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Efficacy of a high quality O_1/Campos foot-and-mouth disease vaccine upon challenge with a heterologous Korean O Mya98 lineage virus in pigs

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ABSTRACT

In 2010 serotype O foot-and-mouth disease virus of the Mya98 lineage/SEA topotype spread into most East Asian countries. During 2010–2011 it was responsible for major outbreaks in the Republic of Korea where a monovalent O/Manisa vaccine (belonging to the ME-SA topotype) was applied to help control the outbreaks. Subsequently, all susceptible animals were vaccinated every 6 months with a vaccine containing the O/Manisa antigen. Despite vaccination, the disease re-occurred in 2014 and afterwards almost annually. This study focuses on the *in vivo* efficacy in pigs of a high quality monovalent commercial O_1/Campos vaccine against heterologous challenge with a representative 2015 isolate from the Jincheon Province of the Republic of Korea. Initially, viral characterizations and r_1 determinations were performed on six viruses recovered in that region during 2014–2015, centering on their relationship with the well characterized and widely available O_1/Campos vaccine strain. Genetic and antigenic analysis indicated a close similarity among 2014–2015 Korean isolates and with the previous 2010 virus, with distinct differences with the O_1/Campos strain. Virus neutralisation tests using O_1/Campos cattle and pig post vaccination sera and recent Korean outbreak viruses predicted acceptable cross-protection after a single vaccination, as indicated by r_1 values, and in pigs, by expectancy of protection. In agreement with the *in vitro* estimates, *in vivo* challenge with a selected field isolate indicated that O_1/Campos primo vaccinated pigs were protected, resulting in a PD50 value of nearly 10. The results indicated that good quality oil vaccines containing the O_1/Campos strain can successfully be used against isolates belonging to the O Mya98/SEA topotype.

1. Introduction

Foot-and-Mouth Disease (FMD) is a highly transmissible and economically devastating vesicular disease of cloven-hoofed animals [1,2]. Its presence severely constrains international trade of livestock and animal products and poses a constant threat to FMD-free countries. The causative agent, FMD virus (FMDV), belongs to the genus Aphthovirus within the Picornaviridae family [2].

There are seven immunologically distinct serotypes, and new variants arise continuously [3–4] that are grouped in intratypic genetic lineages within topotypes. Infection or vaccination with one serotype does not cross-protect against the other serotypes and may fail to protect fully against some strains within serotypes [5,6].

Inactivated vaccines are widely used to control, eradicate and prevent FMD [7,8]. Historically, serotype O vaccine strains can be included within two main groups. One represented by the South American strain O_1/Campos, selected and harmonized for use in the region, as well as by the related viruses: O/Lausanne, OBFS/1860 (UK1967) and O/Kaufbeuren, which were widely used in Europe. The second group, represented by the O/Manisa strain,

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was used mainly in Middle East and Asia, as well as in North and South Africa [9]. However, the vaccine has not provided effective protection against recent viruses from the Middle East [10], requiring the testing of alternative vaccine strains. In this regard, preliminary studies were addressed with some candidate vaccine strains, establishing antigenic correlations through r_1 values [11].

In 2010 a FMDV serotype O, Southeast Asia (SEA) topotype/Myanmar 98 (Mya98) lineage, endemic in Southeast Asia, expanded into most eastern Asian countries [12,13]. In 2010 major outbreaks occurred in the Republic of Korea where the culling of hundreds of thousands of pigs took place [14,15]. A monovalent O/Manisa vaccine was applied to assist in controlling the episode. Subsequently, to help prevent recurrence of the disease, it was mandatory to vaccinate all susceptible animals twice a year with trivalent vaccines containing O/Manisa, A Malaysia 97, and Asia 1 Shamir viruses [16,17].

Despite nationwide immunization, the Mya98 lineage of serotype O reappeared in 2014 and afterwards almost every year [17]. These recurrences revived the controversy over the efficacy of the O/Manisa vaccine [11,15,16], backed by the low or moderate serological relationship between O/Manisa and Korean 2010 stains described in some reports (r_1 value of approximately 0.3) [15,16]. It also reinforced the need to search for an alternative vaccine strain, preferably a well-established and well-characterized strain with a broad antigenic spectrum.

The antigen derived from O_1/Campos strain blended in oil adjuvanted vaccines gave effective and broad immunological coverage against South American strains [18,19]. In addition, the strain was successfully used to assist in controlling a widespread epidemic in pigs caused by serotype O in Taiwan in 1997 [20,21].

In a previous *in vitro* study we suggested that good quality vaccines containing the O_1/Campos strain can be used against representative viruses of three currently circulating topotypes in Asia (SEA, ME-SA and CATHAY), including a 2010 Korean isolate belonging to the SEA topotype, Mya98 lineage [22]. The results supported the application of O_1/Campos vaccines in emergency vaccination programs in pigs in the Republic of Korea, since 2016.

This study extends to the 2014-2015 O/Mya98 Korean viruses the previous *in vitro* assessments and confirms the accuracy of such predictions by an *in vivo* vaccination and challenge study in pigs with a representative 2015 Korean isolate.

2. Materials and methods

2.1. Cell lines and FMDV strains

Baby hamster kidney (BHK)-21 cells were used for all virus related work. O_1/Campos South American vaccine virus was provided by SENASA. Korean isolates were received from the Animal and Plant Quarantine Agency in the Republic of Korea (APQA). Viruses O/SKR/02 D1-2, O/SKR/02 D6-2, O/SKR/02 D11-1, were isolated in the year 2014 and virus O/SKR/84 YDM in 2015 from pig feet tissue and viruses O/SKR/71 GHW, O/SKR/35 LYC in the year 2015 from cattle tongue. For serological assays, viruses were amplified in cell monolayers, clarified and stored at -70 °C. Dulbeccós modified minimal essential medium without serum was used for cell infection. All field viruses were passaged seven times to reach titres around 10^7 $TCID_{50}$.

2.2. Genetic characterization: Phylogenetic analysis

RNA extraction, amplification and sequencing conditions to determine the sequence of the complete VP1-coding region of the isolates were as described [23]. Phylogenetic analysis was performed using the program MEGA, version 7.0 [24], applying the General Time Reversible evolutionary model to construct unrooted trees, with evolutionary distances calculated using the Kimura two-parameter method and a bootstrap resampling analysis performed with 1000 replicates.

2.3. Antigenic characterization

Monoclonal antibody profiling, was determined through a trapping ELISA using a panel of 21 monoclonal antibodies (MAbs), characterized as described [25]. MAbs were raised against FMDV strains O_1/Campos (1H10, 1B9-3, 17, 2B3, 3H10), O_1/Caseros (8G, 3, 74, 69, 2-6F) and O/Taiwan (3A1, 3D1, 4B2, 1A11, 3A2, 2F8, 1B3, 2D4, 1B9, 2C9, 3G10). To obtain a relationship between viruses, coefficient of correlation (cc) of their MAb reactivity values was calculated by applying a linear regression to fit the best straight line. cc values = 1 correspond to identical profiles; cc values close to 0 indicate totally dissimilar antigenic profiles [25].

2.4. In vitro vaccine matching studies

2.4.1. r_1 determination

Virus neutralization (VN) titers against the homologous O_1/Campos vaccine strain and the heterologous field viruses were obtained by two-dimensional assays performed as described in the World Organization for Animal Health (OIE) Manual [26] using pools of five medium to high titer serum samples collected from cattle or pigs vaccinated with a 2 ml dose of O_1/Campos monovalent vaccine. The r_1 values were calculated as the reciprocal serum titer against heterologous virus/reciprocal serum titer against homologous vaccine virus.

2.4.2. Expectancy of protection (EPP)

EPP estimates the likelihood of protection by correlating VN antibody titers in vaccinated animals with clinical protection against challenge with 10,000 infective doses, based on predetermined tables established in cattle for the O_1/Campos vaccine strain [27]. Titers were expressed as $\log_{10}$ of the reciprocal sera dilution. Four different commercial monovalent vaccine batches were assessed using groups of 10 pigs for each batch tested.

2.5. Vaccine formulation and approval

The commercial vaccines (Aftogen Oleo®) were produced by Biogénesis Bagó (Argentina) according to good manufacturing practices as single-in-oil emulsion vaccines (PD50 > 6) using O_1/Campos purified antigen [28]. They were approved by SENASA (Argentine Animal Health Authorities) for safety, purity and potency in swine following local [29,30], OIE [26], European Pharmacopeia (Ph.Eur.) [31], and specific Korean standards before release to the market. The batches were also approved by APQA (Animal and Plant Quarentine Agency) and applied during the 2016 vaccination program in Korea.

2.6. In vivo challenge test

2.6.1. Challenge virus stock production

The work was performed according to the Argentine Animal Ethics Code in the animal facility of SENASA, Argentina. BHK adapted O/SKR/84 YDM virus was passaged twice by intradermal inoculation of $10^{6.8}$ TCID50/ml in the heel bulb of each mayor digit of the left forefoot in 2-month-old seronegative Landrace x Large White pigs (approximately 30 kg) obtained from a commercial farm. Epithelia recovered from the second passage were mortared in DMEM (proportion 1:5), clarified by centrifugation, aliquoted and stored at −80 °C. Aliquots were further titrated.

Fig. 1. Phylogenetic tree showing the genetic relationships of FMDV type O 2014–2015 isolates in Korea. Maximum Likelihood tree was constructed computing the evolutionary distances by the Kimura-two (K-2) parameter model, based on the comparison of the complete region coding for VP1 protein, using the Mega program, version 7.0. Sequences obtained in this work are indicated, together with their accession numbers (▲). Viruses used for comparisons were obtained from GenBank. Relevant lineages and topotypes are indicated with brackets. For reasons of clarity non related topotypes or clades including geographical and chronologically related isolates are shown as compressed branches (filled triangles). A distance of 5% is depicted by the scale.

2.6.2. Pig immunization and challenge

Two-month healthy Landrace x Large White pigs were used. Prior to the study, absence of anti-FMDV antibodies was confirmed by lpELISA, VN [26] and I-ELISA3ABC/EITB [32].

Vaccination and challenge were carried out following the scheme recommended for cattle in the Ph.Eur. [31]. Three groups of six pigs each were distributed separately. Twenty-eight days prior to challenge, pigs were vaccinated intramuscularly in the neck region above the left shoulder with: 2 ml (full dose), 0.5 ml (1/4 dose) or 0.125 ml (1/16 dose). Two unvaccinated pigs were included as controls. Seventy-two hours before challenge they were transferred to OIE level 4 animal facilities. Unvaccinated controls and the three vaccinated groups were kept in two separate rooms with independent air handling facility. The vaccinated groups had no direct contact among them and were provided with separate feed and water troughs. Sedated pigs were treated with local anesthesia and inoculated intradermally in 2 sites in the plantar heel bulb of each major digit of each right foot with 10,000 ID in a total volume of 0.2 ml.

Animals were observed for clinical disease daily for 8 days, and those showing lesions at sites other than the inoculation sites were considered as non-protected. The PD50 values were calculated by the Spearman-Kärber method [33].

3. Results

3.1. Genetic characterization: Phylogenetic analysis

Results from phylogenetic analysis of the complete VP1 gene sequence (Fig. 1) showed that the 2014–2015 Korean isolates belonged to the Mya98 lineage within the SEA topotype. All isolates, independently from the host species from which they were recovered (pigs or cattle) were genetically almost identical, showing homology over 99% among them. When compared to previously reported viruses, they showed the closest relationship with one isolate collected in 2014 and three isolates collected in 2016 in the Republic of Korea, with which they recorded 99% and 98% homology, respectively. The 2014–2015 Korean isolates clustered within a group containing isolates responsible for outbreaks/emergencies recorded in 2010 in Hong Kong, Japan, China, Russia and Republic of Korea, with which they present approximately 95–96% homology. Compared with the strain O_1/Campos the field isolates showed nucleotide differences of approximately 25%, of which 21% were reflected in amino acid changes, representing distances of 17% on average.

3.2. Antigenic characterization

Antigenic relatedness between viruses was established by a rapid and sensitive approach based on the comparative analysis of ELISA reactivity profiles of the viruses with 21 MAbs (Fig. 2) and the cc values derived therefrom (Table 1). The results indicated almost identical patterns among the different 2014–2015 isolates, with cc values close to 1, and a close relationship between them and the 2010 variant recording cc values of approximately 0.8. Average cc values for O_1/Campos vaccine virus when compared to the 2014–2015 Korean isolates were approximately 0.6.

Analysis of reactivity with individual MAbs established clear differences with O_1/Campos. Whereas O_1/Campos showed reactivity with MAbs 1H10, 1B9, 8G, and 2-6F, the Korean 2014–2015 isolates reacted poorly, as did the 2010 isolate. The reactivity with MAb 3 that had been lost in the 2010 virus was recovered in the 2014–2015 isolates. Reactivity with MAbs 17 and 74, known to have the capacity to in vitro neutralize the strain of origin O_1/Campos, was preserved in all the Korean viruses.

The resulting profiles derived from the reactivities with the 11 MAbs produced against the virus O/Taiwan/97 were rather homogeneous between the O_1/Campos and the Korean viruses. Particularly, the weak reactivity of the O_1/Campos virus observed for

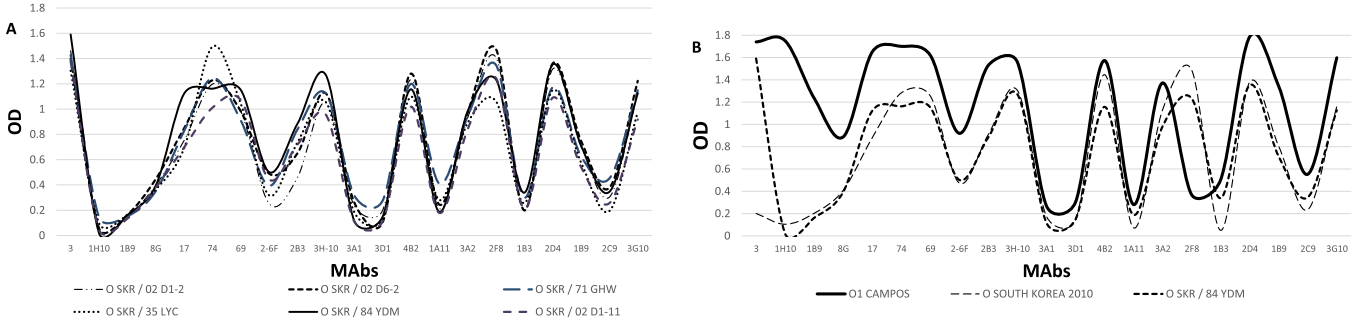

Fig. 2. MAbs profiling of Korean isolates. Korean amples were analyzed by trapping ELISA using a panel of 21 MAbs raised against FMDV strains O_1/Campos (1H10, 1B9-3, 17, 2B3, 3H10), O_1/Caseros (8G, 3, 74, 69, 2-6F) and O/Taiwan (3A1, 3D1, 4B2, 1A11, 3A2, 2F8, 1B3, 2D4, 1B9, 2C9, 3G10). A blank with no virus was included in each test. The OD values obtained with each of the MAbs after subtracting their corresponding blank were plotted. A. Comparative profiles among the six 2014–2015 Korean isolates. B. Comparative profiles between: a representative 2014–2015 Korean virus, a 2010 Korean isolate and the vaccine strain O_1/Campos.

Table 1
Coefficient of correlation of the MAbs reactivity values between the indicated viruses.

	O_1/CAMPOS	O SKR/02 D1-2	O SKR/02 D6-2	O SKR/71 GHW	O SKR/35 LYC	O SKR/84 YDM	O SKR/02 D1-11	O SOUTH KOREA 2010
O_1/CAMPOS	–	0.52	0.53	0.51	0.61	0.62	0.56	0.50
O SKR/02 D1-2		–	0.99	0.97	0.95	0.95	0.97	0.80
O SKR/02 D6-2			–	0.98	0.95	0.96	0.98	0.84
O SKR/71 GHW				–	0.96	0.96	0.97	0.79
O SKR/35 LYC					–	0.95	0.96	0.81
O SKR/84 YDM						–	0.98	0.79
O SKR/02 D1-11							–	0.79
O SOUTH KOREA 2010								–

MAbs 3A1, 3D1, 1A11, 2F8 and 2C9, was also recorded for the 2010 and 2014–2015 Korean isolates, except for MAb 2F8 which showed considerable reactivity.

3.3. In vitro vaccine matching studies

3.3.1. r_1 determination

To infer to what extent the vaccine strain O_1/Campos was able to cross-protect the Korean isolates, r_1 values were calculated. Considering that these viruses were infectious in pigs, r_1 determinations included a pool of sera from vaccinated pigs. Average neutralization titers with the homologous virus O_1/Campos was 2 and 2.4 for the pool of pig and cattle sera, respectively, while for the Korean viruses they ranged between 1.9 and 2.2 for pig and 1.9 to 2.3 for cattle (Fig. 3). Average r_1 values were all above 0.75 and 0.4 for pigs and cattle, respectively, which is higher than the 0.3 cut off, indicating that the O_1/Campos strain is likely to effectively protect against challenge with Korean isolates.

3.3.2. Expectancy of protection

The protective capacity of the vaccine strain was further assessed by EPP, selecting the last virus collected in 2015 from pigs, O/SKR/84 YDM. Four different commercial monovalent vaccine batches were assessed using groups of 10 pigs for each batch tested and results of average VN titers for each batch are given in Table 2. Prior to vaccination all animals were seronegative. Average VN titers against the homologous virus at 21 and 28 DPV reached values $\geq$1.84 and $\geq$1.89, respectively, corresponding to EPP values of at least 85% and 87%. When sera were tested with the heterologous O/SKR/84 YDM virus, average VN titers for the 4 batches were 2.0, 2.05, 1.88 and 1.92, at 21 DPV, and 1.92, 2.24, 2.0 and 2.25 at 28 DPV, corresponding to EPP values of 90.6%, 91.9%, 86.6% and 88.1% at 21 DPV and of 88.1%, 95.5%, 90.6% and 95.6% at 28 DPV. At the individual level, over 85% of the animals gave VN titers $\geq$1.65 (EP P $\geq$ 75%) (data not shown). The results indicated that the vaccine used containing the O_1/Campos strain is likely to confer effective protection against this heterologous strain.

3.4. In vivo challenge test

Further studies were performed to confirm the *in vitro* results through the *in vivo* gold standard challenge test. Unvaccinated control animals showed generalized disease with lesions in the four feet. Only one animal in the full dose group was not protected, whereas in each of the groups receiving 1/16 and 1/4 vaccine dose, two animals developed generalized disease (Table 3). Consequently, the vaccine conferred protection, reaching a PD50 value of 9.96 against heterologous challenge.

Serological response registered no detection of neutralizing antibodies in sera at 0 DPV. At 28 DPV all animals in the three groups had VN antibody titers for both, homologous and heterologous strains, leading to EPP values greater than the one indicative of protection (VN titer $\geq$ 1.65; EPP $\geq$ 75% (Table 3).

4. Discussion

Identifying the most effective vaccine strains to control FMD outbreaks that could spread to new regions is essential for contingency plans. Likewise, the suitability of vaccine strains maintained in strategic antigen reserves should be monitored continuously.

The present study is the first report on the effectiveness of South American FMDV vaccine strain O_1/Campos to confer protection against *in vivo* challenge of pigs with an O Mya98/SEA topotype isolate.

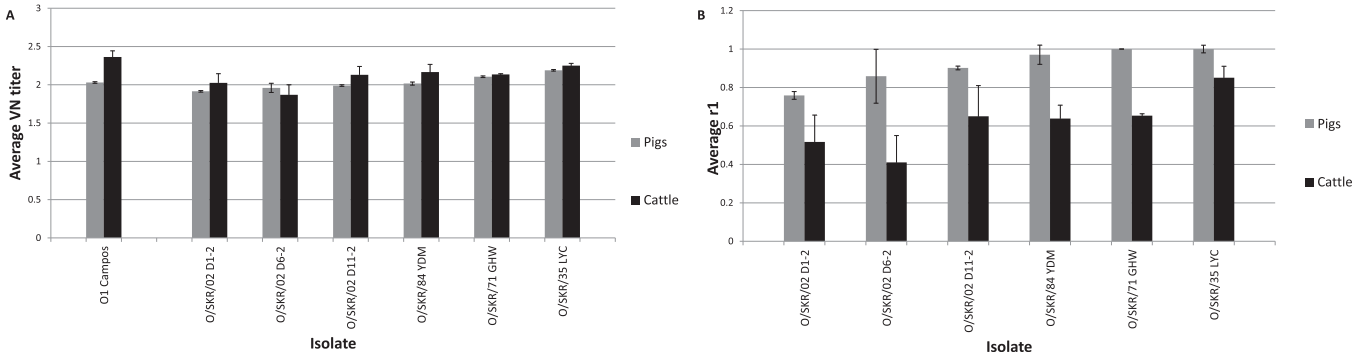

Fig. 3. VN titers and their corresponding r_1 values of O_1/Campos vaccine against Korean strains. A. Homologous and heterologous average VN titers were obtained through at least 2 independent assays for each individual sample with pools of sera derived from pigs and cattle vaccinated with an O_1/Campos monovalent vaccine, collected at 21 and 27 DPV, respectively. B. Average r_1 values were calculated from the individual r_1 values obtained in each of the assays. The interpretation of the results was as described [26]. r_1 values $\geq$0.3 indicate that the field isolate is sufficiently similar to the vaccine strain (the vaccine is likely to confer protection). Bars indicate standard deviation.

Table 2
VN titers and their corresponding EPP estimations for O/SKR/84 YDM strain using FMDV O_1/Campos vaccination in pigs.

Batch	0 DPV		21 DPV				28 DPV			
	O_1/Campos	O/SKR/84 YDM	O_1/Campos		O/SKR/84 YDM		O_1/Campos		O/SKR/84 YDM	
	VN	VN	VN[a] (SD) [b]	**EPP%**[c]	VN (SD)	**EPP%**	VN (SD)	**EPP%**	VN (SD)	**EPP%**
1	< 0.90	< 0.90	2.13 (0.14)	**93.6**	2.00 (0.15)	**90.6**	2.01 (0.31)	**91.0**	1.92 (0.28)	**88.1**
2	< 0.90	< 0.90	1.96 (0.19)	**89.4**	2.05 (0.16)	**91.9**	2.10 (0.17)	**93.1**	2.24 (0.09)	**95.5**
3	< 0.90	< 0.90	1.84 (0.24)	**85.0**	1.88 (0.29)	**86.6**	1.89 (0.24)	**87.0**	2.00 (0.25)	**90.6**
4	< 0.90	< 0.90	2.13 (0.26)	**93.6**	1.92 (0.38)	**88.1**	2.40 (0.07)	**97.3**	2.25 (0.28)	**95.6**

[a] Average value obtained from groups of 10 vaccinated pigs.
[b] SD: Standard deviation.
[c] EPP: Calculated from the mean VN titer of the 10 individual serum samples. An EPP $\geq$75% (VN titer $\geq$1.65) indicates that the vaccine will protect against the homologous vaccine strain.

Table 3
Serological responses and protection to heterologous challenge of pigs vaccinated with O_1/Campos vaccine.

Animal	2 ml dose						0.5 ml dose						0.125 ml dose				
	O_1/Campos		O/SKR/84 YDM				O_1/Campos		O/SKR/84 YDM				O_1/Campos		O/SKR/84 YDM		
	VN titer	EPP %	VN titer	EPP %	Protection	Animal	VN titer	EPP %	VN titer	EPP %	Protection	Animal	VN titer	EPP %	VN titer	EPP %	Protection
26	2.03	91.7	2.29	96.3	P	33	2.13	93.9	2.19	94.0	P	40	2.07	92.6	2.14	93.9	P
27	2.04	91.7	1.85	85.8	P	34	2.12	93.5	1.72	79.3	P	41	1.70	78.2	1.75	81.3	P
28	2.22	95.2	2.23	95.5	P	35	2.08	92.6	1.95	89.4	P	42	1.91	88.1	1.94	88.8	P
29	1.88	86.6	2.20	94.9	NP	36	2.04	91.7	2.18	94.6	P	43	2.14	93.9	2.34	96.7	P
31	1.92	88.1	2.03	91.7	P	38	2.34	96.7	2.43	97.6	NP	44	1.85	85.8	1.84	85.0	NP
32	2.32	96.5	2.31	96.5	P	39	1.74	80.3	1.90	87.4	NP	45	2.17	94.6	2.15	94.2	NP
Average	2.32	91.6	2.15	93.5			2.07	91.4	2.06	90.5			1.97	88.9	2.03	90.0	
SD	0.17	3.85	0.18	4.12			0.20	5.71	0.25	6.69			0.18	6.26	0.22	5.99	
P/NP					5/6						4/6						4/6

SD: Standard deviation.
P: protected.
NP: non protected.

This lineage, endemic in Southeast Asia, has been responsible for recurrent episodes in the Republic of Korea despite systematic vaccination with O/Manisa vaccine. Low r_1 values have been reported between O/Manisa and 2010 Korean viruses [15,16]. Moreover this vaccine strain failed to protect pigs against challenge with the 2010 Korean isolate at 21 DPV [10], which demanded the search for new vaccine strain candidates [11].

This scenario encouraged the evaluation of the protective capacity of the well-established O_1/Campos vaccine strain that has been widely and successfully used not only in South America but also in Asian regions, such as Taiwan (20,21). In addition it is available as a reserve in a number of international FMDV vaccine banks.

Our previous in vitro studies indicated the potential of high quality O_1/Campos vaccine to provide protection against representative isolates of the 3 serotype O topotypes active in Asia, achieving protective immunity after a single vaccination which is especially critical for emergency vaccination. The work included a 2010 Korean isolate (22). In the present study we extended the characterization to Korean viruses isolated during 2014–2015, and confirmed the *in vitro* results for Mya98 isolates with an *in vivo* challenge test in pigs.

Genetic and antigenic characterizations indicated high identity among all 2014–2015 isolates, regardless of their species of origin, with close homology to the 2010 viruses and other strains circulating in East Asia, suggesting that a monovalent high quality vaccine that confers protection against these isolates could by itself control a large proportion of the episodes that takes place in the region.

The observation that the Korean viruses maintained reactivity with two MAbs having the capacity to in vitro neutralize the vaccine strain O_1/Campos could account for the protective response of the O_1/Campos against Korean isolates described in this study. Previous reports showed that animals infected with isolates lacking reactivity with these two MAbs required revaccination to attain satisfactory protection [34–36].

In vitro inferences to predict the likelihood that O_1/Campos vaccinated animals would be protected against challenge were determined by r_1 values. Considering the epidemiological relevance of pigs, and even though vaccine-matching tests traditionally use bovine sera, we also tested pig sera. The r_1 values indicated considerable degree of relatedness between the O_1/Campos and the Korean viruses, suggesting that this vaccine strain is likely to effectively protect cattle and pigs against these isolates. All viruses recorded higher r_1 values for the pig pool than for the cattle pool. This could be explained, considering that immunological responses can be quite different in cattle and pigs, although the mechanisms involved are still unclear [37]. Although r_1 values were all above the established 0.3 cut-off used to infer the protective capacity of the vaccine strain, the values for the individual Korean viruses were variable. Considering the inherent variability of r_1 value determinations [28,38], most likely this variation is not indicative of fluctuating antigenic phenotypes.

EPP estimation was assessed with 4 different vaccine batches against the last pig isolate obtained during the 2015 episode. The results revealed a cross-protective response by the O_1/Campos vaccine at 21 and 28 DPV, with most EPPs above the indicative value for an expected protection [26,27]. Although protection against FMDV is associated with the induction of high levels of circulating neutralizing antibodies, the titers that correspond to protection are difficult to establish universally for various conditions (heterologous viruses, different species, revaccination, different days after vaccination, vaccine potency, etc.). In overall correlations between VN titers and protection were established for cattle, assessing homologous viruses. Nevertheless, international guidelines consider that, when not available for certain target species, cattle data can be endorsed for its use in other species [26,37]. In addition, previous studies with FMDV serotype O indicated that cattle with serum titers $\geq$1.65 (EPP $\geq$ 75%) challenged with heterologous virus were protected [35,36]. Overall VN titers registered in this study were above this threshold.

Due to the limitations of *in vitro* predictions for heterologous protection, particularly for pigs, an *in vivo* study was performed, which is the most direct and reliable method to measure cross-protection [26]. The result of this *in vivo* test indicated effective heterologous protection of O_1/Campos primo vaccinated pigs (PD50 value of nearly 10), which is in agreement with the *in vitro* data.

At the individual level it was observed that, despite having high VN titers some animals were not protected (17% in the full dose group; 28% for all the groups). It should be mentioned that if this would occur at the field level, this proportion would not impede controlling the spread of the disease. Such miss-matches have been described [27,29,39].

The results demonstrate that good quality O_1/Campos vaccines protect Mya98/SEA topotype viruses circulating in East Asia. The obtained PD50 value of over 9 is highly satisfactory considering that a PD50 value >3 is recommended for prophylactic purposes and vaccines formulated to have a homologous potency >6PD50/-dose compensate for a poor match between the vaccine and the field virus [40]. In addition, this study is valuable as an input to

the limited number of *in vitro* and *in vivo* correlation data available to establish heterologous protection in pigs.

Conflict of interest

Espinoza AM and Smitsaart E, employees of Biogenesis Bagó, declare that their judgment and objectivity were not biased by their contractual condition.

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