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Simultaneous immunization of cattle with foot-and-mouth disease (FMD) and live anthrax vaccines do not interfere with FMD booster responses

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ABSTRACT

Foot-and-mouth disease (FMD) vaccination in Argentina is compulsory for most of the cattle population and conducted by certified veterinarians. This organized campaign may facilitate the controlled application of other vaccines against endemic diseases, provided immune responses against FMD are not hindered. There is no published information on the interference of immunity against FMD vaccines when applied together with a live bacterial vaccine. In this study we evaluated if the simultaneous application of a *Bacillus anthracis* live vaccine with a commercial tetravalent oil-based FMD vaccine (FMD-vac) used in Argentina, modifies the antibody booster responses against FMD virus (FMDV) in cattle. Two groups of 16 heifers with comparable liquid phase blocking ELISA (LPBE) titers were immunized with the FMD-vac alone or simultaneously with a commercial attenuated bovine anthrax Sterne strain vaccine (ABV). Serum samples were obtained at 0, 25, 60 and 90 days post vaccination (dpv) and specific antibodies against two FMDV vaccine strains were assessed by LPBE, avidity and IgG-isotype ELISAs. Bovines immunized with FMD-vac or FMDV-V + ABV responded with a boost in the LPBE antibody titers and avidity at 25 dpv, and remained within similar levels up to the end of the study. Animals vaccinated with FMD-vac + ABV had significantly higher LPBE titers at 25 dpv, compared to those immunized with FMD-vac alone; which was due to an increase in IgG2 titers. Overall, antibody titers elicited in both groups were similar and followed comparable kinetics over time. We conclude that the simultaneous application of a live anthrax vaccine with the current FMD tetravalent vaccine used in Argentina in cattle previously immunized against FMD, did not counteract the serological response induced by FMD vaccination.

Introduction

Foot and mouth disease (FMD) is a highly contagious acute vesicular viral disease that affects cloven-hoofed animals. FMD virus (FMDV) belongs to the genus *Aphthovirus* in the *Picornaviridae* family, and includes seven serotypes: O, A, Asia, C, and SAT-1, -2, and -3 [1]. The circulation of FMDV in susceptible livestock imposes severe restrictions on the movement and trade of animals and derived products, causing serious economic loss to the affected countries [2]. FMD is endemic in many parts of Asia, Africa, and South America, where vaccination of susceptible populations is widely used as a major control measure. Commercial formulations usually contain more than one virus strain, as immune responses induced by vaccination are strain-specific [3,4].

The vaccine currently used in Argentina is oil-adjuvanted and contains four FMDV strains of latest regional circulation: O1/Campos/Brazil/58 (O1/Campos), A24 Cruzeiro/Brazil/55 (A24/Cruzeiro), A/Argentina/2001 (A/Arg/01) and C3/Indaial/Brazil/71 (C3/Indaial) [5,6]. FMD vaccination is compulsory and rigorously controlled by the local sanitary authority (SENASA). SENASA provides the virus vaccine strains and vaccination is performed by trained and certified personnel [7], animals are properly identified and cold chain is verified and guaranteed. Vaccines are applied to

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the whole cattle population above the 42° South parallel on fixed schedules. Animals older than 2 years are immunized once a year, while calves aged up to 2 years-old are vaccinated every 6 months. Vaccine efficacy as well as surveillance of vaccine immunity is performed by serology. Strain-specific antibody titers obtained with liquid phase blocking ELISA (LPBE) have been statistically correlated to *in vivo* protection to assess vaccine potency and herd immunity through the estimation of a percentage of expected protection (EPP) [8–12].

The controlled and correct application of vaccines is as important as quality control assessments performed to the vaccine itself. Vaccines may fail in inducing protection if cold chain is not preserved or if the vaccine is not properly applied. These side-issues have major impact when working with livestock. However, gathering all the animals, vaccinators and monitoring cold-chain is difficult to achieve, particularly in large regions, areas of difficult access or extensive production systems. In this scenario, the combination or co-administration of vaccines together with the FMD vaccine appears as a practical and efficient option for immunizing livestock, as long as this practice does not interfere with the immunogenicity conferred by those vaccines applied.

One major pathogen affecting livestock, which also has zoonotic impact, is *Bacillus anthracis*. *B. anthracis* is a Gram-positive bacillus that forms spores that are highly resilient, surviving extremes of temperature, low-nutrient environments, and harsh chemical treatment. This bacterium is the etiologic agent of anthrax, an endemic disease in many countries of Southern Europe, South America, Asia and Africa [13]. In Argentina, livestock is concentrated in seven provinces in the center of the country, with 42 million cows and nearly 2 million rural inhabitants which implies a high risk for anthrax transmission [14]. A surveillance performed in Buenos Aires Province, which represents 32% of farming land and 28% of the livestock stock, revealed that 49% of the farms have had at least one outbreak of bovine *B. anthracis* between 1977 and 2013 [15,16].

The anthrax vaccine currently used for adult bovines in Argentina is based on live spores prepared from the attenuated, capsule-deficient *B. anthracis* Sterne strain (Weybridge no. 34F2). The protective effect of a single dose in adult animals is assumed to last for about 1 year [17], and therefore annual booster vaccinations are recommended for livestock. This schedule can be perfectly coupled with the FMD vaccination program, provided that FMDV titers are not reduced due to the simultaneous vaccination, as this could lead to increase the risk of outbreaks in FMDV-free regions.

There is little information in the literature regarding FMD vaccination efficacy when applied together with other vaccines. In fact, only two publications have addressed the simultaneous application of FMD vaccine (FMD-vac) with other veterinary vaccines in bovines. One study showed that immunization of young calves immunized (by subcutaneous route) against FMD and against infectious bovine rhinotracheitis/adenovirus/parainfluenza-3 (by intranasal route) did not interfere with the serological response against the FMD virus strains included in the vaccine [18]. Another study, however, showed interference between FMD-vac and a vesicular stomatitis virus live vaccine [19]. Altogether, available data indicate that the simultaneous application of FMD vaccines, particularly with live vaccines, needs to be evaluated.

In a first attempt to address the possible interference of *B. anthracis* live vaccine (ABV) with FMD-vac we studied the serological response of cattle that received one dose of FMD-vac or FMD-vac simultaneously with ABV. Due to the fact that all adult animals in the region have vaccine-induced anti-FMDV antibodies, and ABV is only applied in adult animals, we evaluated the serological response to a booster FMD-vac dose (4th dose) applied alone or together with the anthrax vaccine. Our data indicate that the simultaneous application of these vaccines do not modify the serological response profiles to FMD booster vaccination. Moreover, higher titers against FMDV were obtained at 25 days post-vaccination (dpv) when both ABV and FMD-vac were applied, mainly due to an increase in IgG2 antibody titers.

Materials and methods

Animals

Heifers used in this study were from the same farm and had four previous FMD vaccinations, corresponding to FMD campaigns of November 2011, March 2012, November 2012 and March 2013. Thirty-two animals were selected from a herd of 120 heifers according to the levels of antibodies against FMDV (O1/Campos strain) measured by ELISA (LPBE, see below) a week before vaccination. Animal handling, vaccination and serum sampling procedures were previously approved by INTA's Animal Welfare Commission (protocol approval No. 025/2011).

Vaccines

Commercial vaccines were used in this study. The FMD vaccine, referred here as "FMD-vac", was an oil-adjuvanted (water-in-oil) vaccine containing inactivated FMDV from four strains: O1/Campos, A24/Cruzeiro, A/Arg/01 and C3/Indaial and produced by a local manufacturer. This vaccine was approved by SENASA according to the current national regulations [6,20].

The bovine anthrax vaccine "PROVIDEAN CARBUNCLO®" (Tecnovax SA, Buenos Aires, Argentina), here called "ABV", contains non-encapsulated, non-virulent spores of *B. anthracis* $F_2$34 Sterne strain with an antigen payload of 1.8×10^7 spores per dose.

Experimental design

The day of vaccination, 32 animals that had received three previous vaccinations and showing LPBE titers against FMDV O1/Campos ranging from 3.37 to 3.96, were selected from a herd of 120 animals, and randomly distributed in two groups of 16 animals. One group received 2 mL of FMD-vac applied subcutaneously in the left side of the neck (Group FMD-vac). The other remaining 16 animals received the same vaccine and also 2 mL of ABV (also subcutaneously) in the right side of the neck (Group FMD-vac + ABV). Serum samples (2 aliquots of 2 mL each per animal) obtained at 0, 25, 55 and 90 dpv were stored at −20 °C for further serological assessments.

Liquid phase blocking ELISA (LPBE)

Total anti-FMDV O1/Campos and anti-FMDV A24/Cruzeiro antibody responses were assessed in serum samples by LPBE performed as stated by the OIE Manual using a rabbit antiserum to capture inactivated whole 140S viral particles, and a guinea-pig antiserum as detector antibody, both of them strain-specific as described before [21]. Antibody titers were expressed as the reciprocal Log10 of serum dilutions giving the 50% of the absorbance recorded in the virus control wells without serum.

Single dilution avidity ELISA

Avidity assessment of specific antibodies was performed as described before [22]. The Avidity Index (AI) was calculated as the percentage of residual activity of the serum sample after a 20 min urea washing step, relative to that of untreated sample: AI% = (OD sample with urea/OD sample without urea) × 100.