PROPHYLACTIC AND THERAPEUTIC VACCINATION AGAINST INFECTIOUS BOVINE RHINOTRACHEITIS

Fernando R. Spilki[1,2,3*], Alessandra D. Silva[1,2], Mauro Bronzatto[1,2], Paulo A. Esteves[1,2], Ana Cláudia Franco[1,2], Paulo M. Roehe[1,2]

1 - Centro de Pesquisas Veterinárias Desidério Finamor - Fepagro Saúde Animal, Estrada do Conde 6000, Cx. Postal 47, Eldorado do Sul, RS 92990-000, Brazil
2 - Dept° Microbiologia, Laboratorio de Virologia, UFRGS, Porto Alegre, RS 90050-170, Brazil;
*3 – Present address: Animal Virology Laboratory, DMI – IB, Unicamp, Po.Box. 6109, CEP 13083-970, Campinas, SP, Brazil. *corresponding author: spilki@unicamp.br*

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Introduction

During an outbreak of infectious bovine rhinotracheitis (IBR), as is called the upper respiratory disease caused by bovine herpesvirus type 1 (BoHV-1), affected cattle are often treated with antibiotics and supportive therapy to reduce the risk of secondary bacterial pneumonia. Field observations have pointed that vaccination of cattle early in an outbreak with a modified live intranasal IBR vaccine may reduce the number of new cases of IBR (Van Donkersgoed & Babiuk, 1991). In the last 10 years, a new generation of recombinant BoHV-1 vaccines has been developed (Van Oirschot et al., 1996; Franco et al., 2002). Recent reports tell that might be possible for these vaccine viruses to undergo recombination with field viruses and regain its pathogenic phenotype (Schynts at al. 1999, 2003). However, no experiments were conducted to test the occurrence of such phenomenon *in vivo*. In the present experiment, calves were vaccinated with the gE-deleted virus two days after the challenge with a virulent wild type BoHV-1. The clinical and virological findings were compared with those on previously vaccinated and non-vaccinated control animals. In addition, tests were performed in search for possible wild type/vaccine virus recombinants.

Materials and methods

Viruses and cells

The vaccine virus used for immunization of calves is a glycoprotein E (gE)-deleted (gE-negative) recombinant, denominated 265 gE-. The construction and characterization of 265 gE-, which was made from a subtype 2a (BoHV-1.2a) wild type virus (SV265/96) was described elsewhere (Franco et al., 2002). The virus used for challenge was a subtype 1

(BoHV-1.1) strain denominated EVI 123/98 an isolate of BoHV-1 1.1 (D'Arce et al. 2002). Both viruses were multiplied in CRIB-1 cells (Flores and Donis, 1995) to prepare stocks with approximately $10^{8.3}$ 50 % tissue culture infective doses per mL ($TCID_{50}$).

Inoculation of calves and monitoring

Twelve, 2 to 3 months old, Holstein male calves, seronegative for BoHV-1 were purchased from a local dairy farm. Calves were separated in four groups (A, B, C, and D) of 3 animals each and kept in isolation. After twelve days for adaptation to the isolation units, calves in groups A and B were vaccinated with $10^{6.9}$ $TCID_{50}$ of the 265 gE- mutant (2 mL on the right nostril). Group A was vaccinated intranasally (IN), whereas group B was immunized intramuscularly (IM). Fourteen days after immunization, calves in groups A and B, as well as non-vaccinated calves (groups C and D) were challenged with $10^{9.3}$ $TCID_{50}$ of BoHV-1 strain EVI 123/98 in 5 mL of inoculum each nostril. Forty-eight hours later, coinciding with the first febrile peak, animals in group C were inoculated intranasally in the right nostril with the same amount ($10^{6.9}TCID_{50}$) of vaccine virus (265 gE-) used to immunize calves in groups A and B. Calves were clinically examined daily from day 12 before inoculation until day 16 after challenge (ac). Respiratory rates and rectal temperatures were recorded. Clinical monitoring, virological and serological analysis were performed essentially as described in Spilki et al. (2004).

Monitoring of recombination between vaccine and challenge strains

Viruses recovered from infected animals were multiplied in CRIB cells until viral plaques were evident in about 20 % of the monolayer. The isolated plaques were counted for each animal. Cells were then fixed with 4% paraformaldehyde and tested in an immunoperoxidase monolayer assay (IPMA) in which the primary antibody was a monoclonal antibody (Mab) that specifically recognizes the subtype of the challenge virus (BoHV-1.1). Such Mab (Mab 71) does not react with the vaccine virus subtype (BoHV-1.2) (Rijsewijk et al., 1999). Subsequently, infected monolayers were submitted to another IPMA with a pool of anti-gE Mabs (Rijsewijk et al., 2000) as primary antibodies, followed by peroxidase/anti mouse IgG conjugate as above and TMB as substrate. After such double immunostaining, wild type virus-infected cells stained double-coloured (red and blue), whereas cells infected with vaccine virus remained unstained. A recombinant BoHV-1.1 gE-negative plaque would be stained red, whereas BoHV-1.2a, whereas cells infected with gE-positive virus would be stained blue.

Results

The recombinant vaccine virus 265 gE- administered to calves on groups A and B fully protected the calves against challenge 14 days later with strain EVI123/98. In contrast, non-immunized calves (group D) developed clear signs of infectious bovine rhinotracheitis, (Figure 1). In group C, where calves were vaccinated therapeutically, the signs of disease observed were undistinguishable from those seen on non-immunized calves (group D), indicating that vaccination did not confer clinical protection to calves, with the exception of the small period between days 10 and 14 ac. Both previously immunized and therapeutically vaccinated calves excreted less infectious virus and for shorter periods than non-immunized calves (Figure 2). The reduction in virus shedding was at the order of 10^2 to 10^5 $TCID_{50}$ less infectious virus than the amount shed by calves in the non-immunized group (D). Animals from all groups seroconverted after challenge, between 7 and 14 ac. No recombination event was evident in viral plaques as determined by the double staining IPMA.

Discussion

The results obtained in the present study showed that post-exposure vaccination to BoHV-1 had no effect on the severity of clinical signs induced by BoHV-1. The efficacy of preventive vaccination was demonstrated by the challenge of previously vaccinated (IN and IM) calves proven efficacious in preventing clinical disease when administered fourteen days previous to challenge. Nevertheless, therapeutic vaccination did reduce remarkably the amount of virus shedding in acutely infected animals.

A major concern on the use of a live herpesvirus vaccine in infected animals relies on the possibility of recombination between wild type virus and the vaccine virus, as demonstrated previously both *in vivo* and *in vitro* (Schynts et al, 1999; 2004). However, in those studies, the occurrence of recombination was favored by the simultaneous inoculation of both viruses. In another in vitro study, when the viruses were inoculated in cell cultures at different time intervals (Meurens et al., 2004) recombination did not occur. The results obtained here have similarities with the *in vitro* studies of the later study, since the viruses were inoculated into the hosts on different occasions and no detectable recombination events took place between wild type and vaccine viruses.

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Figure 1. Clinical scores measured in previously vaccinated animals (Groups A and D, represented by triangles and empty squares respectively), therapeutically vaccinated calves (Group C, circles) and non –vaccinated calves (Group D, full squares) after challenge with BoHV-1.

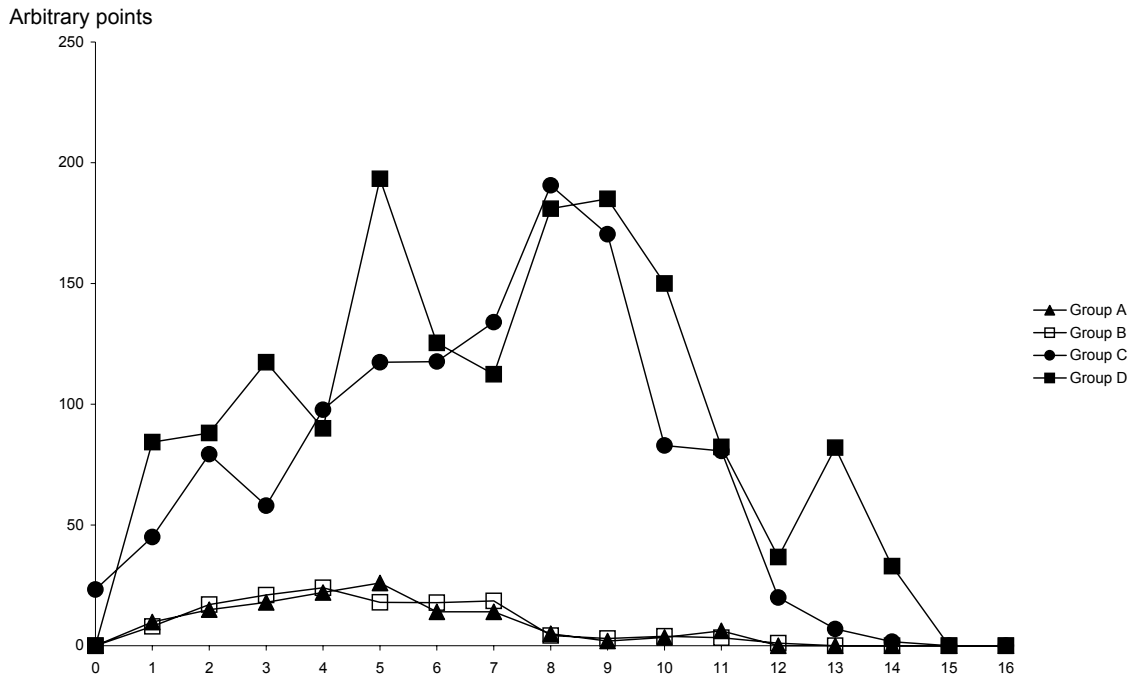

Figure 2. Viral excretion measured in previously vaccinated animals (Groups A and D, represented by triangles and empty squares respectively), therapeutically vaccinated calves (Group C, circles) and non–vaccinated calves (Group D, full squares) after challenge with BoHV-1.