Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Antibodies to Glycoprotein Antigen of Bovine Herpesvirus Type 1

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Infectious Bovine Rhinotracheitis (IBR) is an acute contagious disease of cattle, characterized by the upper respiratory tract infection due to Bovine Herpesvirus Type 1 (BHV-1). Serological diagnosis of BHV-1 is commonly performed using the virus neutralization (VN) test, however, this test has limited use in field surveys because of its great deal of time to be carried out.

It is well known that enzyme-linked immunosorbent assay (ELISA) is particularly useful for rapid and simple diagnosis. For the detection of serum antibodies to BHV-1, and ELISA has been developed using an infected cell lysate antigen and a purified virus [2, 3]. And it was explained that the neutralizing antibodies mainly reacted to glycoproteins of the virus [3-5]. However, glycoprotein purified from BHV-1 has never been used for ELISA. In this report, we describe an ELISA system which uses glycoproteins purified from infected cell lysate for a more specific and higher sensitive antigen than the infected cell lysate antigen or purified virus.

The antigenic glycoprotein was purified from bovine testicle (BT) cell lysates infected with BHV-1. Secondly, BT cells (2×10^6/ml) were cultured with Eagle’s minimum essential medium (MEM) containing 10% goat serum in an incubator at 37°C for 48 hr. Monolayers of BT cells were inoculated with the Los Angeles strain of BHV-1 at a multiplicity of infection (MOI) of 0.01 and incubated at 37°C for 72 hr, at the time when the cytopathic effect was maximal. The cells were harvested and sedimented by centrifugation at 3,000 rpm for 15 min at 4°C. The resulting pellet was resuspended 1:10 (v/v) in 0.05M phosphate buffered saline (PBS, pH 7.2) containing 0.4% Nonidet P-40 and then lysed by stirring overnight at 4°C. The antigen preparation was clarified by centrifugation at 10,000 g for 30 min at 4°C and stored at -70°C until use as cell lysate antigen. The cell lysate antigen was applied to a Lentin-lectin Sepharose 4B (Pharma-
value of 0.08±0.03. A cut-off value of 0.14 (mean+2SD) was selected for evaluating subsequent ELISA results. Thus, ELISA values less than >0.14 were considered positive.

To identify the molecular weights of antigens which reacted to BHV-1 antibodies, Western blotting was performed according to the modified method of Drunen et al. [4]. Antigens were mixed with electrophoresis sample buffer (0.01M Tris hydrochloride [pH 8.0], 1 mM EDTA, 2.5% sodium dodecyl sulfate, 12.5% glycerol, 0.00125% bromophenol blue) at 100°C for 5 min to produce a lysate. Five percent β-mercaptoethanol was added to the sample buffer at reducing condition. The solubilized proteins were electrophoresed in a 4-28% polyacrylamide gradient gel. After electrophoresis, the polypeptides were transferred to a nitrocellulose sheet (pore size; 0.2μm) at 150mA for 16 hr. The electrode solution was 25 mM sodium phosphate buffer (pH 6.8) containing 0.01% sodium dodecyl sulfate. The nitrocellulose transferred proteins was incubated for 1 hr in 0.05M PBS (pH 7.2) containing 0.5% Tween-20. The nitrocellulose was then transferred to a 1:100 dilution of bovine serum in Tween-PBS. After 2 hr the nitrocellulose was washed for 30 min in three changes of Tween-PBS. Subsequently, the nitrocellulose was incubated in a 1:1000 dilution of horseradish peroxidase-linked anti-bovine IgG (Miles Lab., IL) in Tween-PBS. The nitrocellulose was washed as described above and transferred to development solution (0.01M Tris hydrochloride [pH 7.4], 0.025% 3,3'-diaminobenzidine, 0.012% H₂O₂) for 30 seconds. The reaction was stopped by washing the nitrocellulose with distilled water.

The virus neutralizing (VN) test was carried out by two methods. One was the serum dilution method (SDM) using an endpoint dilution procedure described by Carberry [1]. The VN titers with SDM were expressed as the reciprocal of the serum dilution corresponding to the 50% endpoint of neutralization as calculated by the method of Kärber. The other was the plaque reduction method (PRM), which was devised to detect a small amount of antibody. The VN titers with PRM were expressed as the number of plaques formed with 0.1 ml of BHV-1 (the Los Angeles strain, 200 plaque-forming units/0.1 ml) which was incubated for 1 hr at 37°C with an equal volume of sample serum from the cattle inoculated with 1 dose of BHV-1 attenuated, live vaccine (Kyoto Biken Lab., Japan). To determine cut-off titers, serum samples from colostrum-deprived calves were tested and had a mean number of 155±15. A cut-off titer of 125 (mean-2SD) was selected for evaluating subsequent results of VN test with PRM. Thus, VN titers less than 125 were considered positive.

Three antigen preparations were used for the ELISA, a cell lysate antigen, gp-antigen and p-antigen. Using a high titer BHV-1 antiserum, reactivity of each antigen was investigated. Fig. 1 shows that cell lysate antigen (A) and gp-antigen (B) had good specific reactivity, defined as an OD with viral antigen from which an OD with control antigen was subtracted, but p-antigen (C) had little. Furthermore, cell lysate antigen and p-antigen had high background value which was defined as an OD with control antigen, but gp-antigen had little. From these results, it was found that the specific reactivity of cell lysate antigen was due mostly to glycoprotein and the non-specific reactivity to protein. Therefore, we concluded that gp-antigen was more suitable for ELISA.

Western blotting was used to examine the presence of antigen polypeptides recognized by high titer antiserum of animal infected with BHV-1 (Fig. 2). Viral antigen polypeptides recognized by antiserum had molecular weight of 125, 96, 82, 75, 55, 42-46 and 35 K under non-reducing conditions (lane A), and 115, 102, 96, 84, 72, 62, 48-55, 42 and 35 K under reducing conditions (lane C). No specific bands were observed in control antigen (lanes B and D). These results show that gp-antigen has several specific epitopes for antibodies to BHV-1.

To determine whether this ELISA could be applied to the routine serological test, the ELISA values of 81 individual field bovine sera were compared with their VN titer with SDM. The cell lysate antigen and gp-antigen were used in the ELISA. Fig. 3 shows the linear correlation between the ELISA value using cell lysate antigen and the VN titer with SDM (A; correlation coefficient: r=0.69) and between the ELISA value using gp-antigen and VN titer with SDM (B; r=0.78). Gp-antigen gave higher correlation coefficients than cell lysate antigen (p<0.1), most likely because gp-antigen had a lower background antigen and showed more specifically antibodies associated with neutralization than by cell lysate antigen. Several samples with negative VN titer (SDM; <1:2) indicated positive ELISA.
Fig. 1. Reactivity in ELISA of a high titer BHV-1 antiserum to cell lysate antigen (A), gp-antigen (B) and p-antigen (C).
(—: viral antigen, ---: control antigen) a) Dilution of peroxidase-linked rabbit anti-bovine IgG.

Fig. 2. Western blotting analysis of gp-antigen with a high titer antiserum to BHV-1. Lane A: viral gp-antigen under non-reducing condition. Lane B: control gp-antigen under non-reducing condition. Lane C: viral gp-antigen under reducing condition. Lane D: control gp-antigen under reducing condidion. a) Molecular weight of polypeptide (kilo-dalton).

reactions. Therefore, it was necessary to determine whether the ELISA positive reactions were specific or not. To achieve this, we investigated the relationship between the ELISA values and VN titers with PRM of the serum samples from cattle monitored for 28 days after inoculation with BHV-1 attenuated, live vaccine. As shown in Fig. 4, the rising pattern of the ELISA values and the VN titer (PRM) reducing pattern were correlated. However, the VN titer (SDM) rising pattern was less after a few days. Therefore, the ELISA reaction was specific and more sensitive than the VN test with SDM.

In conclusion, the ELISA using gp-antigen was more suitable for VN test than that using cell lysate antigen. No problems with respect to the specificity of this ELISA were found, making it useful for the serological diagnosis of BHV-1.

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Fig. 3. A. Comparison of ELISA values obtained with ELISA using cell lysate antigen to VN titers about 81 field bovine sera. B. Comparison of ELISA values obtained with ELISA using gp-antigen to VN titers about 81 field bovine sera. a) Correlation coefficient.

Fig. 4. Antibody responses of cattle inoculated with BHV-1 attenuated, live vaccine.

REFERENCES

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牛ヘルペス1型ウイルス(BHV-1)感染細胞由来糖蛋白抗原を用いた酵素免疫測定法(ELISA)について(短報)：中島永昭・大石英司・岡部道二・岩本智藏・佐々木文存(京都府研)—BHV-1感染牛精巢細胞可溶化抗原より精製した糖蛋白(GP)及び蛋白(P)抗原についてELISAを試み、細胞可溶化抗原と比較した。その結果、細胞可溶化抗原の有する特異的反応は主としてGP抗原によることが明らかとなった。更に、細胞可溶化抗原を用いた場合より中和抗体と良好に相関するELISA抗体が得られたことから、本GP抗原を用いたELISAは、BHV-1の血清学的診断に有効であると考えられた。