Neutralizing antibodies to bovine herpesviruses types 1 (BHV-1) and 5 (BHV-5) induced by an experimental, oil-adjuvanted, BHV-1 vaccine

Anticorpos neutralizantes contra herpesvírus bovinos tipos 1 (BHV-1) e 5 (BHV-5) induzidos por uma vacina experimental anti-BHV-1 com adjuvante oleoso

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SUMMARY

An experimental oil-adjuvanted, inactivated vaccine against bovine herpesvirus type 1 (BHV-1,1), was produced and evaluated in its capacity to induce neutralizing antibodies against bovine herpesvirus type 1 (BHV-1, subtypes 1.1 and 1.2) and bovine herpesvirus type 5 (BHV-5). Cattle were vaccinated and revaccinated 90 days later. Antibodies were measured at days 0, 30, 90, 120, 180, 270 and 450 days after the first dose of vaccine (DPV). Antibody titres to BHV-1.1 and BHV-1.2 were significantly higher than to BHV-5 throughout the experiment. While all calves seroconverted to BHV-1.1 and BHV-1.2 after the first dose of vaccine, only two out of 23 (8.7%) calves seroconverted to BHV-5. However, after the booster injection all animals seroconverted to the three virus types. At 450 DPV, 79% (15/19 cattle) and 84% (16/19) were still positive for antibodies to BHV-1.1 and BHV-1.2, whereas 50% (10/19) of the calves remained seropositive for BHV-5. It was concluded that although a potent BHV-1 vaccine may induce crossreactive neutralizing antibodies to BHV-5, the levels of such antibodies are significantly lower and of shorter duration than antibodies to BHV-1.1 or BHV-1.2.

UNITERMS: BHV-1; BHV-5; Bovine herpesvirus; Experimental vaccine; Inactivated vaccine; Neutralizing antibodies.

INTRODUCTION

Bovine herpesvirus type 1 (BHV-1), also known as infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) virus, and bovine herpesvirus type 5 (BHV-5) or bovine encephalitis herpesvirus are members of the family Herpesviridae, sub-family Alphaherpesvirinae. BHV-1 has been divided into subtypes BHV-1.1 and BHV-1.2, based on monoclonal antibody and restriction enzyme analysis. BHV-1 is a major pathogen of cattle, widespread in its geographical distribution, affecting cattle of all ages and breeds. Although rarely fatal after post-natal infections, BHV-1 can be responsible for significant economic losses due to reproductive and respiratory disease. On the other hand, data on BHV-5 geographical distribution is scarce. The virus has been more often detected in the southern hemisphere, affecting usually young cattle with low morbidity whereas mortality rates approach 100%. Despite the large number of different BHV-1 vaccines in the market, no commercially available vaccine has been specifically designed to BHV-5. As a consequence to that, a common practice is to vaccinate against BHV-1 when BHV-5 infections are diagnosed, in attempting to reduce mortality. However, the efficacy of such procedure has not yet been determined. Therefore, it is of interest to evaluate whether BHV-1 vaccines would protect against BHV-5 infections. As part of such evaluation, the present study was carried out to examine the neutralizing antibody responses induced to BHV-1.1,2 and BHV-5 by a highly potent antibody inducer, oil-adjuvanted, inactivated BHV-1 vaccine.

MATERIAL AND METHOD

Calves

Thirty calves of mixed European breeds, aged between 8 and 15 months, were used in the vaccination experiments. At the beginning of the experiment, calves had no detectable neutralizing antibodies to BHV-1 (1.1 and 1.2) and BHV-5, as determined by serum neutralization tests. Seven of the calves were kept as unvaccinated controls throughout the experiment.

Virus strains

The BHV-1 strain “Cooper” (subtype 1 or BHV-1.1) was obtained from the Panamerican Centre for Foot and Mouth Disease, Rio de Janeiro, Brazil. The BHV-1 isolate EVI 014 (subtype 2 or BHV-1.2) was isolated in 1993 in our laboratory, from organs of an aborted foetus. The BHV-5 strain EVI 88 was also isolated in our laboratory in 1995 from a calf which died with signs of nonpurulent meningoencephalitis in the state of Mato Grosso do Sul (close to the Pantanal region). Viruses were
characterized as BHV-1 or BHV-5 using a panel of monoclonal antibodies described previously\(^9\) and by restriction enzyme analysis\(^10,14\).

**Cells**

Madin Darby bovine kidney cells (MDBK; ATCC CCL-22) between the 15\(^{th}\) and 25\(^{th}\) passage number were used for the multiplication of viruses and preparation of the vaccine. Cells were multiplied and maintained in Eagle’s minimal essential medium, supplemented with 8\% fetal calf serum (Nutricell), following standard procedures\(^11\). Cells for vaccine production were prepared in 2-litre roller bottles seeded with 300 ml of suspensions containing 2 x 10\(^5\) cells/ ml.

**Vaccine production**

About 16-24 hours after seeding of the cells, the medium was removed and bottles infected with 4 x 10\(^5\) tissue culture infective doses (TCID) of the BHV-1 strain Cooper. After one hour for adsorption at 37\(^\circ\)C, the inoculum was removed, the bottles replenished with E-MEM without fetal calf serum and incubated for 16-24 hours at 37\(^\circ\)C, when cytopathic effect was evident in about 90\% of the monolayers. Bottles were then shaken to remove attached cells and stored at 4\(^\circ\)C for 24 hours. The sediment was then discarded and the infectious titre of the bulk suspension (supernatant) was determined (10\(^{7.25}\) TCID ml\(^{-1}\)) following standard procedures\(^11\). The viral suspension was inactivated with binary ethylenimine (BEI) as described previously\(^2\). The vaccine was prepared as a water-in-oil type emulsion and subjected to usual controls as recommended\(^17\).

**Vaccination of cattle**

Twenty-three calves were vaccinated intramuscularly in the cranial third of the neck with 5 ml of the vaccine. Seven other calves were kept as unvaccinated controls. At day 90 after the first vaccination, all calves received a booster injection. Blood calves were kept as unvaccinated controls. At day 90 after the first dose of vaccine, the titres in different days the method

of orthogonal polinomia was used. The level of significance adopted was 0.05.

**RESULTS**

The mean neutralizing antibody titres induced by the experimental vaccine against BHV-1 and BHV-5 are shown in Tab. 1. At the beginning of the experiment, all animals, including the control unvaccinated cattle, were negative for neutralizing antibodies to the three herpesviruses (BHV-1.1, BHV-1.2 and BHV-5).

At day 30 after the first dose of vaccine (30 DPV), most animals seroconverted to BHV-1.1 and BHV-1.2. However, only one animal became seropositive to BHV-5. At 90 DPV, all vaccinated calves responded with the production of neutralizing antibodies to BHV-1.1 and BHV-1.2, whereas 2/23 showed detectable antibodies to BHV-5. Neutralizing antibody levels were significantly higher to BHV-1.2 than to the other two viruses. At day 30 after the booster injection (120 DPV), all 23 calves had antibodies to the three viruses. However, neutralizing antibody titres were significantly lower to BHV-5 than to BHV-1.1 or BHV-1.2. Titres remained significantly lower to BVH-5 than to both BHV-1 viruses throughout the experiment, up to 450 DPV. On the other hand, after the booster dose of vaccine, no significant differences were detected between the neutralizing antibody titres to BHV-1.1 and BHV-1.2, albeit titres were always about twofold lower to BHV 1.1 than to BHV-1.2.

The number of animals that seroconverted for the three virus types up to day 450 after vaccination is shown in Tab. 2. At 180 DPV, 23/23 calves remained seropositive for both BHV-1.1 and BHV-1.2. At 450 DPV, 79\% (15/19) and 84\% (16/19) of the cattle were seropositive for BHV 1.1 and BHV 1.2, respectively, while 52\% (10/19) of the animals were still seropositive for BHV-5.

None of the seven unvaccinated control calves developed neutralizing antibodies to the three viruses tested throughout the duration of the experiment.

**Statistical analysis**

The results of the neutralizing antibody titres were submitted to the transformation: log (y+10). Statistical analysis was performed using the analysis of variance in a model of subdivided parcels, considering the calves as blocks, the main parcel being the three different viruses; the interaction between virus type and animal, the error. In each subclass, time (in days) and the interaction “days plus virus” were considered. For the comparison between the averages of the neutralizing antibody titres for the different viruses the Student-Newman-Keuls analysis was employed. For the comparison of titres in different days the method


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<td>Mean neutralizing antibody titres and number of cattle seropositive to BHV-1.1, BHV-1.2 and BHV-5, after vaccination with an inactivated, oil-adjuvanted anti-BHV-1 vaccine.</td>
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<td>Days post vaccination</td>
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Serum neutralization tests performed against BHV-1.2 (EVI 014) and BHV-5 (EVI 88) as described in methods, (***) Booster carried out at 90 days post-vaccination. Bold numbers refer to statistically significant differences (p < 0.05). µ: mean neutralizing antibody titre; σ: standard deviation. Titres expressed as the reciprocal of the neutralizing antibody titre.
It was concluded that although a potent BHV-1 vaccine may induce crossreactive neutralizing antibodies to BHV-5, the levels of such antibodies are significantly lower and of shorter duration than antibodies to BHV-1.1 or BHV-1.2. However, it remains to be answered whether BHV-1 vaccination would induce protection to BHV-5 infection or disease. Studies are in progress to examine this question.

ACKNOWLEDGEMENTS

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RESUMO

Foi avaliada a capacidade de uma vacina experimental oleosa inativada contra o herpesvírus bovino tipo 1 induzir anticorpos contra o herpesvírus bovino tipo 1 (BHV-1.1 e BHV-1.2) e herpesvírus bovino tipo 5 (BHV-5). Bovinos foram vacinados com duas doses de vacina, aplicadas com um intervalo de 90 dias. Foi medido o título de anticorpos nos dias 0, 30, 90, 120, 180, 270 e 450 após a primeira vacinação (DPV). Os títulos de anticorpos contra BHV-1.1 e BHV-1.2 foram significativamente maiores que os contra BHV-5, ao longo do experimento. Enquanto todos os animais sorocorreram para BHV-1.1 e BHV-1.2 após a primeira dose de vacina, apenas dois em 23 animais (8,7%) sorocorreram para o BHV-5. No entanto, após o reforço, todos os animais sorocorreram contra BHV-1.1, 1.2 e BHV-5. Aos 450 DPV, 79% (15/19) e 84% (10/19) ainda se encontravam soropositivos para BHV-1.1 e BHV-1.2, enquanto apenas 50% dos animais (10/19) se demonstraram positivos para o BHV-5. Foi concluído que, embora uma vacina com alta capacidade de indução de anticorpos contra o BHV-1 possa induzir anticorpos capazes de neutralizar o BHV-5, os níveis de tais anticorpos são significativamente mais baixos e de menor duração do que aqueles induzidos contra BHV-1.1 e BHV-1.2.

UNITERMOS: BHV-1; BHV-5; Herpesvírus bovino; Vacina experimental; Vacina inativada; Anticorpos neutralizantes.

REFERENCES