Vaccination with Bovine Herpesvirus 1 Inactivated Vaccine Cannot be Considered as a Stimulus of Reactivation

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Abstract

In countries where bovine herpesvirus 1 (BHV-1) infection is highly prevalent, repeated vaccinations of seropositive cattle are recommended to reduce virus circulation with the aim of reaching infectious bovine rhinotracheitis (IBR) free status. In this context, it was of great importance to investigate whether or not vaccination of seropositive latently infected cattle with inactivated vaccine could induce BHV-1 reactivation and re-excretion. Six seronegative calves were intranasally infected with BHV-1 Iowa strain. Four months later, they were randomly allocated in two groups. Four animals were vaccinated twice, four weeks apart, with an adjuvanted inactivated BHV-1 vaccine and two animals were used as controls. After viral inoculation, all calves excreted large amounts of viruses. Following each vaccination, no infectious virus was isolated in the nasal secretions of calves. Three weeks after the second vaccination, all calves were subjected to a five consecutive days dexamethasone treatment to reactivate putative latent virus. Although the magnitude of virus shedding was lower in vaccinated than control calves, all animals re-excreted virus after dexamethasone treatment. This study demonstrates that vaccination of latently infected animals with an inactivated BHV-1 vaccine does not provoke virus re-excretion, and therefore, in our conditions of work, is not considered as a reactivation stimulus.

Key Words: BHV-1, IBR, respiratory disease, vaccination

Introduction

The control of infectious bovine rhinotracheitis (IBR) is now a major concern in the European Union in terms of cattle, semen and embryo trade. Several countries or regions have initiated the eradication or the control of this infection caused by bovine herpesvirus type 1 (BVH-1). The establishment of BHV-1 in a latent state after infection is considered as a major obstacle to control programmes. Control of IBR in countries where BHV-1 infection is highly prevalent must be associated with repeated vaccinations of seropositive latently infected cattle in order to reduce virus circulation. All live attenuated BHV-1 strains tested so far establish latency and can be reactivated and re-excreted (Pastoret *et al.*, 1980; Thiry *et al.*, 1985; Kit *et al.*, 1985; Whetstone *et al.*, 1992). Their use in a control programme with the aim to eliminate BHV-1 infection from a herd, a region or a country, will lead to a widespread latent persistence of vaccine strain in the whole vaccinated population. Considering this aspect of vaccine safety, inactivated vaccines are therefore better suited for IBR control purpose provided that the other safety and efficacy criteria are met.

BHV-1 latent infections can be reactivated by a wide variety of stimuli including transport, parturition, treatment with glucocorticoids (Lemaire *et al.*, 1994). This work deals with a particular aspect of the safety of inactivated vaccines adjuvanted with saponin and aluminium hydrocide. The aim of this work was to investigate whether or not vaccination of seropositive latently infected cattle with this kind of vaccine, could induce BHV-1 reactivation and re-excretion. Indeed vaccination with an adjuvanted inactivated vaccine may lead to local and general secondary reactions which are usually very mild (Cupta *et al.*, 1993) but can be recognized as stressful conditions in cattle. In this study, two vaccinations with an inactivated vaccine did not produce any virus re-excretion in latently infected animals and cannot therefore be considered as a BHV-1 reactivation stimulus.

Materials and Methods

Experimental Design

Six calves (two heifers and four bulls) seronegative for BHV-1 were inoculated intranasally when they were 7 to 8 months old with virulent BHV-1 strain Iowa, with a total dose of 10^7 PFU (plaque forming unit), 1 ml per nostril. Four months later four of them were randomly vaccinated with an inactivated BHV-1 vaccine adjuvanted with saponin and aluminium hydroxide (Bayovac IBR/IPV inactivated vaccine, Bayer). They were injected subcutaneously with 2 ml of vaccine, twice, 4 weeks apart, as recommended by the manufacturer. Two control animals were injected subcutaneously with

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2 ml of saline solution. Three weeks after the second immunisation, all animals were treated with intravenous injections of dexamethasone (0.1mg per kg body weight; Dexavène®, Schering-Plough), for five consecutive days, to reactivate latent virus.

Clinical Observation

Following viral inoculation, each vaccination and dexamethasone treatment, clinical examinations and rectal temperature were recorded for each animal daily for 14 days.

Collection of Nasal Secretions and Virus Isolation

Nasal swabs were taken from each calf daily for 8 days, and then every two days until 16 days after viral inoculation to show virus excretion profiles. To determine putative virus re-excretion consecutive to vaccination, nasal swabs were taken daily on each animal for 14 days after each vaccination. In order to demonstrate latency, nasal swabs were taken on all animals daily for 14 days after the first day of dexamethasone treatment.

The presence of BHV-1 virus in nasal swabs was estimated by plaque assay. The virus titre was expressed in PFU/100 mg of nasal secretion.

Blood Sampling and Serological Test

Blood samples from each animal were taken monthly until one week before viral inoculation. Blood samples were taken from each animal on weeks -1, 0 (virus inoculation), 1, 2, 3, 7, 10, 13, 15, 16, 17 (first vaccination), 19, 21 (second vaccination), 24 (day of the first dexamethasone treatment), 26, 27, 28 and 29 weeks after viral inoculation.

The presence of antibody was detected in the serum by a commercially available ELISA (enzyme-linked immunosorbent assay) test (SERELISA IBR/IPV Ab Bi Indirect, Rhône Mérieux).

Statistical Analysis

Differences between control and vaccinated animals with regard to duration, peak titres and magnitude of virus shedding after dexamethasone treatment were analysed statistically, using the Student's t test. The magnitude of virus shedding was determined by adding the mean titres for each day for each animal in control and vaccinated groups and dividing this total by the number of animals in each group (Mengeling *et al.*, 1992).

Results

Clinical Observations

After BHV-1 inoculation, all calves showed moderate reduction in alertness for 5.5±1.2 days and moderate reduction in appetite for 4.7±1.2 days (mean±s.d.). Hyperthermia (>39.5°C) was observed in all animals for 4.5±0.8 days, starting between day 1 to day 4 after inoculation (fig. 1). Mild to severe nasal discharge was recorded for 16.2±1.6 days. Five animals presented a cough for 6.4±4.2 days. Mild conjunctivitis was recorded in 3 animals for 3 days starting on day 8 after inoculation. Lesions of the nasal mucosae were shown in all animals for 4.8±2.4 days and were visible from day 4 after inoculation.

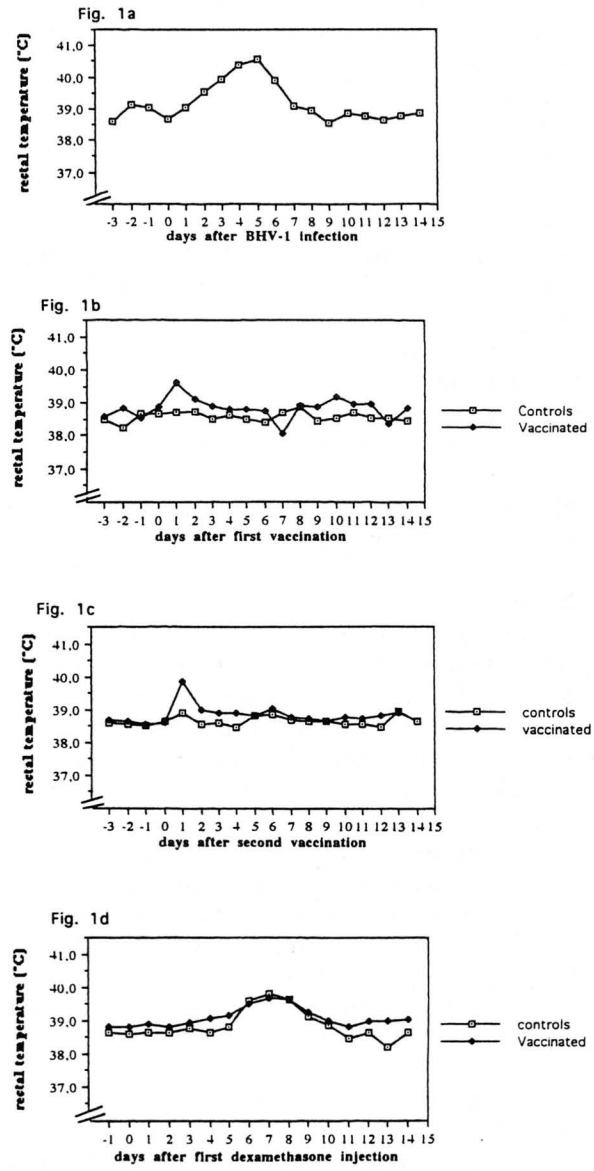

Figure 1. Mean daily rectal temperatures in calves, 1a. after bovine herpes virus 1 infection; 1b. after the first vaccination; 1c. after the second vaccination; 1d. after dexamethasone treatment.

All animals remained in good health within the 14 days following each vaccination. However, following each

vaccination, vaccinated calves showed a rise in rectal temperature the day after vaccination, whereas no hyperthermia was observed in control calves (fig.1).

Following dexamethasone treatment, moderate apathy was observed in control animals from day 6 to day 8 (after the first dexamethasone treatment) and from day 6 to day 7 in vaccinated calves. Elevation of rectal temperature was recorded in all animals from day 5 to day 8 (fig. 1). Nasal discharge was observed in all animals from day 6 to day 11, vaccinated animals presented slight to mild nasal discharge and control animals showed moderate nasal discharge. Large lesions of the nasal mucosae were shown in all animals from day 6 to day 9. They were particularly abundant in control animals. Moderate diarrhoea was observed in all animals from day 6 to day 9.

Virus Isolation

After inoculation, BHV-1 virus replicated in the nasal mucosa of all calves and was isolated from nasal swabs starting from day 1 (5 animals) and day 2 (1 animal) after viral inoculation. Virus was isolated in their nasal secretions for 10.5±2.2 days (means±s.d.). The mean peak virus titre was $10^{6.2\pm0.6}$ PFU/100 mg (mean±s.d.) of nasal secretion on days 4 after experimental inoculation.

No virus was isolated from control and vaccinated animals within 14 days after the first and second vaccinations.

Nevertheless, when animals were treated with dexamethasone, 21 days after the second vaccination, virus was recovered in nasal swabs from all animals. The two control animals re-excreted virus from the noses for 6 days starting on days 3 and 4 after the first dexamethasone treatment. Vaccinated animals re-excreted virus for 5 to 6 days starting on day 4 after the first dexamethasone treatment. Vaccinated animals showed a lower mean peak titre ($10^{5.0\pm0.3}$ PFU/100 mg of nasal secretion on day 7) than unvaccinated animals ($10^{6.3\pm0.6}$ PFU/100 mg of nasal secretion on day 6) (mean±s.e.m.). The magnitude of virus shedding was significantly reduced ($p<0.05$) in vaccinated animals ($10^{3.7\pm0.1}$ PFU/100 mg) in comparison with control animals ($10^{4.8\pm0.4}$ PFU/100 mg) (mean±s.e.m.).

Evolution of BHV-1 Antibody Response

All calves remained seronegative for BHV-1 until BHV-1 infection. BHV-1 antibodies were detectable 14 days after viral inoculation. The BHV-1 antibody level rose very significantly within 6 weeks after infection, reaching a plateau which was maintained for the duration of the experiment (fig. 2). Indeed no significant difference between control and vaccinated animal could be observed following each vaccination and after dexamethasone treatment.

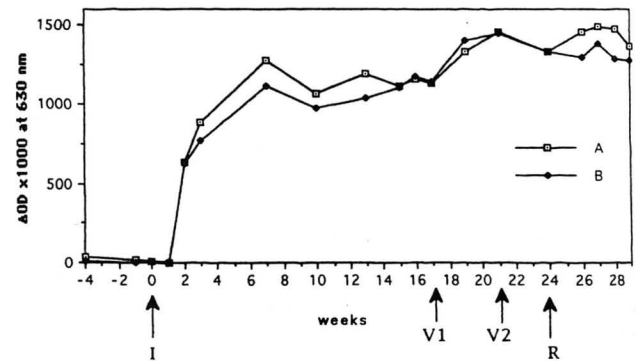

Figure 2. Evolution of antibodies (expressed in difference in optical densities obtained in ELISA) during the experiment. I: infection; V1: first vaccination; V2: second vaccination. R: dexamethasone treatment; A: mean (n=2) of controls; B: mean (n=4) of vaccinated calves.

Discussion

Vaccination of the four latently infected calves with an adjuvanted inactivated BHV-1 vaccine did not induce any virus re-excretion in these animals. It is therefore assumed that reactivation did not occur since re-excretion, its direct consequence, was not detected. Latency of the experimental calves was proven three weeks after the second vaccination by dexamethasone treatment. Although the magnitude of virus shedding was lower in vaccinated than control calves, all animals re-excreted virus after dexamethasone treatment. Therefore, in the conditions of this experiment, vaccination with an inactivated BHV-1 vaccine adjuvanted with saponin and aluminium hydroxide has not been identified as a BHV-1 reactivation stimulus.

These results can be extrapolated to the new generation of inactivated BHV-1 marked vaccines because their formulation do not differ from the tested vaccine with regard to the amount of virus antigen and the nature of adjuvants. This is also reflected by the increase of rectal temperature observed in calves vaccinated with glycoprotein E-negative BHV-1 inactivated vaccine the day after vaccination (Kaashoek *et al.*, 1995). This rise in body temperature was also recorded in this experiment in vaccinated animals following each vaccination.

The results obtained in this study suggest that the use of inactivated vaccines on seropositive latently infected animals cannot provoke virus re-excretion and subsequent virus spreading. Therefore, these vaccines can be safely used for repeated immunisations of seropositive animals which are the main targets in IBR control programmes.

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Editor's note: Professor Etienne Thiry was the recipient of the 1996 PfizerAward for Outstanding Contribution to the Advancement of Knowledge in the Origin and Treatment of Bovine Diseases Caused by Microorganisms, presented at the XIX World Buiatrics Congress.

Abstract

Immunohistochemical method for detecting lesions in the prostate gland of bulls treated with diethylstilbestrol-dipropionate

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Hyperplastic and metaplastic lesions in the prostate gland of bulls are used as morphological evidence for the illegal administration of oestrogens or compounds with oestrogenic activity, like stilbenes. Exogenous and endogenous androgens can suppress the effects of these drugs, resulting in suspect or negative results. The detection of epithelial changes immunohistochemically with cytokeratin antibodies appeared to facilitate the diagnosis of hyperplasia and metaplasia in the prostate. A specific and sensitive method for detecting such lesions in formalin-fixed, paraffin-embedded prostate samples has been developed with the commercially available cytokeratin monoclonal antibody 34βE12, and applied to bulls treated with diethylstilbestrol-dipropionate. The highly reproducible staining pattern of 34βE12 can be used for the detection of hyperplasia and metaplasia in the prostate glands of animals treated illegally with oestrogens.