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Field evaluation of safety during gestation and horizontal spread of a recombinant differential bovine herpesvirus 1 (BoHV-1) vaccine[1]

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ABSTRACT.- Spilki F.R., Silva A.D., Batista H.B.C.R., Oliveira A.P., Winkelmann E., Franco A.C., Porciúncula J.A. & Roehe P.M. 2005. [**Field evaluation of safety during gestation and horizontal spread of a recombinant differential bovine herpesvirus 1 (BoHV-1) vaccine**.] *Pesquisa Veterinária Brasileira 25(1):54-58.* Instituto de Pesquisa Veterinária Desidério Finamor, Fepagro-Saúde Animal, Cx. Postal 47, Eldorado do Sul, RS 92990-000, Brazil. E-mail: proehe@ufrgs.br

Bovine herpesvirus type 1 (BoHV-1) is recognized as a major cause of respiratory, reproductive disease and abortion in cattle. Vaccination is widely applied to minimize losses induced by BoHV-1 infections; however, vaccination of dams during pregnancy with modified live virus (MLV) vaccines has been occasionally associated to abortions. We have previously reported the development of a BoHV-1 recombinant virus, constructed with basis on a Brazilian BoHV-1 (Franco et al. 2002a) from which the gene coding for glycoprotein E (gE) was deleted (gE-) by genetic manipulation. Such recombinant has been previously evaluated in its potential as a differential vaccine (gE- vaccine) that allows differentiation between vaccinated and infected animals. Here, in the first part of the present study, the safety of the gE- vaccine during pregnancy was evaluated by the intramuscular inoculation of $10^{7.4}$ tissue culture 50 % infective doses ($TCID_{50}$) of the virus into 22 pregnant dams (14 BoHV-1 seronegative; 8 seropositive), at different stages of gestation. Other 15 pregnant dams were kept as non-vaccinated controls. No abortions, stillbirths or fetal abnormalities were seen after vaccination. Seroconversion was observed in both groups of previously seronegative vaccinated animals. In the second part of the study, the potential of the gE- vaccine virus to spread among beef cattle under field conditions was examined. Four heifers were inoculated intranasally with a larger amount ($10^{7.6}$ $TCID_{50}$) of the gE- vaccine (to increase chances of transmission) and mixed with other sixteen animals at the same age and body condition, in the same grazing area, at a population density equal to the average cattle farming density within the region (one cattle head per 10,000 m^2), for 180 days. All animals were monitored daily for clinical signs. Serum samples were collected on days 0, 30, 60 and 180 post-vaccination. Seroconversion was observed only in vaccinated heifers. These results indicate that, under the conditions of the present study, the gE- vaccine virus did not cause any noticeable harmful effect on pregnant dams and on its offspring and did not spread horizontally among cattle.

INDEX TERMS: Bovine herpesvirus 1, BoHV-1 recombinant gE- vaccine.

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RESUMO.- [Avaliação a campo da segurança para vacas prenhes e capacidade de disseminação horizontal de uma vacina diferencial recombinante contra o Herpesvírus Bovino tipo 1 (BoHV-1).] Infecções pelo herpesvírus bovino tipo 1 (BoHV-1) são importantes causas de doença respiratória, reprodutiva e abortos em bovinos. A vacinação é freqüentemente empregada para minimizar as perdas produzidas pela infecção. Todavia, a imunização de vacas durante a prenhez com algumas vacinas contendo vírus vivo modificado (MLV) pode ocasionalmente causar abortos. Em trabalho prévio, nosso grupo desenvolveu uma vacina recombinante de BoHV-1 construída a partir de um isolado brasileiro de BoHV-1 (Franco et al., 2002a) do qual o gene que codifica para a glicoproteína E (gE) foi artificialmente deletado. Tal recombinante (gE-) vem sendo avaliado como vacina diferencial, isto é, capaz de permitir a diferenciação entre animais vacinados e infectados. No presente estudo, o potencial de disseminação do vírus recombinante foi avaliado em um rebanho de gado de corte, em condições de campo. Para tanto, a segurança da vacina gE- quando aplicada durante a prenhez foi avaliada pela inoculação intramuscular de $10^{7,4}$ doses infectantes para 50% dos cultivos celulares ($DICC_{50}$) do vírus em 22 fêmeas prenhes (14 previamente soronegativas e 8 previamente soropositivas para BoHV-1) em diferentes fases da gestação. Outras 15 vacas prenhes foram mantidas como controles não-vacinados. Não ocorreram abortos, natimortos ou anormalidades fetais em nenhum dos grupos. Soroconversão foi observada nas fêmeas vacinadas previamente soronegativas. Em um segundo experimento, 4 novilhas foram inoculadas pela via intranasal com $10^{7,6}$ $DICC_{50}$ do vírus recombinante, sendo mantidos em contato com 16 novilhas em uma área de campo, a uma densidade de 1 animal por hectare. Os animais foram monitorados quanto à presença de sinais clínicos; amostras de soro foram coletadas nos dias 0, 30, 60 e 180 após a vacinação. Soroconversão foi observada apenas nos animais vacinados e não nos contatos. Estes resultados indicam que, nas condições do presente estudo, a vacina gE- não tem efeitos deletérios para fêmeas gestantes nem para seus fetos e não se dissemina horizontalmente no rebanho.

TERMOS DE INDEXAÇÃO: Herpesvírus bovino tipo 1, vacina recombinante gE- contra BoHV-1.

INTRODUCTION

Bovine herpesvirus type 1 (BoHV-1) has been associated with a number of different clinical manifestations in cattle, such as infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis/ infectious pustular balanoposthitis (IPV/ IPB). The most striking effect of BoHV-1 infection is its capacity to interfere in gestation, often leading to termination of pregnancy, with serious economical consequences (Guy & Potgieter, 1985; Miller et al. 1991, Siebert et al. 1995a, Turin et al. 1999). To minimize such losses, both conventional modified live or inactivated as well as recombinant vaccines have been widely used (Kleiboeker et al. 2003, Turin et al. 1999).

One of the recent strategies for the development of BoHV-1 vaccines relies on the deletion of non-essential genes from the viral genome. Such deletions allow the distinction between wild type virus-infected and vaccinated animals, provided that a serological test capable of recognizing antibodies to the deleted protein is available (Belknap et al. 1999, Flores et al. 1993, Franco et al. 2002a). Such vaccines are often referred to as "differential vaccines" (Wentink et al. 1993). Recently, we constructed a glycoprotein E (gE)-negative BoHV-1 recombinant, based on an autochthonous Brazilian strain of BoHV-1. Such recombinant is intended for use as an attenuated, differential vaccine (Franco et al. 2002b) and, as such, was shown to be safe and efficacious for calves (Franco et al. 2002a), yet allowing differentiation between vaccinated and infected animals. An important drawback occasionally found on other MLV is that those may also lead to embryonic, fetal death and abortions (Miller et al. 1989, McFelly et al. 1968, Mitchell 1974, Whetstone et al. 1986). Therefore, it is essential to investigate whether any new vaccine candidate would bring undesirable side effects if eventually administered during gestation. Another important issue on MLVs is its potential to spread within the herd (Pastoret et al. 1980). This is an undesirable side effect, since the vaccine virus may perpetuate within herds (Hage et al. 1996). Therefore, it would be of interest to examine whether the differential vaccine virus might spread within a herd.

In the present study, it was initially aimed to determine the safety of the gE- vaccine for pregnant dams. Subsequently, the potential of the gE- vaccine to spread within a herd under typical beef cattle field conditions was examined.

MATERIALS AND METHODS

Multiplication of the gE- vaccine virus

The construction of the recombinant vaccine virus (265gE-), which gave rise to the gE-negative vaccine (gE- vaccine), was described previously (Franco et al. 2002a). The virus was multiplied in CRIB-1 cells (Flores & Donis 1995). BoHV-1 strain EVI 123/98, a typical representative of BoHV-1.1 isolated in Brasil (D'Arce et al. 2002), was multiplied in CRIB-1 cells and used for serum neutralization (SN) assays. Cell cultures were maintained in Eagle's minimal essential medium (EMEM) supplemented with 5 % to 10 % fetal bovine serum (FBS; Nutricell), 2 mM glutamine and antibiotics (100 IU/ml penicillin and 100 mg/ml streptomycin) following standard procedures.

Safety for pregnant dams

Dams and immunization. Thirty seven pregnant dams of mixed European beef breeds were used in the experiment. Twenty two, 2 to 4 years-old dams, were vaccinated intramuscularly (IM) on the side of the neck with 3 mL of a suspension containing $10^{7.4}$ $TCID_{50}$ of the gE- vaccine virus in EMEM. Fourteen pregnant dams in different stages of gestation were seronegative for BoHV-1 at the start of the experiment. Another group consisted of eight BoHV-1-seropositive pregnant dams an additional group of 15 pregnant dams were kept as non-vaccinated controls. From the control group, at the start of the experiment, seven dams were BoHV-1 seronegative and 8 were seropositive for BoHV-1. The stage of pregnancy was determined by rectal palpation and confirmed by the date of parturition. Table 1 shows the stages of pregnancy of dams within different groups.

Vaccine virus spread in a seronegative herd

Animals and vaccine virus inoculation. Twenty Aberdeen Angus heifers, aged 18 months, all seronegative for BoHV-1, were selected from the stock of the institution of origin of the authors. Four heifers were inoculated by nasal instillation (IN) of 3 mL of a viral suspension containing $10^{7.6}$ $TCID_{50}$ of the gE- vaccine virus. The animals

Table 1. Serological status to bovine herpesvirus type 1 (BoHV-1) and approximate stage of pregnancy of dams vaccinated (or not) with the recombinant gE-negative vaccine

Group	Serological at day 0 status	1st trimester	2nd trimester	3rd trimester
Vaccinated	Seronegative	4[a]	5	5
	Seropositive	3	2	3
Non-vaccinated	Seronegative	1	3	3
	Seropositive	2	2	4

[a] Number of pregnant dams in that stage of gestation.

were observed daily for clinical signs. Serum samples were collected on days 0, 30, 60 and 180 days post-vaccination (DPV). Seroneutralization assays (SN) were performed as described below. Any seronconversion to BoHV-1 during this period was assumed as induced by the vaccine virus. The animals were kept under field conditions throughout the experiment, in a grazing area of 10.000 m^2, at a density of 1 animal per 10.000 m^2 for six months. Serum samples were collected from the dams by caudal or jugular venipuncture on days 0, 40 and 80 post-vaccination (PV). Samples were also taken from the calves born from the dams under study, during the first 2 weeks of life. Sera were tested in serial twofold dilutions in a standard BoHV-1 neutralizing antibody test against strain EVI 123/ 98 (Franco et al. 2002).

Statistical analysis

The results were statistically evaluated by analysis of variance (ANOVA); the least significance difference for $p = 0.05$ was determined. Statistical analysis was performed with Data Analysis Supplement for Excel[tm] (Office XP for Windows[tm], Microsoft Corp., USA). The term "significant" (statistically significant) in the text means $p = 0.05$.

RESULTS

Safety for pregnant dams

No embryonic deaths, abortions and stillbirths were detected in any vaccinated dam throughout the experiment. Likewise, no reproductive abnormalities were detected on the group of non-vaccinated dams. Seroconversion was observed in vaccinated dams that were seronegative at the start of the experiment, as demonstrated by SN (Fig.1). On the other hand, previously seropositive dams had no significant alterations in their serum neutralizing antibody titres (Fig.1).

Vaccine virus spread in a seronegative herd

All four animals vaccinated IN developed a strong immune response against BoHV-1, as measured by SN assays. Only mild clinical signs, characterized by light serous discharges from days 1 to 7 PV, were observed on vaccinated heifers. In contrast, no seroconversion was detected on "in contact" cattle. These results demonstrate that the vaccine virus was not capable of spreading from vaccinated to contact animals.

DISCUSSION

Although vaccination with MLV for IBR virus is recognized as an efficient way to improve herd immunity to BoHV-1 infections

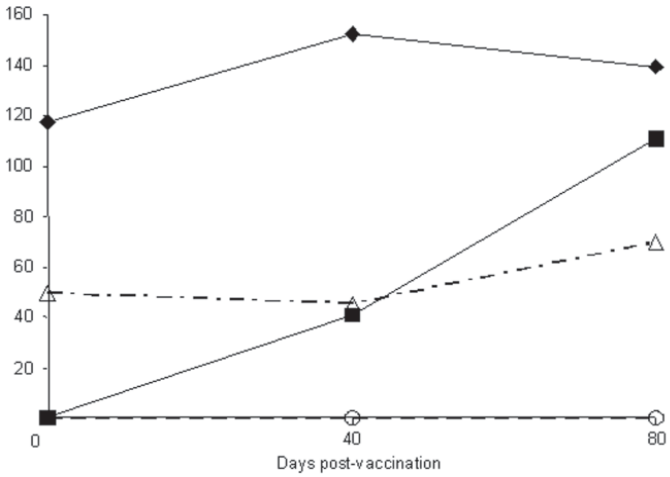

Fig.1. Neutralizing antibody titres (geometric mean) in dams vaccinated (or not) with the bovine herpesvirus type 1 (BoHV-1) gE-negative vaccine. Black losenges: vaccinated, previously seropositive animals; White triangles: non-vaccinated, previously seropositive animals; Black squares: vaccinated, previously seronegative animals; Blank circles: non-vaccinated, seronegative animals.

(Wentik et al. 1993, Siebert et al. 1995a), the use of this kind of vaccines during pregnancy may result in fertility problems such as early embryonic deaths, abortions and stillbirths (Lomba et al. 1976, Whetstone et al. 1986).

In order to examine the effect of the gE- vaccine during pregnancy, in the first experiment of the present study seronegative and seropositive dams were vaccinated intramuscularly with a gE- vaccine. Vaccination was performed via IM in order to increase the chances of the virus reaching the conceptuses. Despite the inoculation of a large dose of vaccine virus ($10^{7.4}$ $TCID_{50}$) no detectable harmful effect was observed, neither on pregnant dams nor on its offspring, demonstrating its safety for application during pregnancy, at least under the conditions of the present study. Another gE- vaccine (Siebert et al. 1995b) had been also evaluated on pregnant cattle, with similar results. However, other recombinants with a functional gE gene retained its abortigenic capacity (Miller et al. 1995). Such studies, when examined comparatively with the one here reported, suggest that there may be a link between the apparent lack of ability to reach and/or cause fetal damage might be specifically linked to the removal of the gE gene. Further studies should be able to determine whether gE in fact plays a significant role leading to abnormalities during gestation.

In addition to being apparently safe for pregnant dams, the gE- vaccine was capable of inducing high levels of neutralizing antibodies on vaccinated dams. This is beneficial for the passive transfer of antibodies to the newborn, as shown for other herpesviruses (Casal et al. 2004) and also in response to other viruses (Roehe 1991). In fact, some of the calves born to vaccinated dams in the present study had higher levels of neutralizing antibodies than their own dams. Others have speculated that higher antibody titres in newborns were associated to intrauterine infection with the vaccine virus (Lomba et al. 1976). In our view, a more likely possibility is that a physiological concentration of immunoglobulins in the colostrum would allow more effective

transfer of these to the newborn, as also pointed out by others (Odde 1988, Roehe 1991, Ellis et al. 1996).

Interestingly, vaccination of previously seropositive dams led to no significant rise in antibody levels after immunization. In fact, neutralizing antibody levels in such animals showed a tendency to decline at 80 DPV. As neutralizing antibody levels in such dams were already relatively high, it is possible that the vaccine virus could have been inactivated by the host's defense mechanisms, such as shown for pseudorabies virus (PrV) in swine (Zuckermann et al. 1998).

The route of inoculation might also play a role in nasal virus spread. In the experiment designed to detect nasal virus spread, the inoculation was performed via IN and with a larger amount of virus, since this could increase the possibility of shedding. Transmission following IM inoculation is much less likely to occur (Siebert et al. 1995b, Mars et al. 2000). Despite IN inoculation, no transmission of the vaccine virus to herdmates was detected. The sixteen "in contact" animals kept as sentinels did not seroconvert to BoHV-1 up to six months after vaccination. This was probably a result of the poor replication of the gE- virus in the host. Viral spread within a herd is not dependent on the herd size, but is directly related to the agent's ability to replicate efficiently in the host and be shed to contacts (Bouma et al. 1995, Hage et al. 1996). We have previously demonstrated (Franco et al. 2002a), that the gE- virus evaluated here replicates to very low titres in calves, as has also been shown for another gE- strain (Kaashoek, 1995, Strube et al. 1995, Mars et al. 2000). Such poor replication does not favor efficient transmission, as apparent in the experiment here described. Therefore, at the cattle density employed here, it seems that the gE- vaccine would not spread within the herd.

The experiments reported here suggest that the gE- vaccine was not hazardous to dams vaccinated during gestation. In addition, it did not spread horizontally to herdmates under usual beef cattle farming conditions usually employed for this region. These studies will be extended in the future to evaluate the efficacy of the gE-deleted vaccine in preventing abortions following challenge of pregnant dams with wild type BoHV-1.

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