Vaccine 34 (2016) 4140–4144

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Protection induced by a commercial bivalent vaccine against Foot-and-Mouth Disease 2010 field virus from Ecuador

Hernando Duque [a], Jose Naranjo [c], Consuelo Carrillo [a], Alexandra Burbano [b], Javier Vargas [b], Lisa Pauszek [a], Ian Olesen [a], Manuel J. Sanchez-Vazquez [c], Ottorino Cosivi [c], Rossana M. Allende [c,*]

[a] Foreign Animal Disease Diagnostic Laboratory (FADDL), APHIS, USDA, Plum Island Animal Disease Center (PIADC), Greenport, NY, USA
[b] Agencia Ecuatoriana de Aseguramiento de Calidad del Agro – AGROCALIDAD, Av. Interoceánica km. 14½, La Granja MAGAP, Tumbaco, Ecuador
[c] Pan-American Foot-and-Mouth Disease Center – PANAFTOSA/PAHO, Av. Governador Leonel de Moura Brizola 7778, Duque de Caxias, RJ CEP 25045-002, Brazil

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 22 June 2016
Accepted 24 June 2016
Available online 6 July 2016

Keywords:
Foot-and-Mouth Disease
Vaccine matching
Expectancy of protection
Vaccination
Disease control

ABSTRACT

Foot-and-Mouth Disease serotype O circulated endemically in Ecuador for many years, with an upsurge occurring in 2009. This manuscript describes retrospectively *in vitro* and *in vivo* laboratory studies to predict the field effectiveness of a commercial FMD vaccine to protect against the field strain, and explains the key actions and epidemiological strategies followed by the country to control the disease.

The results established that the use of a good quality oil vaccine, manufactured with strains that were isolated long ago: O1 Campos Br/58 and A24 Cruzeiro Br/55; combined with the correct epidemiological strategies, are useful to control field strains when used in periodic biannual vaccination campaigns.

Published by Elsevier Ltd.

1. Introduction

Foot-and-Mouth Disease (FMD) was first detected in Ecuador in 1956 as serotype A, then in 1962 the first outbreak of serotype O occurred [1]. Historically, serotype A has had a sporadic presence in the country, with its last detection being in 2002. Serotype O has been endemic almost from its first incursion. Between 2000 and 2008, an average of 33 outbreaks associated with serotype O were reported in Ecuador per year [2]. In 2009, an upsurge in the occurrence of FMD took place across the country, with a peak of 109 outbreaks detected that year, as recorded with the Continental FMD Surveillance System.[1] The greatest peaks of outbreaks occurred over the months of May and June, six-month after the previous vaccination cycle (November to mid-December).

The Veterinary Authority of Ecuador (VEA) requested technical cooperation to PANAFTOSA to support their National FMD Eradication Program, particularly in assisting with the response to the recent increase of FMD clinical cases. Lack of success in FMD control was attributed to a deficient employment of the FMD control program. In particular this was due to a weakness of animal movement restrictions and a poor implementation of the vaccination campaign. These two activities were mainly managed by the private sector with poor regulation by the official authorities.

FMD vaccines in use in Ecuador are BEI inactivated with oil adjuvant, and formulated with O_1 Campos and A_{24} Cruzeiro strains. Considering the relevance of vaccination to control the disease, it was of paramount importance to verify to what extent the vaccine strains currently used in Ecuador were capable of controlling the disease outbreaks occurring in 2010. Upon performing *in vitro* studies with the 2010 FMD field virus, as well as the epidemiological analysis of the field situation in Ecuador; PANAFTOSA recommended revaccination of the cattle population with the bivalent commercial vaccine to control field outbreaks. In contrast, another OIE FMD reference laboratory suggested the development of an autologous vaccine as their recommendation [3]. Moreover, results obtained by *in vivo* challenge were published indicating the lack of appropriate protection of a monovalent O1 Campos experimental vaccine against the field viruses circulating in Ecuador [4].

The VAE followed the recommendations from PANAFTOSA to carry on a vaccination campaign using the former bivalent commercial vaccine and incorporated a number of key actions and control strategies to progress in the FMD control.

The situation of opposing vaccine strain recommendations for the control of a field virus described above is likely to be repeated in the FMD scenarios worldwide. This study will help to contextu-

* Corresponding author at: Pan-American Foot-and-Mouth Disease Center – PANAFTOSA-PAHO/WHO, Av. Governador Leonel de Moura Brizola 7778, Duque de Caxias, RJ CEP 25045-002, Brazil.
E-mail address: rallende@paho.org (R.M. Allende).

[1] Continental FMD Surveillance System (SIVCONT)-PANAFTOSA-PAHO/WHO.

http://dx.doi.org/10.1016/j.vaccine.2016.06.073
0264-410X/Published by Elsevier Ltd.

alize the balance between laboratory work, field activities and epidemiological strategies when countries encounter similar situations upon decisions of which vaccine to use. This paper explains the *in vitro* tests performed by PANAFTOSA, as well as the *in vivo* challenge studies performed at PIADC by USDA. Additionally, the paper describes the key actions taken by the VAE to fight against the disease and the evolution of occurrence of FMD in the country.

2. Materials and methods

2.1. Virus strains and typing

In September 2010, PANAFTOSA received and analyzed nine vesicular epithelium samples previously identified as FMDV serotype O by the Ecuadorian laboratory. Virus typing was performed by ELISA as previously described [5]. Field samples were processed and passaged twice in BHK-21 Clone 13 cells and supernatants were used for sequencing of the VP1 coding region and determination of the serological relationship (r_1 value) by complement fixation [6]. A sample identified as 036-331 (r_1 0.45), was collected on June 4th, 2010 from a FMD outbreak which occurred in Orellana Province in Ecuador (O/Orellana-036/ Ecuador 2010).This sample was selected for further studies of r_1 value and vaccine matching tests. FMDV O_1 Campos from the PANAFTOSA repository collection was also used for determining the r_1 value. Simultaneously, five separate samples of tongue epithelium homogenates were received in Plum Island Animal Disease Center (PIADC) for similar purposes of serological, genetic and antigenic characterization of the Ecuador 2010 field strains of FMDV, including the O/Orellana-051-350/Ecuador 2010. All samples were fully characterized by virus isolation, real time RT-PCR, Ag ELISA and cross-neutralization assays. All five specimens were directly sequenced from the epithelial homogenate without adaptation to tissue culture to obtain the full-length nucleotide sequence of the protein coding region (ORF).

2.2. Nucleotide sequencing

For obtaining the VP1 sequences, the RNA extraction was performed using Trizol reagent (Invitrogen) following the manufacturer's protocol. PCR amplification and sequencing were performed as described elsewhere [7]. For full ORF sequence of O/Orellana-051-350/Ecuador 2010 the long distance cDNA and overlapping PCR DNA fragments technique was used as described in [8]. The purified material was used for sequencing reaction using the Big Dye Terminator kit 3.1 (Applied Biosystems) according to manufacturer's procedure. VP1 sequences were edited manually and aligned using the program BioEdit, version 5.0.2.1. For the full-length ORF sequence, the Sequencher software was used for sequence assembly. Finally, for performing alignments and for the comparative analysis of the sequences with those available in GenBank, MEGA 6.0 software was used [9].

2.3. Vaccines

For the *in vivo* cross protection study the conventional, commercial BEI inactivated, water in oil, bivalent vaccine O_1 Campos/A_{24} Cruzeiro manufactured by VECOL in Colombia was used. For the *in vitro* studies to predict the expectancy of protection by EPP calculation, the bovine sera panels used were prepared with conventional, water in oil, trivalent vaccine O_1 Campos/A_{24} Cruzeiro/C_3 Indaial, BEI inactivated and manufactured by PANAFTOSA.

2.4. Determination of serological relationship "r_1"

The r_1 value was used to estimate the antigenic relatedness of the vaccine O1 Campos strain and the field isolate. The reciprocal serum titer against heterologous virus/reciprocal serum titer against homologous virus was determined by complement fixation test (CF) [6] and virus neutralization (VN) assays. CF was performed in tubes as previously described [10] using guinea pig FMDV O_1 Campos antisera. One dimensional microplate neutralization tests were performed as previously described [11]. Briefly, a virus preparation containing 2000 tissue culture infectious doses/mL was mixed volume to volume with serial dilutions of sera. Mixtures were incubated for 1 h at 37 °C and then 100 μL of each mixture was inoculated into four wells each in microplates with BHK-21 C13 cell monolayers. A bovine sera panel composed of cattle sera collected 30 days post vaccination (dpv) and 30 days post booster vaccination (dpr), with a trivalent commercial vaccine, were analyzed by VN against vaccine and field strains. Antibody titers were calculated as the $\log_{10}$ of the reciprocal antibody dilution required for 50% neutralization ($TCID_{50\%}$).

2.5. Assessment of expectancy of protection (EPP)

Sera panels from 30 vaccinated cattle or 30 revaccinated cattle were used to estimate the protection offered by the vaccine against the field virus by the EPP (likelihood that vaccinated cattle would be protected against a challenge of 10,000 bovine infective doses of virus) [12,13]. The EPPs were determined by using the liquid phase blocking ELISA (LPBE) and the VN assays. LPBE tests were carried out according to the method previously described [10]. Sera panels were titrated in ELISA against FMDV O_1 Campos and FMDV field strain O/Orellana-036/ Ecuador 2010. Titers were expressed as $\log_{10}$ of the reciprocal sera dilution giving an OD value equal to the 50% of mean OD value of antigen control. The VN assay was performed as described above and a group of 10 revaccinated cattle sera were used to estimate the EPP. The EPP value for each individual serum was obtained using the EPP table generated by PANAFTOSA (table available upon request). A mean of individual EPPs was then calculated.

2.6. Cross protection experiment

The protection induced by the bivalent O_1 Campos, A_{24} Cruzeiro vaccine against a serotype O/Orellana-051-350/Ecuador 2010 field strain was tested by protection against generalized foot infection (PGP) [14]. Twenty-two Holstein cross-bred steers 300–400 lb, were housed in a large animal room in the BL3 Ag facilities at PIADC. All animals were tested free of FMDV antibodies. All procedures were conducted humanely according to the NIH Guide for the Care and Use of Laboratory Animals and preapproved by the institutional animal care and use committee. The bovines were randomly distributed in three different groups and challenged after either single vaccination or double vaccination. Group 1: (10 bovines) received one full dose of vaccine (2.0 mL) at day 0 and another 2.0 mL booster dose 14 days later. Group 2: (10 bovines) received one full dose of vaccine (2.0 mL) on the same day group 1 received the booster dose. Group 3: (2 bovines) were the unvaccinated control animals. All of the bovines comingled in the same room for the duration of the experiment. The vaccine used in this experiment was a conventional, commercial water in oil, bivalent vaccine O_1 Campos/A_{24} Cruzeiro similar, but a different production batch, from the FMD vaccine that was used in the outbreak area in Ecuador. The EPP for the O_1 Campos strain was performed by the Colombian vaccine regulatory authorities resulting in 89.9% EPP. The challenge virus was derived from tongue epithelium sample 051-350, collected in the province of Orellana Ecuador in 2010