



## SHORT COMMUNICATION

### First Isolation and Genetic Characterization of Bovine Herpesvirus 1 From Cattle in Pakistan

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#### ABSTRACT

The study was aimed to isolate BoHV-1 circulating in Lahore, Pakistan and its genetic characterization. For this purpose, blood samples were collected from different areas of district Lahore through convenient sampling technique. Out of 100 blood samples, 69 (69%, 95% CI: 58.86-77.66) samples have shown seropositivity against BoHV-1 through ELISA. For the isolation of BoHV-1, nasal swab samples were collected from 69 seropositive cattle and buffalo. Out of 69 nasal swab samples, only 6 samples have shown the visible cytopathic effect and confirmed through nested polymerase chain reaction by targeting glycoprotein B (gB). The phylogenetic analysis showed that local isolates showed similarity to subtype 1.1 of BoHV-1 and had 99-100% homology with Cooper strain. It was concluded that BoHV-1.1 is being circulating in Pakistan and further studies are needed which will help for clear understanding of virus characterization and development of effective local vaccine.

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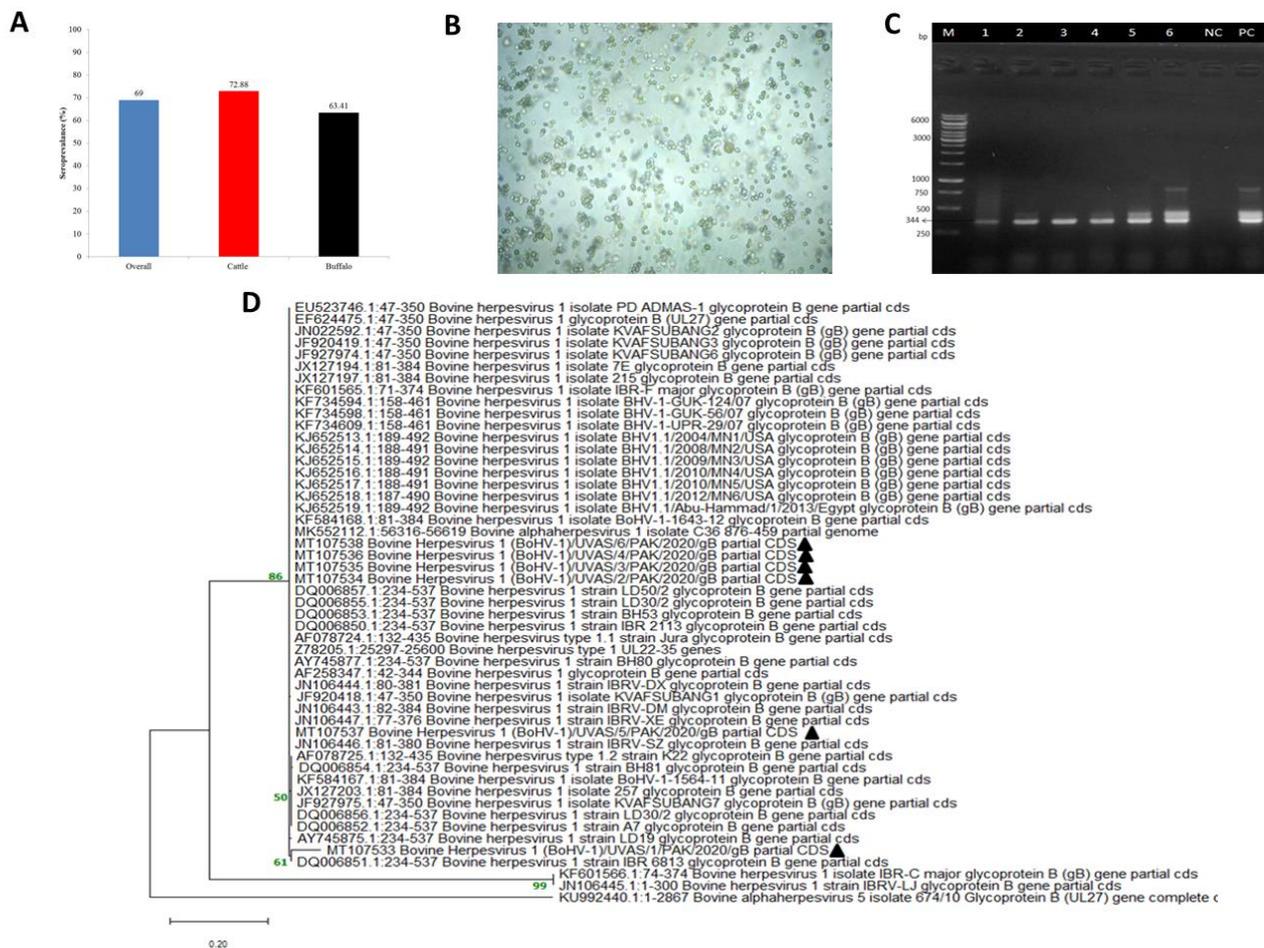
#### INTRODUCTION

The livestock industry of Pakistan has the potential to export meat and dairy products. The government is planning to increase the international trade of livestock. The animals that are to be free of BoHV-1 are only eligible for export to most countries. The virus presence restricted the trade of animals as well as their products. Preliminary epidemiological study indicated a high seroprevalence (69-70%) of BoHV-1 infection in the local cattle and buffalo populations (Shabbir *et al.*, 2013). BoHV-1 infection, also known as Infectious Bovine Rhinotracheitis (IBR), is one of the major respiratory and reproductive infections in cattle and buffalo, which leads to production losses due to pneumonia, genital disorders, encephalitis, infertility, abortions and immunosuppression (Jones and Chowdhury, 2010). BoHV-1 is an enveloped DNA virus that belongs to Family *Herpesviridae* and Subfamily *Alphaherpesvirinae*. Alphaherpesviruses (Albayrak *et al.*, 2020, Patil *et al.*, 2016). During primary infection, BoHV-1 replicates in the nasal epithelium and then invades the sensory nerve endings of the trigeminal nerve in the nasopharynx. Subsequently, the virus is transported to trigeminal ganglia (TG) where it establishes lifelong latency. Periodically, BoHV-1 reactivates from

latency, which results in anterograde axonal transport of virus or viral particles to the axon termini/nerve endings in the primary infection sites, nasopharynx resulting in nasal virus shedding and transmission and maintenance of the virus in the susceptible cattle and buffalo population (Jones, 2016). The purpose of this study was to isolate Pakistani BoHV-1 and an understanding of its genetic relationships with other bovine alphaherpesviruses isolates. This is the first study regarding the isolation and genetic characterization of Pakistani BoHV-1 isolate. These types of studies are needed for the determination of the BoHV-1 strain circulating in Pakistan which will be helpful for development of an effective local vaccine.

#### MATERIALS AND METHODS

**Collection of samples:** In this study, 100 blood samples were collected from different localities of district Lahore (Harbanspura, Ravi chowk, Gwala colony, Shalimar tehsil, Jallo park, Jallo more and Cantonment tehsil) through convenient sampling technique. In this study, the target population was cattle and buffalo with a history of respiratory and reproductive disorders. Nasal swab samples were also collected from seropositive cattle and buffaloes in transport media for virus isolation.



**Fig. 1:** First Isolation and genetic characterization of Pakistani BoHV-1. A) Seropositivity of BoHV-1 in Cattle and Buffalo B) Observation of cytopathic effect on MDBK cells under inverted microscope C) Nested PCR for the detection of BoHV-1 targeting glycoprotein B (gB). Line M showed 1kb ladder (Thermo Fisher Scientific, Massachusetts, USA); Line 1, 2, 3, 4, 5 and 6 are the local isolates (MT107533, MT107534, MT107535, MT107536, MT107537 and MT107538 respectively) showed 344bp; Line NC is the negative control and Line PC is the positive control D) BoHV-1 phylogenetic analysis. The phylogenetic maximum likelihood tree for the glycoprotein B (gB) of the Bovine Herpesvirus genome was generated by using the MEGA X software. Sequences generated in this study were as follows: MT107533, MT107534, MT107535, MT107536, MT107537, MT107538. Sequences not generated in this study were derived from GenBank® and were as follows: EU523746, EF624475, JN022592, JF920419, JF927974, JX127194, JX127197, KF601565, KF734594, KF734598, KF734609, KJ652513, KJ652516, KJ652517, KJ652518, KJ652519, KF584168, MK552112, DQ006857, DQ006855, DQ006853, DQ006850, AF078724, Z78205, AY745877, AF258347, JN106443, JN106447, JN106446, AF078725, DQ006854, KF584167, JX127203, JF927975, DQ006856, DQ006852, AY745875, DQ006851, KF601566, JN106445 for BoHV-1; and KU992440.1 for BoHV-5.

**Determination of BoHV-1 antibodies by ELISA:** For the detection of antibodies against Bovine herpesvirus-1, all the serum samples were tested through commercially available ID Screen® IBR gE competition ELISA kit (ID.Vet, Grabels, France) as per the manufacturer's recommendations. The Kit is able to detect IgG antibodies against the gE protein of BoHV-1. In this kit, there was a competition between subject antibody and kit provided anti-antibodies (competitive ELISA).

**Virus isolation:** Virus isolation was performed according to the procedure mentioned in the OIE manual, 2010 (Manual, 2010). At least, three consecutive passages were carried out until the cytopathic effect (CPE) was observed.

**Nested Polymerase Chain Reaction:** Nested polymerase chain reaction (PCR) was performed for the confirmation of BoHV-1 isolates by targeting glycoprotein B (gB). Total Viral DNA was extracted by using a commercially available GF-1 Viral Nucleic acid DNA extraction kit (Vivantis, Selangor, Malaysia) as per the

recommendations of the manufacturer. The purity of extracted DNA was checked through NANO Drop 1000 (ThermoFisher, Massachusetts, USA). Then DNA was subjected to PCR using previously published two sets of primers targeting gB region and predicted to produce PCR amplicons of 468 and 344 bp for external and internal primers respectively (Rocha *et al.*, 1998). Nested PCR was performed by using commercially available Green Taq Master Mix (Thermo Fisher Scientific, Massachusetts, USA). The reaction mixture was prepared and applied for conditions as described previously (Rocha *et al.*, 1998). The amplified products were visualized by making 1% agarose gel.

**Phylogenetic Analysis:** The PCR amplicons were sent for sequencing to a commercial supplier. The obtained sequences were deposited in GenBank® under accession number MT107533, MT107534, MT107535, MT107536, MT107537 and MT107538. The reported sequences were downloaded from GenBank® for comparing with obtained sequences. The phylogenetic

maximum likelihood tree for the glycoprotein B (gB) of the bovine herpesvirus genome was generated by using the MEGA X software.

**Statistical analysis:** In this study, the lower and upper limits of the 95% confidence interval were calculated according to a method described by Robert Newcombe, 1998 (Newcombe, 1998).

## RESULTS AND DISCUSSION

In the present study, 69% (95% CI: 58.86-77.66) seropositivity rate was detected in the targeted area (Figure 1A). In this study, more seropositivity was found in case of cattle [n=43/59, (72.88%, 95% CI: 59.51-83.25)] as compared to buffaloes [n=26/41, (63.41%, 95% CI: 46.91-77.43)] (Figure 1A). The prevalence of BoHV-1 noted in this study is very close to the prevalence noted previously on a single dairy herd with an outbreak of abortion due to mixed infection involving different pathogens, major damages due to BoHV-1 (Shabbir *et al.*, 2013). However, our study results are in concordance with findings of research conducted in India (68.9%) but these results are not in concordance with Algeria (31.17%) which revealed high level of seroprevalence in subcontinent but lower in Europe (Derrar *et al.*, 2019; Nandi *et al.*, 2011). The possible reasons for these disagreements could be due to the housing of different breeds of cattle imported from other countries, availability of better diagnostic facilities, and regular screening of animals.

Virus isolation was performed by collecting nasal swab samples from 67 seropositive cattle and buffaloes. Out of 67 nasal swab samples, only 6 samples showed the visible cytopathic effect which was confirmed by rounding of cells as a grape-like cluster (Figure 1B). Confirmation of PCR was observed by 344bp amplified product on agarose gel electrophoresis (Figure 1C). The results of phylogenetic analysis showed that local isolates of BoHV-1 have more similarity with reported sequences of India, Iran, China, Egypt, Israel, Belgium, USA, Sweden and Switzerland (Figure 1D). All isolated sequences matches to the subtype 1.1 and 99-100% homology with reference Cooper strain of BoHV-1. The subtypes prevalent in the current study matches to studies conducted in subcontinent (Patil *et al.*, 2016). Similar

subtypes was isolated from another study conducted in Turkey (Albayrak *et al.*, 2020). These are the first nucleotide sequences of BoHV-1 deposited in GenBank® that circulating in Pakistan. Further studies of more viruses isolated from different localities of Pakistan are needed with such molecular characterization which will be helpful for clear understanding of the virus epidemiology and preparation of effective local vaccine.

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**Author's contribution:** Conceptualization, MR, SR; methodology, HR, MR and SR; formal analysis, HR, MR, and AG; writing original draft preparation, HR and MR; writing, review and editing, HR, MR, AG, AR, FNA, SR.

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