Vaccine 32 (2014) 1882–1889

Contents lists available at ScienceDirect

Vaccine

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

Protection to homologous and heterologous challenge in pigs immunized with vaccine against foot-and-mouth disease type O caused an epidemic in East Asia during 2010/2011

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ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form 14 January 2014
Accepted 21 January 2014
Available online 10 February 2014

Keywords:
Foot-and-mouth disease
Inactivated vaccine
O–SEA topotype
Heterologous challenge
Homologous challenge

ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious infectious disease, and the use of vaccines is known to be effective for its prevention. In 2010/2011, there was an epidemic of the South East Asia (SEA) topotype in East Asian countries. We adapted the SEA topotype virus isolated in November 2010 in Korea in cells to analyze the characteristics of the virus and evaluate its possibility as a vaccine. After cell culture adaptation, the FMD virus particle 146S was purified to develop an inactivated oil vaccine for SEA or other topotypes. To measure its immunogenicity, pigs were inoculated with the experimental vaccine at different concentrations of the antigen. The results indicated that the groups immunized with at least 7.5 μg antigen were protected from homologous challenge. The immunized pigs were also protected against heterologous virus (ME–SA topotype) challenge. The genetic variations between the two field isolates and the adapted vaccine strains were identified in six amino acids by complete genome sequencing.

1. Introduction

Foot-and-mouth disease (FMD) is an economically important disease because it is highly contagious, infects many cloven-hoofed animals (such as cattle, sheep, and pigs), and there is no treatment method; thus, stamping out policies are implemented in most countries once animals have been infected. The pathogenic FMD viruses (FMDVs) are classified into small icosahedral viruses in the *Aphthovirus* genus in the Picornaviridae family [1]. There are seven serotypes of FMDVs (O, A, C, SAT1, SAT2, SAT3, and Asia1). The O and A serotypes frequently occur globally, but the Asia1 serotype occurs restrictedly in Asian countries. Among these, the O type is the most widespread. Infection with one serotype does not confer immunity against another.

Animals may have different protective abilities for different topotypes even if they belong to the same serotype of FMD. Therefore, various types of appropriate vaccines should be developed [2,3]. Among the seven serotypes known thus far, the O types are most widespread throughout the world. Serotype O is divided into eight topotypes: ME–SA (Middle East–South Asia), EURO–SA (Europe–South America), CHY (Cathay), SEA (South East Asia), ISA (Indonesia)-I, ISA-2, EA (East Africa), and WA (West Africa) [4]. Among topotypes, ME–SA, SEA, and Cathay topotypes are virus types that prevail in the Asian region. The SEA topotype occurred in FMD-susceptible animals, such as cattle and swine, in the East Asian region in 2010–2011. Most animals show severe typical symptoms, regardless of the species, and this disease has had a grave effect in many Asian countries, such as Korea, Japan, and North Korea. In this outbreak, about 3.5 million swine and cattle were culled, and the direct economic losses alone amounted to 3 billion US$ [5]. FMD spread in Korean livestock rapidly in late 2010, but nationwide vaccinations prevented its further expansion. The decline in the frequency of FMD occurrences was observed in pigs 3 weeks after the vaccinations and in cattle 2 weeks after vaccination [6]. In order to gain the same neutralizing antibody level through vaccination, pigs require a longer period of immunity than cattle [7]. The immunological relationship between O Manisa vaccine and field strains was relatively low or moderate (r value of approximately 0.3). Effective vaccination using O Manisa during the 2010–2011 outbreaks in Korea was controversial. The development of a new vaccine using field isolates caused large-scale

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http://dx.doi.org/10.1016/j.vaccine.2014.01.067

outbreaks was required. In particular, since more viruses are discharged from pigs than from cattle in general, vaccines should be developed that can be effectively used for pigs.

However, despite these large-scale FMD occurrences, a vaccine targeted to the SEA topotype has not yet been commercialized. We therefore tried to develop vaccines using O–SEA-topotype viruses isolated in Korea since effective vaccines are required that can accurately protect against this topotype or others when it occurs. The virus antigens were purified to analyze the properties of the vaccine strains, and immunogenicity and challenge tests were conducted to evaluate efficacy of the experimental vaccines.

2. Materials and methods

2.1. Viruses and cells

We used baby hamster kidney (BHK) 21, swine kidney (IBRS-2), and fetal goat tongue epithelial (ZZ-R) cell lines maintained at the Animal, Plant, and Quarantine Agency (QIA). We received kindly bovine calf kidney (LF-BK) cells from the Plum Island Animal Disease Center (USA). BHK21, IBRS-2, and LF-BK cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Corning, USA) with 5% fetal bovine serum (FBS; Gibco, USA). ZZ-R cells were sustained in DMEM F-12 (Corning) with 10% FBS. Cells were grown at 37 °C with 5% CO_2 in a humidified atmosphere.

Seed viruses for the production of virus antigens used in the vaccines were obtained from FMD viruses isolated in swine vesicles of two regions (Andong in Gyeongbuk province, Yangju in Gyeonggi province) in Korea in November and December 2010. To adapt the viruses to in vitro culture and increase the viral titers, the virus was subjected to serial passages in suckling mice and ZZ-R cells and was subsequently adapted to BHK21 cells. Infected cells were frozen within 24 h and subjected to three cycles of freezing–thawing steps to release the viral particles. The virus was harvested by centrifugation at 13,500 rpm for 5 min at 4 °C, and the supernatant was stored at −70 °C until used.

FMDV strains O/Andong/SKR/2010 and O/YJ/SKR/2010 for the SEA topotype, O Manisa (O1/Manisa/Turkey/69) and O/SKR/2002 for the ME–SA topotype, and O/ASP/Cathay (phenotype of Genbank accession HQ412603) for the Cathay topotype were used for the cross-virus neutralization test (VNT) or challenge test.

2.2. Virus adaptation and purification of virus particles (146S)

The virus isolate O/Andong/SKR/2010 from Andong was subjected to serial passages in suckling mice five times, in ZZ-R cells three times, and in BHK21 cells 15 times, for a total of 23 serial passages (named as AD-P, M5Z3B15); the virus O/YJ/SKR/2010 from Yangju was subjected to serial passages in mice seven times, in ZZ-R cells three times, and in BHK21 cells 14 times, for a total of 24 serial passages (named as YJ-P, M7Z3B14).

To produce FMDV antigens, BHK21 cells were inoculated with the viruses when the cells formed a monolayer in a 175-cm^2 T-flask. When a 100% cytopathic effect (CPE) was formed 24 h later, the viruses were kept in a freezer at −70 °C. The viruses were harvested, binary ethyleneimine-inactivated, concentrated using polyethylene glycol (PEG)-6000, and then purified with sucrose density gradient centrifugation in an ultracentrifuge [8]. The viruses were concentrated using Amicon Ultracentrifuge filters (100 kDa) and were exchanged with TN buffer (50 mM Tris [pH 7.6], 100 mM NaCl). The 146S virus particles were quantified at 259 nm using a spectrophotometer [9].

2.3. Immunization of pigs by experimental vaccines

The purified 146S antigen was mixed with Montanide ISA 206 VG (Seppic, Paris, France), an oil adjuvant, at a mixing ratio of 1:1 to make vaccines in the form of water in oil in water, and the vaccines were kept refrigerated at 4 °C for 1 day. All experimental animals were cared for according to the animal management guidelines of the QIA. The two or five of 3-month-old pigs with FMD antibody-free per group were vaccinated intramuscularly in the neck with an experimental vaccine containing the AD-P strain or the commercial vaccine (Merial Co. Ltd, Pirbright, UK) containing the O Manisa strain. For the negative control, PBS mixed with the adjuvant through the same method was used. We designed experiment 1 (2, 5, 20 μg per dose) for the immunity level (Supplement Table 1) and experiment 2 (7.5, 10, 15 μg per dose) for immunity and challenge (Table 1). The virus challenge for the second group was conducted at 30 days after the first inoculation. The neutralizing antibody level for the cross VNT was tested using the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Animal Health Organization.

2.4. Challenge test of immunized pigs

The pigs of experiment 2 (7.5, 10, 15 μg per dose) were immunized with the experimental (AD-P) vaccine for 4 weeks. All pigs of the experiment were challenged with the virus O/Andong/SKR/2010 ($10^{5.0}$ $TCID_{50}$/0.1 ml) on each footpad (0.1 ml/animal) to identify protective effects with clinical signs (Table 1 and Fig. 1) at 30 days post vaccination (DPV). To identify protective effects to the heterologous virus, the 10-μg group of the experiment was challenged with virus O/SKR/2002 ($10^{5.0}$ $TCID_{50}$/0.1 ml) of the ME–SA topotype on each footpad (0.1 ml/animal) at 49 DPV (Table 2 and Fig. 2). The pigs' clinical scores were based on the sum of each FMD lesion or sign (maximum score =16) according to the following parameters. Clinical score was determined by the addition of points distributed by the method of Alves et al. [10] as follows: an elevated body temperature of 40 °C (1 point), 40.5 (2 points), or 41 (3 points); reduced appetite (1 point), or no food intake and food left over from the day before (2 points); lameness (1 point) or reluctance to stand (2 points); presence of heat and pain after palpation of the coronary band (1 point), or not standing on the affected foot (2 points); vesicles on the feet, dependent on the number of feet affected and with a maximum of 4 points; visible mouth lesions on the tongue (1 point), gums, or lips (1 point) or snout (1 point), with a maximum of 3 points. The scores of each pig were recorded daily. After the challenge inoculation, nasal discharges and serum were monitored for 10–13 days by collecting them at one-day intervals; viruses were detected using real-time RT-PCR [11].

PrioCHECK FMDV NSP (Prionics AG, Schlieren-Zurich, Switzerland), an ELISA for the detection of FMD virus non-structural protein (NSP) antibodies in serum samples of pigs, was employed to detect NSP antibodies.

2.5. Sequence analysis

Viral RNA extraction was performed using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). cDNA was synthesized with SuperScript III™ (Invitrogen, Karlsruhe, Germany) and amplified by a Phusion™ Hot Start (Thermo) kit. The complete genome was sequenced by a sequencing service company (Macrogen Corporation, Seoul, Korea) using sequencing primers based on GenBank

Table 1
Summaries of protective effects and virus neutralizing antibody level after experimental FMD vaccination in pigs according to antigen dose.

Vaccinated groups (virus, μg)	Pig ID	Serum neutralizing antibody titers (days post vaccination, log)						Virus detection by real-time RT-PCR for 10 days after challenge		NSP antibody detection for 13 days after challenge	Protective effects after challenge[a]	
		0	7	14	21	28	49[b]	Nasal swabs	Sera		Clinical score[c]	Protection based on clinical signs
Vaccinated group 1 (AD-P, 15 μg)	46	<1.2	1.7	1.5	1.3	1.8	nd[d]	–	–	–	0	Yes
	47	<1.2	1.6	1.7	1.9	2.2	nd	–	–	–	0	Yes
	48	<1.2	<1.2	1.7	1.6	2.1	nd	–	–	–	0	Yes
	49	<1.2	<1.2	1.5	1.5	1.9	nd	–	–	–	0	Yes
	50	<1.2	1.2	1.4	1.3	1.5	nd	–	–	–	0	Yes
Vaccinated group 2 (AD−P, 10 μg)	51	<1.2	<1.2	1.3	2.0	2.0	2.0	–	–	–	0	Yes
	52	<1.2	1.4	2.0	1.5	2.1	2.7	–	–	–	1	Yes[e]
	53	<1.2	<1.2	1.7	2.1	2.3	2.3	+	–	–	0	Yes
	54	<1.2	1.2	1.7	1.8	1.7	2.9	–	–	–	0	Yes
	55	<1.2	1.4	1.7	2.1	2.1	2.4	–	+	–	0	Yes
Vaccinated group 3(AD−P,7.5 μg)	62	<1.2	<1.2	1.7	2.1	2.5	nd	–	–	–	0	Yes
	63	<1.2	1.3	1.8	2.1	2.5	nd	–	–	–	0	Yes
	64	<1.2	1.4	1.8	2.0	2.0	nd	–	–	–	1	Yes[e]
	65	<1.2	1.6	1.8	1.8	2.3	nd	–	–	–	0	Yes
	66	<1.2	1.4	1.4	1.8	2.3	nd	–	–	–	1	Yes[e]
Control group	19	<1.2	<1.2	<1.2	<1.2	<1.2	nd	+	+	–	13	No
	20	<1.2	<1.2	<1.2	<1.2	<1.2	nd	+	+	+[f]	13	No

[a] Challenged (O/Andong/SKR/2010) at 30 DPV(day post vaccination) and examined until 13 DPC.
[b] 21 DPC (day post challenge).
[c] Clinical scores were based on the sum of each FMD lesion or sign (maximum score = 16) according to the method of Alves et al. [10] based on the addition of points.
[d] Not done.
[e] Vesicle lesion on the site injected with challenge virus excepted.
[f] NSP antibody was detected after 8DPC.

KC503937. Multiple alignments were analyzed using the Clone MgrSuite (Sci-Ed software, NY, USA).

3. Results

3.1. Virus replication and sequence changes in the cell-adapted virus

To develop vaccines using the two SEA topotype viruses O/Andong/SKR/2010 and O/YJ/SKR/2010 isolated in the field, the viruses were subjected to serial passages in mice or cells 23–24 times. The purpose of this was to enhance growth properties through appropriate and stable culture in antigen-producing cells for the vaccines; the resultant viruses were used as seed viruses (named as AD-P, YJ-P). As the virus titers were enhanced and individual viruses were subjected to passages seven times and 12 times, virus replication with a constant titer was maintained in BHK21 cells. One-step growth of viruses was completed within 24 h, and AD-P and YJ-P were replicated to the highest titer at 16 h and 12 h post infection, respectively. AD-P plaques were larger than YJ-P plaques (Supplement Fig. 1). The inactivated antigen for vaccine preparation was inoculated three times in the ZZ-R cell, and we could not find any live virus. The safety of the vaccine in vivo was simultaneously checked with the pig vaccination, and the pigs did not show any clinical signs for 4 weeks.

As a result of passages to animals and cells, changes in the nucleotide sequences of the viruses were induced. The antigenic site 1 as the VP1 major epitope of SEA topotypes showed high similarity with that of the ME–SA topotype (Table 3). However, the site of the Cathay topotype had relatively low similarity of 76.5–82.4% from that of the SEA topotype. In particular, the largest changes were observed in the VP2 and VP1 parts of the structural proteins. Six amino acids were changed in the coding regions, and the viruses showed a similarity of 99.74% (2326/2332) to the viruses originally derived (Supplement Table 2). Particularly in 807 aa (VP1) of the P1 region of the two-candidate vaccine viruses, amino acid glutamic acid (E) was changed into lysine (K) in an identical pattern through passages in BHK21 cells. Only one amino acid residue of VP1 containing antigenic site 1 of AD-P was critical; this was located at 208 amino acid position of VP1 (Table 3).

3.2. Protection after homologous virus challenge

Neutralizing antibodies (>1:16) against the FMDV SEA topotype were detected in only part (2/3) of the groups vaccinated with a low dose (2 and 5 μg) and were detected in all (3/3) of the groups vaccinated with high-dose (20 μg) purified AD-P or YJ-P 146S antigens from 14 days after vaccination (Supplement Table 1).

The 7.5-, 10-, and 15-μg groups were inoculated with the O/Andong/SKR/2010 challenge virus on day 30 after the first vaccination. In one or two of the pigs vaccinated with 7.5 and 10 μg of the antigens, lesions with vesicles on only the injected site of one leg formed from the third or fourth day after the homologous challenge (Table 1 and Fig. 1). In the pigs vaccinated with the antigens at low or high dose, the formation of neutralizing antibodies and protective effect against challenges by viruses was in proportion to the antigen concentrations (Table 1 and Supplement Table 1).

High levels of neutralizing antibodies were detected in the groups vaccinated with high-dose antigens (7.5, 10, 15, 20 μg) from day 14 after vaccination; clinical signs except vesicle lesions in the inoculated leg in the 7.5-μg groups were not identified after the direct challenge inoculation. The group inoculated with the O–ME–SA topotype vaccine (a commercial vaccine using the O Manisa strain) showed relatively low levels of antibodies against O–SEA topotype viruses. The negative control animals showed typical FMD symptoms from 2 days post challenge (DPC), and viremia and virus excretion were detected from 1 DPC (Table 1 and Fig. 1). However, viruses in pigs (no. 53, 55) of the 10-μg group were only detected from serum or nasal discharges for a short time (3 or 6 DPC). A few (no. 52, 64, and 66) of the vaccinated pigs had vesicle lesions on the injected site of the footpad from 3∼4 DPC (Fig. 1).

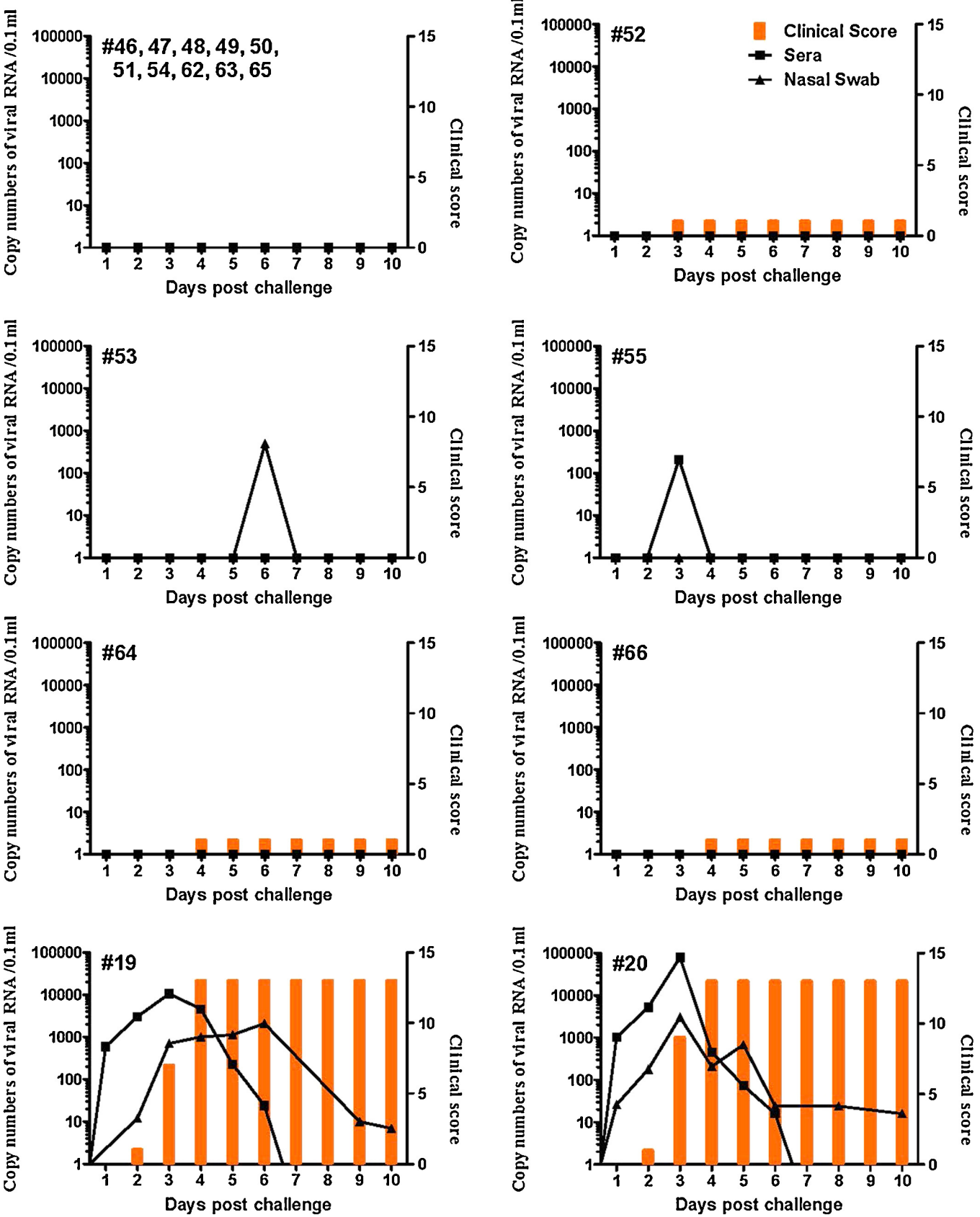

Fig. 1. Virus excretion and clinical score in pigs immunized with the experimental vaccine according to dose after homologous virus (O/Andong/SKR/2010) challenge. Clinical score was determined by the addition of points distributed as follows: an elevated body temperature of 40 °C (1 point), 40.5 (2 points), or 41 (3 points); reduced appetite (1 point) or no food intake and food left over from the day before (2 points); lameness (1 point) or reluctance to stand (2 points); presence of heat and pain after palpation of the coronary band (1 point) or not standing on the affected foot (2 points); vesicles on the feet, dependent on the number of feet affected and with a maximum of 4 points; visible mouth lesions on the tongue (1 point), gums or lips (1 point), or snout (1 point), with a maximum of 3 points.

Table 2
Summaries of protective effects and virus neutralizing antibody level in vaccinated pigs after heterologous virus (O/SKR/2002) challenge.

Vaccinated groups (virus, μg)	Pig ID	Serum neutralizing antibody titers against O/SKR/2002 (days post-vaccination, log)							Virus detection by real-time RT-PCR for 10 days after challenge		NSP antibody detection for 10 days after challenge	Protective effects after challenge[a]	
		0	7	14	21	28	49[a]	56[b]	Nasal swabs	Sera		Clinical score[c]	Protection based on clinical signs
AD-P, 10 μg	51	<1.2	<1.2	<1.2	1.2	<1.2	1.3	2.0	–	–	–	0	Yes
	52	<1.2	<1.2	<1.2	<1.2	1.3	1.3	2.1	–	+	–	0	Yes
	53	<1.2	<1.2	<1.2	<1.2	1.2	1.2	1.8	–	+	–	0	Yes
	54	<1.2	<1.2	<1.2	<1.2	<1.2	1.7	1.8	–	–	–	0	Yes
	55	<1.2	<1.2	<1.2	1.2	<1.2	1.2	1.8	–	–	–	0	Yes
Control	76	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	–	+	–	6	No
group	77	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	+	+	–	7	No

[a] Challenged (O/SKR/2002) at 49 DPV(day post vaccination) and examined until 10 DPC(day post challenge).
[b] Seven DPC.
[c] Clinical scores were based on the sum of each FMD lesion or sign (maximum score =16) according to the method of Alves et al. [10] based on the addition of points.

3.3. Protection after heterologous virus challenge and serological relationship

The groups immunized with 10 μg of SEA topotype (AD-P) were challenged with the O/SKR/2002 virus belonging to the ME–SA topotype on day 49 after the first vaccination. No vaccinated pig showed any clinical signs or lesions at the injected site (Fig. 2). Neutralizing antibodies (>1:16) against O/SKR/2002, ME–SA topotype were detected in only part (3/5) of the groups at the time of virus challenge and were detected in all (5/5) of the groups 7 DPC (Table 2).

The serological relationship between the two vaccine candidates of the SEA topotype showed a high *r* value (>0.9) that was relatively higher than the ME–SA (0.45–0.52) or CHY (0.13–0.19) topotype strains (Table 4). The matching value of ME–SA (O Manisa) to SEA topotype (0.24–0.33) was similar to the CHY topotype (0.25).

When the O Manisa vaccine was administered to the pigs, the neutralizing antibody against O/Andong/SKR/2010 and O/SKR/2002 showed a relatively lower level than that against O Manisa (Supplement Fig. 2). Interestingly, the neutralizing antibody against O/SKR/2002, ME–SA topotype to the same group as O Manisa showed a relatively lower level than that against the O/Andong/SKR/2010, SEA topotype (Supplement Fig. 3). However, all pigs immunized with AD-P were completely protected against O/SKR/2002 (Fig. 2).

4. Discussion and conclusions

The SEA topotype that belongs to FMDV serotype O has become more frequent in the Asian region since 2006 [12]. The SEA topotype occurs in Hong Kong and China in the southern Asian regions. In April 2010, it occurred on a large scale in Korea and Japan. The VP1 sequence of viruses was isolated in 2010 in Korea, and the viruses originating in Hong Kong showed at least 97.8% similarity [13]. Since SEA and ME–SA viruses are still circulating in the Asian region, vaccines based on these viruses are required.

To protect against the SEA topotype of the virus, O Manisa (O1/Manisa/Turkey/69), which is the ME–SA topotype, can be used [14]. However, O Manisa (GenBank: AJ251477.1) and O/Andong/SKR/2010 show a relatively large genetic difference, with a similarity rate of 83.5% in the VP1 sequence, which is the most important antigenic site. When the O Manisa vaccine in Korea was administered, pigs showed relatively lower immune responses compared to cattle. The reason for this is assumed to be the difference in the responsiveness of swine immune cells to the special antigenic structure of the FMDV topotype [15]. Although vaccines so far have been developed based on cattle, considering the recent characteristics of the prevalence of the SEA topotype—which showed severe clinical symptoms in all susceptible hosts regardless of species in 2010–2011—vaccine virus strains that are more suitable for pig, which play a major role as an amplifying host, should be developed. In the outbreak case of November 2010 in Korea, pigs were mainly infected. Even though a vaccine-matching test should use bovine sera, we tested vaccine–matching using pig sera and a challenge to pigs to reflect the field situation. In some countries, vaccine strains suitable to the field situation have already been developed as custom-made vaccines, such as Philippines 98, O Geshur, and O-3039 for prevailing viruses. Therefore, vaccines should be developed in advance to be prepared for the recurrence of the SEA topotype.

To produce vaccines in an FMD-free country, all conditions should be safe because of the possibility of leaks of pathogenic viruses since live viruses need to be cultured in large amounts [16]. For safer vaccine production, the pathogenic characteristics of vaccine strains should be changed so that the vaccine viruses cannot be transmitted or will have low pathogenicity. Through preliminary experiments, decreased pathogenicity was identified in viruses subjected to 17–19 serial passages in animals and cells (data not shown). However, this will be verified through more accurate experiments. Even when vaccine viruses secured through serial passages were used, the original immunological characteristics as antigens of the prevailing SEA topotype field viruses were not induced to change, and this fact was proven by the formation of neutralizing antibodies and the protection against virus challenge.

Most FMDVs grow more easily under monolayer culture conditions in tissue culture flasks than in suspension cell culture [17,18]. However, to produce large-scale vaccines, the establishment of suspension cell culture conditions using BHK21 cells and adaptation to the conditions are required. In the present experiment, viruses in which CPE was identified were appropriately replicated in monolayer cell culture through successive serial passages, but the efficiency of the production of antigens for the vaccine was not high. Therefore, further studies are necessary for the production stages to create stable BHK21 cell cultures that will enable suspension culture [19,20].

Once animals were FMD-vaccinated in the field, immune antibodies were formed 2 weeks later in cattle and 4 weeks later in pig [7]. When pigs vaccinated with 2 or 5 μg antigens were challenged, FMD symptoms, such as fever, were identified in some pigs 1–2 days later [21,22], and not all pigs were protected by inoculation at these amounts [23]. In the present experiment, pigs vaccinated with 10 μg did not show FMD clinical symptoms in homologous or heterologous virus challenge except vesicle lesions at the injected site of one pig; however, the viruses were detected in their nasal

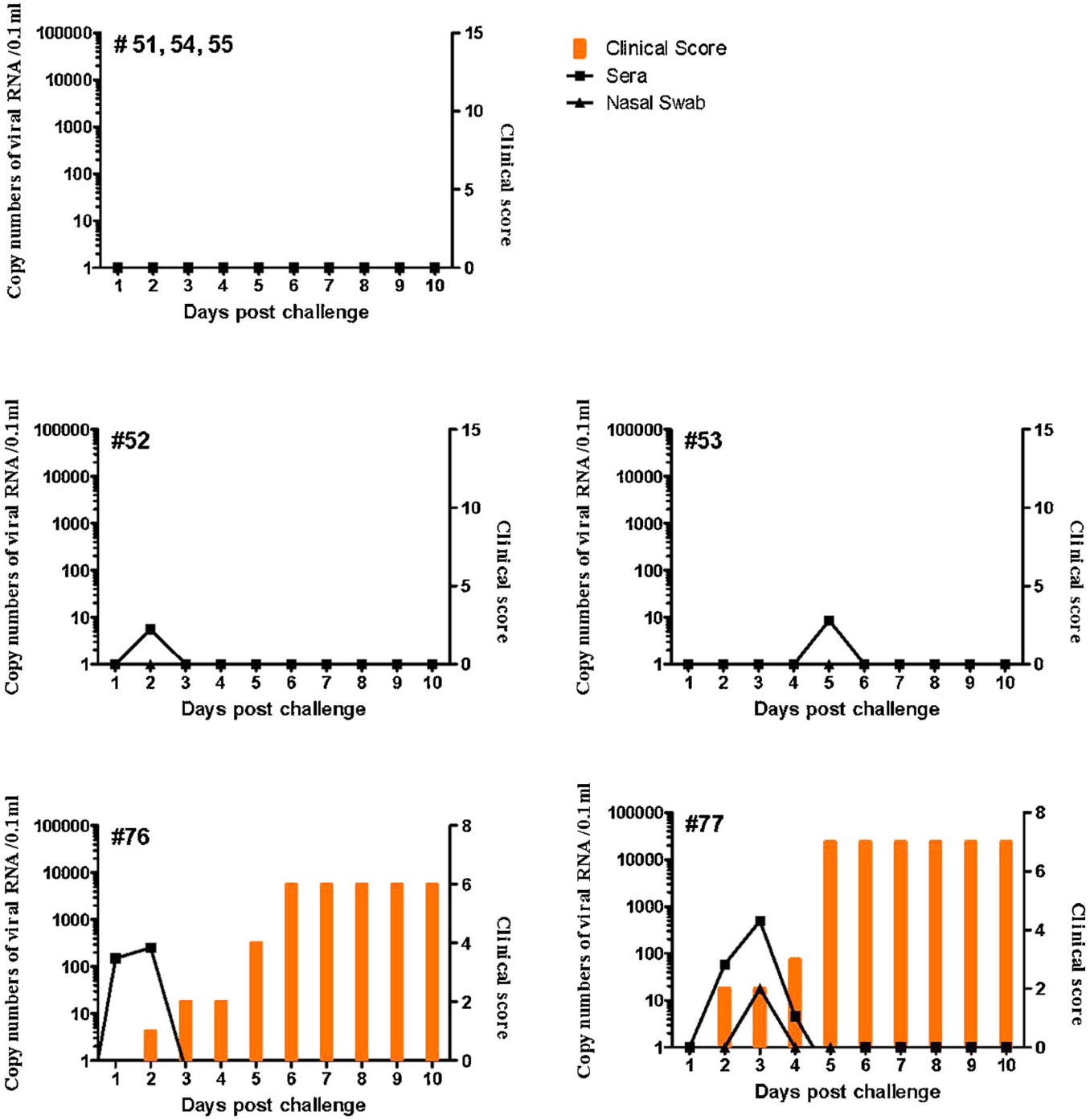

Fig. 2. Virus excretion and clinical score in pigs immunized with the experimental vaccine according to dose after heterologous virus (O/SKR/2002) challenge. Clinical score was determined by criteria of Fig. 1.

discharge or serum. NSP antibody did not show after heterologous virus challenge in the 10-μg group. One possible reason for this is that the experiment was terminated at 10 DPC before elicitation of the NSP antibody.The pigs vaccinated with 15 μg were completely protected from FMD clinical symptoms, including no vesicle lesions at the injected site; furthermore, the viruses were not detected in the nasal discharge or serum in any of these pigs. The level of neutralizing antibodies and protective effects do not completely match up each other. Situations in which animals are protected even when the levels of antibodies are low may be assumed to occur because cell-mediated immune responses are also present [24].

Although the 146S antigens used as vaccines are generally inoculated in a range of 1 to 10 μg, in the case of O serotype antigens, larger amounts are required to obtain the same potency compared to other serotypes [25]. Since antigen concentrations do not perfectly coincide with potency, the correlation between the concentration of 146S antigen and potency cannot be easily determined when the concentration is greater than 10 μg [26]. In our experiment, animals vaccinated with 2 or 5 μg of 146S antigens partially produced neutralizing antibody, whereas those vaccinated with 7.5–15 μg antigens produced protectable antibody and were clinically protected from homologous or heterologous challenge, indicating that the effect of vaccination improved at

Table 3
Similarity of VP1 major antigenic sites among the new vaccine and wild-type strains of three topotypes circulating in the Asia region.

Topotypes	Strains	Virus type	Similarity of VP1 antigenic site 1			GenBank accession
			VP1 143-162(A)	VP1 200-213(B)	Similarity (%, A + B)	
SEA	O/Andong/SKR/2010	Wild type	NVRGDLQVLAQKAARPLPTS	RHKQKIVAPVKQSL	ID[a]	KC503937
SEA	AD-P	Vaccine	NVRGDLQVLAQKAARPLPTS	RHKQKIVA**S**VKQSL [b]	97.0 (33/34)	KF501486
SEA	O/YJ/SKR/2010	Wild type	NVRGDLQVLAQKAARPLPTS	RHKQKIVAPVKQSL	100 (34/34)	KF501487
SEA	YJ-P	Vaccine	NVRGDLQVLAQKAARPLPTS	RHKQKIVAPVKQS**S**	97.0 (33/34)	KF501488
SEA	O/KOR/1/2010	Wild type	NVRGDLQVLAQKAA**W**PLPTS	RHKQKIVAPVKQSL	97.0 (33/34)	HM143846
ME–SA	O Manisa	Vaccine	NVRGDLQVLAQKAAR**A**LPTS	RHKQKIVAPVKQ**L**L	94.1 (32/34)	AY593823
ME–SA	O/SKR/2002	Wild type	NVRGDLQVLAQKAAR**T**LPTS	RHKQKIVAPVKQ**L**L	94.1 (32/34)	AY312589
ME–SA	O/SKR/2000	Wild type	NLRGDLQVLTQKAARTLPTS	RHKQKIVAPVKQLL	88.2 (30/34)	AY312587
Cathay	O/ASP/Cathay(O/YM/YN/2000)	Wild type	NVRGDLQVLAQK**TEKT**LPTS	RHKQRIVAP**AQ**QL**L**	76.5 (26/34)	HQ412603
Cathay	O-TW-185-97	Wild type	NVRGDLQVLAQKA**E**R**T**LPTS	RH**E**Q**M**IVAPAKQLL	82.4 (28/34)	GQ292726

[a] ID, Identity.
[b] Underlined and bold letters show different amino acids with O/Andong/SKR/2010.

Table 4
Serological relationships between experimental vaccination groups through the cross-virus neutralization test (VNT) by three topotype viruses.

Vaccination groups	Virus strains for VNT (*r* value[a])			
	SEA topotype		ME–SA topotype	Cathay topotype
	O/Andong/SKR/2010	O/YJ/SKR/2010	O Manisa	O/ASP/Cathay
AD-P	ID[b]	0.92	0.52	0.19
YJ-P	0.97	ID	0.45	0.13
Manisa	0.24	0.33	ID	0.25

[a] *r* values were calculated by division between reciprocal arithmetic titers (serum titer against viruses used for cross VNT/serum titer against vaccine viruses in the vaccination groups, in 28 days post vaccination).
[b] ID, identity.

higher antigen concentrations. Furthermore, this result from the matching test showed that the experimental SEA vaccine matched the ME–SA strain but not the CHY topotype.

Nonstructural protein coding regions that play important roles for virus replication show fewer nucleotide mutations than structural protein coding regions [27]. In particular, VP1 is a region exposed on the surfaces of viral capsids. It is one of the capsid proteins that determines the infectivity of host cells and range of hosts [28]. In accordance with previous reports, FMDVs adapted to cells show many nucleotide mutations in their structural protein regions. In the present study, the protective effects of experimental vaccines using cell-adapted virus to protect pigs against the SEA and ME–SA topotypes could be observed. These results can be used as preliminary data for future commercial vaccine production.

Acknowledgments

We thank the staff of the Foot and Mouth Disease Division and the Foreign Animal Disease Division at the Animal and Plant Quarantine Agency (QIA). This research was supported by a grant from the QIA's National Animal Disease Research Project.

Conflict of interest: None.

Grant sponsor: Supported by the Animal and Plant Quarantine Agency, Anyang, Gyeonggi, Republic of Korea.

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.2014.01.067.

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