



## SHORT COMMUNICATION

### First Report on the Detection and Molecular Characterization of Bovine Herpesvirus 1 from a Clinical case of Infectious Bovine Rhinotracheitis in Pakistan

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#### ABSTRACT

Bovine herpesvirus 1 (BHV-1) is a noteworthy reason for many Cattle/ Buffalo diseases. Infectious bovine rhinotracheitis (IBR) is one of the diseases which are caused by the BHV-1. In the present study a cow which was suspected of IBR was examined. The animal was suffering from fever and respiratory distress along with rhinitis (red nose), inappetence, and dyspnea. The nasal mucosa and muzzle were distinctly inflamed, with nasal discharge. DNA from blood samples and nasal swabs were subjected to nested PCR using glycoprotein B gene (gB) Primers. The samples were found positive for BHV-1 gB gene. Sequencing and phylogenetic analysis revealed close similarities with other BHV-1 gB gene sequences. The accession numbers assigned to this pioneer sequences in GenBank are MT449510 (BHV-1-Pak 1) and MT449511 (BHV-1-Pak 2). In this study, we reported for the first time the detection of DNA of BHV-1 through nested PCR assay and molecular characterization of BHV-1 gB gene in Pakistan. This study will be useful in further diagnoses of BHV-1 in Pakistan and in development of BHV-1 vaccine to reduce economical losses due to IBR.

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#### INTRODUCTION

Bovine herpesvirus 1 (BHV-1) belongs to family *Herpesviridae*, subfamily *Alphaherpesvirinae* and genus *Varicellovirus*. BHV-1 is a double-stranded DNA virus with a genome of 135–140 kbps in size. The virus is subdivided into different sub-types including BHV-1.1, 1.2a, 1.2b (Biswas *et al.*, 2013). BHV-1 is linked with the diversity of clinical disorders in the cattle including upper respiratory tract and reproductive tract. BHV-1 is endemic in both wild and domestic ruminants of tropical, subtropical, and temperate regions of the sector i.e. the USA, Europe, Asia, Australia and most of the international locations in Africa which include South Africa, Ruanda-Urundi, Egypt, Nigeria, Kenya, Tanzania, Zambia, Namibia (Graham 2013; Yazici *et al.*, 2015).

BHV-1 causes infectious bovine rhinotracheitis (IBR) in cattle and buffaloes. Lower milk productions, respiratory tract problems and abortion are the conditions of animals suffering from IBR, which may lead to death

and cause great economic losses to the livestock industry and small level farmers (Lojkić *et al.*, 2011). IBR is an acute infection characterized by sudden onset of fever, salivation, rhinitis, conjunctivitis, inappetence, and dyspnea. In acute cases, respiratory distress increases and open-mouth breathing is evident. Primary BHV-1 infection can lead to secondary bacterial infection which later can cause bronchopneumonia and death in complicated cases. The shedding of virus in the secretions may be or may not be accompanied with clinical symptoms (Muykens *et al.*, 2007). The reproductive system may also become involved manifested by infertility problems, abortion, and birth defects (Nandi *et al.*, 2009). BHV-1 attains latency in sensory ganglion. During acute phase of the disease viremia occurs and viral DNA can be isolated from blood, body secretions and tissues. BHV-1 diagnosis and isolation is possible by using nasal swabs, vaginal swabs, placental parts of aborted fetus, ovary of infected animal and various vital organs. The widespread nature of BHV-1 among dairy

cattle pointed out the need for additional field and the laboratory examinations to recognize proper immunization or biosecurity measures to avoid infection and protect pregnant susceptible cows, thus minimizing economical effect of these diseases in a country (Elhassan, *et al.*, 2015).

Pakistan has a huge livestock population such as cattle, buffaloes, sheep, and goat. Majority of the human population rely upon livestock and agriculture for their livelihood. Infectious diseases are continuing threat to livestock industry in Pakistan and worldwide. IBR caused by BHV-1 is one of the major reasons of many clinical conditions in livestock. At present very limited information is available on molecular characterization of BHV-1 in Pakistan. The present study describes for the first time the detection of BHV-1, sequencing and phylogenetic analysis of BHV-1 gB gene from a cow suffering from IBR.

## MATERIALS AND METHODS

A six years old Holstein Friesian was brought to the clinical facility of Faculty of Veterinary and Animal Sciences (FV&AS), PMAS Arid Agriculture University Rawalpindi for checkup, diagnosis and treatment purposes. Animal was showing signs and symptoms related to respiratory tract infection and suspected of IBR case. Blood sample and nasal swabs were collected from the infected animal by certified veterinary clinicians/practitioners without giving any harm to the animal. Blood was taken in EDTA containing tube whereas nasal swabs were suspended in PBS solution. DNA was extracted using wizprep™ gDNA mini kit (Korea) for blood sample and One-4-All Genomic DNA Kit (Bio Basic Canada Inc.) for swab samples, as per instruction of the manufacturer and stored at -20 °C. Nested PCR was performed using extracted DNA and two sets of primers specific for BHV-1 gB gene. For first PCR of nested PCR Ext F 5' CACGGACCTGGTGGACAAGAAG-3 and Ext R 5'CTACCGTCACGTGCTGTGTACG-3' were used and for the second PCR "Int F 5'AGCCGAGTACCTGCGCAG-3' and Int R 5'AGCCGAGTACCTGCGCAG-3' were used. BHV-1 bacterial artificial chromosome (Raza *et al.*, 2016) was used a positive control. In a total volume of 50 µL reaction mix, 100ng/µL DNA was used to perform PCR. PCR conditions include; 10 minutes at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 58°C, 1 min at 72°C and final extension for 7 minutes at 72°C. These PCR products will be used for second PCR (nested PCR). The PCR reaction conditions were similar as in the first step except the annealing temperature which was changed to 56°C in the second step. The PCR products (344 bp) of second step of nested were separated on 1.8% agarose gels.

For gene sequencing, the positive PCR products were sent to MacroGen® Korea. Sequences derived from this study were subjected to NCBI BLAST to find similar sequences in the GenBank database. Phylogenetic analysis was also performed using MEGA7 with neighbor joining method.

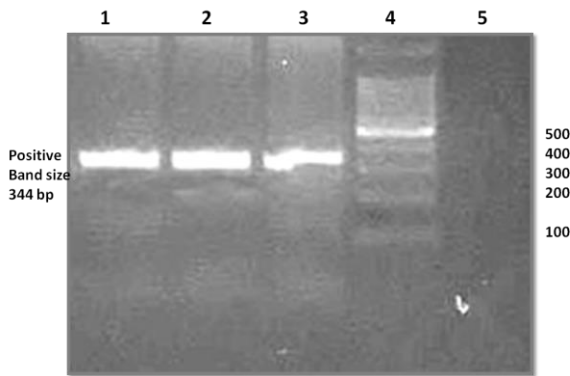
## RESULTS AND DISCUSSION

A six years old Holstein Friesian was brought to the clinical facility of FV&AS, PMAS Arid Agriculture University Rawalpindi. Animal was restless and showed clinical signs which include Fever, coughing, depression, loss of appetite, hyperaemia of the mucosa, mucosal lesions, and drop in milk production. Because of respiratory tract problem animal was feeling difficulty in breathing and there was nasal discharge. According to sign and symptoms animal was expected to have IBR. Nested PCR confirmed the presence of viral DNA. Both the samples (blood and nasal swab) have shown bands of approximately 344 bp of gB gene with nested PCR (Fig. 1). Sequencing analysis of the positive PCR products also confirmed that both sequences were from BHV-1 gB gene.

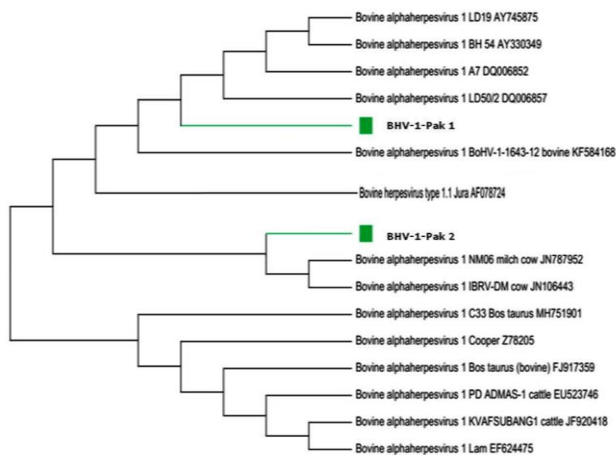
BHV-1 is a latent virus which attains latency in sensory ganglion and shedding of virus occurs in the acute stage of the disease or when animal is in stress. Viral antigens can only be detected in nasal swab, vaginal swab, tissues and whole blood during the acute phase of the infection. In the present study blood and nasal swab samples were found positive for BHV-1 DNA using nested PCR (Fig. 1). Nested PCR is a very sophisticated method that can detect certain DNA sequences in a variety of samples and is widely used in diagnostics and validation of BHV-1 infection. Lojkic *et al.* (2011) performed nested PCR specific for gB and gC genes of BHV-1 using nasal swab and lung samples. All nasal swab samples and a lung sample were found positive with both sets of primers (Lojkić *et al.*, 2011). Nezzal *et al.* (2017) demonstrated through conventional PCR that among 50 serologically positive BHV-1 samples, from both cattle and buffaloes, 37 (74.00%) samples were positive for the presence of viral genetic material. Presence of BHV1 was highest in abomasal contents followed by whole blood and nasal swabs. Elhassan *et al.* (2015), studied the infection outline of BHV-1 in dairy cattle in the Sudan. Out of 140 samples obtained from the dairy flocks which expressed predominantly higher rate of abortion and the infertility, 11% were found positive using nested PCR method. Whereas using antigen capture ELISA only one sample from vaginal swab, 5 samples from placental material and one sample from whole blood were found positive. These results indicated that PCR is a good alternative for the rapid detection of the BoHV-1.

In the present study the sequences of the gB gene region of positive PCR products were also analyzed and compared with 15 sequences reported by other researcher using NCBI-Blast. Sequences were also submitted to GenBank and their assigned accession numbers were; MT449510 (BHV-1-Pak 1) and MT449511 (BHV-1-Pak 2). The DNA sequence analysis and phylogenetic analysis have shown that the gB gene sequence obtained in the current study showed 99 to 100% homology in the sequence of the BHV-1 gB gene sequences worldwide.

BHV-1 strains isolated from Brazil (AY745875, AY330349, DQ006852 and DQ006857), China (JN78724 and JN106443), Israel (KF584168), Sweden (AF078724) make one complex whereas USA (MH751901), India (EU523746 and JF920418), Switzerland (Z78205), Norway (FJ917359) and Belgium (EF624475). Sequences MT449510



**Fig. 1:** Fragment of BHV-1 gB gene amplified from blood and Nasal swab of cattle infected with IBR. Positive samples are showing bands of approximately 344 bp; Lane 1: Blood sample; Lane 2: Nasal swab sample; Lane 3: Positive control; Lane 4: 100bp DNA marker; Lane 5: Negative control.



**Fig. 2:** Phylogenetic tree for fragments of sequences of BHV-1 gB gene. The green squares are showing two of the samples (from blood and nasal swab) isolated in the present study from IBR infected cow.

(BHV-1-Pak 1) and MT449511 (BHV-1-Pak 2) obtained from this study were present in the first complex of the tree (Fig. 2). Phylogenetic analysis of the present study sequences has also shown that BHV-1 has no geographic separation. These analyses also revealed similarities of sequences of this study to the sequences from neighboring countries; India and China. Free movements of animals across the borders lead to the transmission of virus to neighboring countries. The other transmission routes might include free trading of meat and movement of people from one country to other country. The spread of respiratory viruses is very fast as we are experiencing COVID 19 (Corona virus disease 19) pandemic worldwide (Lai *et al.*, 2020). Poor managerial

practices and unhygienic condition are the most common reasoning in a sense to spread these diseases either in human beings or animals. Despite the fact that BHV-1 is mostly benign and the self-proscribing, it can also affect in a huge drop in the milk productivity, growth in the vulnerability of mammary gland for bacterial type mastitis and culling of efficient cattle because of chronic type mastitis (Moeller *et al.*, 2013).

Many clinical symptoms due to IBR infection have not been identified and are present in various diagnoses; it is often overlooked by a veterinarian. The findings of present study indicate that further research for the diagnosis of BHV-1 using sensitive molecular techniques like nested PCR, is needed to protect the livestock industry in Pakistan.

**Authors contribution:** AR arranged funding, conducted experiments, analyzed data and wrote manuscript. BJ and SA performed sampling and conducted experiments. SR provided positive control. MAAS, AY, SUR and AS helped in taking sample, analyzing data and writing manuscript.

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