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Development and statistical validation of a guinea pig model for vaccine potency testing against Infectious Bovine Rhinothracheitis (IBR) virus

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ABSTRACT

Infectious Bovine Rhinothracheitis (IBR) caused by bovine herpesvirus 1 (BoHV-1) infection is distributed worldwide. BoHV-1 either alone or in association with other respiratory cattle pathogens causes significant economic losses to the livestock industry. The aim of this work was to validate a guinea pig model as an alternative method to the current BoHV-1 vaccine potency testing in calves. Guinea pigs were immunized with two doses of vaccine, 21 days apart and sampled at 30 days post vaccination (dpv). BoHV-1 antibody (Ab) response to vaccination in guinea pigs, measured by ELISA and virus neutralization (VN), was statistically compared to the Ab response in cattle. The guinea pig model showed a dose–response relationship to the BoVH-1 antigen concentration in the vaccine and it was able to discriminate among vaccines containing 1 $\log_{10}$ difference in its BoHV-1 concentration with very good repeatability and reproducibility (CV $\leq$ 20%). A regression analysis of the Ab titers obtained in guinea pigs and bovines at 30 and 60 dpv, respectively, allowed us to classify vaccines in three potency categories: "very satisfactory", "satisfactory" and "unsatisfactory". Bovines immunized with vaccines corresponding to each of these three categories were experimentally challenged with BoVH-1 virus, the level of protection, as measured by reduction of virus shedding and disease severity, correlated well with the vaccine category used. Data generated by 85 experiments, which included vaccination of calves and guinea pigs with 18 reference vaccines of known potency, 8 placebos and 18 commercial vaccines, was subjected to statistical analysis. Concordance analysis indicated almost perfect agreement between the model and the target species for Ab titers measured by ELISA and almost perfect to substantial agreement when Ab titers were measured by VN. Taken together these results indicate that the developed guinea pig model represents a novel and reliable tool to estimate batch-to-batch vaccine potency and to predict efficacy of killed BoHV-1 veterinary vaccines.

1. Introduction

Infectious bovine rhinothracheitis (IBR) and pustular vulvovaginitis (IPV) are respiratory and reproductive diseases of domestic and wild cattle caused by bovine herpesvirus 1 (BoHV-1) [1–3]. The disease presents a respiratory form, including coughing, nasal discharge and conjunctivitis. Signs can range from mild to severe, depending on the presence of secondary bacterial pneumonia, with the development of dyspnea. In the absence of bacterial pneumonia, recovery generally occurs 4–5 days after the onset of signs. Because virus latency is a normal sequel to BoHV-1 infection, and antibody (Ab) response after infection seems to be life-lasting, any seropositive animal should be considered as a potential carrier and intermittent shedder of the virus, with the exception of young calves with passive maternal Ab and non-infected cattle vaccinated with killed vaccines [1].

BoHV-1 infection is distributed worldwide affecting domestic and wild ruminants [2–7]. BoHV-1 either alone or in association with other respiratory cattle pathogens is the cause of significant economic losses in the livestock industry. However, after the implementation of strict control programs, the disease has been eradicated from Nordic European countries (Norway, Finland

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and Sweden), Austria, Denmark, and part of Italy. Currently, other European countries are under compulsory or voluntary eradication programs, all involving the application of inactivated or live "marker" vaccines, based on the deletion of BoHV-1 gE or gD viral glyproteins. In the rest of the world, classical attenuated and killed BoHV-1 vaccines are commonly applied [1,8].

In South American countries like Argentina, Brazil and Uruguay BoHV-1 infection is endemic [7,9,10] and vaccination is not mandatory. In Argentina, due to regulatory restrictions, only killed vaccines can be used to prevent viral diseases in cattle. Several conventional combined inactivated vaccines containing BoHV-1 together with other viruses (bovine viral diarrhea virus, bovine parainfluenza type 3 virus and bovine respiratory syncytial virus) and bacterial pathogens (*Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus sommi*, *Moraxella bovis*, *Moraxella ovis*, *Campylobacter fetus-fetus*, *Campylobacter fetus-venerealis* and *Leptospira* sp.) have been used for many decades in routine vaccination protocols to prevent bovine respiratory and reproductive diseases in cattle. These multivalent vaccines were designed to control a sanitary problem of complex etiology. However, the potency and efficacy against each antigen contained in some of these combined formulations is unclear and further studies need to be carried out to properly address this issue.

Specifically for BoHV-1 vaccine approval, international regulatory policies recommend to evaluate vaccine quality in a potency test conducted in seronegative calves [1,11]. A BoHV-1 vaccine must prevent the development of severe clinical signs and markedly reduce virus shedding after experimental challenge. Bovine trials are cumbersome, expensive and time consuming, particularly, in countries like Argentina, where BoHV-1, as well as other viral infections are endemic [10,12,13]. The difficulty in finding seronegative bovines, from BoHV-1 free herds, to be used in vaccine potency tests, pose the need for the developing standardized and harmonized tests in laboratory animals. The availability of a laboratory animal model would enable the regulatory authority and vaccine manufacturers to carry out batch-to-batch release tests on a routine basis in a less time consuming and less expensive way.

Although some vaccine manufactures have reported the use of guinea pigs as internal quality test to evaluate their vaccines [14] a validated method for vaccine potency testing in laboratory animals possessing a demonstrated concordance with the target species, is not yet available [15–17]. Such a properly validated vaccine potency test especially designed for combined vaccines including inactivated viruses is also required in the US and the European Union and could be globally used to control viral vaccines applied in cattle.

Although several ELISA tests were developed to determine BoHV-1 Ab and probed to be more sensitive and specific than the viral neutralization (VN) test [1,18], the latter is still considered the gold standard technique used for vaccine potency testing [1,11].

The general aim of this project is the development and statistical validation of a guinea pig model to be used for veterinary vaccine potency testing. The model has been specifically designed to evaluate the immunogenicity against the viruses currently included in combined vaccines for cattle (BoHV-1, bovine parainfluenza type 3, bovine viral diarrhea virus, bovine sincitial virus, bovine rotavirus and bovine coronavirus).

In the present paper we report the statistical in-house validation of a guinea pig model as a method for potency testing of inactivated IBR vaccines. The validation involved the study of the kinetic of the Ab response in the animal model and the target species, a regression analysis applied to the dose–response curve to define categories for vaccines qualification, a concordance analysis between the laboratory animal model and the natural host confirmed with a BoHV-1 experimental challenge in the latter. Results obtained indicted that the Ab titers of immunized guinea pig constitute a useful predictive tool of vaccine efficacy in cattle.

2. Material and methods

2.1. Bovine: vaccination and sampling

A total of 553 male and female beef calves (Aberdeen Angus, Hereford, and their crossbreeds), 6–12-month-old, were included in the study. Vaccination trials were conducted in 12 beef farms located in Buenos Aires, Argentina. Herds without previous history of vaccination against BoHV-1 were selected. As BoHV-1 infection is endemic in Argentina, vaccines were evaluated in BoHV-1 seronegative animals from BoHV-1 free herds, and also in seronegative and seropositive calves from BoHV-1 endemic herds, in order to consider the variability of the real target population [1,19]. In the trials conducted in BoHV-1 endemic farms, the number of positive and negative animals was randomly distributed in each treatment group so as to initiate the study with statistically similar pre-vaccination mean Ab titers and variances among groups. In every trial, bovines were vaccinated with two doses of vaccine, 30 days apart, as recommended in the label of each of the 22 commercial vaccines tested. Vaccines were administered by the subcutaneal route with doses of 5 or 3 ml according to manufacturersĭ recommendations. Vaccine formulated for model validation and named as "reference vaccines" were applied following same time intervals and dose volumes as commercial vaccines. Blood samples for serum extraction were collected by puncture of the jugular or coccygeal vein. Animals were sampled at 0, 30 and 60 days post-vaccination (dpv). Control groups included placebo and non-vaccinated animals. Groups of calves that received two doses of placebo formulated with culture media (without virus) emulsified in oil-adjuvant were assayed in eight occasions including the dose–response trials in which the animals were sampled until 90 dpv. In addition, in order to have vaccinated and non-vaccinated groups exposed to similar natural conditions, a group of non-vaccinated calves was included in every bovine trial conducted in each of the 12 farms that participated in this study. A bovine trial was only considered valid and included in the statistical analysis if no seroconversion was detected in the non-vaccinated and placebo control groups. Most experiments were carried out blinded (veterinarian, laboratory technician). Seroconversion was defined as a 4-fold increase in antibody titer for both ELISA and VN.

2.2. Guinea pigs: vaccination and sampling

The laboratory animal model for BoHV-1 vaccine potency testing was developed using a total of 497 guinea pigs (*Cavia porcellus*), SiS Al, around 350–400 g weight. After various preliminary assays, using different vaccination routes, dose volume and intervals between doses (data not shown), the immunization protocol was standardized as follows: a minimum of 5 guinea pigs were vaccinated with two doses of vaccine, 21 days apart, by the intramuscular route, in the hind-leg. The volume of the dose administered to the guinea pigs, corresponded to 1/5 of the volume of the dose given to bovines [20]. For the dose–response study serum samples were obtained at 0, 30 and 60 dpv in order to obtain the kinetic of the Ab response in the lab animal model. For concordance studies guinea pigs were sampled at 0 and 30 dpv. Blood extraction was conducted by cardiac puncture under anesthesia, following ECVAM recommendations for animal welfare [21]. The protocol was approved by the CICV y A, INTA Ethical Committee (CICUAE). In each immunization assay, at least three negative control animals (non-vaccinated or vaccinated with placebo) were included. Guinea pigs experimental groups were coded and serologic analyses were carried out blinded.