Indian Journal of Animal Sciences **89**(9): 923–926, September 2019/Article
https://doi.org/10.56093/ijans.v89i9.93749

Stress due to vaccination may induce re-activation of latent bovine herpesvirus-1 in cattle

B M CHANDRANAIK[1], POOJA GOPIKUMAR[2], AMITHA REENA GOMES[3], POORVI REDDY[4], POOJAPPA NANDINI[5], M SHOBHA RANI[6], M D VENKATESHA[7] and S M BYREGOWDA[8]

Karnataka Veterinary Animal and Fisheries Sciences University, Bengaluru, Karnataka 560 024 India

Received: 12 February 2019; Accepted: 5 March 2019

ABSTRACT

This study evidences that, stress due to vaccination can cause re-activation and clinical outbreak of bovine herpesvirus-1 in latently infected cattle. Three to four days following mass-vaccination of cattle against Foot and Mouth Disease in Karnataka of India, symptoms of acute respiratory distress, conjunctivitis and vulvo-vaginitis were observed in vaccinated cattle in many villages of the state. Nasal and ocular swabs were collected from 25 ailing cattle in six villages of Hassan district of Karnataka. Upon bacteriological analysis, the samples were found negative for *Pasteurella multocida*. The serum samples from ailing animals were positive for bovine herpesvirus-1 (BoHV-1) antibodies by Indirect-ELISA. The swab samples were found for BoHV-1 by PCR targeting glycoprotein-C gene of BoHV-1. The swab samples when subjected to virus isolation in MDBK cells yielded characteristic CPE of bunch of grape like clustering of cells by fifth passage. PCR targeting conserved region on glycoprotein-C gene on DNA extracted from cell-culture supernatants showing CPE confirmed the presence of BoHV-1. Nucleotide sequencing of the PCR amplicon showed that the BoHV-1 isolated during this study shared 100% sequence identity with BoHV-1 isolates from India, Switzerland and Brazil. This study emphasizes that stress due to vaccination can cause re-activation and clinical outbreak of bovine herpesvirus-1 in latently infected cattle.

Key words: Bovine herpesvirus-1, Latency, Re-activation, Vaccination stress, Virus isolation

Bovine herpesvirus-1 (BoHV-1), a member of the subfamily *Alpha herpesvirinae,* in the order *Herpesvirales,* causes diverse ailments in cattle (Patil *et al*. 2016). Following infection and local replication, BoHV-1 gains access to terminal sensory nerves and then transported intra-axonally in a retrograde manner to neuronal cell body in trigeminal and sacral ganglion where lifelong latency is established. Latency is characterised by minimal viral gene expression, absence of lytic viral proteins and absence of any virus production (Jones 2003, Majumder *et al.* 2015). The virus gets re-activated under stress conditions (Chandranaik *et al.* 2010). Though, BoHV-1.1 is the major subtype circulating in cattle worldwide, recent descriptions of other sub-types complexes the understanding of viral pathogenesis. Through this paper we report that, stress due to vaccination can induce re-activation of latent BoHV-1 and cause clinical outbreak in cattle.

Present address: [1,3,6,7]Scientist (drbmchandranaik@gmail.com, argomes@gmail.com, Shobarani@gmail.com, mdv@gmail.com), [4,5]Laboratory Technician (poorvireddy@gmail.com, nandini.poojar@gmail.com), [8]Director (smbyregowda @gmail.com), Institute of Animal Health and Veterinary Biologicals, Hebbal, Bengaluru. [2]MSc Scholar (poojag @gmail.com), Department of Microbiology, St Josephs College, Bengaluru.

MATERIALS AND METHODS

History of the disease and source of sample: Three to four days following mass-vaccination of cattle against Foot and Mouth Disease (FMD) in Karnataka, India, symptoms of acute respiratory distress, conjunctivitis and vulvo-vaginitis were observed in vaccinated cattle. Blood samples, nasal and ocular swabs were collected from 25 ailing cattle in six villages of Hassan district of Karnataka.

Bacteriological culturing: The swab samples collected from ailing animals were subjected for bacteriological culture to see if the samples contain *Pasteurella multocida* as per standard microbiological procedures of Quinn *et al.* (2011).

Enzyme linked immuno sorbent assay: Enzyme linked immuno sorbent assay (ELISA) was employed on serum samples of all 25 ailing animals. Avidin-Biotin ELISA kits supplied by Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Bengaluru, India, were used. 1:30,000 dilution of biotinylated anti IgG and 1:15,000 dilution of Avidin-HRPO conjugate were used during the test.

DNA extraction: DNA was extracted from swabs collected in ailing animals using DNA extraction kit (Nucleo

Spin tissue-Macherey-Nagel, Germany), following the procedure outlined by the kit manufacturer.

Polymerase chain reaction: The DNA extracted from clinical samples were subjected to PCR using the primers and protocols described by Engelenburg *et al.* (1993) and Chandranaik *et al.* (2010). The primers were targeted to amplify the conserved region on glycoprotein C (gC) gene of BoHV-1. The primers included gC-F: 5'-CTG-CTG-TTC-GTA-GCC-CAC-AACG-3' and gC-R:5'-TGT-GAC-TTG-GTG-CCC-ATG-TCGC-3'.

The PCR reaction was carried out in 25 µl reaction mixture containing a final concentration of 10 mM Tris (pH 9.0), 50 mM KCl, 1.9 mM $MgCl_2$, 0.2 mM deoxy nucleoside triphosphates, 1.0 µl each of primer (10 pm/µl), 1 U of Taq Polymerase and 5.0 µl template DNA. The PCR reaction included initial denaturation at 95°C for 1 min; followed by 35 cycles of denaturation at 95°C for 1 min, annealing a 60°C for 1 min, extension at 72°C for 1 min; and a final extension for 4 min at 72°C.

Cell lines: Madin Darby Bovine Kidney (MDBK) cells (National Centre for Cell Sciences, Pune, India) were grown in DMEM growth media supplemented with 5% fetal calf serum.

*Processing and infection to cells:*The nasal swabs were agitated in PBS, pooled and were filter sterilized in 0.45 µ syringe filters. Of filter sterilized samples (200 µl) were infected on to MDBK cells by adsorption method as described by OIE (Anonymous 2010). The samples were given five passages.

Virus neutralisation index: The virus isolate thus obtained were confirmed by neutralisation test using BoHV-1 specific antiserum procured from PD_ADMAS, Bengaluru, India. The virus titration, neutralisation and determination of neutralisation index (NI) of virus isolates were carried out as per standard protocols (Anonymous 2010, Reed and Muench 1938). The BoHV-1 virus isolate obtained from Project Directorate on Animal Disease Monitoring and Surveillance, Bengaluru, India, was used as positive control.

Phylogenetic analysis: DNA was extracted from cells showing CPE and subjected for PCR targeting amplification of conserved region on gC gene of BoHV-1. The PCR product was gel purified and subjected for nucleotide sequencing at Bioserve Pvt. Ltd, Hyderabad, India. The deduced nucleotide sequences were BLAST analysed and were aligned with published BoHV-1 sequences deposited in GenBank. Phylogenetic analysis was done using MEGA-6 software and tree was constructed using the Neighbour Joining method (Tamura *et al.* 2013).

RESULTS AND DISCUSSION

The animals from which the samples were collected showed symptoms of high fever, acute respiratory distress with nasal discharge and hurried respirations, conjunctivitis with lacrimation and a few had lesions of vulvo-vaginitis. Upon bacteriological cultural examinations, the nasal swabs did not yield growth of *Pasteurella multocida.* The serum samples from all the 25 ailing animals from which samples were collected were found positive for BoHV-1 antibodies by Avidin-Biotin ELISA. The DNA extracted from swab samples collected from 25 ailing animals yielded specific amplicon of 173 bp when subjected for gC gene based PCR, indicating presence of BoHV-1 in ailing animals.

The lesions of acute respiratory distress and vulvo-vaginitis are the classical symptoms of BoHV-1 infections in cattle (Benoit *et al.* 2007, Ranganatha *et al.* 2013, Chandranaik *et al.* 2014). Detection of BoHV-1 antibodies in all ailing animals showing clinical symptoms of BoHV-1 infection correlated with previous findings, recording high sero-prevalence of IBR in Indian cattle (Ganguly and Mukhopadhay 2010, Nandi *et al.* 2010, Chandranaik *et al.* 2014). It is important to note that India does not practice any vaccinations against BoHV-1 in cattle.

When the nasal swab samples were pooled and subjected for virus isolation in MDBK cells, at third passage cells showed changes of rounding and vacuolations, which extended by fourth passage and full blown CPE was observed at fifth passage. The CPE at fifth passage comprised of appearance of vacuolations by 24–48 h post infection, rounding of cells and thread like cellular elongations by 48–72 h post infection, with characteristic 'bunch of grapes' like aggregation developed by 72 h, finally leading to complete destruction of cell sheet by 96–120 h (Fig. 1).

The CPE of "bunch of grapes" like appearance of virus infected cells observed during this study was in correlation with previous BoHV-1 isolation reports (Chandranaik *et al.* 2010, Ranganatha *et al.* 2013, Shapouri *et al.* 2016).

The isolate was neutralised by BoHV-1 specific serum obtained from PD_ADMAS, Bengaluru, India, with

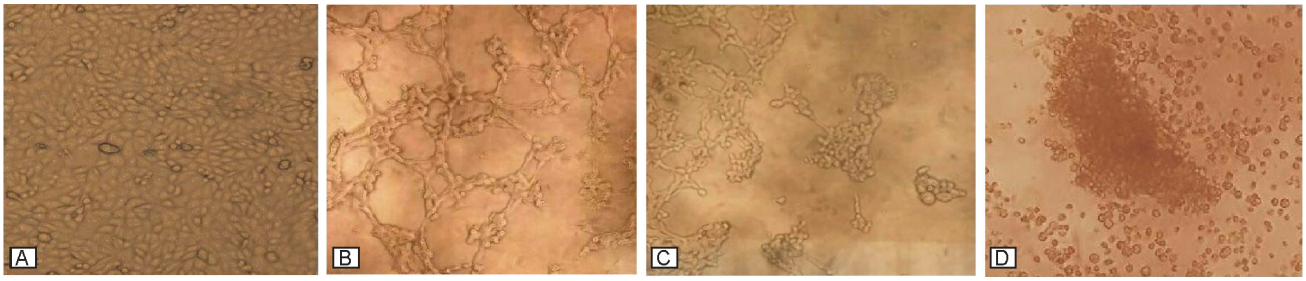

Fig. 1. Cytopathic changes produced by BoHV-1 isolate at 5th passage in MDBK cells.

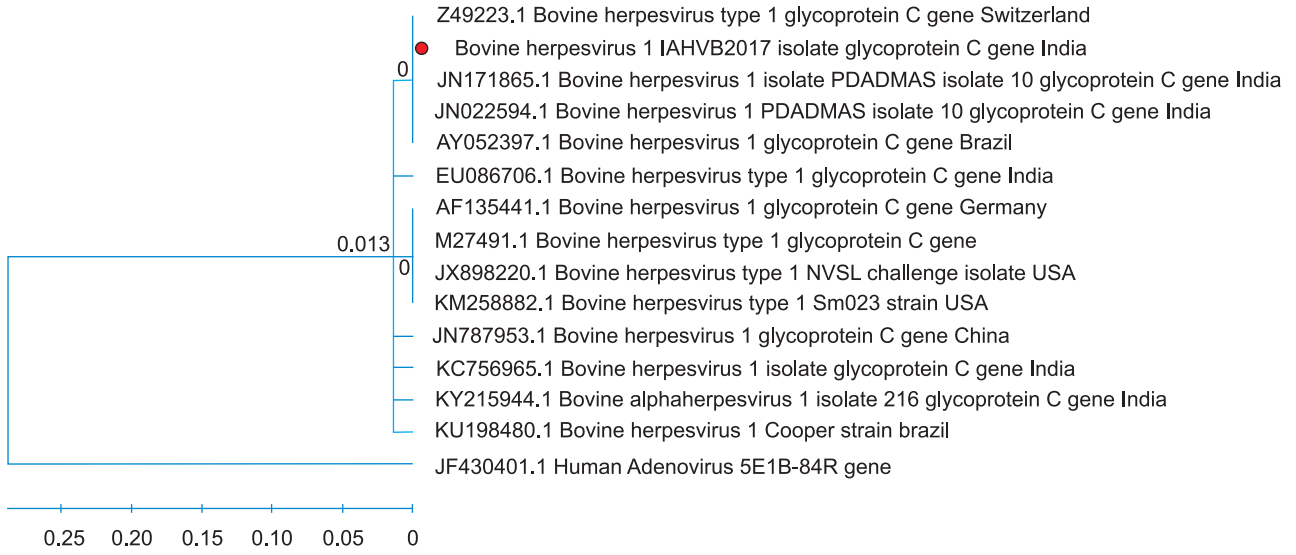

Fig. 2. Phylogenetic analysis of gC gene of BoHV-1 isolate from cattle.

neutralisation index (NI) of more than 1.5. The titre of the virus isolate was log 10^4TCID$_{50}$/0.1ml. As per OIE, to declare isolate as BoHV-1, the isolate should have a neutralisation index of at least 1.5 (Anonymous 2010, Chandranaik *et al.* 2014) and the isolate obtained in this study was confirmed as BoHV-1 with NI of more than 1.5.

The DNA extracted from the fifth passage cells showing CPE when subjected to PCR targeting Glycoprotein C gene of BoHV-1 yielded a specific amplicon of 173 bp confirming the virus as BoHV-1. After PCR confirmation the isolate was named as Bovine herpesvirus-1- IAHVB 2017.

Phylogenetic analysis of partial gC gene (sequenced PCR product) showed the isolate had highest sequence identity with BoHV-1 isolates from Switzerland, Brazil and Indian BoHV-1 isolates deposited in GenBank (Fig. 2), conclusively confirming the virus involved in the current disease outbreak. The sequencing results are in correlation with the previous findings who have reported that BoHV-1.1 is the most common subtype of herpes virus circulating and causing outbreaks in India (Ranganatha *et al.* 2013, Chandranaik *et al.* 2014, Patil *et al.* 2016). The limitation of this study was that only partial gC gene of 173 bp sequence was targeted for PCR as well as sequencing and complete gene sequencing could have given better picture of genetic variations of current BoHV-1 isolate in relation to circulating isolates in India and abroad.

The biggest challenge in herpes viral infections is the carrier status they induce in the animals (Chandranaik *et al.* 2010). During primary infection BoHV-1 establishes latency in sensory neurons of trigeminal and sacral ganglions. Once infected, the animal becomes lifelong carrier of BoHV-1 (Lovato *et al.* 2003). Latency of BoHV-1 involves complicated virus-host interaction which plays a critical role in pathogenic potential of the virus. The ability of the virus to reactivate from latency is responsible for recurrent disease and its transmission. In this study vaccination had possibly caused stress in animals that had resulted in re-activation of BoHV-1 in latently infected cattle, resulting in clinical outbreaks. When the animal is under stress, it leads to release of epinephrine from sympathetic nervous system and glucocorticoids from adrenal gland. An increase in the levels of corticosteroids results in re-activation of virus in latently infected cattle. Corticosteroids may have a role in altering the neuronal cell environment by activating cellular genes that enable reactivation of virus from latency by down regulating the expression of LR transcripts that suppressed viral gene expression during the period of latency. During reactivation from latency, productive viral gene expression is readily detected in sensory neurons, LR gene expression declines, and infectious virus can be detected in nasal or ocular swabs (Benoit *et al.* 2007, Jones 2003, Shapouri *et al.* 2016).

Through this study we demonstrate that re-activation of latent virus occurs following stressful conditions animal undergoes after vaccination leading to shedding of virus which plays an important role in perpetuation and transmission of the virus.

REFERENCES

Anonymous. 2010. Infectious bovine rhinotracheitis, pp. 752–67. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Chapter 2.4.13.

Benoit M, Julein T, Philippe K, Frédéric S and Etienne T. 2007. Bovine herpes virus 1 infection and infectious bovine rhinotracheitis. *Veterinary Research* **48**: 181–209.

Chandranaik B M, Chethana C S, Shivaraj and Renukaprasad C. 2010. Isolation of BHV-1 from bovine semen and application of real time PCR. *Veterinarski Arhiv* **80**: 467–75.

Chandranaik B M, Rathnamma D, Patil S S, Ranganatha S, Isloor S, Renukaprasad C and Prabhidas K. 2014. Epidemiology of bovine herpes virus-1 under different housing practices in cattle breeding stations. *Indian Journal of Animal Sciences* **84**: 103–07.

Engelenburg V, Maes R K, Oirschot J T V and Rijsewijk F A M. 1993. Development of a rapid and sensitive polymerase chain

reaction assay for detection of bovine herpesvirus type 1 in bovine semen. *Journal of Clinical Microbiology* **31**: 3129–35.

Ganguly S and Mukhopadhay. 2010. Serological survey of infectious bovine rhinotracheitis. *Indian Veterinary Journal* **87**: 711–12.

Jones C. 2003. Herpes simplex virus type 1 and bovine herpesvirus 1 latency. *Clinical Microbiology* **16**: 79–95.

Lovato L, Inman M, Henderson G, Doster A and Jones C. 2003. Infection of cattle with a Bovine herpesvirus 1 strain that contains a mutation in the Latency-Related gene leads to increased apoptosis in trigeminal ganglia during transition from acute infection to latency. *Journal of Virology* **77**: 4848–57.

Majumder S, Ramakrishnan M A and Nandi S. 2015. Infectious bovine rhinotracheitis: An indian perspective. *International Journal of Current Microbiology and Applied Sciences* **4**: 844–58.

Nandi S, Manish M and Manoj K. 2010. Sero-surveillance of infectious bovine rhinotracheitis in buffalo bulls. *Indian Veterinary Journal* **87**: 544–45.

Patil S S, Prajapati A, Hemadri D, Suresh K P, Desai G and Reddy G B. 2016. Phylogenetic analysis of glycoprotein B gene sequences of bovine herpesvirus 1 isolates from India reveals the predominance of subtype 1.1. *Veterinary World* **9**: 1364–69.

Quinn P J, Markey B K, Leonard F C, Fitzpatrick E S, Fanning S and Hartigan P J. 2011. *Veterinary Microbiology and Microbial Diseases*. 2nd edn. Wiley-Blackwell Publications, United Kingdom.

Ranganatha S, Rathnamma D, Patil S S, Chandranaik B M, Isloor S, Veeregowda B M, Narayanabhat M and Srikala. 2013. Isolation and molecular characterization of Bovine Herpes Virus-1 by polymerase chain reaction. *Indian Journal of Animal Research* **47**: 40–43.

Reed L V and Muench H. 1938. A simple method of estimating fifty per cent end points. *American Journal of Hygiene* **27**: 493–97.

Shapouri S, Rad G and Hajikolaei H. 2016. Isolation of Bovine herpes virus-1 from latently infected cattle in Ahvaz. *Iranian Journal of Ruminant Health Research* **1**: 11–20.

Tamura K, Stecher G, Filipski A and Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725–29.