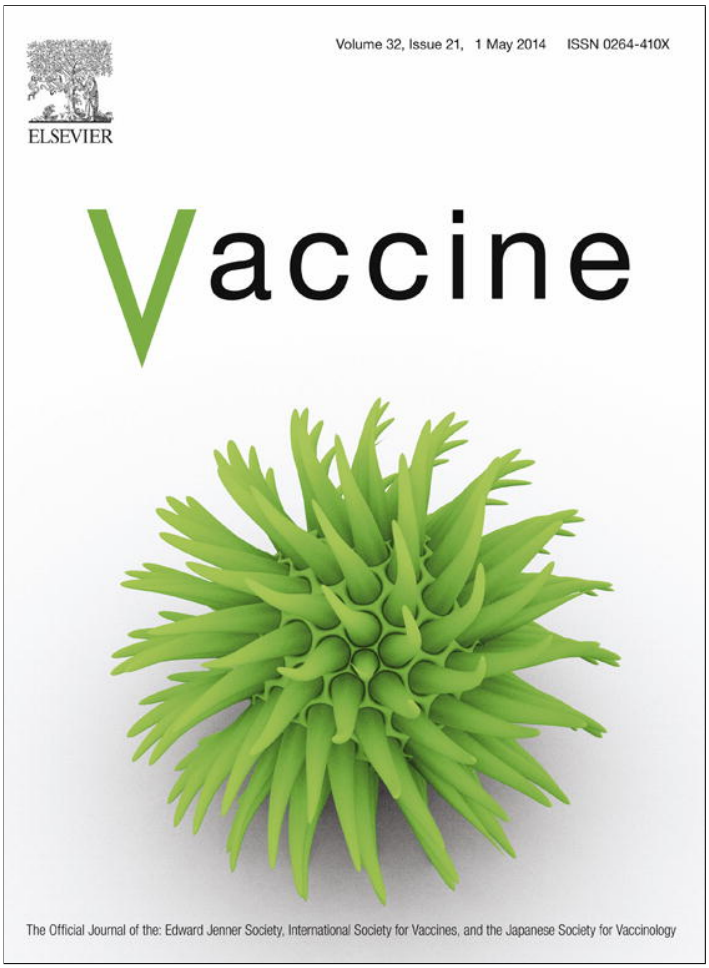

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Emergence of antigenic variants of Foot-and-Mouth Disease Virus serotype O in Ecuador and preliminary evaluation of a field strain as a vaccine candidate

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ABSTRACT

Foot-and-Mouth Disease Virus serotype O has been circulating regularly throughout most provinces of Ecuador, one of the two South American countries that still remain endemic, although satisfactory vaccination coverage was reported. This study concentrates in the characterization of isolates collected during 2008–2011, focusing particularly on the antigenic and immunogenic relationships of the field viruses with the O_1/Campos vaccine strain in use in the region and with an experimental vaccine formulated with a representative strain of the 2010 epidemic. The results established that antigenically divergent variants poorly protected by the vaccine in use emerged and co-circulated in a limited period of time. A monovalent vaccine formulated with the representative 2010 strain elicited high antibody titers and protected against challenge with homologous virus. In addition, cross-reactive antibodies to predominant viruses in the region were established. In overall this study indicates the ability of the virus to diversify under field conditions in which a vaccine strain with poor match is applied, and the potential of the selected 2010 field virus as a vaccine candidate for incorporation into strategic antigen banks and/or for addition to current formulations for systematic vaccination, in order to prevent the emergence of even more divergent isolates in the future.

1. Introduction

Foot-and-Mouth Disease (FMD) is a highly contagious and economically devastating vesicular disease of cattle and other cloven-hoofed animals [1,2], and severely constrains international trade of livestock and animal products. The causative agent, FMD virus (FMDV), belongs to the genus *Aphthovirus* within the *Picornaviridae* family [2]. It is present as seven immunologically distinct serotypes, O, A, C, Asia 1, South African Territories (SAT) 1, SAT 2 and SAT 3, in circulation worldwide and new variants arise continuously [2–4]. Only serotypes O, A and C have been recorded in South America. Infection or vaccination with one serotype does not cross-protect against other serotypes and may also fail to protect fully against other strains of the same serotype [5,6].

Inactivated vaccines are widely used to control, eradicate and prevent FMD [7,8]. Selection of vaccine strains that are as immunogenic and cross reactive as possible is essential not only for systematic vaccination programs but also for the incorporation to strategic FMDV inactivated frozen antigens for rapid formulation into vaccines for use in case of an emergency (i.e. antigen banks) [9]. These banks are important in FMD-free countries/zones without vaccination as well as in countries/zones where vaccination is practiced where storing antigens from strains different from those included in the vaccine in use should be considered.

In South America vaccines are formulated with selected strains harmonized for use in the region: O_1/Campos, A_{24}/Cruzeiro and most of the Southern Cone countries comprise also virus C_3/Indaial.

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The variant A/2001 is also included in vaccine formulations in Argentina [6]. In principle, these strains are able to give a satisfactory immunological coverage when systematic vaccination is applied. At present most of the countries/regions have their status recognized by the World Organization for Animal Health (OIE) as FMD-free either with or without vaccination. Endemic countries are restricted to Venezuela where FMD types O and A have been acting yearly and Ecuador where, since the year 2002, only FMD type O episodes have been reported [10,11].

During 2009 and 2010 frequent epidemics of FMDV were reported throughout most provinces of Ecuador. The reported control measures were mainly oriented to ring vaccination campaigns and control of animal movements, using a bivalent FMDV vaccine, containing both O_1/Campos and A_{24}/Cruzeiro strains. Nevertheless, the annual pattern of FMDV occurrence observed reflected low levels of herd immunity against the active circulating strains. The genetic and immunogenic characteristics of the circulating strains indicated the emergence of distinct viruses resistant to neutralization/protection [10].

In 2011 new epidemic waves occurred, which prompted the need to further characterize the field strains. This paper studies viruses acting during this epidemic and extends the studies to more isolates of the 2009–2010 epizooties and to a virus collected during episodes in the year 2008, focusing particularly on the antigenic and immunogenic/protective relationships of the field viruses with the O_1/Campos vaccine strain. Additionally, the effectiveness of an experimental vaccine prepared with a representative strain of the 2010 epidemic was evaluated.

2. Materials and methods

2.1. Virus strains

Samples were collected in the following Ecuadorian provinces (supplementary file): 11-2008: Esmeraldas; 39-2009 and 46-2010: Napo; 169-2009: Imbabura; 10-2010, 23-2010 and 31-2011: Tsáchila; 18-2011: Pichincha and 15-2011: Guayas. They were assayed directly from epithelium and/or after passages in baby hamster kidney (BHK-21) cells (clone 13). Vaccine strains O_1/Campos/Brazil/58 (O_1/Campos) used throughout this study and O/Paraguay/2011 belong to the Argentine National Service of Agrifood Health and Quality (SENASA) reference collection. Viruses 169-2009 and 46-2010 were representatives of the 2009 and 2010 epidemics, respectively [10].

2.2. Antigenic characterization

Antigenic profiling was performed with a panel of 20 monoclonal antibodies (MAbs) raised against FMDV strains O_1/Campos (1H10, 1B9-3, 17, G8, 2B3, 3H10), O_1/Caseros (3, 74, 69, 2-6F) and O/Taiwan (3A1, 3D1, 4B2, 1A11, 3A2, 1B3, 2D4, 1B9, 2C9, 3G10). Reactivity with reference strains and field isolates, established through a trapping ELISA, and coefficients of correlation of ELISA reactivity between samples were determined as described [12].

2.3. Determination and interpretation of r_1 values

The antigenic relatedness of the vaccine virus and field isolates was estimated according to the r_1 value: reciprocal serum titer against heterologous virus/reciprocal serum titer against homologous virus. Titers were obtained by two-dimensional virus neutralization (VN) assays [13,14]). A pool of five serum samples from cattle vaccinated with a monovalent vaccine containing O_1/Campos vaccine strain or 46-2010 virus (see Section 2.5), collected 27–30 days post vaccination (DPV) was used in the VN assay against the homologous and heterologous viruses. The interpretation of the results was as described [15]. r_1 values greater than 0.3 indicate that the field isolate is sufficiently similar to the vaccine strain and that the use of the vaccine is likely to confer protection against challenge with the field isolate.

2.4. Assessment of expectancy of protection (EPP)

EPP estimates the likelihood that vaccinated cattle would be protected against a challenge of 10,000 infective doses [14], and was obtained by reference to predetermined tables of correlation established for the O_1/Campos vaccine strain between clinical protection and serological titers, determined by liquid phase blocking competitive ELISA (lpELISA) [16] or VN [17]. Sera used in lpELISA or VN test were obtained from two groups of 16 cattle involved in *in vivo* trials, bled at 30 DPV with a full dose of an experimental vaccine O/Ecuador/46-2010 (see Section 2.6).

2.5. Vaccine formulation and potency assessment

FMDV 46-2010 strain was propagated in BHK-21 suspension cell cultures. Infected cell culture supernatants were collected, clarified and inactivated twice with binary ethyleneimine [18]. Inactivated supernatants were concentrated and partially purified by ultrafiltration. Vaccines were prepared as single water-in-oil emulsions consisting of 60% oil phase (mineral oil and emulsifier) and 40% aqueous phase (inactivated antigen) [19]. Production of FMDV antigen and vaccine formulation was performed at SENASA facilities either in the high containment or clean areas for the work with infective virus or with inactivated antigen, respectively. Vaccine efficacy was evaluated at 30 DPV by live virus challenge (see Section 2.6), lpELISA/EPP and VN/EPP.

2.6. Protection against Podal Generalization (PPG) test

PPG trials were carried out as described [20]. Briefly, Hereford breed cattle, aged 18–24 months and free from FMDV antibodies, were used for the trials. They belonged to the FMD-free zone in Argentina, the South Patagonia Region, where vaccination is not practiced. A full dose of the experimental vaccine containing a total antigenic mass of 20 μg of 146S of the 46-2010 strain was used. After vaccination, animals remained in isolated premises until challenge, which was carried out in animal facilities under biosecurity conditions. Challenge was performed by inoculation of 10,000 suckling mouse lethal dose 50% (SMLD 50%) by the intradermolingual route. Two unvaccinated cattle were included in the trial as controls. Seven days after challenge, the animals were examined for podal lesions of FMD. Animals were considered unprotected when typical FMD lesions developed at least in one foot. All the unvaccinated control animals showed podal lesions caused by the disease. A vaccine batch is approved for licensing if at least 12 out of the 16 animals are found to be protected [16].

3. Results

3.1. Antigenic characterization

ELISA assay typed the 2008–2011 isolates as serotype O. Further characterization was performed by testing the reactivity by ELISA of the three FMDV field strains collected in 2011 and the sample recovered in 2008 against a panel of 20 MAbs. Patterns obtained were compared with the profile of the prototype vaccine strain O_1/Campos and with those patterns registered for the 29 viruses collected during the 2009–2010 epidemics, which were encompassed in two main groups: group 1 included the 19 viruses active in the year 2009 and the first two viruses isolated in 2010 (2009

pattern); group 2 comprised the other 8 viruses recovered in the year 2010 (2010 pattern) [10].

Through the analysis of the MAbs profiling (Fig. 1) and the individual coefficient of correlation values (Table 1) it was possible to establish the co-circulation in the year 2011 of viruses with three different MAb reactivity patterns with poor relatedness with the vaccine strain O_1/Campos (Fig. 1A). Two profiles were quite related to the 2009 and 2010 patterns, and the third one was rather unique. Profile of virus 31-2011 was similar to the 2009 pattern, with a coefficient of correlation close to 1 with respect to its representative virus (169-2009). This pattern was already present at least since 2008, as revealed by sample 11-2008 (coefficient of correlation of 0.97 with the 169-2009 virus). The profile of virus 18-2011 was more associated to the 2010 pattern revealing against its representative strain (46-2010) a coefficient of correlation of 0.72. Virus 15-2011 revealed a rather distinctive profile with a coefficient of correlation close to 0.5 when compared against O_1/Campos and the 2009 pattern, and of 0.25 when evaluated against the 2010 pattern. Among the three viruses circulating in the year 2011, coefficients of correlation ranged from 0.50 to 0.60. When compared with the O_1/Campos virus, they exhibited values ranging between 0.29 and 0.55.

The analysis of the reactivity with the individual MAbs included in the panel established clear-cut differences between Ecuadorian isolates and the vaccine strain O_1/Campos. As reported for the 2009–2010 viruses [10], whereas the reference strain O_1/Campos had a high level of reactivity with MAbs 1H10, 17, G8 and 74, the last three of them having the capacity to *in vitro* neutralize the strain of origin, viruses 11-2008 and 31-2011, as well as virus 18-2011, corresponding respectively to the 2009 and 2010 patterns, showed no reactivity with those MAbs (Fig. 1B and C). Likewise, strain 15-2011 showed no reactivity with MAbs 1H10 and G8, but maintained the reactivity with MAbs 17 and 74 (Fig. 1D). Additionally this strain lost reactivity with MAbs 3 and 1B9-3. Samples 11-2008 and 31-2011 reacted identically to the 2009 representative virus with the individual MAbs, except that the former isolate presented an additional decline in reactivity with MAb 1B9-3. Sample 18-2011 showed a profile similar to the 2010 pattern, but with an augmented deviation with respect to the vaccine strain, as it failed to react with MAbs 3 and 1B9-3, in addition to the 8 that had been already lost in the 2010 pattern (coefficients of correlation with the O_1/Campos of 0.47 and 0.29 for strains 46-2010 and 18-2011, respectively). Strain 15-2011, which presented a distinctive pattern, registered differences in reactivity with 4 or 8 MAbs when compared to the 2009 and 2010 patterns, respectively.

3.2. r_1 values of O_1/Campos vaccinated animals

Table 2 shows the antigenic relatedness of field isolates against the vaccine strain (r_1 values) assessed by VN test with sera from animals vaccinated with the O_1/Campos vaccine strain. Studies were carried out with a pool of five medium to high titer sera, as recommended [21]. These sera were confronted with the O_1/Campos vaccine strain, and with the virus isolates collected between the years 2008 and 2011.

Average neutralization titer with the homologous virus O_1/Campos was 2.31, while for the Ecuadorian viruses average values were as low as 0.93 (for virus 46-2010) with a maximum titer of 1.77 for sample 11-2008. Values for r_1 were calculated for each individual test. Average values were all below the 0.3 cut off, with values ranging from 0.06 to 0.22, indicative of low level of neutralization of the variants prevalent in the field by the vaccine strain O_1/Campos. In overall the results indicated a low degree of relatedness between the O_1/Campos vaccine strain in use and the field viruses, suggesting that the vaccine strain is unlikely to effectively protect against the field isolates.

3.3. Experimental vaccine O/Ecuador/46-2010

Considering the poor matching of the currently in use vaccine containing the O_1/Campos virus with the 2008–2011 Ecuadorian isolates, and the lack of *in vivo* protection observed even after revaccination for strain 46-2010 [10], a vaccine containing the latter isolate, representative of the 2010 epidemics, was developed. The 46-2010 strain was adapted to grow in BHK-21 suspension cultures proving to give high infectious titer, adequate inactivation rate and high yield of 146S particles.

Protection data (Table 3) of the O/Ecuador/46-2010 vaccinated animals showed that of the 16 cattle vaccinated with one dose of the experimental vaccine, 14 were protected (87.5%) against challenge with virus 46-2010 (cattle 290–305), but only two (12.5%) of 16 animals (cattle 306–321) were protected when challenged with the O_1/Campos strain. Comparable conclusions can be drawn by indirect tests such as lpELISA/EPP and VN/EPP when confronted with the homologous 46-2010 or with the O_1/Campos strains. As can be seen lpELISA/EPP tested against the homologous virus reached values of 86.9% and 85.9% when assessed with the sera collected prior to challenge with the 46-2010 and O_1/Campos challenged groups, respectively, and in that same order VN/EPP were 88.84% and 85.69%. In contrast, when the sera were confronted with the O_1/Campos virus, lpELISA/EPP and VN/EPP gave values indicative of poor protection: 53.9% and 60.92%, respectively.

3.4. Antigenic spectrum of 46-2010 strain

In vitro vaccine matching studies were carried out in order to infer to what extent the vaccine strain 46-2010 was able to protect the Ecuadorian field isolates and relevant heterologous strains. The neutralizing titer and r_1 values obtained with a pool of five sera from animals vaccinated with the O/Ecuador/46-2010 vaccine when confronted with the homologous and heterologous viruses are shown in Table 4.

Average VN titer with the homologous virus 46-2010 was 2.92 and for most Ecuadorian viruses, values were in overall above 2.40, rendering relatively high r_1 values for most isolates, indicative of appropriate protection. Only isolate 15-2011 recorded an average titer of 1.99 (r_1: 0.16). Values obtained for the O_1/Campos and for a representative virus of the Southern Cone emergencies (O/Paraguay/2011), reached titers of 1.76 and 1.73, respectively, resulting in r_1 of 0.08.

4. Discussion

Awareness of the strains prevailing, their distribution/evolution and particularly, assessment of the probable efficacy of the vaccine strain in use to control the disease is of utmost importance. Appropriate vaccine strain replacement/inclusion is an important element for the application of vaccination programs in FMD-affected areas, as well as for the establishment and maintenance of vaccine antigen reserves to be used in the event of new incursions.

This study establishes the antigenic characteristics of viruses acting during 2008–2011 in Ecuador extending previous characterization of viruses circulating in 2009–2010 [10], and explores the effectiveness of an experimental vaccine containing a representative virus of the 2010 epidemic.

Sample characterization established three distinct antigenic variants co-circulating during 2011, quite different from the vaccine strain (r_1 values below 0.3), suggesting that the generation of variants responded to the lack of immunological pressure. This field situation points to poor immunity, despite having reported over 90% coverage during the 6-month vaccination cycles. Although there could be several explanations to the apparent vaccine failure,

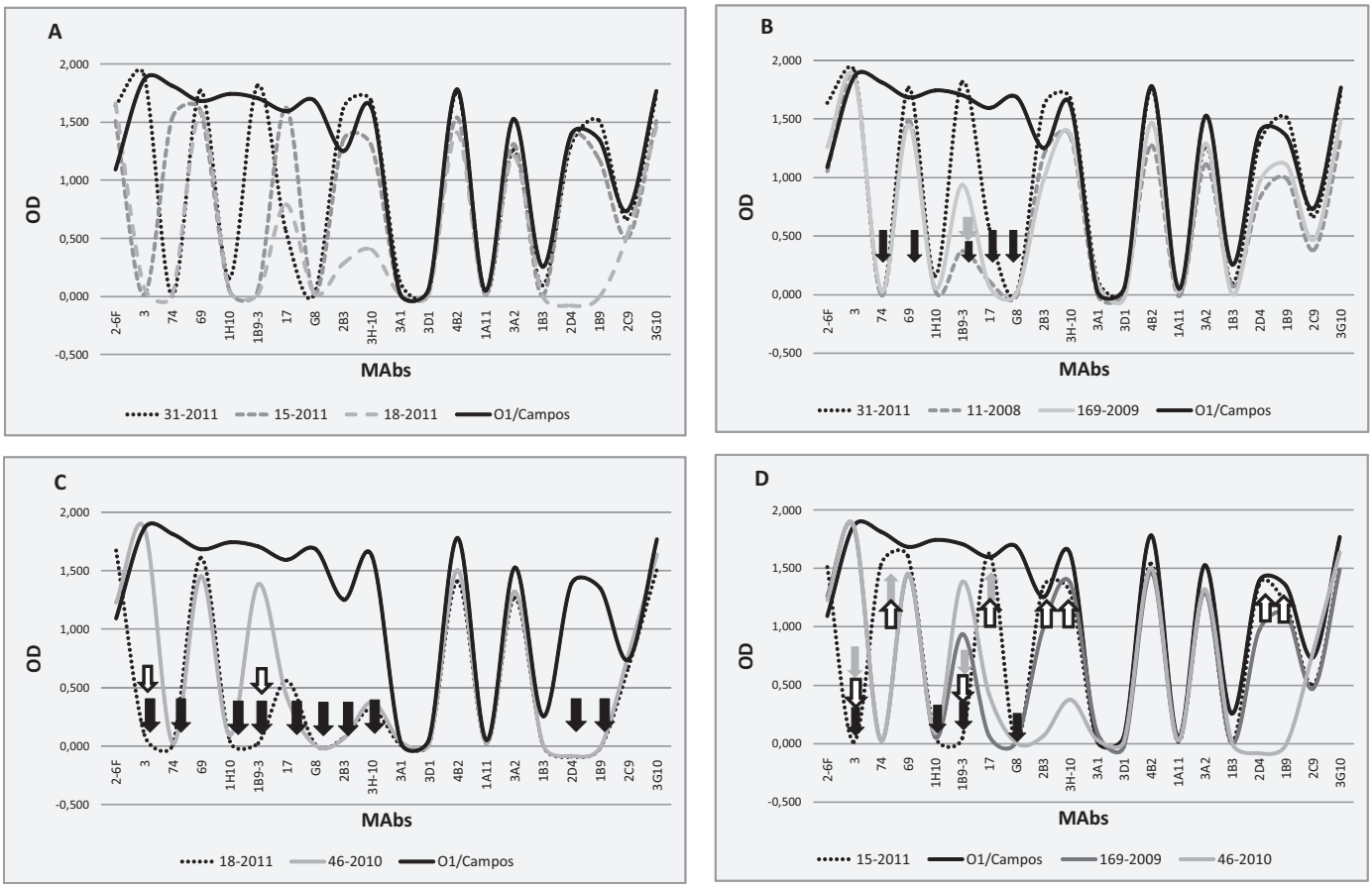

Fig. 1. MAbs profiling of field isolates from the 2008 and 2011 outbreaks in Ecuador. Field samples were analyzed by trapping ELISA using a panel of 20 MAbs indicated on the *x*-axis. A blank with no virus was included in each test. OD values obtained with each MAb after subtracting their corresponding blank were plotted. (A) Profiles of the 2011 viruses and the O_1/Campos vaccine strain. (B) Comparative profiles of viruses 31-2011 and 11-2008 with the O_1/Campos vaccine strain and the 2009 pattern. (C) Comparative profile of virus 18-2011 with the O_1/Campos vaccine strain and the 2010 pattern. (D) Comparative profile of virus 15-2011 with the O_1/Campos vaccine strain and the 2009 and 2010 patterns. Differences in reactivity of the 2008 and 2011 viruses with the vaccine strain (black arrows); with the 2009 pattern (gray arrows); and with the 2010 pattern (empty arrows) are depicted. Lost or augmented reactivity is illustrated by arrows facing downward or upward, respectively.

Table 1
Coefficient of correlation of the MAb reactivity values between the indicated viruses (see Fig. 1).

	O_1/Campos	169-2009	46-2010	11-2008	15-2011	18-2011	31-2011
O_1/Campos	1.00	0.54	0.47	0.51	0.49	0.29	0.55
169-2009	–	1.00	0.76	0.97	0.48	0.56	0.96
46-2010	–	–	1.00	0.68	0.25	0.72	0.71
11-2008	–	–	–	1.00	0.51	0.53	0.91
15-2011	–	–	–	–	1.00	0.60	0.51
18-2011	–	–	–	–	–	1.00	0.50
31-2011	–	–	–	–	–	–	1.00

one possibility is that systematic vaccination in endemic regions with a vaccine strain which matches poorly with the field virus may not be sufficient to confer protection, reinforced by the rapid waning of immunity which could occur with poorly matched viruses [22]. Such situation could be underestimated, since there are no studies on population immunity confronting the sera from vaccinated animals against heterologous viruses, which could give input to the risk imposed by applying vaccines with strains that poorly matched with the field strains. The emergence of antigenically distinct viruses escaping neutralization under sub-neutralizing conditions has been previously reported [23].

The results together with the field circumstances supported the need to study the ability of a new vaccine virus to improve the protection conferred by the vaccine. In fact, updating of vaccine strains is important in order to achieve levels of protection which can impair the selection of new variants resistant to neutralization. In addition, in endemic areas where vaccination is practiced, younger animals are more susceptible to infection, especially around weaning when maternal protection wears away, strengthening the need to apply vaccines that better match the field strain.

We selected strain 46-2010 for vaccine candidate based on the biological characteristics and the limited relationship to the vaccine strain, which demonstrated very low protection even after revaccination [10]. Moreover it belongs to the genetic lineage with the highest evolutionary fitness recorded for the viruses that circulated in Ecuador since 2002 [10,24].

Satisfactory protection against challenge with the homologous virus was attained with the monovalent O/Ecuador/46-2010 vaccine. Moreover, all Ecuadorian strains studied were expected to be protected, except for virus 15-2011. It should be mentioned that this virus shows a distinctive MAb profile, which is rather distant from the 2010 pattern, and belongs to a unique genetic lineage

Table 2
VN titers and their corresponding r_1 values using FMDV O_1/Campos vaccination.

HV	O_1/Campos sera									Av. r_1
	Test 1			Test 2			Test 3			
	VN titer			VN titer			VN titer			
	O_1/C	HV	r_1	O_1/C	HV	r_1	O_1/C	HV	r_1	
11-2008	2.63	1.74	0.13	2.46	1.83	0.23	2.44	1.74	0.2	**0.19**
39-2009	2.25	1.54	0.19	2.29	1.57	0.19	2.34	1.78	0.28	**0.22**
169-2009	2.32	1.49	0.15	2.41	1.5	0.12				**0.14**
10-2010	2.34	1.52	0.15	2.25	1.42	0.15	2.29	1.55	0.18	**0.16**
23-2010	2.31	1.14	0.07	2.32	1.03	0.05				**0.06**
46-2010	2.17	0.87	0.05	2.15	0.94	0.06	2.18	0.98	0.06	**0.06**
15-2011	2.32	1.5	0.15	2.41	1.22	0.06				**0.11**
18-2011	2.41	0.85	0.03	2.46	1.39	0.09	2.18	1.29	0.13	**0.08**
31-2011	1.91	0.96	0.11	2.46	1.28	0.07	2.18	1.32	0.14	**0.11**
O/San Pedro/Par/11										**0.13**[a]

HV, heterologous virus; O_1/C, O_1/Campos; Av, average of the different assays.
[a] Published in Maradei et al. [25].

Table 3
Evaluation of O/Ecuador/46-2010 vaccine.

Challenged with 46-2010 virus					Challenged with O_1/Campos virus							
Bovine	PPG	Antigen 46-2010			Bovine	PGP	Antigen O_1/Campos			Antigen 46-2010		
		lpELISA titer	VN titer	VN/EPP(%)			lpELISA titer	VN titer	VN/EPP(%)	lpELISA titer	VN titer	VN/EPP(%)
290	P	3.6	2.68	98.91	306	NP	1.46	1.53	67.84	1.83	2.2	94.9
291	P	1.84	1.87	86.63	307	NP	1.49	1.26	45.58	2.01	1.92	88.08
292	P	2.87	1.85	85.84	308	NP	1.79	1.66	75.81	2.54	2.25	95.78
293	P	2.59	2.46	97.77	309	NP	1.38	1.26	45.58	1.62	1.35	53.81
294	P	2.8	2.43	97.62	310	NP	1.73	1.34	52.17	2.46	2.04	91.65
295	P	2.7	2.3	96.28	311	NP	1.4	1.26	45.58	1.94	1.57	70.65
296	P	1.76	1.83	85.02	312	NP	1.81	1.28	47.22	2.8	2.25	95.78
297	P	2.02	1.78	82.32	313	NP	1.89	1.8	83.26	2.49	2.32	96.51
298	P	2.94	2.45	97.77	314	NP	1.44	1.28	47.22	1.73	1.98	90.01
299	P	2.02	2.13	93.86	315	NP	1.38	1.26	45.58	1.51	1.31	50.52
300	P	2.71	1.8	83.26	316	NP	1.81	1.43	60.27	2.62	1.94	88.76
301	P	2.98	2.24	95.5	317	P	3.37	2.04	91.65	3.6	2.46	97.77
302	P	2.12	1.83	85.02	318	NP	1.67	1.35	53.81	2.33	1.66	75.81
303	NP	1.1	1.6	72	319	P	2.74	2.17	94.57	3.6	2.46	97.77
304	NP	2	1.59	72	320	NP	1.62	1.52	66.39	2.49	2.14	93.86
305	P	2.18	2.04	91.65	321	NP	1.46	1.33	52.17	2.11	1.96	89.4
Mean titer		2.39	2.06				1.78	1.49		2.36	1.99	
EPP (%)		86.9[a]		88.84[b]			53.9[a]		60.92[b]	85.9[a]		85.69[b]

P, protected; NP, non-protected.
[a] The lpELISA/EPP was calculated from the average lpELISA titers, according to the Argentine Animal Health Service (SENASA) Resolution No. 351/06 [26].
[b] The mean VN/EPP was calculated from the VN/EPPs obtained for each individual serum [17].
An EPP ≥ 75% (lpELISA titer ≥ 2.11 and VN titer ≥ 1.65) is an indication that the vaccines will protect against the homologous vaccine strain [27].

Table 4
VN titers and their corresponding r_1 values using FMDV O/Ecuador/46-2010 vaccination.

HV	O/Ecuador/46-2010 sera									Av. r_1
	Test 1			Test 2			Test 3			
	VN titer			VN titer			VN titer			
	46-2010	HV	r_1	46-2010	HV	r_1	46-2010	HV	r_1	
O1/C	2.87	1.78	0.08	2.84	1.9	0.11	2.9	1.6	0.05	**0.08**
11-2008	3.11	2.91	0.63	2.94	2.97	1.07				**0.85**
169-2009	2.85	2.71	0.72	2.89	2.57	0.48				**0.6**
10-2010	3.11	2.73	0.42	2.73	2.53	0.63				**0.52**
23-2010	3.03	3.11	1.20	2.98	2.97	0.98				**1.09**
15-2011	2.81	1.66	0.07	3.11	2.11	0.1	2.73	2.21	0.3	**0.16**
18-2011	3.00	2.96	0.91	2.9	2.8	0.79				**0.85**
31-2011	2.94	2.42	0.3	3.00	2.41	0.26	2.81	2.32	0.32	**0.29**
O/Paraguay/2011	3.11	1.69	0.04	2.73	1.77	0.11				**0.08**

HV, heterologous virus; Av, average.

with poor field presence at least for the past 10 years, with only one isolate recovered in 2005 (unpublished results).

It is expected that the inclusion of this new vaccine virus, antigenically close to the predominant circulating variants, could restrict the emergence of even more divergent isolates in the future. In fact, the replacement or inclusion of new variants in vaccine formulations has been previously documented. For example, during the emergencies of serotype A viruses which occurred in already free regions of the Southern Cone of South America during 2000–2001, Argentina included type A 2001 in their vaccines for emergency vaccination, which helped attain a rapid control of the disease. This strain is still present in the vaccines used in this country and was included in international vaccine banks [6].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.02.092.

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