I.B.R-I.P.V.) virus in a stud of bulls. Vet Rec, 88: 292-297

Jain L, Kanani AN, Purohit JH, Joshi CG and Rank DN *et al.*, 2009. Detection of bovine herpes virus-1 (BHV-1) infection in breeding bulls by serological and molecular methods and its characterization by sequencing of PCR products. Buffalo Bulletin, 28(2): 76-84

Jain V, Parihar AK, Upadhayay AK and Kumar M. 2006. Seroprevalance of IBR among bovines of Garwal region. IndianVet J, 83: 340-342

Kaashoek MJ, Moerman A, Madic J, Rijsewijk FAM and Quak J *et al.*, 1994. A conventionally attenuated glycoprotein E-negative strain of bovine herpesvirus type 1 is an efficacious and safe vaccine. Vaccine, 12: 439–444

Kataria RS, Tiwari AK, Gupta PK, Mehrotra ML and Rai A *et al.*, 1997. Detection of bovine herpesvirus 1 (BHV-1) genomic sequences in bovine semen inoculated with BHV-1 by polymerase chain reaction. Acta Virol, 41(6): 311-315

Kendrick JW, Gillespie JH and Mcentee K, 1958. Infectious pustular vulvovaginitis of cattle. Cornell Vet, 48: 458 495.

Khan, OA, 2004. Seroprevalence of Infectious Bovine Rhinotracheitis in Gujarat State. M.V.Sc. thesis submitted to G.A.U, Sardarkrushinagar.

Kiran KK, Ravi P and Prabhudas K, 2005. Infectious bovine rhinotracheitis-National survey of IBR antibodies by AB-ELISA kit. Annual Report of Project Directorate on Animal Disease Monitoring and Surveillance. ICAR, Bangalore, pp 7-10

Kiran KK, Savita P, Rajeswari S and Krishnamsetty P, 2007. Seroprevalence of infectious bovine rhinotracheitis in Karnataka. Indian Vet J, 84(6): 569-572

Kramps JA, Magdalena J, and Quak J, 1994. A simple, specific, and highly

sensitive blocking enzyme-linked immunosorbent assay for detection of antibodies to bovine herpesvirus 1. J Clin Microbiol, 32:2175–2181

Kramps JA, Quak S, Weerdmeester K and Van Oirschot JT, 1993. Comparative study on sixteen enzymelinked immunosorbent assays for the detection of antibodies to bovine herpesvirus 1 in cattle. Vet Microbiol, 35: 11–21

Kramps JA, Banks M, Beer M, Kerkhofs P and Perrin M *et al.*, 2004. Evaluation of tests for antibodies against bovine herpesvirus 1 performed in national reference laboratories in Europe. Vet Microbiol, 102:169–181

Madbouly HM, Tamam AM and Abd-El-Gaid BS, 2008. Isolation and identification of bovine herpes virus -1 (BHV-1) from semen of foreign breeds bulls. Bs Vet Med J, 18(2): 22-27

Madin, SH, York, CJ, Mckercher, DG, 1956. Isolation of the Infectious Bovine Rhinotracheitis Virus. Science. 124, 721722.

Magyar G, Tanyi J, Hornyak A and Batha A, 1993. Restriction endonuclease analysis of Hungarian bovine herpesvirus isolates from different clinical forms of IBR, IPV and encephalitis. Acta Vet Hung, 41: 159–170

Mallick BB, 1986. Importance of bovine herpes virus 1 in India. Procidings of National Symposium on current status of herpes virus in Man and Animals, HAU, Hissar, pp 54-59

Manickam R and Mohan M, 1987. Seroepidemiological studies on Infectious bovine rhinotracheitis (IBR) viral abortions in cows. Indian J Anim Sci, 57: 959-962

Masri SA, Olson W, Nguyen PT, Prins S and Deregt D, 1996. Rapid detection of bovine herpesvirus-1 in the semen of infected bulls by a nested polymerase chain reaction assay. Can J Vet Res, 60 (2): 100-107

Mars MH, Bruschke CJ and van Oirschot JT, 1999. Airborne transmission of BHV1, BRSV, and BVDV among cattle is possible under experimental conditions. Vet Microbiol, 66(3): 197-207

Mehrotra ML, 1977. Isolation of respiratory viruses from cattle and their possible role in gental disorders. Ph. D. thesis. Agra. Univ. Agra

Mehrotra ML, Rajya BS and Kumar S, 1976. Infectious bovine rhinotracheitis (IBR) keratoconjunctivitis in calves. Indian J Vet Pathol, 1: 70-73

Mehrotra ML, Shukla DC and Sharma AK, 1987. Experimental bovine herpes virus 1 infection in hill bulls. Indian J Anim Sci, 57: 359–365

Mehrotra ML, Singh KP, Khanna PN and Shukla DC, 1994. Isolation of BHV-1 from an outbreak of abortion in an organized herd. Indian J Anim Sci, 64 (5): 341-354

Metzler AE, Matile H, Gassmann U, Engels M and Wyler R, 1985. European isolates of bovine herpesvirus 1: a comparison of restriction endonuclease sites, polypeptides and reactivity with monoclonal antibodies Arch. Virol, 85(1-2): 57- 69

Miller, NJ, 1955. Infectious necrotic rhinotracheitis of cattle. J Am Vet Med Assoc, 126(939): 463 467.

Miller JM, 1991. The effects of IBR virus infection on reproductive function of cattle. Symposium on IBR virus. Vet Med, 1: 95-98

Misra PK and Misra A, 1987. Infectious bovine rhinotracheitis virus infection and infertility in cows, heifers and bulls. Ind J Anim Sci, 57: 267-271

Misra V, Blumenthal RM and Babiuk LA, 1981. Proteins Specified by bovine herpesvirus 1 (infectious bovine rhinotracheitis virus). J Virol, 40(2): 367-378

Mohan Kumar, KM, Rajasekhar M and Krishnappa,G, 1994. Isolation of infectious bovine rhinotracheitis virus in Karnataka. Ind Vet J, 71(2): 109 113

Nandi S, Pandey AB, Audarya SD and Suresh I, 2004. Serological evidence of bovine herpes virus 1 antibodies in cattle and buffaloes from different states of India. Indian J Comp Microbiol Immunol Infect Dis, 25: 87–89

Nandi S and Kumar M, 2010. Serological evidence of bovine herpesvirus- 1 (BoHV-1) infection in yaks (Peophagus grunniens) from the National Research Centre on Yak, India. Trop Anim Health Prod, 42(6): 1041 1042.

Pandey AB, Mehrotra ML, Verma RP and Pati US, 2000. Investigation of an outbreak of infectious pustular balanoposthitis in breeding bulls. Indian J Vet Res, 9: 27–37

Pandita N and Srivastava RN, 1993. A study on seroepizootiology of BHV- 1 in Haryana. Ind J Virol, 9(31): 31-38

Pandita N and Srivastava RN, 1995. Dot immune binding assay for detection of bovine herpes virus-1 (BHV-1) antibodies. Ind J Virol, 11: 27-29

Patel, DM, 1983. Studies on some aspects of gestation and abortion in Surti buffaloes. M.V.Sc. thesis submitted to G.A.U, Sardarkrushinagar

Rana SK, Kota SNLS, Samayam PNR, Rajan S and Srinivasan VA, 2011. Use of real-time polymerase chain reaction to detect bovine herpesvirus 1 in frozen cattle and buffalo semen in India. Vet Ital, 47(3): 313- 322

Rajesh JB, Tresamol PV and Saseendranath MR, 2003. Seroprevalence of infectious bovine rhinotracheitis in cattle population of Kerala. Indian Vet J, 80: 393- 396

Rajkhowa, S, Rajkhowa, C, Rahman, H, Bujarbaruah, KM, 2004. Seroprevalence of infectious bovine rhinotracheitis in mithun (Bos frontalis) in India. Rev Sci Tech, 23(3): 821 829

Reghuvanshi S, Kumar M and Singh JP, 2006. Avidin biotin ELISA for seromonitoring of infectious bovine rhinotracheitis in cattle and buffaloes. Indian J Vet Med, 26: 49-51

Regina GS, 2014. Biosecurity, Bovine Viral Diarrhoea Virus (BVDv), and Bovine Herpesvirus-1 (BoHV-1): Epidemiological investigations in Irish Dairy Herds. A dissertation submitted to the University of Limerick, November, 2014

Riegel CA, Ayers VK and Collins JK, 1987. Rapid, sensitive, competitive serologic enzyme-linked immunosorbent assay for detecting serum antibodies to bovine herpesvirus type 1. J Clin Microbiol, 25: 2418–2421

Reisinger L and Reimann H, 1928. Beitrag ZurAtiologic des lachenausschlages des Rindes.Wine Tirrarztl Mch, 15: 249-261

Renukaradhya GJ, Rajasekhar M and Raghavan R, 1996. Prevalence of infectious bovine rinotracheitis in southern India. Review Scientific Tech, 15 (3): 1021-1028

Rocha MA, Barbosa EF, Guimaraes SEF, Neto ED and Gouveia AMG, 1998. A high sensitivity-nested PCR assay for BHV-1 detection in semen of

naturally infected bulls. Vet Microbiol, 63: 1-11

Rola J, Polak M and Zmudzinski J, 2003. Amplification of DNA of BHV 1 isolated from semen of naturally infected bulls. Bull Vet Inst Pulaway, 47: 71–75

Rock DL, Lokensgard J, Lewis T and Kutish G, 1992. Characterization of dexa methasone induced reactivation of latent bovine herpesvirus 1. J Virol, 66: 2484-2490

Rock DL, Beam SL and Mayfield JE, 1987. Mapping bovine herpesvirus type 1 latency related RNA in trigeminal ganglia of latently infected rabbits. J Virol, 61: 3827-383

Rychner JJ, 1841. Bujatrik oder Systematisches Handbuch der äusserlichen und innerlichen Krankheiten des Rindviehes. Aufl Chr Fischer Verlag Bern, 2: 514-517

Sachin SP, Chetan DM, Niraj KS, Arvind AS and Mohini S *et al*., 2014. Rapid detection of bovine herpesvirus 1 in bovine semen by loop-mediated isothermal amplification (LAMP) assay. Arch Virol, 159: 641–648

Santurde G, Silva ND, Villares R, Tabares E and Solana A *et al*., 1996. Rapid and high sensitivity test for direct detection of bovine herpesvirus-1 genome in clinical samples. Vet Microbiol, 49: 81–92

Satyanarayana K and Suri Babu T, 1987. Comparative evaluation of enzyme linked immunosorbent assay (ELISA) and indirect haemagglutination (IHA) test in the detectionof antibodies to Bovine herpes virus-1.(BHV-1) in cattle. Ind J Comp Microbiol Immunol Infect Dis, 8: 31-32

Schroeder RJ and Moy SMD' 1954 . An acute respiratory infection of dairy cattle. J Am Vet Med Assoc, 125: 471 - 472 .

Schynts F, Baranowski E, Lemaire M and Thiry E, 1999. A specific PCR to differentiate between gE negative vaccine and wild type bovine herpesvirus type 1 strains. Vet Microbiol, 66: 187–195

Selvaraj J, Murali Manohar B, Balachandran C, Kiran Kumar KK and Gajendran MR, 2008. Current status of seroprevalence of Infectious bovine rhinotracheitis using avidin-biotin ELISA in she buffaloes. Tamilnadu J Vet Anim Sci, 4(1): 33-34

Sinha BK, Mishra KK, Singh AP and Kumar R, 2003. Seroprevalence of infectious bovine rhinotracheitis in Bihar. Proceedings of the 4th Asian Buffalo Congress on Buffalo for Food Security and Rural Employment, 2: 17

Singh BK, Ramakant and Tongaonkar SS, 1983. Adaptation of Infectious bovine rhinotracheitis virus in Madin Darby bovine kidney cell line and testing of buffalo sera for neutralizing antibodies. Indian J Comp Microbiol Immunol Infect Dis, 4: 6-8

Singh BK, Ramakant and Tongaonkar SS, 1985. Serological survey of infectious bovine rhinotracheitis (IBR) in dairy cattle. Indian J Anim Sci, 55: 843-847

Singh BK, Ramakant, Tongaonkar SS, Roy Choudhury PN and Mukherjee F, 1986 (a). Serological and virological examinations of infectious bovine rhinotracheitis (IBR) in dairy bulls. National Symposium on current status of Herpesvirus infections in Man and Animals in India held at HAU, Hissar, pp 65-69

Singh BK, Sreenivasan MA, Tongaonkar SS, Kant R and Choudhari PNR, 1986 (b). Isolation of infectious bovine rhinotracheitis virus from semen and aborted materials of dairy cattle. Indian J Anim Sci, 56: 823–826

Smits CB, Van Maanen C, Glas RD, De Gee AL and Dijkstrab T *et al*., 2000. Comparison of three polymerase chain reaction methods for routine detection of bovine herpesvirus 1 DNA in fresh bull semen. J Virol Methods, 85: 65–73

Sulochana S, Pillai RM, Nair GK and Abdulla PKR, 1982. Serological survey on the occurrence of infectious bovine rhinotracheitis in Kerala. Indian J Comp Microbiol Immunol Infec Dis, 3: 7-11

Sunder J, Rai RB, A.Kundu, Jeyakumar Sakthivel, 2005. Incidence and prevalence of livestock diseases of Andaman and Nicobar Islands. Indian J Anim Sci, 75(9): 1041- 043

Suresh KB, Sudharshana KJ and Rajasekhar M, 1999. Seroprevalence of infectious bovine rhinotracheitis in India. Indian Vet J, 76: 5-9

Suri Babu T, Mallick BB and Das SK, 1984. Prevalence of infectious bovinerhinotracheitis virus (BHV-1) antibodies in bovines. Ind Vet J, 61: 195-200

Saha T, Guha C, Chakraborty D, Pal B and Biswas U *et al.*, 2010. Isolation and Characterization of BoHV-1 from Cattle in West Bengal, India, Iranian J Vet Sci Tech, 2 (1): 1-8

Tiwari AK, Kataria RS, Butchaiah G and Prasad N, 2000. A simple method for the detection of BHV-1 from infected MDBK cells by polymerase chain reaction Indian Vet J, 77: 98-102

Tousimis AJ, Howells WV, Griffin TP, Porter RP and Cheatham WJ *et al.*, 1958. Biophysical characterization of infectious bovine rhinotracheitis virus. Proc Soc Exp Biol Med, 99(3): 614 617.

Wiedmann M, Brandon R, Wagner P, Dubovi EJ and Batt CA, 1993. Detection of bovine herpesvirus-1 in bovine semen by a nested PCR assay. J Virol Methods, 44(1): 129-139

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