

## Prokaryotic expression of a truncated form of bovine herpesvirus 1 glycoprotein E (gE) and its use in an ELISA for gE antibodies<sup>1</sup>

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**ABSTRACT-** Oliveira S.A.M., Brum M.C.S., Anziliero D., Dellagostin O., Weiblen R. & Flores E.F. 2013. **Prokaryotic expression of a truncated form of bovine herpesvirus 1 glycoprotein E (gE) and use in an ELISA for gE antibodies.** *Pesquisa Veterinária Brasileira* 33(1):41-46. Setor de Virologia, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Avenida Roraima 1000, Santa Maria, RS 97105-900, Brazil. E-mail: [eduardofurtadoflores@gmail.com](mailto:eduardofurtadoflores@gmail.com)

This article describes the expression of a truncated form of bovine herpesvirus 1 (BoHV-1) glycoprotein E (gE) for use as immunodiagnostic reagent. A 651 nucleotide fragment corresponding to the amino-terminal third (217 amino acids) of BoHV-1 gE - that shares a high identity with the homologous BoHV-5 counterpart - was cloned as a 6×His-tag fusion protein in an *Escherichia coli* expression vector. A soluble protein of approximately 25 kDa purified from lysates of transformed *E. coli* was recognized in Western blot (WB) by anti-6xHis-tag and anti-BoHV-1 gE monoclonal antibodies. In addition, the recombinant protein was specifically recognized in WB by antibodies present in the sera of cattle seropositive to BoHV-1 and BoHV-5. An indirect ELISA using the expressed protein as coating antigen performed comparably to a commercial anti-gE ELISA and was able to differentiate serologically calves vaccinated with a gE-deleted BoHV-5 strain from calves infected with BoHV-1. Thus, the truncated gE may be useful for serological tests designed to differentiate BoHV-1/BoHV-5 infected animals from those vaccinated with gE-negative marker vaccines.

INDEX TERMS: BoHV-5, bovine herpesvirus, vaccine, DIVA, recombinant protein.

**RESUMO.- [Expressão procariota de uma forma truncada da glicoproteína E (gE) do herpesvírus bovino tipo 1 e uso em ELISA para anticorpos contra a gE.]** Este trabalho relata a expressão de uma forma truncada da glicoproteína E (gE) do herpesvírus bovino tipo 1 (BoHV-1) para uso em imunodiagnóstico. Um fragmento de 651 pares de bases (pb) correspondente ao terço amino-terminal (217 aminoácidos) da gE do BoHV-1 – que compartilha uma alta identidade com a gE do BoHV-5 – foi clonada como proteína de fusão com cauda 6x de histidina em um vetor de expres-

são em *Escherichia coli*. Uma proteína solúvel de aproximadamente 25 kDa purificada de lisados de *E.coli* foi reconhecida em Western blot (WB) por anticorpos monoclonais anti-6xHis-tag e anti-gE. Além disso, a proteína recombinante purificada foi reconhecida em WB por anticorpos presentes no soro de animais soropositivos ao BoHV-1 e BoHV-5. Um ELISA indireto utilizando a proteína recombinante como antígeno apresentou performance comparável a um ELISA gE comercial e foi capaz e diferenciar sorologicamente animais vacinados com uma cepa gE-negativa de BoHV-5 de animais infectados com o BoHV-1. Portanto, a gE truncada pode ser útil em testes sorológicos diferenciais para uso conjunto com vacinas com marcador antigênico gE para o BoHV-1 e BoHV-5.

TERMOS DE INDEXAÇÃO: BoHV-5, herpesvírus bovino, vacina diferencial, proteína recombinante.

### INTRODUCTION

Bovine herpesvirus type 1 (BoHV-1) and BoHV-5 are large, enveloped DNA viruses belonging to the family *Herpesvi-*

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*ridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus* (Roizman et al. 1992). BoHV-1 and BoHV-5 are closely related genetically, antigenically and display an extensive serological cross-reactivity (Bratanich et al. 1991, Vogel et al. 2002, Delhon et al. 2003, Del Medico Zajac et al. 2006). Bovine herpesvirus type 1 is distributed worldwide - with the exception of some European countries that eradicated the infection - and is associated with a variety of clinical conditions, including respiratory disease (infectious bovine rhinotracheitis, IBR), reproductive system disease (vulvovaginitis or balanopostitis IPV/IBP) and abortions (Kahrs 2001). BoHV-5 is the agent of herpetic meningoencephalitis, a severe and important disease of cattle in South American countries, where numerous outbreaks are reported every year (Salvador et al. 1998, Rissi et al. 2007).

A number of BoHV-1 vaccines are available in South America, the majority based on inactivated virus. Few vaccines contain BoHV-5 antigens in their formulation, none of them based on live virus (E.F. Flores, unpublished). The antigenic similarity and serological cross-reactivity between BoHV-1 and -5 has led to the assumption that vaccines containing either virus would confer protection upon homologous and heterologous virus challenge (Del Medico Zajac et al. 2006, Brum et al. 2010a, Anziliero et al. 2011). Following the trend of North America and Europe, South American countries are now embarking on the development and use of BoHV-1 DIVA (for *differentiating infected from vaccinated animals*) vaccines (Brum et al. 2010a). Herpesvirus DIVA vaccines are usually based on viral strains deleted in genes encoding an envelope, non-essential glycoprotein (Van Oirschot 1996, Ackermann & Engels 2006, Van Drunen Littel-van den Hurk 2006). Hence, the serological response to vaccination can be distinguished from that induced by natural infection by the use of ELISA tests for antibodies to the deleted protein (Van Oirschot 1999, Van Drunen Littel-van den Hurk 2006). The non-essential envelope glycoprotein E (gE) has been largely used as the antigenic marker of BoHV-1 differential vaccines (Kaashoek et al. 1995, Van Oirschot 1999, Van Drunen Littel-van den Hurk 2006). In addition to provide an antigenic marker, gE deletion from BoHV-1 and BoHV-5 genomes contributes for virus attenuation (Kaashoek et al. 1995, 1998, Chowdhury et al. 2000, Brum et al. 2010b).

Towards the development of DIVA vaccines for use in South America, a gE-deleted BoHV-1 based on a Brazilian BoHV-1 strain has been constructed (Franco et al. 2002). In addition, our group recently described the construction of a BoHV-5 strain lacking gE and thymidine kinase (tk) genes (Brum et al. 2010b). In order to provide a serological companion test for these vaccine strains, we herein describe the expression of a truncated form of BoHV-1 gE and its evaluation as a coating antigen in an indirect ELISA for gE antibodies.

## MATERIALS AND METHODS

### Cells, virus and bacterial strains

A MDBK-derived cell line named CRIB (Flores and Donis 1995) was used for virus amplification. Cells were grown on minimal essential medium (MEM), containing ampicillin (1.6mg/L),

streptomycin (0.4mg/L), amphotericin (2mg/L), supplemented with 10% fetal bovine serum (Cultilab, Brazil). The virus used for gE cloning was the BoHV-1.2 isolate SV56/90 (Weiblen et al. 1992, Silva et al. 2007). The viruses used in the characterization of the recombinant proteins were Brazilian BoHV-1 (SV-265 and 265gE-) and BoHV-5 (SV507/99) strains described elsewhere (Franco et al. 2002, Silva et al. 2007). *Escherichia coli* strain BL21 (DE3) was used for initial cloning, sequencing and maintenance of DNA fragment. For expression of the recombinant protein, *E. coli* BL21 (DE3) RP codon plus were used. *E. coli* was grown in Luria-Bertani (LB) media (Sambrook 2001).

### Gene amplification and cloning

Total DNA was extracted from CRIB cells infected with BoHV-1 SV56/90 using DNAzol Reagent (*Invitrogen*). A fragment of 651 bp of the gE coding region was amplified by PCR using the following primers: forward 5' AAACCCGCATATGGCTTCGGTCGACACGGTCTTCA and reverse 3' GTCGAAGGATCCAGACTGCAGACCACCGATAG. These primers contain restriction sites for *NdeI* and *BamHI*, respectively. The PCR conditions were as follows: initial denaturation for 15 min at 95°C, and 30 cycles of 30 sec at 95°C, 30 sec at 60°C and 45 sec at 72°C, followed by an extension of 10 min at 72°C. The 651 bp amplicon was digested with *NdeI* and *BamHI* (*Invitrogen*) and ligated to the pET16b vector (*Novagen*) after digestion with the same enzymes. Ligation products were used to transform *E. coli* BL21 DE(3) RP codon plus by heat shock (Sambrook 2001). The recombinant clones were selected and plasmid DNA was extracted and characterized by digestion with restriction enzymes and PCR amplification.

### Expression and purification of recombinant gE fragment

*E. coli* strain BL21 (DE3) transformed with pET16b-gE was grown in LB broth supplemented with ampicillin (100 mg/ml) at 37°C with agitation. When the log phase was reached ( $OD_{600}=0.6$ ), expression was induced with 0.3mM isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) for 3 h. To monitor protein expression, *E. coli* lysates were resolved in a 12% SDS-PAGE and the gel was stained with Coomassie blue. For protein purification, selected clones were grown, IPTG-induced and bacterial pellet was collected. The purification was done by affinity chromatography using HisTrap™ HP 1ml columns prepacked with precharged Ni Sepharose™ using the ÄKTAprime™ automated liquid chromatography system (GE Healthcare). Fractions containing recombinant proteins were identified by SDS-PAGE and quantified by comparison with different concentrations of bovine serum albumin (BSA). Stocks of the purified protein typically contained approximately 0.25 mg/ml. The antigen was stored at -80°C for further use.

### Antibodies and immunoblot analysis

To check the initial expression of the fusion protein, lysates of transformed and induced *E. coli* were submitted to SDS-PAGE, blotting and probing with an anti-His-tag Mab. For Western blot (WB) analysis, 2.5 $\mu$ g of purified recombinant protein was used per well, along with lysates of *E. coli* BL21 (non-transformed, transformed and induced). Gels were blotted onto Nitrocellulose Supported Transfer Membrane (Gibco®) using a semi-dry system (Bio-Rad), using transfer buffer containing 25mM Tris (pH = 8.3), 192 mM glycine and 20% methanol at 100 V for 1 h at 25°C. The blotted membrane was blocked with 5% (w/v) non-fat dry milk in TRIS buffer saline tween 20 (TBST buffer) (0.5 M NaCl, 0.02 M Tris pH = 8.5, 0.05% Tween 20) for 1 h at room temperature (RT). The primary antibodies used to probe the membranes were as follows: a commercial anti-His tag mouse monoclonal antibody (MAb); an anti-BoHV-1 gE MAb (kindly provided by Dr. Geoffrey

Lecthworth) diluted 1:200 in TBST (incubated 1 h at 37°C); sera of cattle experimentally infected with BoHV-1 or BoHV-5 (Anziliero et al. 2011) diluted 1:1000 in TBST (incubated overnight at 4°C). After incubation with the primary antibodies, the membranes were washed three times with TBST (10 min each) and incubated with peroxidase conjugated anti-mouse or anti-bovine IgG antibodies (Sigma Inc.) at an 1:2400 dilution in TBST for 1 h at 37°C. The blots were then washed three times with TBST and reactions were developed with chemiluminescent substrate (Thermo Scientific) and exposed to an X-ray film.

### Indirect anti-gE ELISA

An indirect ELISA for gE antibodies (thereafter named ELISA gE<sup>r</sup>) was set up using the recombinant protein to coat the plates. For this, 96-Well Microtiter Plates (Costar®) were coated with 100 µl of purified gE diluted at 1 µg/mL in carbonate-bicarbonate buffer (pH 9.5) and incubated at 4°C overnight. Plates were then washed with phosphate-buffered saline containing 0.05% Tween-20 (PBS-T) and blocked with blocking solution (0.5% skim milk in PBS-T) at 25°C for 2 h. Subsequently, plates were incubated with serum samples diluted 1:8 in blocking solution at 37°C for 1 h. The plates were washed five times and incubated for 1 h at 37°C with 100 µl of an anti-bovine IgG-Alkaline Phosphatase conjugate (Sigma, Inc., diluted 1:15.000). After washing, a chromogenic substrate (10mg/mL of 4-nitrophenylphosphatase) was added and the plates were incubated at room temperature for 20 min. Then, 0.5% EDTA was added to stop the colorimetric reaction. Optical density (OD<sub>405nm</sub>) was read using a Molecular Devices Microplate Reader Model Spectramax M5. In each plate, standard gE positive and negative samples were added in duplicates, as positive and negative controls, respectively. The optimal antigen concentration, serum and secondary antibody dilution were defined as 1 µg/mL; 1:8 and 1:15.000, respectively. For determination of the cut-off value, 118 bovine sera obtained from a BoHV-1-free herd (tested negative for BoHV-1/5 antibodies by virus-neutralizing assay [VN]) were tested in duplicate by the ELISA gE<sup>r</sup> and by a commercial BoHV-1 gE-Antibody Test Kit (IDEXX). The cut-off value was defined as the mean of negative samples OD<sub>405nm</sub> plus three standard deviations (OD mean+3SD/99.8% confidence). On the basis of endpoint values of negative samples, the cut-off value (OD<sub>405nm</sub>) was 0,465 (mean +3SD). Based on this data we defined an interval of results, classified as: negative (OD ≤465), inconclusive/suspect (OD 466-565) and positive samples (OD<sub>405</sub> ≥566). For validation of the test, 152 bovine sera were tested in duplicate and in parallel by the ELISA gE<sup>r</sup> and by the commercial BoHV-1 gE-Antibody Test Kit. The sensitivity and specificity of the ELISA gE<sup>r</sup> were calculated according to Jacobson (1999). Samples with OD within the "suspect" limits were not included in the calculation of sensitivity and specificity.

### Ability of the ELISA gE<sup>r</sup> to detect anti-gE antibodies

Next, the ability of the ELISA gE<sup>r</sup> to specifically detect anti-gE antibodies and, thus, to differentiate serologically infected animals from animals immunized with a gE-negative strain was investigated. Serum samples from 34 calves lacking BoHV-1/BoHV-5 antibodies were obtained at day zero; at day 42 days after intramuscular immunization with a gE-negative recombinant BoHV-5 and 30 days after challenge with a gE-positive BoHV-1 strain (Anziliero et al. 2011). All sera were tested in duplicate by the ELISA gE<sup>r</sup>, by the ELISA gE IDEXX and by VN. Virus-neutralizing (VN) tests were performed according to standard protocols, using 96-well plates and testing two-fold dilutions of sera against a fixed virus titer (100-200 TCID<sub>50</sub>) and MDBK cells as indicators.

## RESULTS

The cloned gE fragment is 651 bp in length and encodes a polypeptide of 217 amino acids, corresponding to the amino-terminal third of gE. The recombinant fragment has an estimated MW of 24 kDa. The 6×His-tag fused to the amino-terminus confers an additional 0.66 kDa. Thus, the predicted mass of the expressed protein plus the histidine tag would have approximately 25 kDa. SDS-PAGE examination of lysates of *Escherichia coli* revealed a discrete protein band of approximately 25 kD among the proteins of transformed bacteria (Fig.1A, lanes 3 and 4). The product of transformed, IPTG induced *E. coli* submitted to purification through a Ni column resulted in an abundant protein of approximately 25 kDa (Fig.1A, lane 5), with the same migration pattern of the protein visualized in *E. coli* lysates (lanes 3 and 4). The purified protein (and the other bands as well) correspond to the expected mass of the cloned fragment plus the histidine tag. Therefore, transformation of *E. coli* with the plasmid pET16b containing a fragment of the gE gene fused to a 6×His tag resulted in expression of a protein of approximately 25 kDa, corresponding to the expected size. Western blot (WB) of *E.coli* lysates probed with an anti-His monoclonal antibody (Mab) revealed a discrete band of approximately 25 kDa, corresponding to the expected mass of the fusion protein (Fig.1B, lane 2).

In order to confirm the identity of the 6×His tagged protein, *E. coli* lysates (non-transformed, transformed and IPTG-induced) were submitted to SDS-PAGE, blotted onto a nitrocellulose membrane and probed with an anti-BoHV-1 gE Mab. The result of this WB is shown in Figure 2. The gE-Mab recognized a protein of approximately 25 kDa in ly-

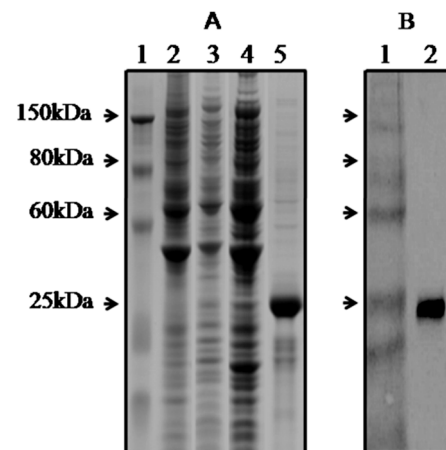


Fig.1. Expression of a truncated form of BoHV-1 gE as a 6x-His tagged fusion protein in *Escherichia coli*. (A) Coomassie blue stained SDS-PAGE gel. Lane 1: Molecular weight marker; lane 2: lysate of non-transformed *E. coli*; lane 3: transformed, non-induced *E. coli*; lane 4: transformed and IPTG-induced *E. coli*; lane 5: purified protein. (B) Western blot. *E. coli* lysates were separated by SDS-PAGE, blotted onto a nitrocellulose membrane and probed with an anti-His-tag Mab followed by incubation with an anti-mouse IgG HRP-conjugated antibody. Reaction was revealed with chemiluminescent substrate and captured in an X-ray film. 1. Molecular weight marker; 2. Lysate of transformed IPTG-induced *E.coli*.

sates of transformed *E. coli*, induced or not (lanes 3 and 4), failing to bind to lysates of non-transformed bacteria (lane 2), as expected. The anti-gE Mab also reacted strongly with the purified recombinant protein (Fig.2, lane 5). These results demonstrate that the expressed protein is indeed the fusion protein containing the expected gE fragment. Furthermore, the expressed fragment contains a likely linear epitope that is recognized by the anti-gE Mab.

As the recombinant protein fragment is intended to be used in immunodiagnostic tests, we next investigated whether it would be recognized by anti-gE antibodies present in the sera of seropositive cattle. Following SDS-PAGE and blotting, the membrane containing the purified protein was probed with sera obtained from calves experimentally infected with BoHV-1 or BHV-5. These sera harbored virus-neutralizing (VN) antibody titers of 1024 and 256, respectively (Anziliero et al. 2011). The result of this WB is shown in Figure 3. Antibodies present in both sera reacted specifically with a protein of approximately 25 kDa, corresponding to the expressed protein (Fig.3, lanes 2 and 3). The weaker signal observed upon incubation with the BoHV-5 antiserum (lane 3 versus lane 2) might reflect its lower antibody titer (VN 256 versus 1024), a suboptimal binding of antibodies to the BoHV-1 gE, or a combination of both. No reaction was observed by probing the membrane with bovine pre-immune serum (lane 1), attesting the specificity of the reaction. Thus, the recombinant gE fragment seems to

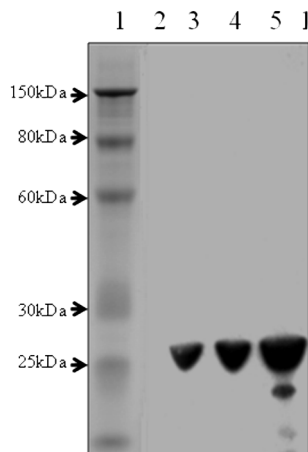


Fig.2. Western blot of the recombinant protein recognized by an anti- BoHV-1 gE Mab. Proteins from *E. coli* lysates were separated by SDS-PAGE, blotted onto a nitrocellulose membrane and probed with a gE-specific Mab followed by incubation with an anti-mouse IgG HRP-conjugated antibody. Lane 1: MWM; lane 2: *E. coli* lysate; lane 3: *E. coli* transformed with the plasmid pET16b-gE; lane 4: *E. coli* transformed and induced by IPTG induced; lane 5. Purified protein.

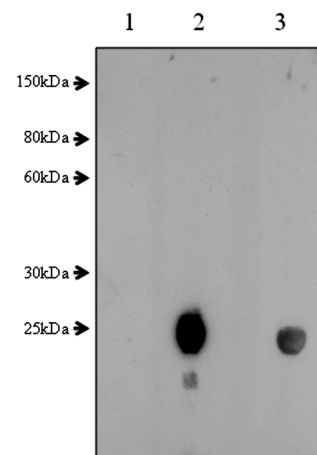


Fig.3. Recognition of the recombinant protein by antibodies present on sera of BoHV-1 and BoHV-5 seropositive cattle by Western immunoblot. The purified protein was submitted to SDS-PAGE, blotted onto a nitrocellulose membrane and probed with bovine preimmune serum (lane 1); or sera of cattle seropositive to BoHV-1 (lane 2) or BoHV-5 (lane 3) as primary antibodies, followed by incubation with an anti-bovine IgG HRP-conjugated antibody. Reaction was revealed with chemiluminescent substrate and captured in an X-ray film.

retain gE epitopes recognized by antibodies to BoHV-1 and BoHV-5 present in bovine immune sera.

An indirect ELISA for gE antibodies (thereafter named ELISA gE<sup>r</sup>) was then set up using the recombinant protein to coat the plates. Testing 152 bovine sera in parallel by the ELISA gE<sup>r</sup> and by a commercial ELISA revealed a sensitivity of 79,2% and specificity of 85,4% (not shown). Although both parameters should be improved, these results are encouraging towards the use of the truncated gE fragment in serological tests for gE antibodies. In particular, the relatively high OD values yielded by negative samples, probably reflecting inadequate purification of the recombinant protein could be lowered by improving the purification and/or blocking steps.

The ability of the ELISA gE<sup>r</sup> to specifically detect anti-gE antibodies and to differentiate serologically infected animals from animals immunized with a gE-negative strain is shown in Table 1. At the day of vaccination (day zero), all calves were negative in both ELISAs and harbored VN titers <2. At day 42pv, all vaccinated calves had seroconverted to the vaccine BoHV-5 strain, presenting VN titers from 2 to 8 (GMT 1.8). As expected, these animals remained negative for gE antibodies, as ascertained by both ELISAs. Thirty days after challenge with a gE-positive BoHV-1 (SV56/90), 32 out of 34 calves had developed gE antibodies. From these, 28 were positive in the ELISA gE<sup>r</sup> and 27 in the com-

**Table 1. Ability of the indirect ELISA gE<sup>r</sup> to differentiate animals vaccinated with a gE-negative BoHV-5 mutant from BoHV-1 infected animals**

	Day of vaccination (day zero)			Day 30 post-vaccination with BoHV-5gEΔ			Day 30 post-challenge with WT BoHV-1		
	ELISA gE <sup>r</sup>	ELISA IDEXX	VN	ELISA gE <sup>r</sup>	ELISA IDEXX	VN	ELISA gE <sup>r</sup>	ELISA IDEXX	VN
Negative	34	34	34	34	34	0	6	7	-
Positive	0	0	0	0	0	34 (GMT=1,8)	28	27	34 (GMT=5,8)

mercial ELISA (Table 1); two samples remained negative in both tests. VN titers in these animals at day 30pi ranged from 16 to 1024 (GMT 5.8). As the experiment was discontinued, we were unable to retest the negative animals at a larger interval to search for late gE seroconversion, as described by Brum et al. (2010b). Thus, the developed ELISA gE<sup>r</sup> was able to specifically detect gE antibodies and to differentiate serologically animals vaccinated with a gE-negative strain from those infected with gE-positive BoHV-1. Moreover, the results obtained with the ELISA gE<sup>r</sup> were comparable to those obtained with a commercial BoHV-1 gE antibody kit.

## DISCUSSION

Recombinant strains to be used in antigenically marked BoHV-1 and BoHV-5 vaccines have been developed in the last decade in Brazil. Our collaborators first reported the construction of a gE-deleted recombinant BoHV-1 intended to be used as a vaccine strain (Franco et al. 2002). As BoHV-1 and BoHV-5 co-circulate in Brazilian cattle (Silva et al. 2007), a commercial vaccine for use in the country would require the inclusion of both viruses. To this end, we constructed a double deletion BoHV-5 gE- and tk- mutant (BoHV-5gEΔTKΔ, Brum et al. 2010a). Hence, a gE-specific ELISA – for serological differentiation of vaccinated from naturally infected animals - would be required as a companion test. Commercial gE-antibody kits are available in several countries, yet they are relatively expensive and subjected to laborious and time-consuming import procedures. Therefore, aiming at producing an immunoenzymatic companion test for our differential, gE-deleted BoHV-1 and BoHV-5 strains, a fragment of BoHV-1 gE was cloned and expressed. Based on the similarity between BoHV-1 and BoHV-5 gE homologues (Chowdhury et al. 1999, Delhon et al. 2003), it was expected that the recombinant protein would cross-react with BoHV-5 antibodies and, thus, would serve for immunodiagnostic for both viruses.

Glycoprotein E of BoHV-1 is a 575 amino-acid, type I transmembrane glycoprotein with a calculated molecular weight (MW) of 61 kDa and an apparent MW of 92 kDa (Fitzpatrick et al. 1989). Deletion of gE gene does not affect the ability of BoHV-1 to replicate *in vitro* or *in vivo*, and has adverse effect on virus immunogenicity (Kaashoek et al. 1996). In addition, gE induces a fast and long-lasting immune response for at least 3 years (Kaashoek et al. 1996). These properties have candidated gE as a suitable target for deletion towards the production of antigenically marked herpesvirus strains (Kaashoek et al. 1996, Van Oirschot 1999, Van Drunen Littel-van den Hurk 2006).

Instead of expressing the whole protein, a gE fragment corresponding to the amino-terminal third of the protein was expressed. The amino-terminal domain is exposed on the surface of virions and is also expressed on the plasma membrane of infected cells (Fitzpatrick et al. 1989). Fortunately, the truncated form of gE seemed to retain critical epitopes as it was recognized by a gE specific Mab (Fig.2, lane 5) and by antibodies present in the sera of BoHV-1 seropositive animals (Fig.3) - a critical property towards its use for diagnostic purposes. In addition, the recombinant

protein fragment was recognized by gE antibodies present on sera of BoHV-5 seropositive cattle (Fig.3).

The recognition of the expressed fragment by BoHV-1 and BoHV-5 antisera was somewhat expected. BoHV-1 and BoHV-5 homologue gE - and the amino terminal domain as well - share high nucleotide and amino acid similarity (Delhon et al. 2003). This similarity has been demonstrated by nucleotide sequencing and by cross-reactivity with polyclonal antiserum and with several MAbs – including the Mab used herein (S.I.Chowdhury, unpublished, E.F. Flores, unpublished). Furthermore, gE antibodies present in sera of cattle experimentally challenged with BoHV-5 (Anziliero et al. 2011) or immunized with an inactivated BoHV-5 vaccine (Brum et al. 2010b) cross-reacted with BoHV-1 antigens in a commercial ELISA kit for BoHV-1 gE antibodies with sensitivity and specificity undistinguishable from that of BoHV-1 antibodies (Brum et al. 2010b, Anziliero et al. 2011). Hence, the recognition of the recombinant gE fragment by BoHV-1 and BoHV-5 antisera would enable its use in ELISA tests - as a companion test for BoHV-1 and BoHV-5 differential gE-deleted vaccines - in cattle populations where both viruses co-circulate.

In order to assess the suitability of the expressed protein fragment as antigen in an immunodiagnostic test for gE antibodies, an indirect ELISA (ELISA gE<sup>r</sup>) was standardized and the results were compared with a commercial gE-antibody kit. The preliminary data showed that the purified protein is suitable for use in such ELISA, as it was recognized by sera from naturally infected animals. As the validation of the assay used a limited number of samples (n= 152, being 52 VN positive and 100 VN negative), testing a higher number of sera and adjusting some assay conditions would be necessary to increase its sensitivity (79.4%) and specificity (85.4%).

The ELISA gE<sup>r</sup> also performed comparably to the commercial ELISA kit in the detection of gE antibodies in sera of calves challenged with BoHV-1, allowing their differentiation from calves vaccinated with the BoHV-5gEΔTKΔ strain (Table 1). Thus, the truncated gE fragment seems suitable for use in immunoenzymatic tests, allowing for specific detection of gE antibodies and, therefore, the serological differentiation of animals vaccinated with gE-negative marked vaccines. A few samples remained negative for gE antibodies in either or both ELISAs at day 30 after challenge, probably reflecting delayed seroconversion to gE as observed earlier (Brum et al. 2010), or a low sensitivity of the test. As the differential ELISAs are mainly intended to be used for herd screening - rather than for individual diagnosis – a few false-negative samples scattered among seropositive animals would have little impact on diagnosis and control at herd level. Retesting these sera 30 to 45 days later would probably yield positive gE antibody results in most samples. Increasing the sensitivity of the test would also accomplish this objective.

In summary, this article reports the expression of a truncated form of BoHV-1 gE that appears suitable for use as immunodiagnostic reagent, especially for the development of a companion ELISA for gE-deleted BoHV-1 and BoHV-5 vaccines.

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