Braz. J. vet. Res. anim. Sci.,
São Paulo, v. 38, n. 4, p. 184-187, 2001.

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

Sylio Alfredo PETZHOLD[1]; Paulo Estanislao RECKZIEGEL[1]; José Antonio Pires PRADO[1]; João Carlos TEIXEIRA[1]; Vera Beatriz WALD[2]; Paulo Augusto ESTEVES[1]; Fernando Rosado SPILKI[1]; Paulo Michel ROEHE[3]

CORRESPONDENCE TO:
Paulo Michel Roehe
Departamento de Microbiologia do Instituto de Ciências Básicas da Saúde da UFRGS
Av. Bento Gonçalves, 9090
90050-170 – Porto Alegre – RS
e-mail: proehe@orion.ufrgs.br

1- Equipe de Virologia do Centro de Pesquisa Veterinária Desidério Finamor (CPVDF), Fundação Estadual de Pesquisa Agropecuária (FEPAGRO)
2- Departamento de Clínica da Faculdade de Veterinária da UUFRGS – RS
3- Departamento de Microbiologia do Instituto de Ciências Básicas da Saúde da UFRGS – RS

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*[15]. BHV-1 has been divided into subtypes BHV-1.1 and BHV-1.2, based on monoclonal antibody and restriction enzyme analysis[5,14]. 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%[5,8,10,18,20]. 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[19] and by restriction enzyme analysis[5,14].

Cells

Madin Darby bovine kidney cells (MDBK; ATCC CCL-22) between the 15th and 25th 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 $10^{5,5}$ tissue culture 50% infective doses (TCID) of the BHV-1 strain Cooper. After one hour for adsorption at 37ºC, the inoculum was removed, the bottles replenished with E-MEM without fetal calf serum and incubated for 16- 24 hours at 37ºC, when cytopathic effect was evident in about 90% of the monolayers. Bottles were then shaken to remove attached cells and stored at 4ºC 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[13]. 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 samples were collected at days 0, 30, 90, 120, 180, 270 and 450 after the first dose of vaccine.

Neutralizing antibody titres

Neutralizing antibody levels were measured against the homologous vaccine virus (Cooper strain), against the isolate EVI 014 and against the BHV-5 strain EVI 88. Antibodies were titrated by the constant virus-varying serum dilution as described[7], with 100 TCID of each virus against twofold serum dilutions (from 1/ 2 onwards).

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 subparcel, 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 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.

Table 1

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.

	Days post vaccination						
	0	30	90**	120	180	270	450
Antibodies to:							
BHV-1.1	< 2	**7.26**	**3.87**	107.83	36.13	23.67	10.90
		4.71	6.68	100.19	45.39	22.95	11.17
BHV-1.2	< 2	10.25	9.60	201.83	72.43	28.18	22.32
		5.5	9.86	159.79	90.59	29.41	11.04
BHV-5	< 2	**0.17**	**0.40**	**30.3**	**9.88**	**4.84**	**3.58**
		0.83	1.43	29.07	10.12	4.82	4.73

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.

Table 2

Number of cattle seropositive to BHV-1.1, BHV-1.2 and BHV-5, after vaccination and boostering with an inactivated, oil-adjuvanted, anti-BHV-1 vaccine.

	Days post vaccination						
	0	30	90	120	180	270	450
Seropositive to:							
BHV-1.1	0/23	21/21**	23/23	23/23	23/23	21/21	15/19**
%	0	100	100	100	100	100	78.9
BHV-1.2	0/23	21/21	23/23	23/23	23/23	20/21	16/19
%	0	100	100	100	100	95.2	84.2
BHV- 5	0/23	1/23	2/23	23/23	17/23	17/21	10/19
%	0	4.3	8.7	100	73.9	80.9	52.6

Booster vaccination carried out at day 90 post-vaccination. Percentages refer to number of seropositive over total number of vaccinated cattle. ** Some samples were not available in that particular sampling, thus the variation in number of examined sera.

DISCUSSION

In countries of the southern hemisphere, the number of outbreaks of encephalitic disease from which BHV-5 has been isolated have increased substantially over the last few years[12,18,20]. Studies with paraffin embedded sections have shown that BHV-5 may have been cause of mortality in the past[8,9], but only at present its importance as a major pathogen is being recognised in the field. Due to the unavailability of a specific BHV-5 vaccine, vaccination with BHV-1 vaccines in farms where BHV-5 associated disease occurred has become a common practice in attempting to reduce losses. However, to date, there is very little experimental data to support such practice[6]. Moreover, as BHV-5 encephalitis is a disease of low morbidity, it is difficult to evaluate the efficacy of such a procedure under field conditions. In view of that, we aimed to examine the neutralizing antibody profile of cattle vaccinated with a highly potent BHV-1 vaccine.

Antibody levels to BHV-1.2 were consistently (about twofold), despite not always significantly (Tab. 1) higher than those to the BHV-1.1 homologous vaccinal virus strain. It was not possible to determine the cause of such unexpected finding. It might be hypothesized that BHV-1.2 strain was more efficiently neutralized than the homologous vaccinal virus, perhaps due to some form of antigen presentation where antibodies had a stronger affinity for the former than for the latter. However, explanations for such findings remain merely speculative.

It is well known that the induction of neutralizing antibodies does not warrant protection. Protection against infection associated to vaccination would only be consistently evaluated in a vaccination and challenge trial. The feasibility of such trial in this particular case suffers from the lack of a virus strain that would consistently reproduce clinically evident encephalitis under experimental conditions. Such difficulty has also been encountered by others[4,3,6]. Nevertheless, quantitation of neutralizing antibodies remains as an indirect means of comparison, and as such it was used here to provide a measure of the extent of crossreactivity of the immune responses to bovine herpesviruses 1.1, 1.2, and 5. Although neutralizing antibodies are only a part of several mechanisms activated in the host's immune response to herpesviruses[16], antibody analysis has also been adopted in previous studies for comparisons of BHV-1 and BHV-5 immunogenicity[4]. From the results obtained in the present work, it was clear that crossreactive neutralizing antibodies to BHV-5 were in fact developed after two injections with the highly antibody inducer, BHV-1 vaccine. When detected, crossreactive anti-BHV-5 antibodies were always at levels below those attained for BHV-1.1 and BHV-1.2. Therefore, although some crossreactive immune response was obtained, the results suggest that calves may not be as prepared to face a BHV-5 challenge as they would to BHV-1.1 or BHV-1.2. Other authors have suggested that the rare occurrence of BHV-5 in countries where BHV-1 vaccination is practiced might be a consequence of such immunization[6]. This would be in agreement with field observations that, in South American countries, BHV-5 has been usually detected in farms where vaccination to BHV-1 was not performed. The observations in the present work add in favour to this hypothesis. As no BHV-5 vaccine is available so far, when BHV-5 episodes of disease are detected, it might be advisable to proceed to BHV-1 vaccination in attempting to minimise BHV-5 associated losses. Nevertheless, as clinically evident BHV-5 infections are not the rule, the cessation of cases of encephalitis after BHV-1 vaccination may not be related to crossprotection induced by the vaccine. The apparent benefit from vaccination might be coincidental with the sporadic nature of the disease. Therefore, it is difficult to assess the efficacy of BHV-1 vaccination on BHV-5 infections under field conditions. Other authors concluded that vaccination or immunity to BHV-1 was protective against the development of mild BHV-5-induced lesions in the brains of calves[6]. However, they did not succeed in reproducing clinically noticeable encephalitic disease in BHV-5 infected control calves. Moreover, we have detected considerable genomic and antigenic variability while examining more than 30 distinct BHV-5 isolates so far[1]. Such differences may add to the variables involved in protection induced by vaccination. Thus, the actual efficacy of protection to BHV-5 after immunization with BHV-1 remains a matter to be examined in the future, preferably upon vaccination and challenge experiments. Such experiments must involve challenge with BHV-5 strains capable of inducing clinically evident disease to allow appropriate interpretation of the consequences of immunization.

CONCLUSIONS

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

This study was carried out with financial support from FAPERGS, CNPq and PRONEX. P.M. Roehe is a research fellow from the Brazilian National Research Council (CNPq).

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 soroconverteram para BHV-1.1 e BHV-1.2 após a primeira dose de vacina, apenas dois em 23 animais (8,7%) soroconverteram para o BHV-5. No entanto, após o reforço, todos os animais soroconverteram contra BHV-1.1, 1.2 e BHV-5. Aos 450 DPV, 79% (15/19 animais) 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 contra 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

1- ALMEIDA, R. S.; MELO, S. V.; SILVA, T. C.; OLIVEIRA, L. G.; LEMOS, R. A.; ROEHE, P. M. Produção de anticorpos monoclonais contra o herpesvírus da encefalite bovina (BHV-5). **Pesquisa Veterinária Gaúcha**, v. 4, p. 65-70, 1998.

2- BAHNEMANN, H.; AUGÉ DE MELLO, P.; ABARACON, D.; GOMES, I. Immunogenicity in cattle of foot-and mouth disease vaccines inactivated with binary ethylenimine. **Bulletin Office of International Epizooties**, v. 81, p. 1335-1343, 1974.

3- BELKNAP, E. B.; COLLINS, J. K.; AYERS, V. K.; SCHULTHEISS, P. C. Experimental infection of neonatal calves with neurovirulent bovine herpesvirus type 1.3. **Veterinary Pathology**, v. 31, p. 358-365, 1994.

4- BRATANICH, A. C.; SARDI, S. I., SMITSAART, E. N.; SCHUDEL, A. A. Comparative studies of BHV-1 variants by in vivo - in vitro tests. **Journal of Veterinary Medicine Series** B, v. 38, p. 41-48, 1991.

5- BULACH, D. M.; STUDDERT, M. J. Comparative genome mapping of bovine encephalitis herpesvirus, bovine herpesvirus 1, and buffalo herpesvirus. **Archives of Virology**, v. 113, p. 17-34, 1990.

6- CASCIO, K. E.; BELKNAP, E.; SCHULTHEISS, P. C.; AMES, A. D.; COLLINS, J. K. Encephalitis induced by bovine herpesvirus 5 and protection by prior vaccination or infection with bovine herpesvirus 1. **Journal of Veterinary Diagnostic Laboratory Investigation**, v. 11, p. 134-139, 1999.

7- DEREGT, D.; CHO, H. J.; KOZUB, G. C. A comparative evaluation of two sensitive serum neutralization tests for bovine herpesvirus 1 antibodies. **Canadian Journal of Veterinary Research**, v. 57, p. 56-59, 1993.

8- D'OFFAY, J. M.; ELY, R. W.; BALDWIN, C. A.; WHITENACK, D. L.; STAIR, E. L.; COLLINS, J. K. Diagnosis of encephalitic bovine herpesvirus type 5 (BHV-5) infection in cattle: virus isolation and immunohistochemical detection of antigen in formalin-fixed bovine brain tissues. **Journal of Veterinary Diagnostic Laboratory Investigation**, v. 7, p. 247-251, 1995.

9- ELY, R. W.; D'OFFAY, J. M.; RUEFER, A. H.; CASH, C. Y. Bovine herpesviral encephalitis: a retrospective study on archived formalin-fixed, paraffin-embedded tissue. **Journal of Veterinary Diagnostic Laboratory Investigation**, v. 8, p. 487-492, 1996.

10- FRENCH, E. L. Relationship between infectious bovine rhinotracheitis and a virus isolated from calves with encephalitis. **Australian Veterinary Journal**, v. 38, p. 555-556, 1962.

11- FRESHNEY, I. (Ed.) **Animal cell culture**: a practical approach. 2.ed. Glasgow: Oxford University Press, 1992.

12- HEINLEIN, A.; METZLER, A. E.; WEIBLEN, R.; BARRIOS, P.; SCHUDEL, A. A.; RODRIGUEZ, M. Molecular characterization of South American bovine herpesvirus-1 isolates with monoclonal antibodies and SDS-PAGE. **Journal of Veterinary Medicine Series B**, v. 40, p. 125-130, 1993.

13- HOUSE, J. A.; BAKER, J. A. Bovine herpesvirus IBR-IPV. The antibody virus neutralization reaction. **Cornell Veterinarian**, v. 61, p. 320-335, 1971.

14- METZLER, A. E.; SCHUDEL, A. A.; ENGELS, M. Bovine herpesvirus 1: molecular and antigenic characteristics of variant viruses isolated from calves with neurological disease. **Archives of Virology**, v. 87, p. 205-217, 1986.

15- MURPHY, F. A.; GIBBS, E. P.; HORZINEK, M. C.; STUDDERT, M. J. **Veterinary Virology**, 3.ed. New York: Academic Press, 1999.

16- NASH, A. A.; CAMBOROPOULOS, P. The immune response to herpes simplex virus. **Seminars in Virology**, v. 4, p. 181-186, 1993.

17- **Organización Panamericana de la Salud**. Producción, control de calidad y uso de vacunas con adyuvante oleoso contra la fiebre aftosa. In: ———. **Programa de Adestramiento en Salud Animal para America Latina**. Washington, Organización Panamericana de la Salud, 1987.

18- RIET-CORREA, E.; VIDOR, T.; SCHILD, A. L.; MÉNDEZ, M. C. Meningoencefalite e necrose do córtex cerebral em bovinos causada por herpesvirus bovino-1. **Pesquisa Veterinária Brasileira**, v. 9, p. 13-16, 1989.

19- ROEHE, P. M.; SILVA, T. C.; NARDI, N. B.; OLIVEIRA, L. G.; ROSA, J. C. A. Diferenciação entre os vírus da rinotraqueíte infecciosa bovina (BHV-1) e herpesvírus da encefalite bovina (BHV-5) com anticorpos monoclonais. **Pesquisa Veterinária Brasileira**, v. 17, p. 41-44, 1997.

20- SALVADOR, S. C.; LEMOS, R. A. A.; RIET-CORREA, F.; ROEHE, P. M.; OSÓRIO, A. L. Meningoencefalite em bovinos causada por herpesvírus bovino-5 no Mato Grosso do Sul e São Paulo. **Pesquisa Veterinária Brasileira**, v. 18, p. 76-83, 1998.

Received: 26/04/2001
Accepted: 22/01/2002