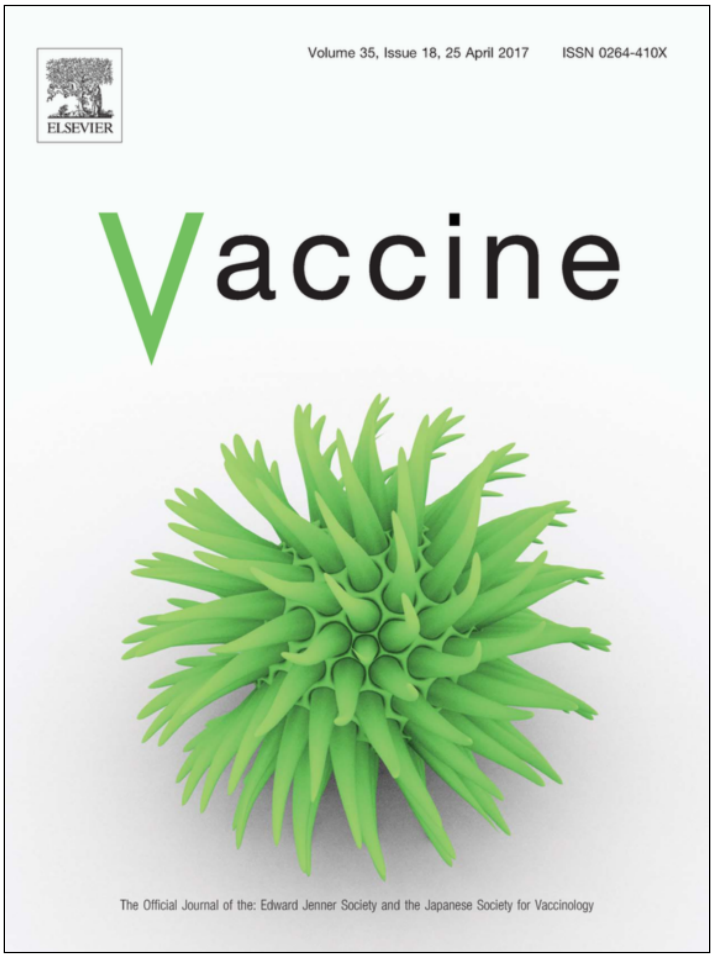

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Short communication

Antigenic and immunogenic spectrum of foot-and-mouth disease vaccine strain O_1 Campos against representative viruses of topotypes that circulated in Asia over the past decade

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Dedicated to the memory of Dr. Claudia Perez Beascoechea (1962–2015).

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ABSTRACT

Identifying vaccine strains to control outbreaks of foot-and-mouth disease virus that could spread to new regions is essential for contingency plans. This is the first report on the antigenic/immunogenic relationships of the South American O1/Campos vaccine strain with representative isolates of the three currently active Asian type O topotypes. Virus neutralization tests using O1/Campos post-vaccination sera derived from cattle and pigs predicted for both species acceptable cross-protection, even after single vaccination, established by r1 values and by expectancy of protection using monovalent or polyvalent vaccines. The results indicate that effective oil vaccines containing the O1/Campos strain can be used against Asian isolates, expanding the scope of O1/Campos strain included in vaccine banks to control emergencies caused by Asian viruses, even on single-dose vaccination, and to cover the need of effective vaccines in Asia during systematic vaccination.

1. Introduction

Foot-and-mouth disease (FMD) is a highly infectious disease of cloven-hoofed animals caused by a small, positive-sense RNA virus, FMD virus (FMDV), belonging to the Genus Aphthovirus, Family Picornaviridae. It exists as seven immunologically distinct serotypes, O, A, C, Asia 1, South African Territories (SAT)1, SAT2 and SAT3. Within each serotype variants arise continuously, resulting in distinct genetic groups known as topotypes [1], which in overall are associated with particular regions. Infection or vaccination with one serotype does not cross-protect against others and may fail to protect fully against some strains within serotypes.

This emphasizes the need of identifying vaccine strains that provide broad-range protection.

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 O/Manisa vaccine strain was used mainly in Middle East and Asia, as well as in North and South Africa [2]. However, recently-circulating viruses in the Middle East were not covered by this strain, requiring the development of O/Pan Asia-2 vaccines [3].

The antigen derived from the O_1/Campos strain blended in oil-type vaccines gave broad immunological coverage against South American strains, as assessed following criteria defined for systematic vaccination, which include revaccination [4]. Given the globalization of trade and the "vaccination to live" policy [5], and consequently the relevance of antigen/vaccine banks, it is important to identify vaccine viruses capable of eliciting effective cross-protection following a single vaccination, to achieve responses as rapidly as possible during emergencies. This evaluation should include extra-continental isolates.

FMD is endemic in much of Asia where 3 topotypes have been identified: South East Asia (SEA), Middle East South Asia (ME-SA) and Cathay. Spread of viruses from these regions has caused devastating epidemics during the past decade, even reaching very distant regions and impacting extra-continental countries which had been free of the disease for decades [6,7].

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This study focuses on the antigenic and immunogenic relationships between the Asian viruses and the O_1/Campos South American vaccine strain and on the predictive protective capacity of the latter against isolates related to the three active Asian topotypes.

2. Materials and methods

Viruses O_1/Campos/Brasil/58 (O_1/Campos) and O/Taiwan/97 (O/Taiwan) were provided by SENASA. Viruses O/South Korea/13/2010 (O/SK), O/Iran/2005 (O/Iran) and O/Manisa, were provided by the FMD Reference Laboratory at Pirbright.

Monoclonal antibody profiling was performed through a trapping ELISA using a panel of 21 monoclonal antibodies (MAbs), and coefficients of correlation (CC) between samples were determined as described [8].

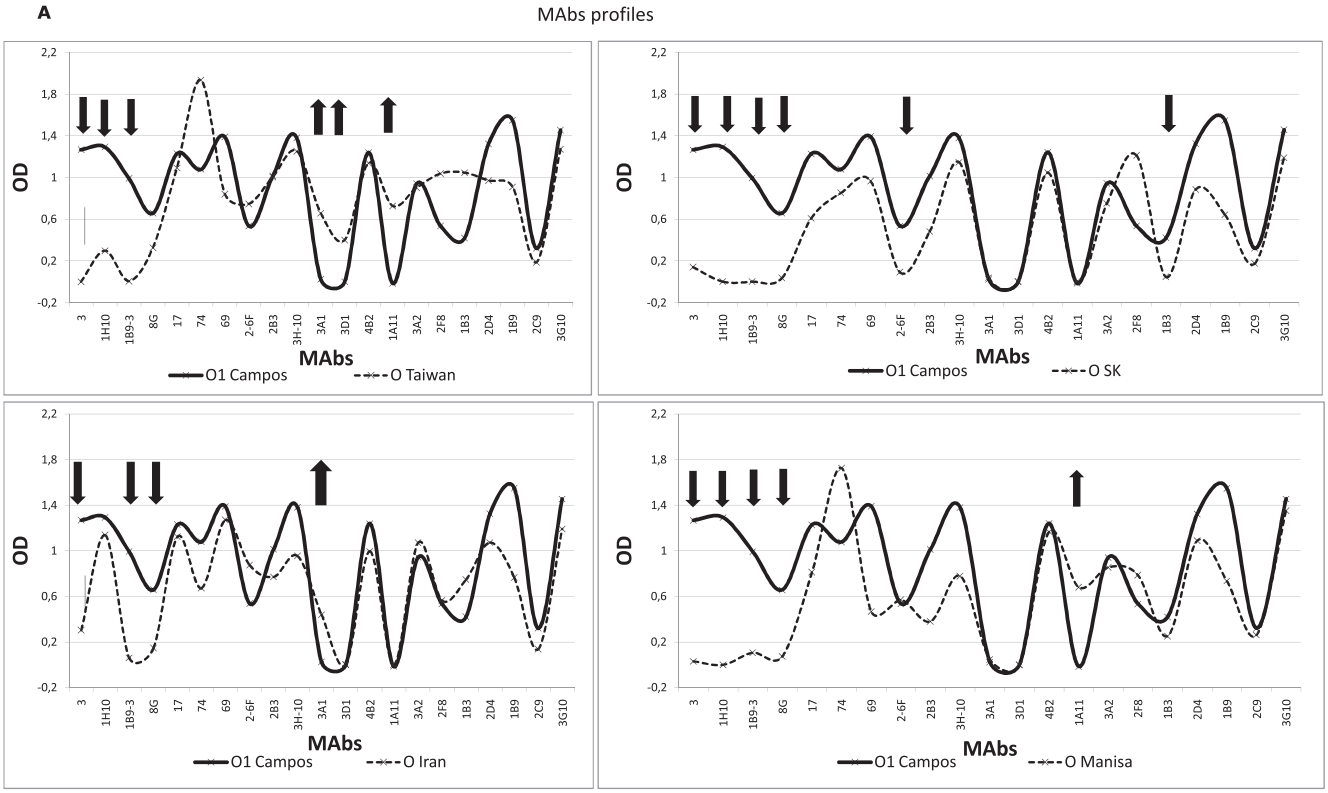
Determination of r_1 values and assessment of expectancy of protection (EPP) were as described [9,10]. The standard deviation (SD) was calculated to determine the variability within each trial.

Vaccines were prepared by Biogénesis Bagó with antigens obtained from BHK-21 suspension cell cultures. Antigens were inactivated with binary ethyleneimine, purified and formulated as water-in-oil single emulsion. The oil phase consisted of mineral oil and emulsifiers derived from mannitol, sorbitan and purified oleic acid. They were approved in safety, purity and potency, in the target species by the Animal Health Authorities in Argentina [11,12].

3. Results and discussion

Three viral strains were selected for this study: O/Taiwan, O/SK, and O/Iran, respectively representative of the three FMDV O topotypes Cathay, SEA and ME-SA that have been circulating in recent years in Asia. Also included was the O/Manisa vaccine strain (details in supplementary file).

Antigenic relatedness between the Asian strains and the O_1/Campos vaccine virus obtained through MAbs profiling is shown in Fig. 1. MAbs profiles (A) and the individual CC values (B) indicated distinctive profiles with varying levels of relatedness between individual Asian viruses and O_1/Campos. CC values ranged between 0.3 (O/Taiwan) and 0.7 (O/Iran). Analysis of reactivity with individual MAbs established that all Asian viruses showed clear-cut differences with O_1/Campos reflected in changes in 4–6 of the 21 MAbs analyzed. Interestingly, reactivity with MAbs 17 and 74, known to have the capacity to in vitro neutralize the strain of origin, was preserved in all the Asian viruses. This could account



B

Coefficient of correlation between samples

	O1 Campos	O Taiwan	O Manisa	O SK	O Iran
O1 Campos	-				
O Taiwan	0.3	-			
O Manisa	0.4	0.9	-		
O SK	0.6	0.7	0.8	-	
O Iran	0.7	0.6	0.5	0.6	-

Fig. 1. MAbs profiling of Asian isolates. Asian samples 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. (A) OD values obtained with each of the MAbs after subtracting their corresponding blank were plotted. Differences in reactivity of the Asian isolates with the vaccine strain (black arrows) are depicted. Lost or augmented reactivity is illustrated by arrows facing downward or upward, respectively. (B) Coefficient of correlation (CC) of the MAb reactivity values between the indicated viruses, calculated by plotting the absorbance values of the samples to be related and applying linear regression to fit the best straight line. CC values = 1 correspond to identical samples, and increased differences in antigenic profiles will result in CC values approaching to 0.

for the protective response of the O_1/Campos against Asian isolates described below. Previous reports showed that field isolates presenting lack of reactivity with these two MAbs elicited a rather poor protective response, particularly after single vaccination [13–15]. Three of the 11 MAbs produced against the virus O/Taiwan did not react with O_1/Campos virus. This lack of reactivity

Table 1
VN titers and their corresponding r_1 values using FMDV O_1 Campos vaccination.

Virus	Test	O_1/Campos	O/Iran		O/Manisa		O/SK		O/Taiwan	
		VN titer[a]	VN titer	r_1[b]	VN titer	r_1	VN titer	r_1	VN titer	r_1
Pool 1	1	2.38			2.19	0.65			1.92	0.35
Pool 1	2	2.40	2.11	0.51	2.14	0.55	1.95	0.35		
Pool 1	3	2.44	2.18	0.55			2.00	0.36	1.84	0.25
Average				**0.53**		**0.6**		**0.36**		**0.3**
SD				*0.03*		*0.07*		*0.01*		*0.07*
Pool 2	1	2.62					2.31	0.49		
Pool 2	2	2.47					2.09	0.42	2.09	0.42
Pool 2	3	2.51					2.19	0.48	2.15	0.44
Average								**0.46**		**0.43**
SD								*0.04*		*0.01*
Pool 3	1	2.15	1.99	0.69	2.07	0.83			1.92	0.59
Pool 3	2	2.24			2.03	0.62	1.84	0.4	1.84	0.4
Pool 3	3	2.36	2.17	0.65			2.16	0.63		
Average				**0.67**		**0.72**		**0.52**		**0.49**
SD				*0.03*		*0.15*		*0.16*		*0.13*

Bold and italics were included to highlight final interpretation of results.
Pool 1: Pool of five serum samples from cattle vaccinated with a O1 Campos monovalent vaccine, collected at 27 days post vaccination (DPV).
Pool 2: Pool of five serum samples from cattle vaccinated as in pool 1, collected at 28 days post revaccination (DPRV) at 27 DPV.
Pool 3: Pool of five serum samples from pigs vaccinated with a O1 Campos monovalent vaccine, collected at 21 DPV.
SD: Standard deviation.
[a] Titers were obtained by two-dimensional VN assays using pools of five serum samples from vaccinated or revaccinated cattle and from vaccinated pigs with a 2 ml dose of O_1/Campos monovalent vaccine.
[b] Values $\geq$ 0.3: isolate sufficiently similar to the vaccine strain (the vaccine is likely to confer protection).

Table 2
VN titers and their corresponding EPP estimations for Asian strains using FMDV O_1 Campos vaccination.

Species	Vaccine	DPV/DPRV	Virus									
			O_1/Campos		O/Iran		O/SK		O/Taiwan		O/Manisa	
			VNT[a] *(SD; CI+/−)*[b]	***EPP%***[c]	VNT *(SD; CI+/−)*	***EPP%***	VNT *(SD; CI+/−)*	***EPP%***	VNT *(SD; CI+/−)*	***EPP%***	VNT *(SD; CI+/−)*	***EPP%***
Bovine	Monovalent 1[d]	27 DPV	2.22 *(0.17; 0.09)*	***95.0***	2.23 *(0.23; 0.13)*	***95.4***	1.66 *(0.41; 0.23)*	***75.8***	2.19 *(0.18; 0.10)*	***94.8***	1.88 *(0.32; 0.18)*	***86.6***
	Monovalent 1[d]	60 DPV	2.22 *(0.18; 0.10)*	***95.0***	2.24 *(0.18; 0.10)*	***95.5***	1.67 *(0.39; 0.22)*	***76.4***	2.21 *(0.29; 0.16)*	***95.0***	2.01 *(0.29; 0.16)*	***91.0***
	Monovalent 2[d]	28 DPRV	1.98 *(0.30; 0.19)*	***90.0***	1.87 *(0.18; 0.08)*	***86.2***	1.81 *(0.15; 0.07)*	***84.2***				
	Trivalent 1[d]	28 DPV	2.01 *(0.28; 0.13)*	***91.0***	2.03 *(0.24; 0.11)*	***91.7***	1.61 *(0.27; 0.12)*	***73.3***				
	Trivalent 1[e]	28 DPRV	1.91 *(0.25; 0.11)*	***88.0***	2.17 *(0.18; 0.07)*	***94.6***	2.22 *(0.18; 0.07)*	***95.2***				
	Trivalent 2[e]	79 DPRV	2.30 *(0.18; 0.08)*	***96.4***			2.05 *(0.25; 0.12)*	***92.2***				
Swine	Monovalent 3[e]	21 DPV	2.23 *(0.11; 0.04)*	***95.3***	2.31 *(0.15; 0.06)*	***96.4***	2.02 *(0.21; 0.09)*	***91.1***	2.08 *(0.24; 0.10)*	***92.6***	2.01 *(0.20; 0.08)*	***91.0***
	Monovalent 4[e]	30 DPV	2.09 *(0.15; 0.12)*	***92.8***	2.00 *(0.16; 0.06)*	***90.8***	1.81 *(0.17; 0.07)*	***84.2***				
	Monovalent 4[e]	30 DPRV	2.32 *(0.14; 0.11)*	***96.5***	2.21 *(0.21; 0.08)*	***95.0***	1.95 *(0.30; 0.13)*	***89.1***				
	Tetravalent 1[e]	30 DPV	2.18 *(0.13; 0.11)*	***94.6***	2.06 *(0.22; 0.09)*	***92.4***	1.94 *(0.16; 0.06)*	***89.0***				
	Tetravalent 1[e]	30 DPRV	2.43 *(0.03; 0.02)*	***97.3***	2.38 *(0.03; 0.01)*	***97.1***	1.98 *(0.16;0.06)*	***90.0***				

Bold and italics were included to highlight final interpretation of results.
Trivalent vaccines contained viruses O1 Campos, C3 Indaial and A24 Cruzeiro.
Tetravalent vaccines contained the same strains as the trivalent vaccines and additionally the A 2001 viral strain.
[a] VNT: VN titer.
[b] SD: standard deviation; CI: 95% confidence interval.
[c] EPP: calculated from the mean VNT of the 16 individual serum samples. An EPP $\geq$ 75% (VNT $\geq$ 1.65) indicates that the vaccine will protect against the homologous vaccine strain.
[d] Experimental vaccines.
[e] Commercial vaccines.

was also observed for the three MAbs in O/SK and for two of them in O/Iran and O/Manisa.

To infer to what extent the vaccine strain O_1/Campos was able to cross-protect the representative Asian viruses, r_1 values were calculated as the reciprocal virus neutralization (VN) titer against heterologous virus/reciprocal VN titer against homologous virus using pools of five serum samples from cattle vaccinated with O_1/Campos monovalent vaccine collected at 27 days post vaccination (DPV) [9]. Considering that many of these viruses were quite active in swine, r_1 determinations from pig sera collected at 21 DPV were also included. As can be seen in Table 1, average r_1 values were all above the 0.3 cut off, resulting in 0.53, 0.60, 0.36 and 0.30 for cattle sera and in 0.67, 0.72, 0.52 and 0.49 for pig sera for the O/Iran, O/Manisa, O/SK and O/Taiwan, respectively. These results indicated considerable degree of relatedness between these strains and the vaccine strain O_1/Campos and suggested that the South American vaccine strain is likely to protect against these extra continental strains. Higher r_1 values were obtained with a pool of cattle sera collected at 28 days post revaccination (DPRV) for viruses O/SK and O/Taiwan. All Asian viruses registered higher r_1 values for pigs than for cattle, fact which is particularly relevant in the case of virus O/Taiwan which is porcinophylic.

The protective capacity of the vaccine strain was also established by EPP, which correlates VN antibody titers in vaccinated animals with protection against challenge with 10,000 infective doses. Since application of vaccine for emergency use needs to be as rapid as possible, EPPs were assessed not only with experimental monovalent vaccines, but also with commercial vaccines containing O_1/Campos. Results revealed for cattle, cross-protective responses by the O_1/Campos vaccine with most EPPs well above the indicative value for an expected protection (75%), (Table 2). For virus O/SK, an increased EPP was observed after revaccination, which was significant in the case of the commercial vaccine ($p < 0.00001$). As in the case of r_1, EPP values tended to be higher in pigs than in cattle, which was significant for virus O/SK ($p = 0.0074$) when comparing monovalent vaccines.

Although the limit of serum titer that corresponds to antibody pass-level for protection is difficult to establish universally for the various conditions, including different species, previous studies indicated that animals with serum titers $\geq$1.65 (EPP 75%) challenged with heterologous virus were protected [13,15]. VN titers registered in this study were way above this threshold. In addition international guidelines consider that, when not available for certain target species, cattle data can be endorsed for its use in other species [9].

Vaccine potency can, partially, compensate for differences between field and vaccine strains [2,16]. Thus, vaccine matching results presented in this work refer to manufacturing conditions and potency evaluation used in South America, showing high levels of repeatability and reproducibility [11,17]. Extrapolating these conclusions to different conditions, including potency determinations by PD50, with non-comparable performance parameters [17], is hampered by the limited existence of comparative studies between these two methodologies [18].

4. Conclusion

This is the first report indicating that the O_1/Campos strain induces satisfactory cross-protection against Asian isolates, and that vaccines containing this strain prepared as referred and with satisfactory potency, could be used, not only during routine vaccination for effective vaccination in Asia, but also to achieve immunity after a single vaccination. This is especially relevant for understanding the scope of antigens maintained in antigen/vaccine banks for emergency vaccination and very advantageous during systematic use in order to prevent the emergence of antigenically distinct viruses escaping neutralization under sub-neutralizing conditions. Protection after a single dose is especially remarkable, considering that, even intra-regional viruses, occasionally required revaccinations to attain satisfactory protection [13–15]. These results are in line with previous findings on the successful use of the O_1/Campos vaccine strain to aid in the control of a devastating epidemic in pigs of serotype O in Taiwan in 1997 [19,20].

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.03.026.

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