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Development of a recombinant protein-based ELISA for detection of antibodies against bovine herpesvirus 6 (BoHV6)

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Abstract

Introduction: Bovine herpesvirus 6 (BoHV6) belongs to the *Herpesviridae* family, *Gammaherpesvirinae* subfamily and *Macavirus* genus. It is common in cattle, but was also detected in American bison (*Bison bison*) and water buffalo (*Bubalus bubalis*). The aim of the experiment was to develop an ELISA for serological examination of cattle sera for the presence of anti-BoHV6 specific antibodies. **Material and Methods:** Viral DNA from a BoHV6-positive cow was amplified by qPCR and the resulting fragments of the *gB* and *gH* genes encoding glycoproteins B and H (gB and gH) were cloned into the pLATE52 vector to express recombinant gB (rgB) and gH (rgH) in Rosetta (DE3) *E. coli*. The expressed recombinant proteins were used as antigens in the developed ELISA. **Results:** The proteins expressed had the expected molecular weight. A total of 143 sera were examined, and 141 of them were positive, according to the chosen cut-off values of 9% and 10% for the sample-to-positive ratios of the rgB and rgH antigens, respectively. **Conclusion:** The rgB and rgH recombinant antigens of BoHV6 were successfully expressed in *E. coli* and successfully used in a newly developed ELISA.

Keywords: BoHV6, recombinant glycoprotein B (rgB), rgH, protein expression, ELISA.

Introduction

Bovine herpesvirus 6 (BoHV6) belongs to the *Herpesviridae* family, *Gammaherpesvirinae* subfamily and *Macavirus* genus (3, 5). Other members of this genus are alcelaphine herpesviruses 1 and 2 (AlHV1 and AlHV2), caprine herpesvirus 2 (CpHV2), hippotragine herpesvirus 2 (HiHV2), ovine herpesvirus 2 (OvHV2) and suid herpesviruses 3, 4 and 5 (SuHV3, SuHV4 and SuHV5). Historically, the virus was detected for the first time by Rovnak *et al.* (15) in 1998 in DNA isolated from peripheral blood mononuclear cells (PBMCs) and B-lymphoma cells using the pan-herpesvirus PCR which was developed by VanDevanter *et al.* (18). Phylogenetical analyses of the pan-herpesvirus amplicon showed the identity at the amino acid (aa) level to BoHV1, BoHV4, AlHV1, and OvHV2 to be from 42 to 70%, respectively. This new virus was named BLHV (bovine lymphotropic herpesvirus). This common name was changed to bovine herpesvirus 6 (BoHV6) as a result of the findings of the International Committee on Taxonomy of Viruses (ICTV) (3, 5).

The viruses in the *Gammaherpesvirinae* subfamily predominantly infect lymphoblastoid cells, where they replicate and establish latency. Members of the subfamily are also known to possess oncogenic potential (7). While highly prevalent in dairy cattle, BoHV6 is not considered to be pathogenic, and no serious disease has been associated with this virus yet (4, 15). Banks *et al.* (1) showed the presence of BoHV6 in vaginal exudates and vaginal swabs from dairy cows with a history of post-partum metritis (27% in both types of samples). Gagnon *et al.* (9) proved the presence of BoHV6 DNA polymerase gene (DPOL) sequences in 3.8% of examined aborted bovine foetuses, and a further 3.8% of them were confirmed to be infectious bovine rhinotracheitis cases. Wüthrich *et al.* (19) used viral metagenomics and different PCR assays to detect potential viruses in the aetiology of non-suppurative encephalitis, and detected BoHV6 in this study in 9 out of 29 brain tissue samples from cattle (31%) but in none of the control tissues (0/49). A neuropathological survey concerning the prevalence of neuroinfectious diseases in cattle in Switzerland showed the presence of BoHV6

DNA in 6 out of 73 examined samples of brainstems with lesions (2.8%) (17). A study by de Oliveira *et al.* (6) in which 314 DNA samples isolated from the blood of water buffaloes (*Bubalus bubalis*) were used showed the presence of BoHV6 in 7 of them. This finding and a report by Collins *et al.* (4) of the virus in sheep and bison proved that BoHV6 is not only a pathogen of cattle, but may also infect other Bovidae. A cross-European study of the presence of gammaherpesviruses (GHVs) in cattle lung, spleen, bronchial lymph node and tongue tissues was carried out in Switzerland, Finland, Germany, the United Kingdom and Belgium. These tissues were examined from 448 cattle (348 necropsied and 100 slaughtered animals) to detect OvHV1, BoHV6, Bison LHV and CpHV2 by means of quantitative PCR (qPCR). Only BoHV6 was determined by this method, with a mean frequency of 32% and frequency ranging between 22% and 42% according to the geographical origin of the sample (14).

The full length genome of BoHV6 and its organisation was discovered by next-generation sequencing (11). As it does in the other GHVs, the BoHV6 genome consists of linear, double-stranded DNA with unpaired, complementary nucleotides at each site (13). It is 144,898 base pairs (bp) long and contains 77 genes, which in the majority represent homologues of conserved *Rhadinovirus* genes. The structure of the BoHV6 genome is similar to that of the other known full-length genomes of *Macavirus* members (AlHV1, AlHV2 and OvHV2), including, like those others, a unique coding region of low guanine and cytosine content and a repetitive region of high guanine and cytosine content. This repetitive region occurs in the genome in at least ten copies. The gene for glycoprotein B (gB), which is open reading frame (ORF) 8, is 2,571 bp long and it encodes 857 amino acids. It is located on the left side of the genome in block I, sequentially positioned at nucleotides 35,068–37,638. The gene for gH (ORF22) is 2,208 bp long and it encodes 736 amino acids. It is located on the left side of the genome in block II, sequentially positioned at nucleotides 54,476–56,683 (11). Glycoprotein B is the most highly conserved entry glycoprotein in herpesviruses and is necessary for viral entry by membrane fusion. Alone, gB is not sufficient for either viral entry or virus-free cell–cell fusion. Its location in the virion envelope and on the surface of infected cells marks out gB as the most important target for the host immune response. Glycoprotein H is essential for viral entry and cell–cell fusion. However, it is active only in a complex with glycoprotein L, which is responsible for transport and folding of gH. The complex of these two proteins combines with gB for viral entry. The gH/gL complex can induce syncytia formation in transfected cells in the absence of any other viral proteins and is a major target of virus-neutralising antibodies (10, 16).

The aims of this study were the expression of recombinant gB (rgB) and gH (rgH) proteins in order to use them as ELISA antigens, and the development of an ELISA incorporating these antigens to detect antibodies specific to bovine herpesvirus 6.

Material and Methods

Serum samples. Bovine serum samples (n = 143) were selected randomly from material collected during serological monitoring for enzootic bovine leukosis performed by the Veterinary Inspectorate as stipulated by national legislation (Ordinance of the Ministry of Agriculture and Rural Development of March 5, 2008).

DNA isolation. Viral DNA was isolated from 5×10^6 PBMCs of a cow confirmed positive for BoHV6 by qPCR (12) using a Blood Quick Pure Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions.

Nested PCR. To amplify BoHV-6 DNA fragments, 0.5 µg of DNA was used as a template in separate reactions containing 0.5 mM of each the primers specific for the *gB* (gBF/gBR) and *gH* (gHF/gHR) genes (Table 1), 0.2 mM of deoxynucleoside triphosphate (Thermo Scientific, Vilnius, Lithuania), 4 µL of 5× Phusion HF buffer, 0.4 U of Phusion HF DNA Polymerase (Thermo Scientific) and nuclease free water (Thermo Scientific) to a final volume of 20 µL. The thermal profile of the reaction was as follows: 98°C for 30 s; 35 cycles of 98°C for 10 s, 60°C for 20 s and 72°C for 1.5 min, and a final elongation step at 72°C for 10 min. The products of the first reaction were reamplified using the same conditions with pL52gBF/pLgBR and pL52gHF/pL52gHR primers (Table 1).

Table 1. Primers[1)] used in this study for amplification of bovine herpesvirus 6 DNA fragments

Primer	Genome position[2)]	Primer sequence (5′→3′)	Use
gBF	35446–35467	AACCTTATCCCGTACATGTTTC	PCR
gBR	37722–37691	CAAAGACCAACATGCCGCCAAA	PCR
gHF	54713–56173	GAGTCTGGCTTGAATGACGATC	PCR
gHR	56021–54767	GGGGTCAGTAATGCAGGGCCTA	PCR
pL52gBF[3)]	35668–35689	**GGTTGGGAATTGCAA**TCTAACATCACGGTGGACCTTA	nested PCR, cloning
pL52gBR	37056–37036	**GGAGATGGGAAGTCATTA**GCTGCTTAAGCGCTTCTCTTC	nested PCR, cloning
pL52gHF	54767–54787	**GGTTGGGAATTGCAA**TACAAAGTAGACAAAGAAGCT	nested PCR, cloning
pL52gHR[4)]	56021–55090	**GGAGATGGGAAGTCATTA**TGATTCTTTGTCTACTTTGTA	nested PCR, cloning
LICfor5[5)]	296–316	TAATACGACTCACTATAGGG	sequencing, colony PCR
LICrev	559–538	GAGCGGATAACAATTTCACAGG	sequencing, colony PCR

[1)] – All primers used in this study were synthesised by Genomed, Warsaw, Poland; [2)] In the genome of the Pennsylvania 47 isolate of bovine herpesvirus 6, GenBank accession number NC_024303; [3)] Containing a specific sequence (in bold) at the 5′ end enabling annealing to the overhang in a vector; [4)] Containing a specific sequence (in bold) at the 5′ end enabling annealing to the overhang in a vector; [5)] Used in the search for the correct size inserts and sequencing; F, for – forward; R, rev – reverse

Cloning of amplified DNA to the expression vector. Products of the PCR conforming to the expected size were purified from agarose gel using a Gel and PCR Clean-up Kit (Macherey-Nagel) and cloned into a pLATE52 vector which was part of the aLICATOR LIC Cloning and Expression Kit 4 (Thermo Scientific) according to the manufacturer's instructions. Ligation products were used to transform *E. coli* NZY5a (NZYTech, Lisbon, Portugal). The presence of inserts of the expected size was confirmed by a colony PCR with LICfor/LICrev primers and sequencing. Briefly, an individual colony was picked up with a sterile pipette tip and transferred to a replica culture plate to save the clone for repropagation. The remaining cells were resuspended in 20 µL of reaction mixture containing 0.3 mM of each of the LICfor and LICrev primers (Table 1), 0.2 mM of each deoxynucleoside triphosphate (Thermo Scientific), 1× Pol C buffer and 0.5 U of OptiTaq DNA Polymerase (EURx Ltd., Gdańsk, Poland). The thermal profile of the reaction was as follows: 95°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1.5 min, and a final elongation step at 72°C for 10 min. After the PCR was complete, 5 µL of each reaction was subjected to electrophoresis on a 1.5% agarose gel (data not shown).

Plasmid isolation and transformation of expression cells. Isolation of pLate52gB and pLate52gH recombinant plasmids was carried out after overnight culture of transformed *E. coli* NZY5a cells in Luria-Bertani (LB) medium with 100 µg/mL penicillin (Polfa Tarchomin, Warsaw, Poland) using a NucleoSpin Plasmid Kit (Macherey-Nagel) according to the manufacturer's instructions. Chemically competent for expression *E. coli* Rosetta (DE3) cells (Novagen, Merck, Darmstadt, Germany) were transformed with 0.1 pmol of isolated plasmids by a standard technique.

Expression and purification of recombinant proteins. Flat-bottomed Erlenmeyer flasks containing 100 mL of MagicMedia *E. coli* Expression Medium (Gibco Inc., Billings, MT, USA) and 100 mg/mL penicillin were inoculated with 5 mL of overnight culture of recombinant *E. coli* Rosetta (DE3) cells in LB and incubated at 37°C for 24 h with continuous shaking at 300 rpm. Thereafter liquid cultures were centrifuged for 15 min at 3,600 rpm at 8°C. The supernatant was discarded and the wet bacterial pellet was frozen at −70°C or was used directly for protein purification using a Protino Ni-IDA Protein Purification Kit (Macherey-Nagel). Briefly, 1 g of bacterial pellet was resuspended in 4 mL of 1× lysis, equilibration and wash (LEW) buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0) containing 1 mg/mL lysozyme and was incubated on ice for 30 min with continuous mixing. Next, the clear, viscous liquid was treated on ice with a series of ten 30 s ultrasound pulses to disrupt cell walls. Then the tubes were centrifuged at 10,000 g for 30 min at 4°C. The supernatant was collected and transferred to prepacked columns with nickel iminodiacetic acid (Ni-IDA) resin for protein purification by gravity flow. Columns were washed twice with 2 mL of 1× LEW buffer. Recombinant proteins were eluted four times from the resin using 0.5 mL of 250 mM imidazole in 1× LEW buffer. The protein concentration was measured spectrophotometrically in a Pearl Nanophotometer (Implen, München, Germany).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. Purified gB and gH proteins were separated in denaturing conditions through 10% polyacrylamide gel in a tris(hydroxymethyl)aminomethane-glycine running buffer (25 mM tris, 192 mM glycine and 0.1% SDS) for 2 h in the presence of PageRuler Prestained Protein Ladder molecular weight marker (Thermo Scientific). The gels were either stained using PageBlue Protein Staining Solution (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions or were transferred wet for 1 h at 100 V onto Amersham Protran Nitrocellulose Blotting Membrane (GE Healthcare Life Science, now Cytiva, Freiburg im Breisgau, Germany) in a running buffer (25 mM Tris, 192 mM glycine and 20% methanol). After that, the membranes were immersed for 15 min in tris-buffered saline and Tween 20 (TBST) buffer (100 mM Tris, 150 mM NaCl and 0.05% Tween 20, pH 7.4). Next the membranes were cut into 5-mm-wide strips. Each strip was incubated for 1 h at room temperature with the examined serum diluted 1:100 in a blocking buffer (1% bovine serum albumin (BSA) in TBST) on a horizontal shaker. After three 10-min washes in TBST, the strips were covered with protein G-peroxidase conjugate (Sigma Aldrich Chemie, Steinheim, Germany) diluted 1:5,000 in a blocking buffer and incubated for 1 h at room temperature. After the last three washes, the reaction was developed using 4-chloro-1-naphthol (Sigma-Aldrich, St. Louis, MO, USA) as the substrate for peroxidase.

Recombinant gB and gH ELISA. Purified gB and gH proteins were diluted to 5 µg/mL in sodium carbonate-bicarbonate buffer (Sigma Aldrich Chemie). To each well of two rows of a 96-well Cliniplate EB plate (Thermo Fisher Scientific, Vantaa, Finland), 100 µL of gB or gH antigen was loaded alternately. The plates were covered and incubated overnight at 4°C. After incubation, the plates were washed three times with PBST (Gibco BRL/Life Technologies, Paisley, United Kingdom). Examined sera and control sera were diluted 1:100 in a blocking buffer (1% BSA in PBST) and added to two wells for each antigen. The plates were covered and incubated for 1h at room temperature. After washing three times with PBST, 100 µL of protein G-peroxidase conjugate diluted 1:5,000 in the blocking buffer was added to each well of the plates. Incubation with conjugate was maintained for 1 h at room temperature. Next, after washing again three times, 100 µL of tetramethylbenzidine substrate (SERVA Electrophoresis, Heidelberg, Germany) was added to each well and the plates were incubated for 20 min at room temperature in the dark. The reaction was

stopped by adding 100 µL of the stopping solution (0.5 M H_2SO_4) to each well and the plates were read at 450 nm in an automated 800 TS plate reader (BioTek Instruments, Winooski, VT, USA). Because each sample and the controls were tested in duplicate, first the mean optical density (OD 450nm) was calculated and the results are presented as sample-to-positive ratios (S/P = (OD sample − OD negative control)/(OD positive control − OD negative control) × 100%).

Results

The first aim of this study was the amplification of *gB* and *gH* internal fragments (lacking the N- and C-termini) by nested PCR with the respective primers (Table 1). The products of the reaction were 1,389 bp long for *gB* and 1,254 bp long for *gH* (Fig. 1).

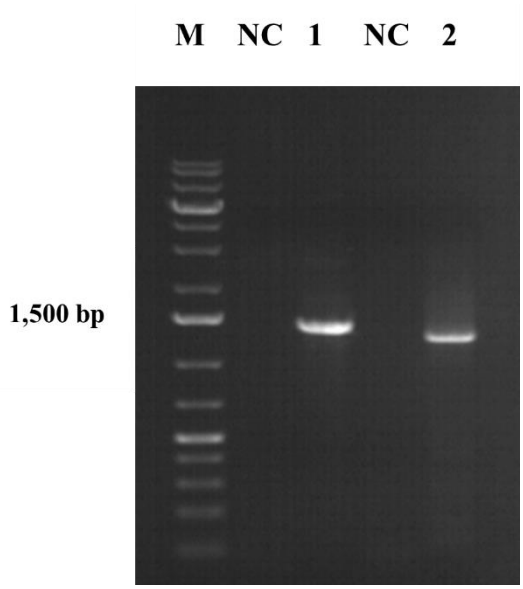

Fig. 1. Electrophoresis in 1% agarose gel of nested PCR amplification products of bovine herpesvirus 6 glycoprotein-coding genes. M – DNA size marker GeneRuler 1 kb Plus DNA ladder (Thermo Scientific, Vilnius, Lithuania); NC – negative control; 1 – 1,389 bp fragment of *gB* gene; 2 – 1,254 bp fragment of *gH* gene

The *gB* sequence codes 463 aa (N-201 → C-664) and that of *gH* codes 418 aa (N-98 → C-516). Both nested PCR products were successfully cloned into the pLATE52 expression vector with an N-terminal 6× His-tag. They also had overhangs at the 5′ and 3′ ends which allowed ligation-independent cloning (LIC), where T4 polymerase is used in the reaction. In the cloning reaction only deoxyguanosine triphosphate is included, causing the 3′→5′-exonuclease and 5′→3′-polymerase activities to equilibrate at the first occurrence of cytosine in the complementary strand. Covalent bond formation at the vector–insert junctions occurred within the cell to yield circular plasmids. The use of the MagicMedia medium allowed the expression of recombinant proteins without induction of the cells with isopropyl β-D-1-thiogalactopyranoside. Purification of recombinant gB and gH from 1 g of pelleted bacteria was achieved. The expected molecular weight of rgB and rgH was calculated from an available full genome sequence (the Pennsylvania 47 isolate logged in GenBank under accession number NC_024303) using the Protein Molecular Weight Calculator tool (https://www.bioinformatics.org/sms/prot_mw.html). These analyses resulted in molecular weights of 53.53 kDa for rgB and 48.08 kDa for rgH. The molecular weight of both proteins was confirmed by SDS-PAGE at the expected values, and satisfactory purity of rgB and rgH was indicated in subsequent electrophoresis runs (Fig. 2).

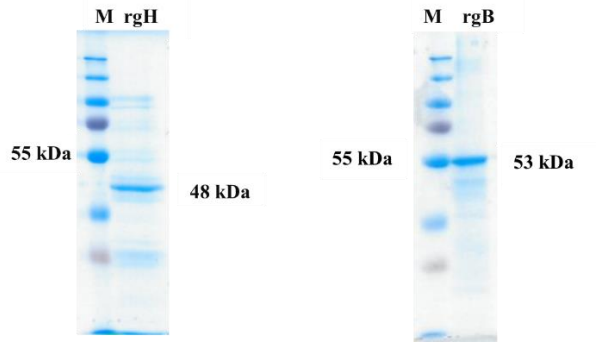

Fig. 2. Electrophoresis of purified recombinant gH (48 kDa) and gB (53 kDa) proteins of bovine herpesvirus 6 in 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis in denaturing conditions. M – PageRuler Prestained Protein Ladder molecular weight marker (Thermo Scientific, Vilnius, Lithuania)

A Western blot was performed to assess the immunogenicity of the recombinant proteins. Fig. 3 shows that the expressed rgB and rgH reacted with positive bovine sera and bands of expected molecular weight were present on the strips. The lack of a colour reaction was typical for negative sera.

In the final step, 143 serum samples were tested by the newly developed ELISA. Figs 4 and 5 show the distribution of S/P values, ranging from 0.73 to 167.3 for the rgB antigen and from 2.7 to 176.4 for rgH.

For both antigens, most seropositive sera (approximately 60% of the tested samples) showed S/P values above 40%. Scatter plot analysis (Fig. 6) of ELISA results showed an excellent relationship (R^2 = 0.9961) between the S/P values obtained for rgB and rgH antigens when all sera were tested.

The cut-off value for positive sera was calculated using the OD values obtained from 34 independent repeats of the negative control serum. The calculated average OD values increased by six standard deviations were 0.113 for rgB and 0.134 for rgH. These values corresponded to S/P ratios of 9% and 10% for rgB and rgH, respectively. Under these conditions, 141 sera were treated as positive, while only 2 were negative for both antigens. The second cut-off value for positive samples

was calculated using a different formula based on S/P values of negative control serum (3 × average OD value increased by three standard deviations). This new cut-off with values of 0.204 for rgB and 0.237 for rgH corresponded to an S/P ratio of 16% for both antigens. Under these conditions 137 sera were positive, while the number of negative ones increased to 6.

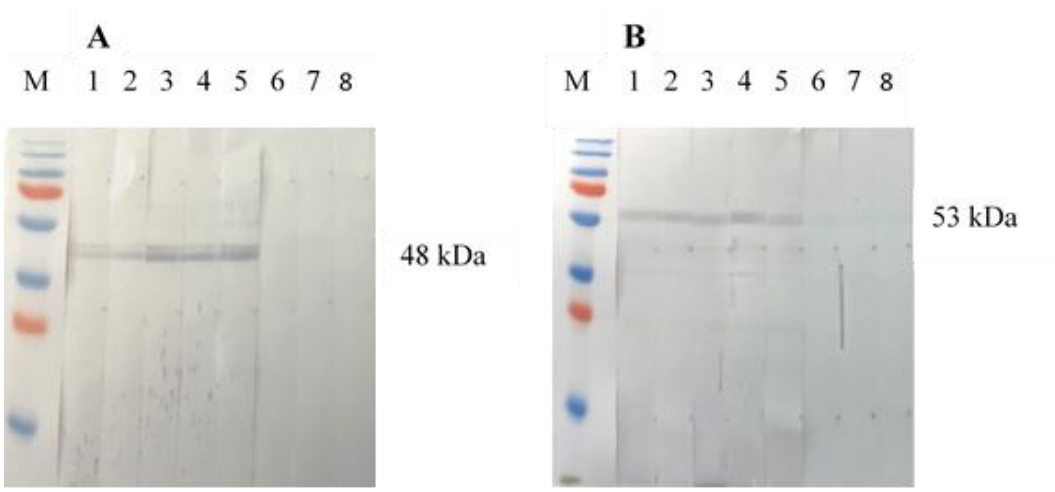

Fig. 3. Western blot detection of recombinant gH (A) and recombinant gB (B) proteins of bovine herpesvirus 6 with bovine serum samples. Lines 1–4 – field positive sera; line 5 – positive control serum; line 6 – negative control serum; lines 7–8 – field negative sera; M – PageRuler Prestained Protein Ladder molecular weight marker (Thermo Scientific, Vilnius, Lithuania)

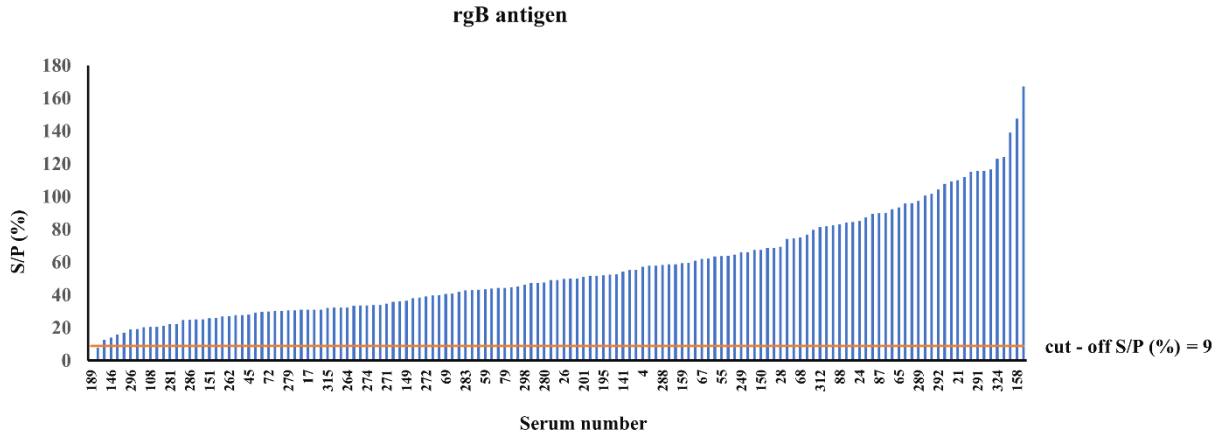

Fig. 4. Distribution of sample-to-positive ratio (S/P) values among bovine sera tested for bovine herpesvirus 6 with recombinant glycoprotein B (rgB) antigen

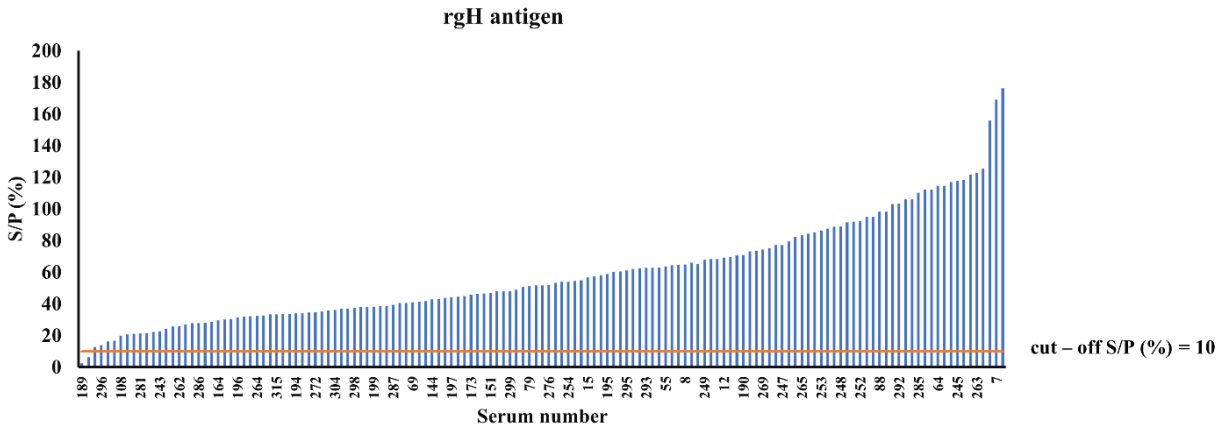

Fig. 5. Distribution of sample-to-positive ratio (S/P) values among bovine sera tested for bovine herpesvirus 6 with recombinant glycoprotein H (rgH) antigen

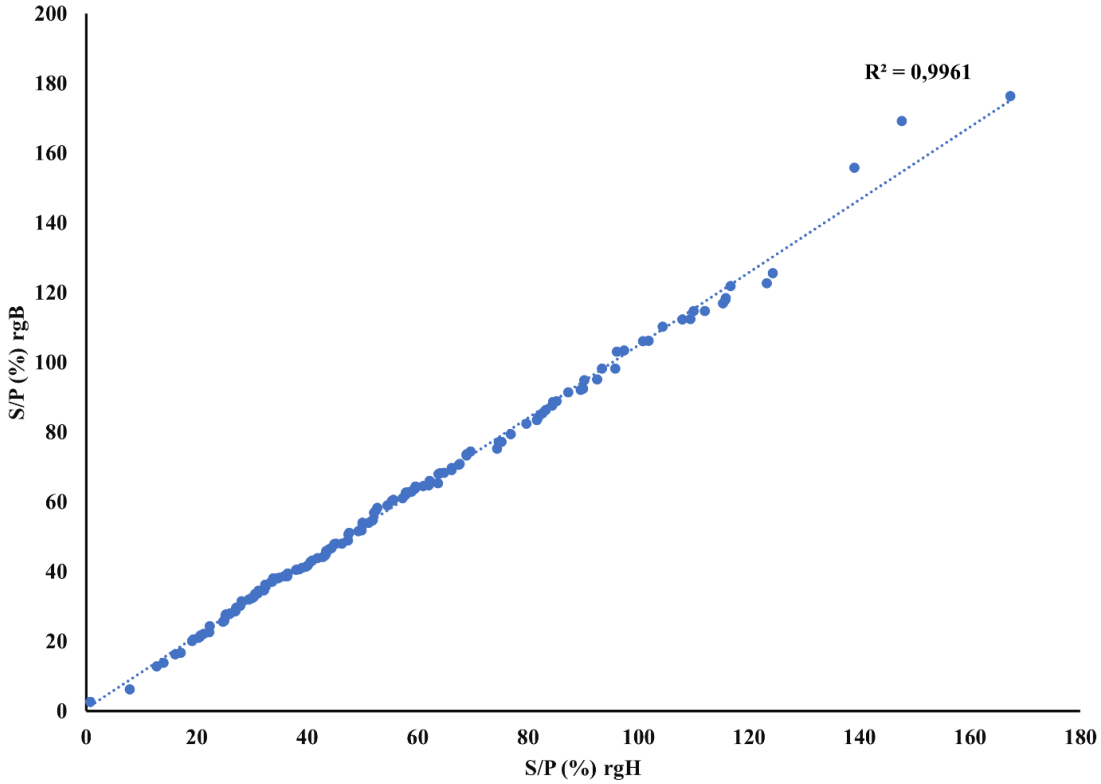

Fig. 6. Scatter plot analysis of ELISA results showing relationship between bovine herpesvirus 6 recombinant gB and recombinant gH (rgB and rgH) antigens. S/P – sample-to-positive ratio

Discussion

An ELISA based on the two recombinant glycoproteins rgB and rgH was developed to examine cattle for the presence of anti-BoHv6 antibodies. Glycoprotein B is the most conserved envelope protein among herpesviruses, and is the first target of the host immune system (15), so it is logical to use it as an antigen in serological tests, whether in-house or commercial. Brema *et al.* (2) used rgB to detect antibodies against porcine lymphotropic herpesviruses-1, -2 and -3 (presently named SuHV3, SuHV4 and SuHV5), in an experiment cloning the N-terminal and C-terminal parts into His-tagged or GST-tagged vectors. In their study, the N-terminal part of rgB revealed a higher immunogenicity than the C-terminal part and was used as an antigen in ELISA. In our study another strategy was adopted. As is known, the genes of the envelope glycoproteins contain sequences coding a signal peptide (N-terminus), an external part and a transmembranal part (C-terminus). The internal part of the *gB* and *gH* genes was amplified for both proteins and cloned into an expression vector to avoid problems with solubility or periplasmic space secretion (8). In this study both proteins were expressed in soluble form and were purified in native conditions using Ni-IDA prepacked columns.

In the newly developed ELISA, the optimal conditions were as follows: the antigen concentration was 0.5 µg/well, the serum dilution was 1:100 and the conjugate dilution was 1:5,000. Protein G-peroxidase was used as a conjugate, which made examination possible not only of cattle but also of other Bovidae. As a cut-off value, an S/P ratio of 9% was taken for serum reactions with rgB and a ratio of 10% for serum reactions with rgH. Applying these values resulted in only 2 negative sera out of 143. The strategy pursued in this study was the use of two antigens instead of gB only, to more easily classify positive sera (S/P above the cut-off for both antigens), negative sera (S/P below the cut-off for both antigens) or doubtful sera (S/P equal to the cut-off for both antigens). In this study all sera reacted almost identically, which is seen in Fig. 6, and we did not obtain any doubtful results. The more stringent qualification proposed for negative and positive sera (three mean values of negative sera plus three standard deviations) did not change the final results materially. The high prevalence of anti-BoHV6 antibodies in the examined sera may be an artefact of the particular examined material. Although all sera used in this study were selected randomly from specimens collected during serological monitoring for enzootic bovine leukosis, they also could all have been positive for bovine leukaemia virus (BLV). Rovnak *et al.* (15) showed that the prevalence of coinfection of BLV with BoHV6 reached 100% in BLV-positive lymphocytotic cattle and 91% in BLV-positive asymptomatic individuals. Because of this, further investigation is needed, and more sera should be examined, especially from BLV-free herds. Another question to keep in mind is the role of protein glycosylation in reactivity with sera. Glycoprotein B has five potential N-glycosylation sites

and gH only has four. The analysis of fragments of the *gB* and *gH* genes amplified and cloned into the expression vector showed four potential N-glycosylation sites in *gB* and two in *gH*. The influence of glycosylation on the findings of serological examination of cattle for anti-BoHV6 antibodies will be the aim of the next study.

In conclusion, rgB and rgH recombinant antigens of BoHV6 were successfully expressed in *E. coli* and successfully used in a newly developed ELISA for serological examination of cattle sera for the presence of specific anti-BoHV6 antibodies.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: None required.

References

1. Banks M., Murphy A.M., Frossard J.P., Crawshaw T.R., Twomey D.F.: Bovine lymphotropic herpesvirus and non-responsive post-partum metritis in dairy herds in the UK. Vet J 2008, 176, 248–250, doi: 10.1016/j.tvjl.2007.02.005.
2. Brema S., Lindner I., Goltz M., Ehlers B.: Development of a recombinant antigen-based ELISA for the sero-detection of porcine lymphotropic herpesviruses. Xenotransplantation 2008, 15, 357–364, doi: 10.1111/j.1399-3089.2008.00495.x.
3. Carstens E.B., Ball L.A.: Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2008). Arch Virol 2009, 154, 1181–1188, doi: 10.1007/s00705-009-0400-2.
4. Collins J.K., Bruns C., Vermedahl T.L., Schiebel A.L., Jessen M.T., Schultheiss P.C., Anderson G.M., Dinsmore R.P., Callan R.J., DeMartini J.C.: Malignant catarrhal fever: polymerase chain reaction survey for ovine herpesvirus 2 and other persistent herpesvirus and retrovirus infections of dairy cattle and bison. J Vet Diag Invest 2000, 12, 406–411, doi: 10.1177/104063870001200503.
5. Davison A.J., Eberle R., Ehlers B., Hayward G.S., McGeoch D.J., Minson A.C., Pellet P.E., Roizman B., Studdert M.J., Thiry E.: The order Herpesvirales. Arch Virol 2009, 154, 171–177, doi: 10.1007/s00705-008-0278-4.
6. de Oliveira C.H.S., de Oliveira F.G., Gasparini M.R., Galinari G.C.F., Lima G.L., Fonseca Jr.A.A., Barbosa J.D., Barbosa-Stancioli E.F., Leite R.C., dos Reis J.K.P.: Bovine herpesvirus 6 in buffaloes (Bubalus bulalis) from the Amazon region. Brazil Trop Anim Health Prod 2015, 47, 465–468, doi: 10.1007/s11250-014-0733-z.
7. Engels M., Ackerman M.: Pathogenesis of ruminant herpesvirus infections. Vet Microbiol 1996, 53, 3–15, doi: 10.1016/s0378-1135(96)01230-8.
8. Francis D.M., Page R.: Strategies to optimize protein expression in *E. coli*. Curr Protoc Protein Sci 2010, 5, 5.24.1-5.24.29, doi: 10.1002/0471140864.ps0524s61.
9. Gagnon C.A., Allam O., Drolet R., Tremblay D.: Detection of bovine lymphotropic herpesvirus DNA in tissues of a bovine aborted fetus. Can Vet J 2010, 51, 1021–1022.
10. Heldwein E.E., Krummenacher C.: Entry of herpesviruses into mammalian cells. Cell Mol Life Sci 2008, 65, 1653–1668, doi: 10.1007/s00018-008-7570-z.
11. Jia J., Delhon G., Tulman E.R., Diel D.G., Osorio F.A., Wen X., Kutish G.F., Rock D.L.: Novel gammaherpesvirus functions encoded by bovine herpesvirus 6 (bovine lymphotropic virus). J Gen Virol 2014, 95, 1790–1798, doi: 10.1099/vir.0.66951-0.
12. Kubiś P., Materniak M., Kuźmak J.: Comparison of nested PCR and qPCR for the detection and quantitation of BoHV6 DNA. J Virol Methods 2013, 194, 94–101, doi: 10.1016/j.jviromet.2013.08.006.
13. McGeoch D.J., Rixon F.J., Davison A.J.: Topics in herpesvirus genomics and evolution. Virus Res 2006, 117, 90–104, doi: 10.1016/j.virusres.2006.01.002.
14. Rosato G., Subira A.R., Al-Saadi M., Michalopoulou E., Verin R., Dettwiler M., Nordgren H., Chiers K., Grossmann E., Köhler K., Suntz M., Stewart J.P., Kipar A.: Gammaherpesvirus infections in cattle in Europe. Viruses 2021, 13, 2337, doi: 10.3390/v13122337.
15. Rovnak J., Quackenbush S.L., Reyes R.A., Baines J.D., Parrish C.R., Casey J.W.: Detection of a novel bovine lymphotropic herpesvirus. J Virol 1998, 72, 4237–4242, doi: 10.1128/JVI.72.5.4237-4242.1998.
16. Schwyzer M., Ackermann M.: Molecular virology of ruminant herpesviruses. Vet Microbiol 1996, 53, 17–29, doi: 10.1016/s0378-1135(96)01231-x.
17. Truchet L., Walland J., Wüthrich D., Boujon C.L., Posthaus H., Bruggmann R., Schüpbach-Regula G., Oevermann A., Seuberlich T.: Neuropathological survey reveals underestimation of the prevalence of neuroinfectious diseases in Switzerland. Vet Microbiol 2017, 208, 137–145, doi: 10.1016/j.vetmic.2017.07.027.
18. VanDevanter D.R., Warrener P., Bennet L., Schultz E.R., Coulter S., Garber R.L., Rose T.M.: Detection and analysis of diverse herpesviral species by consensus primer PCR. J Clin Microbiol 1996, 34, 1666–1671, doi: 10.1128/JCM.34.7.1666-1671.1996.
19. Wüthrich D., Boujon C.L., Truchet L., Selimovic-Hamza S., Oevermann A., Bouzalas I.G., Bruggmann R., Seuberlich T.: Exploring the virome of cattle with non-suppurative encephalitis of unknown etiology by metagenomics. Virology 2016, 493, 22–30, doi: 10.1016/j.virol.2016.03.009.