



## SHORT COMMUNICATION

### Locally Prepared Bovine Herpesvirus 1 gE Deleted Vaccine Induced Immunogenicity in Rabbits

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#### ABSTRACT

In this study, bovine herpesvirus 1 gE deleted vaccine was prepared from local isolate, by replacing entire gE coding region with enhanced green fluorescent protein (EGFP) for selection. After transfection of MDBK cells with genomic viral DNA and EGFP-bearing gE-deletion plasmid, fluorescent recombinant clones were produced. Deletion of gE gene was confirmed by PCR. BoHV-1ΔgE recombinants formed smaller plaques but replicated in similar titer and kinetics as compared to parental virus (UVAS/2) on MDBK cell lines. In-vivo study showed that locally prepared BoHV-1ΔgE vaccine induced good immune response. For confirmation of seroconversion of gE, serum samples were also tested by gE competition ELISA. The results showed that all rabbits vaccinated with recombinant BoHV-1ΔgE remained negative in gE competition ELISA. The study concluded that locally prepared BoHV-1ΔgE marker vaccine can be candidate vaccine to prevent BoHV-1 infections in Pakistan.

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#### INTRODUCTION

Infectious bovine rhinotracheitis (IBR) caused by bovine herpesvirus 1 (BoHV-1), is an enveloped DNA virus which belongs to family herpesviridae and subfamily alphaherpesvirinae. On basis of genomic analysis of BoHV-1, it consists of 3 subtypes (BoHV-1.1, BoHV-1.2a, and BoHV-1.2b) that causes respiratory infections and encephalitis, respectively (Biswas *et al.*, 2013). Alphaherpesviruses encoded about eleven envelope glycoproteins in which glycoprotein E play role for cell to cell spreading and its deletion leads to small plaque phenotype (Weiss *et al.*, 2015). To eradicate the BoHV-1, marker vaccination is most important tool to differentiate between vaccinated and infected individuals. Differentiation of infection from vaccination can be achieved by using an antibody detection system for antibody to gE. Animals that have been vaccinated with gE-deleted vaccine and not exposed to infection will test negative for antibody to gE while those animals that have been vaccinated with this vaccine and exposed to infection will test positive for antibody to gE. This differentiation of infected from vaccinated animals (DIVA) strategy has been important in the European

approach to national control of IBR. Animals that have been vaccinated with other conventional IBR vaccines cannot be differentiated from infected animals. Therefore, glycoprotein E deleted marker vaccine have advantage in the form of immunogenic and serologically distinguishable from wild type field strains (Raaperi *et al.*, 2014). In Pakistan, previous study reported 69% seroprevalence of BoHV-1 in Lahore district (Rehman *et al.*, 2021). The presence of this virus limited the trade of animals and their products. Therefore, this study was being designed to develop BoHV-1 recombinant vaccine and to determine its immunogenicity against local strain.

#### MATERIALS AND METHODS

**Source of virus:** The Pakistani BoHV-1 virus strain UVAS/2 isolated from cattle (Rehman *et al.*, 2021) was used as parental virus to develop BoHV-1 gE deleted vaccine.

**Development of pBoHV-1ΔgE plasmid:** The plasmid pBoHV-1ΔgE was developed by replacing open reading frame of glycoprotein E with EGFP gene (Fig. 1A). To develop this plasmid, upstream (gI) and downstream

fragment (Us9) of glycoprotein E were amplified by modifying the restriction sites in previously described primers (Weiss *et al.*, 2015) given in the Table 1. PCR were performed by using commercially available One Taq® Hot Start Master Mix with GC Buffer (NEB, Massachusetts, USA). EGFP sequence was amplified from pHA2 plasmid (Addgene, Watertown, USA) by using primers given in Table 1. PCR amplified products were digested with respective enzymes and cloning was performed between upstream and downstream fragments. The plasmid pBoHV-1ΔgE contained EGFP gene replacing the glycoprotein E (gE) gene.

**Extraction of DNA and transfection:** DNA Extraction was performed by using ultracentrifugation methods as per previously described procedure (Chowdhury *et al.*, 1999). For generation of BoHV-1 gE deleted virus, transfection was performed by full length parental virus DNA, bICP0 plasmid and linearized pBoHV-1ΔgE plasmid into MDBK cells through lipofectamine reagent (Invitrogen Corp., California, USA) as per protocol described earlier (Chowdhury *et al.*, 1999). The plates were observed after 72 hours under fluorescent microscope. The fluorescent plaques selected and applied in MDBK cells for further characterization.

**PCR for confirmation of gE deletion:** For confirmation of gE deletion, PCR for gE gene was performed by using previously published primers (Fuchs *et al.*, 1999). As a control, PCR for unique short segment (US1.67) was performed by previously published primers (Wang *et al.*, 2006).

**In-Vitro Growth Kinetics:** To analyze growth kinetics of recombinant virus with parental virus, in-vitro experiment was performed in which both BoHV-1ΔgE and parental virus (UVAS/2) were infected in MDBK monolayer cells and observed the virus replication every 6 hours till 72 hours post infection. The titrations of virus were done in triplicate for each point time and calculated titers presented in TCID<sub>50</sub>/ml (log<sub>10</sub>) (Ramakrishnan, 2016).

**In-Vivo and Serological Testing:** A total of sixteen male rabbits of about 8 weeks age, seronegative for BoHV-1, were divided into two separate groups, vaccinated and control group (eight each). In vaccinated group, rabbits were inoculated intranasally with BoHV-1ΔgE (10<sup>8</sup>TCID<sub>50</sub>/rabbit) while control group with maintenance media. Serum samples were collected at day 0, 7, 14, 21 and 28 days of post vaccination. Challenge dose of

parental virus UVAS/2 (10<sup>8</sup>TCID<sub>50</sub>/animal) was inoculated intranasally in both vaccinated and control groups at 28 days of post vaccination. Serum samples were collected at day 0, 7, 14 and 21 days of post challenge. The virus neutralization antibody titers were calculated from collected serum samples according to protocol described earlier (Vargas *et al.*, 2016) and converted into geometrical mean titers (GMT log<sub>10</sub>). To confirm the seroconversion against glycoprotein E, collected serum samples (56 days) in both groups were also tested by a commercially available ID Screen® IBR gE competition ELISA kit (ID.Vet, Grables, France) as per protocol described by manufacturer. In-vivo experiment was approved by Institutional Animal Ethics Committee, UVAS, Lahore (approval # DR/497).

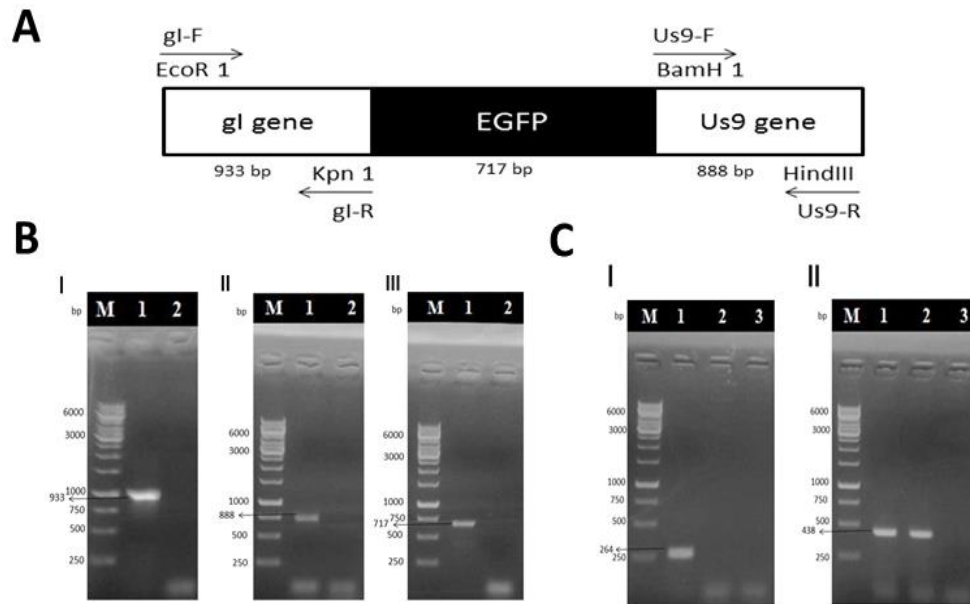
## RESULTS AND DISCUSSION

In the present study, BoHV-1 virus defective in gE gene was developed by homologous recombination between parental viral DNA and linearized pBoHV-1ΔgE EGFP plasmid. After transfection, recombinant virus showed green fluorescent plaques under fluorescent microscope. For confirmation of gE deletion, PCR was performed against gE gene which results in a product of 264 bp in parental virus while no band seen in the gE deleted virus (Fig. 2A). As a control, PCR reactions for US1.67 segment was performed, resulting in 438 bp product in both parental and gE deleted virus (Fig. 2B). On observation of growth pattern in vitro, it showed that BoHV-1ΔgE replicate in same titers as of parental virus (Fig. 2B) which supported study conducted by other authors (Chowdhury *et al.*, 1999; Weiss *et al.*, 2015). On the other hand, recombinant virus created smaller plaques as compared to parental virus (Fig. 2A) which supported previous studies (Chowdhury *et al.*, 1999; Weiss *et al.*, 2015). Results of in vivo experiment showed that locally developed BoHV-1ΔgE vaccine induced good immune response as geometrical mean titer reach up to 1.41±0.05 (log<sub>10</sub>) at 28 day post immunization (Fig. 2C). These titers increased to 1.67±0.06 (log<sub>10</sub>), 2.02±0.05 (log<sub>10</sub>) and 2.19±0.05 (log<sub>10</sub>) at day 7, 14 and 21 post challenge respectively in vaccinated group. In control group, geometrical mean titer was zero up to 28 post vaccination but increased to 1.11±0.05 (log<sub>10</sub>) after 21 days of post challenge (Figure 2C). After virus challenge, virus titer was observed in immunized group as well as in control group in similar kinetics which support the previously conducted studies (Chowdhury *et al.*, 1999; Romera *et al.*, 2014). For the confirmation of seroconversion of gE,

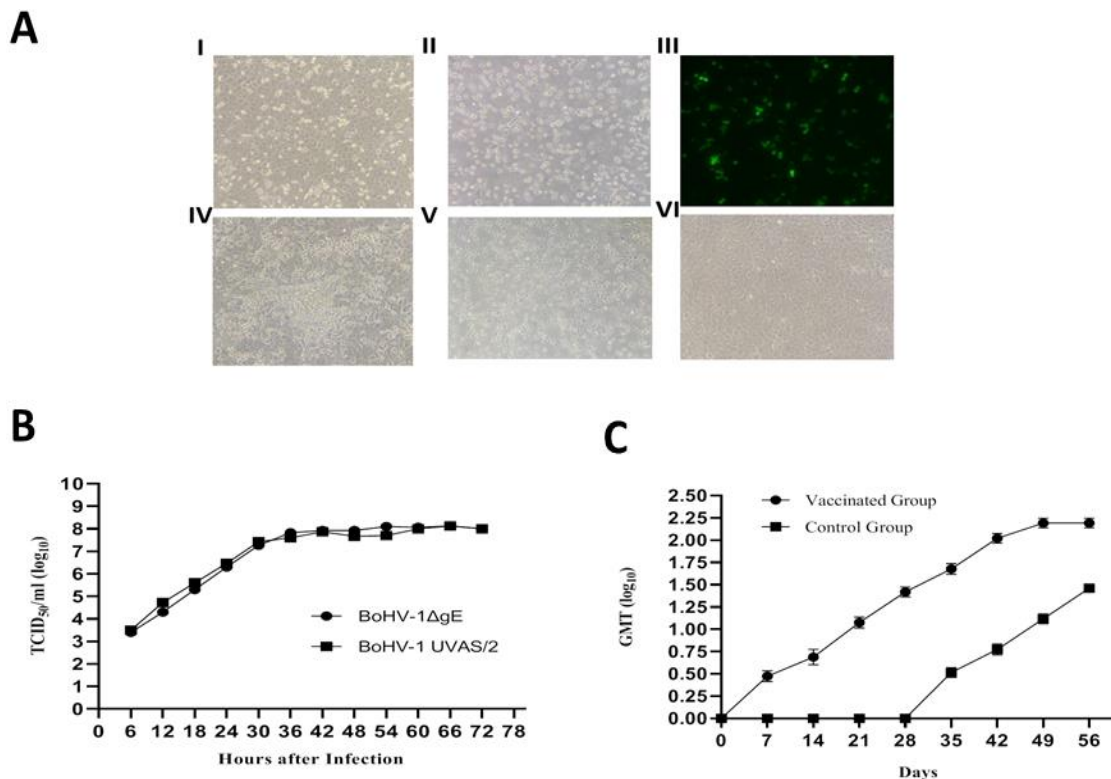
**Table 1:** List of Primers used for PCR reaction

Primer	Name	Sequence (5' to 3')	Product size (bp)
gE Upstream (gl)	gl-F	<b>CAGAATTC</b> GTTTGTACACAGCTTCGG	933
	gl-R	<b>CAGGTACCT</b> GCCAAATGCCCTTTTCG	
gE Downstream (Us9)	Us9-F	<b>CAGGATCC</b> CTGTGCCGTCTGACGGAA	888
	Us9-R	<b>CAAAGCTT</b> GCCCCGAATCCCCTCCTC	
EGFP	EGFP-F	<b>CAGGTACC</b> ATGGTGAGCAAGGGCGAG	717
	EGFP-R	<b>CAGGATCC</b> CTTGTACAGCTCGTCCAT	
gE	gE-F	CTTCGTCGACACGGTCTT	264
	gE-R	CTTTGTGCGCCGTTGAGTGC	
Us1.67	Us1.67-F	AGCGGGCCCTCGTCTCGTAGCC	438
	Us1.67-R	CAGCGCCGGCGTTTGGTCATTTG	

Bold letters indicate restriction sites.



**Fig. 1:** Preparation of BoHV-1 gE deleted virus **A)** Schematic illustration of the development of gE deletion plasmid pBoHV-1 ΔgE-EGFP. **B)** Construction of BoHV-1 gE deletion plasmid. **I)** PCR for upstream fragment. Line M showed 1Kb ladder; Line 1 is the local isolate showed 933bp; Line 2 is the negative control **II)** PCR for downstream fragment. Line M showed 1Kb ladder; Line 1 is the local isolate showed 888bp; Line 2 is the negative control **III)** PCR for EGFP gene. Line M showed 1Kb ladder; Line 1 is the EGFP gene showed 717bp; Line 2 is the negative control **C)** PCR for confirmation of gE deleted virus. **I)** PCR for gE gene. Line M showed 1Kb ladder; Line 1 is the parental virus showed 264bp; Line 2 is the gE deleted virus; Line 3 is the negative control. **II)** PCR for short unique segment (US1.67). Line M showed 1Kb ladder; Line 1 is the parental virus showed 438bp; Line 2 is the gE deleted virus showed 438bp; Line 3 is the negative control.



**Fig. 2:** In-vitro and In-vivo growth properties of recombinant virus in comparison with parental virus **A)** In-vitro growth properties of recombinant virus in comparison with parental virus. **I)** Recombinant virus formed small plaques in MDBK cell monolayer **II)** Cytopathic effect of recombinant virus **III)** Enhanced green fluorescent protein (EGFP) seen in the recombinant virus **IV)** Parental virus formed larger plaques in MDBK cell monolayer **V)** Cytopathic effect of parental virus **VI)** Normal MDBK cell monolayer as a control **B)** In-vitro growth kinetics of BoHV-1ΔgE virus. **C)** Serological response detected in vaccinated and control group.

serum samples collected at 56 days (21 days of post challenge) were tested by gE competitive ELISA. The results showed that all rabbits vaccinated with recombinant BoHV-1ΔgE remained negative in gE competitive ELISA, while positive in case of rabbits

infected with parental virus. This type of serological response is capable to differentiate recombinant BoHV-1ΔgE virus from parental virus which is an important feature of this vaccine for differentiation of naturally infected animals from vaccinated animals (Raaperi *et al.*,

2014). The present study concluded that this locally prepared BoHV-1  $\Delta$ gE vaccine can be used to prevent economic losses caused by BoHV-1 infections but further in-vivo studies in natural host required before implementing on larger scale.

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

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