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

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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.

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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 move-ment 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-

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

and sent to PIADC. Virus extracted from the sample was passaged twice in bovine tongue and a final challenge stock was prepared from the vesicular fluid collected. The titer was calculated by tongue titration in cattle. All bovines were challenged on the same day, group 1 on 21 days post revaccination, group 2 on 21 days post vaccination. Challenge was done by intradermal inoculation in four different sites on the tongue, with a total of 10,000 BID_{50}. Animals were observed under xylazine sedation at 7 and 14 days post challenge (dpc). Animals were considered protected when no vesicular lesions were detected in their feet.

2.7. Key actions and control strategies

During the FMD outbreaks in 2010 and 2011, the VEA established a control strategy to attend all the suspicions, quarantine the affected herds and areas with movement restrictions, and fundamentally undertook ring vaccination around all the detected foci. In 2011, PANAFTOSA facilitated technical cooperation with Ecuador leading to actions being taken at the managerial level to empower the VAE, particularly the National FMD Eradication Program, to perform the activities necessary to eliminate outbreaks. Thus, the FMD control program was reviewed giving responsibility to the VEA in actions directed to FMD control. There was also an improvement in the written guidelines to explain the strategies and actions to detect, follow, and control FMD outbreaks in the field, as well as a reinforcement of the control of animal movement with the incorporation of targeted surveillance strategies to high risk premises. Control of the vaccination campaign was placed under the supervision of the VAE, who strengthened the execution of the biannual vaccination campaigns in cattle of all ages and implemented a strategic additional vaccination targeting young animals.

3. Results

3.1. Virus typing and r_1 value

Field samples were typed as FMDV O_1 by Ag ELISA and confirmed by VP1 sequence analysis. The r_1 values obtained by $CF_{50\%}$ ranged between 0.35 and 0.71, half of the samples having r values between 0. 45 and 0.49 ($n = 8$). Strains within this range of values were considered as the most representative of the field situation. A sample identified as 036-331 (r_1 0.45), collected on June 4th, 2010 from a FMD outbreak in Orellana Province in Ecuador (O/Orellana-036/Ecuador 2010), was selected for further VN studies of r_1 value and vaccine matching tests. The average r_1 values determined by VN test with sera from 30 dpv cattle was 0.158 and with sera from 30 dpr cattle was 0.241.

3.2. Sequencing

Both VP1 and complete ORF nucleotide sequencing demonstrated 98% or greater nucleotide identity between all of the O Ecu 2010 isolates and 100% identity in amino acid sequences. The strain circulating in Ecuador was 91% identical at nucleotide level to the previously published O_1 Campos sequence (AY593819). The main differences were observed in the G-H loop of VP1, with nucleotides deletion which translates to the loss of two amino acids next to the RGD motif of this protein (Fig. 1). Additionally, a double deletion in the coding region corresponding to the host-range related protein 3A, amino acid positions 1565 and 1566, of the ORF was observed (Fig. 2).

A 100% identity was recognized when comparing the VP1 nucleotide sequence from O/Orellana-036/ Ecuador 2010 (GenBank Accession Number KX274464, used for the *in vitro* EPP vaccine matching) and O/Orellana-051-350/Ecuador 2010 (GenBank Accession Number KX35623, used for the *in vivo* cross protection test).

3.3. Expectancy of protection (EPP)

EPP values of 47.85 and 91.40 on the panel of bovine sera were obtained when tested against the Ecuador field virus in the LPBE for 30 dpv and 30 dpr sera respectively. In the VN assay an EPP value of 99.98 was obtained with sera from 30 dpr.

3.4. Cross protection experiment

Clinical evaluation for FMD lesions was performed at 7 and 14 dpc. Unvaccinated control bovines showed lesions in tongue and all four feet at the clinical evaluation at 7 dpc (Table 1). No lesions were found in the feet of bovines in group 1 at 7 dpc, and a single lesion was found on 1 foot of one animal in this group at 14 dpc. Nine out of ten (90%) of the revaccinated bovines were protected against feet generalization at the final clinical evaluation. Fifty percent of bovines that received a single vaccination were protected against feet generalization and only two of them showed generalization in all four feet (Table 1).

3.5. Evolution of FMD in Ecuador (2009–2015)

After the peak of 109 FMD outbreaks in 2009, there was a reduction in 2010 with 42 outbreaks. In response to the outbreak control actions implemented by the VAE a further drop was observed in 2011, with only five outbreaks detected. Since then, no more cases of FMD occurred, with the date of last occurrence being August 2011. The country is currently FMD free and on May 2015 the OIE General Assembly endorsed the recognition of FMD free with vaccination in continental Ecuador and FMD free without vaccination for the Galápagos Islands.

4. Discussion

The results of this report, as well as the recent evaluation of FMD in Ecuador (including its FMD-free international recognition) supports the PANAFTOSA's rationale that the implementation of a vaccination program, using vaccines with appropriate strains and of good quality, likewise an effective control of animal movement, are key issues for the success in controlling the disease. Therefore, we demonstrate here that development of a new vaccine was not necessary for Ecuador in 2010. The development of a new vaccine strain is a lengthy and costly process and the decision of including new strains in vaccine formulations should consider experimental and epidemiological observations. Despite the evidence on the genetic evolution of FMD strains circulating in Ecuador over the years we demonstrated here the capability of the vaccine in use to protect cattle against the disease. Therefore, we propose that implementing adequate vaccination practices is critical to obtain satisfactory protective response against infection with the O/ECU/2010 field strain. Proper protection would only be achieved through adequate vaccine quality control, controlled application conditions such as systematic vaccination campaigns, and good practices of the vaccination program.

There are many examples in literature of the problems encountered by other authors in correlating vaccine coverage *in vivo* with *in vitro* "r_1"values [15–18] and it was also observed in this study. Our VN derived "r" values were definitively in the low range of the predictive scale for protection (between 0.1 and 0.2) which did not mirrored the situation after vaccination in the field. In the past considering the problems observed with vaccine matching
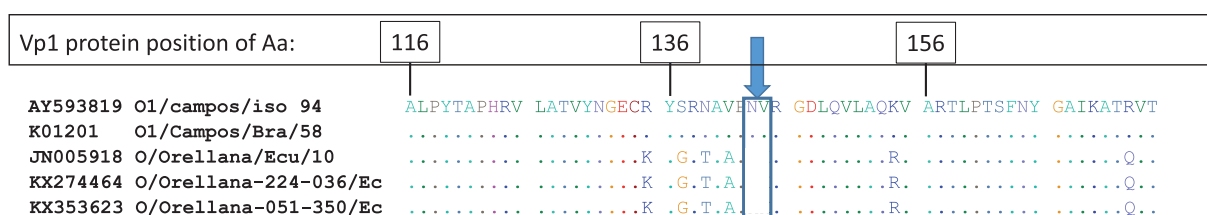
Fig. 1. Amino acid deletion observed next to RGD motif in the FMDV strains from Ecuador.
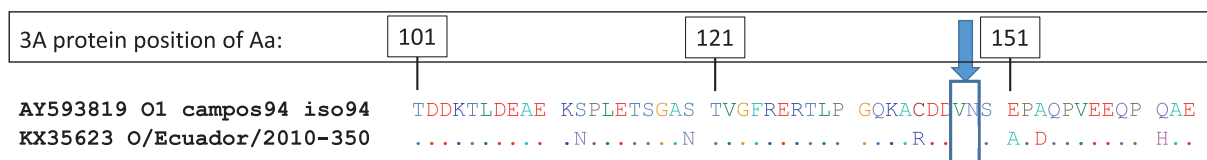

Fig. 2. Amino acid deletions observed in protein 3A in the FMDV strain from Ecuador.

Table 1
Distribution of vaccinated groups, clinical score and protection against generalized foot infection after challenge with 10,000 BID50 of O/Orellana-051-350/Ecuador 2010 virus.

	Vaccination	Animal identification number	Clinical score[*] 7 dpc[**]	Clinical score[*] 14 dpc[**]	Protection (%)
Group 1	Vaccination at 0 days booster at 14 days	25	0	0	90
		26	0	0	
		27	0	0	
		28	0	0	
		29	0	1	
		30	0	0	
		31	0	0	
		32	0	0	
		33	0	0	
		34	0	0	
Group 2	Single vaccination	35	4	4	50
		36	1	1	
		37	0	0	
		38	0	0	
		39	0	0	
		40	0	0	
		41	1	1	
		42	4	4	
		43	0	0	
		44	2	2	
Group 3	Unvaccinated	45	4	4	
		46	4	4	

[*] Clinical score based on number of affected feet.
[**] Days post challenge.

results using r_1 values derived from VN, PANAFTOSA developed the EPP approach to choose the appropriate vaccine viral strain, based on the correlation between antibody titers in vaccinated animals and protection against challenge. The methodology has being applied with success during the last 3 decades. The EPP results reported here closely correlates with results of the *in vivo* challenge experiments, which is still considered the gold standard for vaccine matching. This result is in clear contrast with previously published data [4]. These authors showed only 6% and 18% protection after single and booster vaccination respectively, and claimed that their O_1 Campos vaccine lacked protection against the 2010 field strains from Ecuador.

The success in the control of FMD in South America has been achieved by biannual vaccination using oil vaccine manufactured with strains that were isolated long ago: A24 Cruzeiro/55, O1 Campos/58, and in some countries also C3 Indaial/71. This is an example of how stable the immune protection is, which is provided by old strains, despite the continuous generation of new genetic variants in the field. Such observation strongly suggests that good management practices and good quality vaccines are extremely important when used in periodic biannual vaccination campaigns.

5. Conclusion

This report provides a retrospective analysis of the last occurrence of FMD in Ecuador, the control actions implemented, and their results. It concludes that the application of vaccination campaigns and sanitary measures were effective in controlling the FMD situation in Ecuador, leading to a subsequent suppression of viral circulation throughout the country. In particular, the epidemiological data demonstrates the effectiveness of the use of the commercial vaccines formulated with regionally standardized strains and administrated in the framework of an eradication program strengthened under the concept of good practice. The experience described in this report proves how important it is to consider the laboratory results, together with field-epidemiological information, on the decision-making to choose the vaccine, particularly in cases of emergency.

Conflicts of Interest

None.

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