RESEARCH ARTICLE

Detection and Isolation of Infectious Bovine Rhinotracheitis (IBR) Virus from Clinically Suspected Bovines in Telangana State

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Abstract

Infectious Bovine Rhinotracheatis (IBR) is a viral disease that causes huge losses to dairy economy by causing abortions and other pathogenic effects resulting in drop of production. IBRV circulates as 2 major subtypes; BoHV-1.1 and BoHV-1.2 associated with respiratory symptoms and genital infections, respectively, in bovines. The present study was taken up to detect and isolate BoHV-1 from field cases using conventional PCR, Real-time PCR, followed by isolation in cell cultures. Total of 125 samples comprising of 31 nasal swabs, 14 serum samples, 10 vaginal swabs, 7 raw semen samples and 63 extended semen samples were collected from IBR suspected animals of various organised dairy farms and semen station of Telangana state. All the samples were subjected for virus detection, followed by isolation studies. Of the 125 samples screened by Real-time PCR, 18 extended semen samples, 2 nasal swabs, 1 vaginal swab, and 1 raw semen sample were found positive for BoHV-1 and all the 14 serum samples were negative, which account for a positivity rate of 17.6%. The positive samples were propagated in MDBK cell lines for isolation of BoHV-1. The signs of CPE were seen on the 5th day of inoculation on third passage. The viral harvest was re-confirmed by real time PCR.

Key words: IBR virus, Isolation, MDBK cell line, Real time PCR.

Ind J Vet Sci and Biotech (2025): 10.48165/ijvsbt.21.6.21

Introduction

India is home to 193.46 million cattle and 109.85 million buffaloes with annual production of 221.06 million tonnes of milk (DAHD, 2024). Dairy is the single largest agricultural commodity contributing 5 % of the national economy and employing more than 8 crore farmers directly. India is ranked 1st in milk production contributing 23 % of global milk production (Economic Survey, 2021-22).

Infectious bovine rhinotracheitis (IBR) is a highly contagious infectious disease caused by bovine alpha herpesvirus type 1 (BoHV-1) belonging to the genus *Varicellovirus*, subfamily *Alpha herpesvirinae*, family *Herpesviridae* (Farooq *et al.,* 2019). BoHV-1 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, abortion, infertility, dermatitis and mastitis. In India, the endemic IBR has caused a huge economic loss in the dairy industry due to a drop in milk production, repeat breeding, and abortions. According to Kipyego *et al.* (2020), the dairy industry suffers enormous financial losses due to IBR, a multi-organ infectious and contagious disease of domestic ruminants.

IBR was first identified in India in 1976 by Mehrotra *et al.* (1976), since then it was reported from every state and the disease is now considered to be endemic in the country. Abortions resulting from BoHV-1, which typically occurs in the last trimester of pregnancy are the main source of financial losses for Indian dairy farmers (Yadav *et al.,* 2018).

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How to cite this article: Abhilash, J., Srinivas, M., Kannaki, T.R., Kalyani, P., & Vishveshwar, G. (2025). Detection and Isolation of Infectious Bovine Rhinotracheitis (IBR) Virus from Clinically Suspected Bovines in Telangana State. Ind J Vet Sci and Biotech, 21(6), 114-119.

Source of support: Nil

Conflict of interest: None

Submitted 20/07/2025 **Accepted** 23/08/2025 **Published** 10/11/2025

The prevalence of IBR in bovine herds in different regions of India ranged from 23.94% to 84.5% according to multiple seroprevalence studies conducted by various researchers (Farooq *et al.,* 2012; Kollannur *et al.,* 2014; Patil *et al.,* 2017).

Virus isolation, examination of tissues by fluorescent antibody technique (FAT), antigen detection by enzyme-

linked immunosorbent assay (ELISA), and immuno peroxidase test are some of the methods used in diagnostic laboratories for BoHV-1 detection. The new approaches in diagnosis emphasized mainly on development of nucleic acid techniques for the detection of viruses in clinical specimens. Nucleic acid hybridization and PCR were developed as ideal diagnostic tools for the detection of BoHV-1 in clinical specimens because of their rapidity, sensitivity, and specificity (Majumder *et al.,* 2015; Dima and Abdisa, 2022). Despite regular vaccination against IBR, still there is a huge prevalence of the disease. Understanding the circulating strains of BoHV-1 helps in development of an effective vaccine candidate and devising potent control strategies. Hence, this study was aimed to detect and isolate IBR virus from clinically suspected bovines in Telangana state.

Materials and Methods

Samples Collection from Animals Suspected with IBR

A total of 125 samples comprising of 31 nasal swabs, 14 serum samples, 10 vaginal swabs, 7 raw semen samples and 63 extended semen samples were included in the study. Nasal swabs, vaginal swabs, serum, and raw semen samples were collected from the animals, whereas extended semen samples were collected from frozen semen straws. Nasal swabs, vaginal swabs and raw semen samples were collected in 1 mL sterile PBS and transported to the laboratory with cold chain maintenance. Serum was obtained from blood samples collected aseptically from suspected animals in vacuutainers, centrifuged at 600 x g for 15 min, serum transferred in screw capped plastic vials and transported to the laboratory in cold chain. Frozen semen doses were transported in liquid nitrogen for cold chain maintenance.

Extraction of Viral Nucleic Acid from the Collected Samples

Extraction of viral genomic DNA from extended frozen semen was carried out by following the procedure described in OIE manual of diagnostic tests and vaccines for terrestrial animals (OIE, 2018). Extraction of viral genomic DNA from nasal swabs, serum, vaginal swabs & cell supernatant was done by standard Phenol-chloroform method as described by Sambrook and Russel (2001).

Quantification and Quality Assessment of DNA

DNA was quantified by NanoDrop TM Lite Spectrophotometer (Thermo Fisher Scientific). The UV absorbance was checked at 260 and 280 nm wavelength for determination of sample concentration and purity. Purity of DNA was judged on the basis of OD ratio at 260:280. The DNA having ratio in a range of 1.8 to 2.0 was considered to be of good purity.

Isolation of BoHV-1 in MDBK Cell Lines

Madin-Darby Bovine Kidney (MDBK) cell lines used in the present study were obtained from National Centre for Cell Science, Pune, India and subcultured using T25 flask with healthy confluent monolayer by decanting the spent medium and washing monolayer with 2 mL of 1X PBS gently. After decanting PBS, 1 mL of 0.2 % trypsin was added ensuring that the monolayer is completely covered. The cells were allowed to detach by incubation at room temperature. Three mL of growth medium was added immediately, and the cells were dispersed by repeating pipetting.

Each flask had 5-7 mL of cell suspension to cover the whole surface of the flask. The flasks were incubated in an incubator at 37°C with 5% CO_2 and cells were observed every 24 h to check for formation of confluent monolayer. The monolayers after attaining 70% confluency were used for virus infection.

Propagation of Virus in MDBK Cells

Virus isolation was carried out as per the procedure described in OIE terrestrial manual (OIE, 2018). One mL of extended semen was diluted with foetal bovine serum (free from antibodies against BoHV-1) with antibiotics in 1:10 ratio. Mixed vigorously and left for 30 min at room temperature. Then inoculated 1 mL of the semen/sample mixture onto a monolayer of susceptible cells in a six-well tissue culture plate and incubated for 1 h at 37°C. The mixture was removed and monolayer was washed twice with 2.5 mL of maintenance medium, and then added 2.5 mL of maintenance medium to each well. The plates were observed daily under the microscope for any CPE. If there was no CPE even after 7 days, the cultures were frozen and thawed, clarified by centrifugation, and the supernatant was used to inoculate fresh monolayers. The sample was considered negative if there was no evidence of any CPE after three consecutive passages. The CPEs were noticed as granulation, aggregation, detachment and rounding of the cells in positive samples. After observing complete rounding of monolayer, the cell culture supernatant was collected after centrifugation at 2500 x g for 5 min and stored at 4°C for further use.

Harvesting of Virus and Real-Time PCR for Detection

After 50% detachment of monolayer in infected flasks, cells were lysed by two cycles of freeze/thaw. Then the culture fluid was collected and stored at -20°C. This cell culture supernatant was used for further passage and extraction of nucleic acid if needed.

Real-Time PCR was carried out using Luna® Universal qPCR Master Mix (Cat.No. M3003S; New England Bio Labs) with gB gene-specific primers, IBR OIE Forward 5'-TGTGGACCTAAACCTCACGGT-3' Reverse 5'-GTAGTCGAGCAGACCCGTGTC-3' using Insta Q96-6.0 Real-Time PCR system (HiMedia Laboratories). The Luna® Universal qPCR Master Mix and the working solution of primer set were allowed to thaw completely. Then were mixed gently and spun briefly. The PCR reaction master mix 10 µL was prepared by using Luna® Universal qPCR Master Mix (2X) 5 µL, forward

and reverse primer (10 µM) 0.5 µL each, Template DNA 2.5 µL, and Nuclease-free water 1.5 µL.

The PCR mixture was mixed gently and spun briefly. Then PCR reaction plate was prepared by distributing 9 µL of PCR mixture into each well of MicroAmp® Fast 96-Well Reaction Plate, to that 1 µL of template DNA was added, according to template sheet including No Template Control. The loaded reaction plate was sealed, centrifuged for two min at 1500×g. The plate was transferred to Insta Q96-6.0 Real-Time PCR system and the programme was run according to the following cycling conditions (Table 1).

Results and Discussion

Screening of Samples by Targeting gB Gene using RT-PCR

Out of 125 samples screened (63 extended semen samples, 7 raw semen samples, 31 nasal swabs, 10 vaginal swabs and 14 serum samples), 18 extended semen samples, 2 nasal swabs, 1 vaginal swab, and 1 raw semen samples were found positive for BoHV-1 and all the 14 serum samples were negative for BoHV-1. The representative image of amplification curve showing different Ct values depending on the viral load is shown in Figure 1. Fig. 2 shows the representative image of melt curve where there is a single peak of amplification at melting temperature of 87°C. There is no amplification seen for serum samples.

Isolation and Propagation of BoHV-1 in MDBK Cells

For propagation of PCR positive samples in cell culture, 5 samples (062/2022/DS/3940-354, 063/2023/DS/3304-364, 064/2022/DS/3967-363, 065/2023/DS/3985-38, 074/2023/DS/ 497610-1) were randomly selected from 22 positive samples and were cultured in DMEM media. The MDBK cells with 60% confluency were used for infection. There was no characteristic CPE seen in the first and second passage. On fifth day of the third passage, the cells exhibited characteristic CPE such as enlargement, aggregation and granulation of cells with few cells detached from the surface of the flask (Fig. 3a to 3f). The subculturing of the virus continued till the fourth passage. The virus recovered from the cell culture was confirmed again by real-time PCR. The positive virus stocks were stored at 4°C for short term and at -20°C with glycerol for long term storage.

IBR is one of the significant viral infections of bovine that causes reproductive disorders in addition to other clinical signs and was responsible for most of the abortions in last trimester (Yadav *et al.,* 2018). BoHV-1 is associated with secondary bacterial pneumonia, miscarriages, drop in milk production, establishment of latency in infected animals and

Table 1: RT-PCR cycling conditions

Enzyme activation	PCR (40 cycles)		Melt Curve Stage		
Hold	Denaturation	Annealing/Extension			
10 min	25 sec	45 sec	15 sec	1 min	15 sec
95°C	95°C	60°C	95°C	55°C	95°C

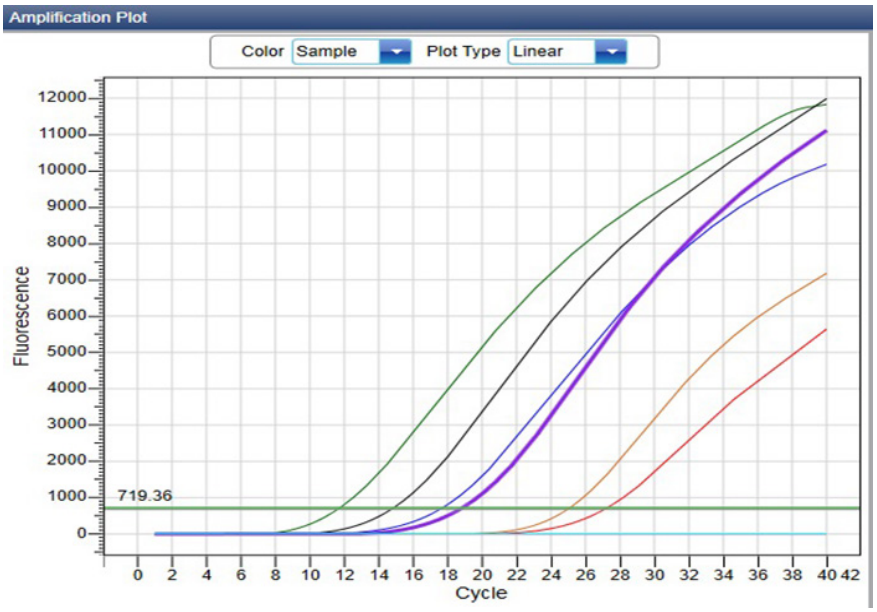

Fig. 1: Representative image of amplification plot for samples screened by SYBR green based real-time PCR. The positive samples show an amplification curve with varying Ct values for each sample. There is no amplification curve for negative samples and no template control.

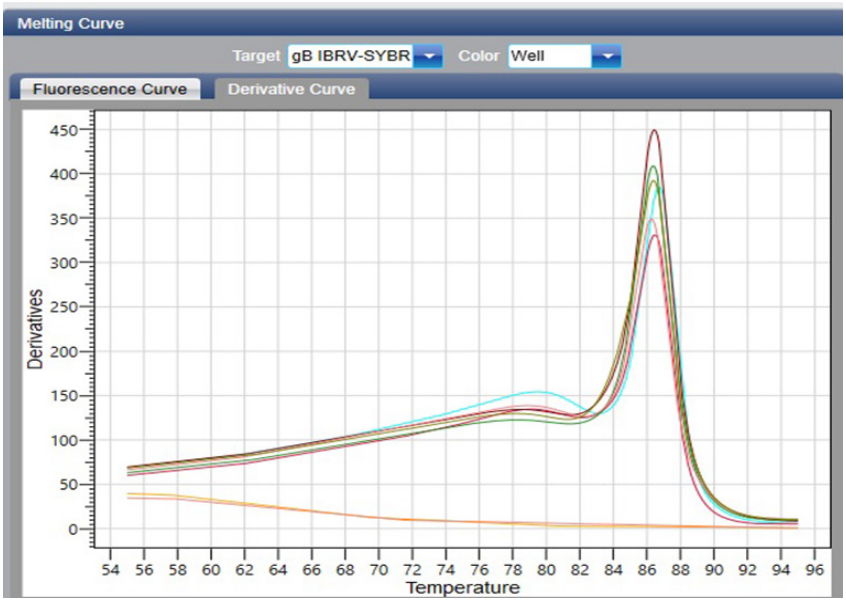

Fig. 2: Representative image of melt curve for samples screened by SYBR green based real-time PCR. The positive samples show a clear single peak with melting temperature of 87°C whereas no peak for negative samples.

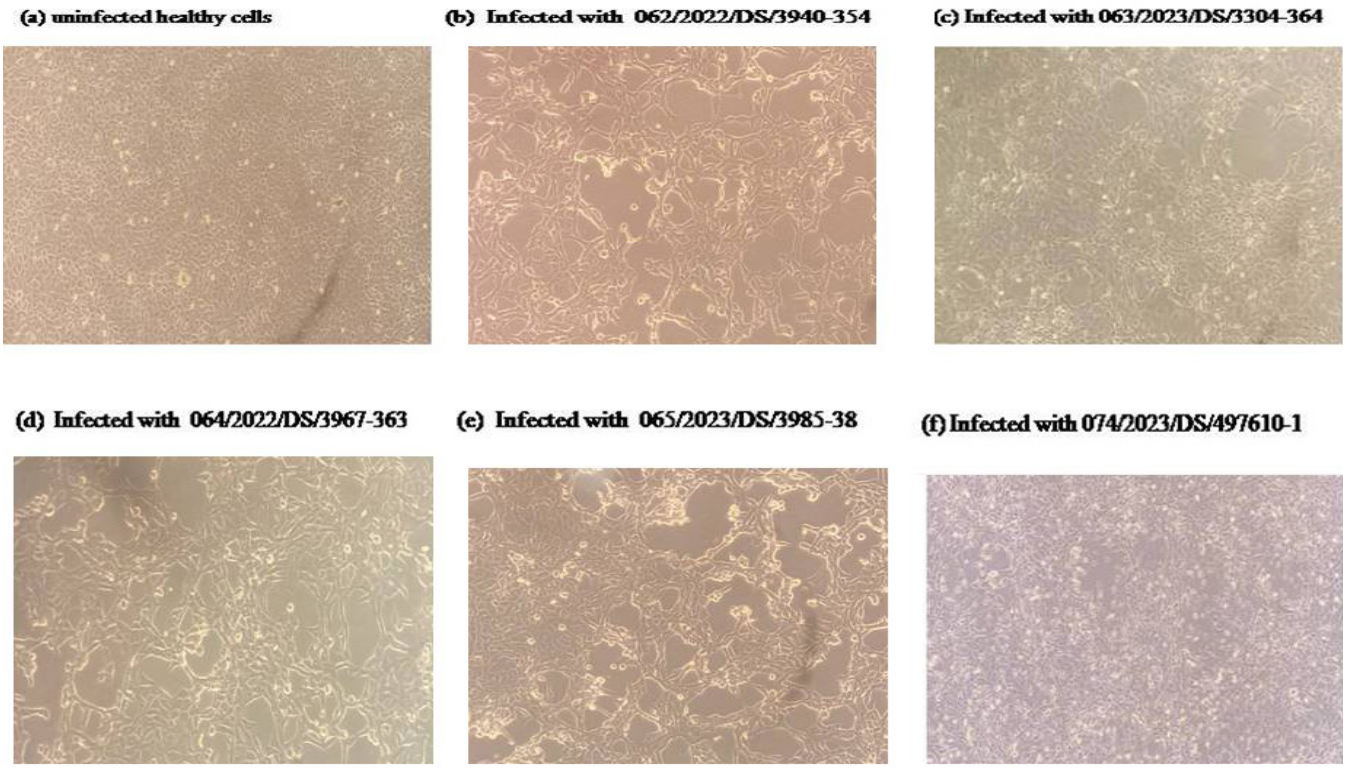

Fig. 3 (a-f): Infection of MDBK cells with IBRV suspected samples. The MDBK wcells at 10x magnification 4 days post infection. a) Healthy MDBK cells, b-f) MDBK cells infected with positive samples.

significant financial losses to the dairy farmers. To improve the productivity of the animal, it is important to focus on disease monitoring system. Hence, there is a need for rapid and cost-effective disease monitoring system to be implemented at point of care. The current study demonstrates the use of molecular techniques like real-time PCR to detect BoHV-1 and isolation of the virus in cell cultures for further studies. As per the previous reports, the BoHV-1 has been isolated from nasal swabs (Peltzer *et al.,* 2021), vaginal swabs (Nettleton and Russell , 2017), blood (Samrath *et al.,* 2016), prepucial washings, semen (Van Oirschot, 1995) and aborted fetal contents (Nandi *et al.,* 2009)

In the present study, we have also attempted to detect the viral genomic DNA from different samples. The DNA was extracted by chelex method from semen samples as per Rana *et al.* (2011), Chandranaik *et al.* (2010), and OIE (2018), and by phenyl chloroform isoamyl alcohol (PCI) method from other samples such as serum, nasal or vaginal swabs and cell culture supernatant (Sambrook and Russel, 2001). We could able to detect the presence of viral DNA in all the samples, except from serum which could be due to absence of virus in those samples.

Several diagnostics techniques have been reported for detection of IBR virus, which include ELISA (Mahajan *et al.,* 2013), VNT (Gur *et al.,* 2019), Agar gel precipitation test (Zeedan *et al.,* 2018), and Indirect Immunofluorescence assay (Dima and Abdisa, 2022). However, the real-time PCR owing to its sensitivity and specificity was considered as the best for diagnosis (Majumder *et al.,* 2015).

Initially, conventional PCR was carried out using primers published by Kibenge *et al.* (1994) targeting the TK gene, which amplifies a product of 183 bp. However, an amplicon size around 220 bp was noticed in our study. We hypothesized that the change in amplicon size might be due to an insertion in TK gene.

The real-time PCR was considered to be the most reliable and rapid technique for screening large sample sizes (OIE, 2010). Hence, the SYBR green based real-time PCR was optimized using the primers targeting the gB gene. Of the 125 samples tested 22 samples were found positive for IBR, which accounts for a positivity rate of 17.6%. Farooq *et al.* (2019) in their review on seroprevalence of IBR in India have noticed that it was ranging from 15% to 90% according to different geographical areas. We could not detect the BoHV-1 in serum. This may be due to the low or absence of viral titre in these samples when compared to positive samples. Most researchers have also detected IBRV DNA from semen samples rather than other tissues/secretions (Dima and Abdisa, 2022). Chandranaik *et al.* (2010) used real time PCR to detect IBR viral DNA from semen samples by targeting gB gene and their results are also in agreement with our study. Annual reports of ICAR-NIVEDI from 2016 to 2020 states that IBR seropositivity in Telangana state varies at following rates in selected period: 36.75 % (2016-17), 20.00% (2017-18), 52.29 % (2018-19) and 27.00% (2019-20) at the same time India's cumulative seropositive rate for 1995-2021 time period stands at 34.51%.

There are reports suggesting the isolation of BoHV-1 from primary and secondary bovine kidney (Biswas *et al.,* 2013), lung or testis cells; cell strains derived from bovine fetal lungs, turbinate or trachea and established cell lines such as the Madin-Darby bovine kidney cell line (MDBK) (OIE, 2018; Dima and Abdisa , 2022).

In the current work, attempts were made to isolate BoHV-1 by passaging in MDBK cells. The CPE was noticeable only after the third passage on day 5 which was characterized by granulation, swelling and aggregation of cells and detachment of few cells. Chothe *et al.* (2018) observed CPE of cell rounding, cell aggregation, and clusters of rounded cell formation using Hematoxylin and Eosin staining, in which the peculiar cell changes were observed. Biswas *et al.* (2013) observed CPE as grape like clusters of round cells around micro plaque in cell culture and syncytia formation. Chandranaik *et al.* (2010) observed CPE as rounding of cells and thread like cellular elongations with characteristic ''bunch of grapes'' like aggregation, finally leading to complete destruction of cell sheet. The isolation rate for IBR virus in cell cultures is usually low because of the sperm cytotoxicity (Rana *et al.,* 2011).

Conclusion

In conclusion, the Real-Time PCR has been demonstrated to be an ideal technique for BHV-1 screening especially for samples with low viral load. This can help to identify latent infections in a herd and thus economic losses can be minimised by containing the spread of infection. This study also concludes the positivity rate of 17.6% IBR in the state of Telangana.

Acknowledgement

We would like to thank Department of Biotechnology, Government of India for providing the opportunity and financial support for this work.

References

Biswas, S., Bandyopadhyay, S., Dimri, U.H., & Patra, P. (2013). Bovine herpesvirus-1 (BHV-1) – a re-emerging concern in livestock: A revisit to its biology, epidemiology, diagnosis, and prophylaxis. *Veterinary Quarterly, 33*(2), 68-81.

Chandranaik, B.M., Shivaraj, C., Kumar, S., & Renukaprasad, C. (2010). Isolation of BHV-1 from bovine semen and application of real time PCR for diagnosis of IBR/IPV from clinical samples. *Veterinarski Arhiv*, *80*(4), 467-475.

Chothe, S.K., Sebastian, A., Thomas, A., Nissly, R.H., Wolfgang, D., Byukusenge, M., Mor, S. K., Goyal, S.M., Albert, I., Tewari, D., Jayarao, B.M., & Kuchipudi, S.V. (2018). Whole-genome sequence analysis reveals unique SNP profiles to distinguish vaccine and wild-type strains of bovine herpesvirus-1 (BoHV-1). *Virology, 522,* 27-36.

DAHD - Department of Animal Husbandry and Dairying (2024). Animal husbandry statistics. Government of India, New Deli.

Dima, C., & Abdisa, K. (2022). Diagnostic techniques for infectious bovine rhinotracheitis: A review. *Journal of Applied and Advanced Research, **7,*** 42-48.

Economic Survey (2021-22). Department of Economic Affairs, GOI. (https://www.indiabudget. gov.in/budget 2022-23/ economicsurvey accessed on 20-02-2024).

Farooq, S., Kumar, A., Chaudhary, S., & Maan, S. (2019). Bovine herpesvirus 1 (BoHV-1) in cattle and buffalo: A review with emphasis on seroprevalence in India. *International Journal of Current Microbiology and Applied Sciences*, *8*(12), 28-35.

Farooq, S., Kumar, A., Chaudhary, S., Patil, C., Banger, Y., & Khasa, V. (2012). Sero-prevalence and risk factor analysis of BoHV-1 in bovines in Haryana state of India. *Indian Journal of Animal Research, 55*(5), 582-587.

Gur, S., Erol, N., Yapıcı, O., Kale, M., Tan, M.T., Turan, T., Çakmak, M.A., Tosun, C., Yılmaz, S., Acar, A., Özenli, I., & Gür, C. (2019). The role of goats as reservoir hosts for bovine herpes virus 1 under field conditions. *Tropical Animal Health and Production*, *51*, 753-758.

Kibenge, F.S., Harris, L.M., McKenna, P.K., Wadowska, D., Yason, C.V. (1994). Amplification of strains of bovine herpesvirus 1 by use of polymerase chain reaction with primers in the thymidine kinase region. *American Journal of Veterinary Research*, *55*(9), 1206-1212.

Kipyego, E.S., Gitau, G., Vanleeuwen, J., Kimeli, P., Abuom, T.O., & Gakuya, D. (2020). Seroprevalence and risk factors of infectious bovine rhinotracheitis virus (type 1) in Meru County, Kenya. *Preventive Veterinary Medicine, 175*,104863.

Kollannur, J.D., Syam, R., & Chauhan, R. (2014). Epidemiological studies on infectious bovine rhinotracheitis (IBR) in different parts of India. *International Journal of Livestock Research, 4*(5), 21-27.

Mahajan, V., Banga, H. S., Deka, D., Filia, G., & Gupta, A. (2013). Comparison of diagnostic tests for diagnosis of infectious bovine rhinotracheitis in natural cases of bovine abortion. *Journal of Comparative Pathology*, *149*(4), 391-401.

Majumder, S., Ramakrishnan, M.A., & Nandi, S. (2015). Infectious bovine rhinotracheitis: An Indian perspective. *International Journal of Current Microbiology and Applied Sciences*, *4*(10), 844-858.

Mehrotra, M.L., Rajya, B.S., & Kumar, S. (1976). Infectious bovine rhinotracheitis (IBR) keratoconjunctivitis in calves. *Indian Journal of Veterinary Pathology*, *1,*70-73.

Nandi, S., Kumar, M., Manohar, M., & Chauhan, R.S. (2009). Bovine herpes virus infections in cattle. *Animal Health Research Reviews*, *10*(1), 85-98.

Nettleton, P., & Russell, G. (2017). Update on infectious bovine rhinotracheitis. *In Practice, 39*(6), 255-272.

OIE (2010). Infectious bovine rhinotracheitis / infectious pustular vulvovaginitis. In: Manual of Standards Diagnostic Tests and Vaccines. Office International des Epizooties (Chapter 2.4.13).

OIE (2018). Manual of diagnostic tests and vaccines for terrestrial animals. Office international des epizooties, Paris, France, pp. 1139-1157.

Patil, S.S., Prajapati, A., Krishnamoorthy, P., Desai, G.S., Reddy, G.B.M., Suresh, K.P., & Rahman, H. (2017). Seroprevalence of infectious bovine rhinotracheitis in organized dairy farms of India. *Indian Journal of Animal Research, 51*(1), 151-154.

Peltzer, D., Tobler, K., Fraefel, C., Maley, M., & Bachofen, C. (2021). Rapid and simple colorimetric loop-mediated isothermal amplification (LAMP) assay for the detection of Bovine alphaherpesvirus 1. *Journal of Virological Methods, 289*, 114041.

Rana, S.K., Kota, S.N., Samayam, P.N., Rajan, S., & Srinivasan, V.A. (2011). Use of real-time polymerase chain reaction to detect bovine herpesvirus 1 in frozen cattle and buffalo semen in India. *Veterinaria Italiana, 47*(3), 313-322.

Sambrook, J., & Russel, D.W. (2001). *Molecular Cloning: A Laboratory Manual*: 3rd edn., Cold Spring Harbor Laboratory Press, New York. USA.

Samrath, D., Shakya, S., Rawat, N., Gilhare, V.R., Singh, F., & Khan, F.F.(2016). Seroprevalence of bovine herpes virus type 1 in cattle and buffaloes from Chhattisgarh. *Journal of Animal Research, 6*(4), 641-644.

Van Oirschot, J. T. (1995). Bovine herpesvirus 1 in semen of bulls and the risk of transmission: a brief review. *Veterinary Quarterly*, *17*(1), 29-33.

Yadav, V., Singh, S.P., Kumar, R., Diwakar, R., & Kumar, P. (2018). A review on current status of infectious bovine rhinotrichitis in India. *International Journal of Current Microbiology and Applied Sciences 7,* 411-426.

Zeedan, G.S.G., Abdalhamed, A.M., Ghazy, A.A., & Ghoneim, N.H. (2018). Serological and molecular identification of infectious bovine rhinotracheitis virus isolation and adaptation in embryonated chicken eggs. *Journal of Antivirals and Antiretrovirals*, *10*(2), 12-17.