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Cross-protective efficacy of the O1 Manisa + O 3039 bivalent vaccine and the O 3039 monovalent vaccine against heterologous challenge with FMDV O/Jincheon/SKR/2014 in pig

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ABSTRACT

After massive foot-and-mouth disease (FMD) outbreaks originated from Jincheon County from Dec. 2014 to Apr. 2015, the effectiveness of the previous FMD vaccine containing only the O1 Manisa as the O antigen, O1 Manisa + A Malaysia 97 + Asia 1 Sharmir trivalent vaccine, was questioned in South Korea, and a change in the O antigen in FMD vaccines was demanded to control the FMD caused by FMDV O/Jincheon/SKR/2014, the O Jincheon strain. Therefore, the efficacies of O1 Manisa + O 3039 bivalent vaccine and O 3039 monovalent vaccine were studied for cross-protection against heterologous challenge with the O Jincheon strain. In this study, the efficacy of the O1 Manisa + O 3039 bivalent vaccine was better than that of the O 3039 monovalent vaccine, even though the serological relationship (r_1 value) between O Jincheon and O 3039 was matched according to the OIE Terrestrial Manual. According to serological test results from vaccinated specific pathogen free pigs, virus neutralization test titers against Jincheon were good estimates for predicting protection against challenge. A field trial of the O1 Manisa + O 3039 bivalent vaccine was performed to estimate the possibility of field application in conventional pig farms, especially due to concerns about the effect of maternally derived antibodies (MDA) in field application of the FMD vaccine. According to the result of the field trial, the O1 Manisa + O 3039 bivalent vaccine was considered to overcome MDA. The results of the efficacy and field trials indicated that the O1 Manisa + O3039 vaccine could be suitable to replace previous FMD vaccines to control the FMD field situation caused by O Jincheon FMDV.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of many cloven-hoofed animals, such as cattle, pigs, sheep and goats. FMD is caused by FMD virus (FMDV), which is a single-stranded, positive sense RNA virus (genus *Aphthovirus,* family *Picornaviridae*), and consists of seven serotypes: O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3 [1]. Among the seven serotypes, O and A have been distributed globally, and the O type occurs most frequently worldwide. Serotype C was assumed to be extinct after the last outbreaks in Brazil and Kenya in 2004 [2–4].

Since 2000, 10 major FMD outbreaks have occurred in the Republic of Korea [5–9]. On Nov. 28th, 2010, a FMD outbreak occurred in Andong city and spread nationwide, affecting 3,748 farms [6,10]. A couple of weeks after the first outbreak in Andong city, Korean authorities renounced the stamping out policy and decided to use commercial FMD vaccines to control the outbreak due to the devastating nationwide spread of FMD [5].

Although FMD vaccines containing the O1 Manisa strain have been successfully used for nationwide mandatory vaccination in South Korea since 2011, the O1 Manisa strain was not effective in controlling the FMD situation of the Jincheon outbreak after Dec 2014 [8]. Therefore, O type strain changes in FMD vaccines were inevitable to reinforce the efficacy of the vaccine. Several vaccine efficacy studies were conducted to select the potential candidates to change the O type vaccine strains to prevent the sporadic FMD outbreaks in Korea.

In this study, the O1 Manisa + O 3039 bivalent vaccine (O1 Manisa + O 3039 vaccine) and O 3039 monovalent vaccine

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(O 3039 vaccine) were evaluated to determine their protective immunity against infection with the O/Jincheon/SKR/2014 (O Jincheon) strain in pigs. Additionally, a field trial of the O1 Manisa + O 3039 vaccine was conducted to evaluate the serological performance of the vaccine in the field.

2. Materials & methods

2.1. Vaccine matching

When FMD occurs in Korea, the FMD samples are sent to the FMD World Reference Laboratory (WRL) at Pirbright, UK. We then receive the vaccine matching results from the test organizations. Vaccine matching was performed in the manner of a two-dimensional neutralization test described previously [11].

2.2. Challenge virus and cells used in the study

FMDV O/Jincheon/SKR/2014 (Mya-98 lineage strain) originated from the FMD outbreak in Jincheon County, Chungcheongbuk-do Province, in 2014. This isolate was propagated and titrated in bovine calf kidney (LF-BK) cells, which were kindly provided by the Plum Island Animal Disease Center (USA). The LF-BK cell line was maintained in Dulbecco's modified Eagle's medium (DMEM; Corning, USA) with 5% fetal bovine serum (FBS; Gibco, USA). Cells were grown at 37 with 5% CO_2 in a humidified atmosphere.

2.3. Experimental animals and vaccines

Animal experiments were conducted in the BSL3 containment facility at the Animal and Plant Quarantine Agency in South Korea. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the Animal and Plant Quarantine Agency of Korea (IACUC No. 2015-267).

Two high-potency commercial vaccines, the O1 Manisa + O 3039 bivalent vaccine (O1 Manisa + O 3039 vaccine) and the O 3039 monovalent vaccine (O 3039 vaccine), were obtained from Merial Company Limited, United Kingdom.

2.4. Vaccine efficacy studies and challenge protocol

Two animal experiments were separately conducted to evaluate the efficacy of two FMD vaccines containing the O 3039 vaccine strain, O1 Manisa + O 3039 vaccine and O 3039 vaccine (Table 1).

In the first study, 20 FMD-free specific pathogen free (SPF) Yucatan pigs (8 weeks old) were used in the experiment to evaluate the efficacy of the O1 Manisa + O 3039 vaccine. Three groups of 5 pigs each (Groups A-C) were vaccinated with one dose (2 ml) and challenged on 7, 14, and 28 days post vaccination (dpv), respectively. One group of 3 pigs (Group D) was unvaccinated and challenged. In the second study, 12 FMD-free SPF Yucatan pigs (8 weeks old) were used in the experiment to evaluate the efficacy of the O 3039 vaccine. One group of 4 pigs (Group E) was vaccinated with one dose and challenged on 28 dpv, and the other group of 4 pigs (Group F) was vaccinated twice with one dose at 8 and 12 weeks old and challenged 14 days after boost vaccination. One group of 2 pigs (Group G) was unvaccinated and challenged (Table 1).

To challenge the experimental group in each study, two naïve pigs, as donor pigs, were challenged by intradermal inoculation with 0.1 ml of challenge virus containing $10^{6.0}$ $TCID_{50}$/ml O Jincheon virus into the heel bulbs. After FMD clinical signs were clearly shown, these donor pigs were kept with experimental pigs for 24 h.

Vaccination was deliberately scheduled so that all animals in each study were challenged by contact infection on the same day. Since the experiment was performed in two steps, each experimental study had unvaccinated control pigs (Group D, n = 3; Group G, n = 2) that were challenged along with the vaccinated groups.

The clinical scores of the pigs were observed daily and recorded using a scoring system described previously [12,13] as follows: an elevated body temperature of 40° C (1 point), 40.5 °C (2 points) or 41 °C (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 point); 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. After virus challenge, nasal swabs and sera were collected at all sequential days from 0 dpc to 8 dpc, then taken at one-day intervals until 14 dpc; viruses were detected using quantitative RT-PCR (qRT-PCR) [14].

2.5. Vaccine field trial

One hundred and seventy-nine crossbred pigs were used for a pilot field trial to evaluate the serological performance of the O1 Manisa + O 3039 vaccine in the field. In the original experimental design of 3 conventional pig farms (JC, GW, and IC), 20 pigs in each farm were planned to be vaccinated once at 8 weeks old, and another 20 pigs in each farm were planned to be vaccinated twice at 8 and 12 weeks old. However, among 3 farms, one farm mistakenly had one more shot of vaccination with FMD vaccine containing the O1 Manisa strain after the prime vaccination, following the routine national vaccination program. In the Korean National Institute of Animal Science (NIAS) and one pig importer farm, 30 and 29 pigs were vaccinated with one dose at 8 and 12 weeks old, respectively. Blood samples were obtained from the jugular vein at 8, 12, 16, 20 and 24 weeks old in the 3 conventional pig farms. In NIAS, blood samples were obtained at 8, 12, 16 and 24 weeks old. In

Table 1
Experimental designs of two FMD vaccine efficacy studies: the O1 Manisa + O 3039 vaccine and the O 3039 vaccine.

Trial[a]	Group	Vaccine strain	No. of animals	Day of vaccination (dpc[b])	Serum collected at (dpc)	Nasal swabs collected at (dpc)
1	A	O1 Manisa + O 3039	5	−7	−28, −21, −14, −7, 0–8, 10, 12, 14	0–8, 10, 12, 14
1	B	O1 Manisa + O 3039	5	−14	−28, −21, −14, −7, 0–8, 10, 12, 14	0–8, 10, 12, 14
1	C	O1 Manisa + O 3039	5	−28	−28, −21, −14, −7, 0–8, 10, 12, 14	0–8, 10, 12, 14
1	D	Unvaccinated	3	–	−28, −21, −14, −7, 0–8, 10, 12, 14	0–8, 10, 12, 14
2	E	O 3039	4	−28	−42, −28, −14, 0–8, 10, 12, 14	0–8, 10, 12, 14
2	F	O 3039	4	−42(prime[c]), −14 (boost[d])	−42, −28, −21, −14, 0–8, 10, 12, 14	0–8, 10, 12, 14
2	G	Unvaccinated	2	–	−42, −28, −14, 0–8, 10, 12, 14	0–8, 10, 12, 14

[a] Two separate animal studies were conducted with two different FMD vaccine, the O1 Manisa + O 3039 vaccine and the O 3039 vaccine.
[b] dpc; day postchallenge.
[c] prime: day of the prime vaccination.
[d] boost: day of the boost vaccination.

the pig importer farm, blood samples were obtained at 8, 12 and 16 weeks old.

2.6. Serological analysis

The neutralizing antibody titers against the O 3039 strain or the O Jincheon strain were measured as specified in the OIE Terrestrial Manual 2017 [15]. The positive criterion of the virus neutralization test (VNT) was designated 1:32 in this study. PrioCHECK FMDV SP O ELISA (Prionics AG, Schlieren-Zurich, Switzerland) and VDPro FMDV NSP AB ELISA (Median, Chuncheon, South Korea) kits were employed to detect antibodies against FMDV structural protein (SP) O and nonstructural protein (NSP) in serum samples of pigs, respectively.

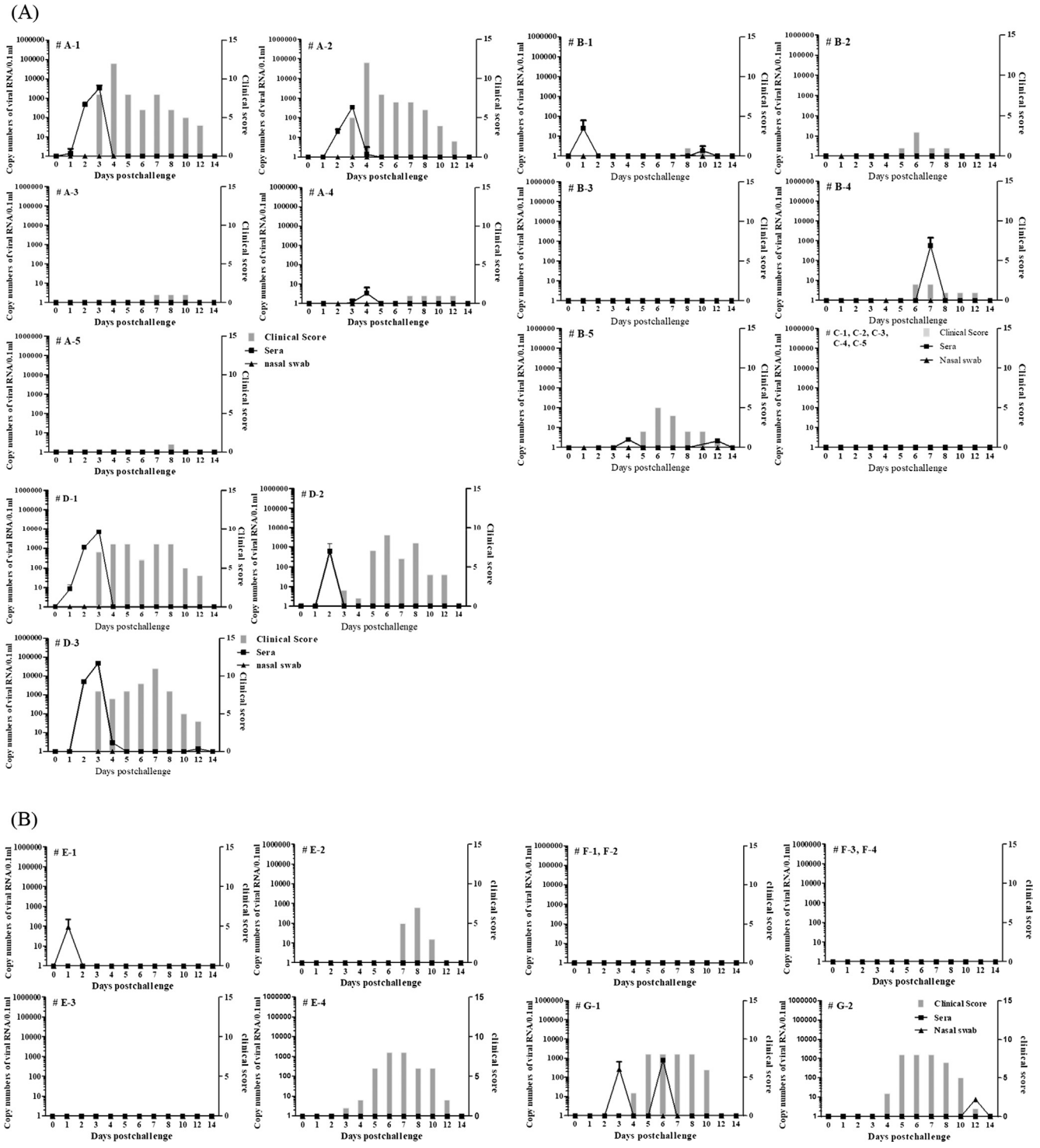

Fig. 1. The changes in clinical scores and FMDV RNA levels in pigs immunized with the O1 Manisa + O 3039 vaccine or the O 3039 vaccine after contact challenge with donor pigs inoculated with the O/Jincheon/SKR/2014 strain. FMDV RNA levels in sera and nasal swabs were measured by qRT-PCR from 0 to 14 days after challenge. (A) O_1 Manisa + O 3039 vaccine (A: 7 dpv, B: 14 dpv, C: 28 dpv, D: unvaccinated), (B) O 3039 vaccine (E: 28 dpv, F: 42 (prime) dpv, G: unvaccinated). # number at the top left of each graph indicates the pig ID. Error bars represent standard error of the mean (SEM).

3. Results

3.1. Vaccine matching

According to results of the FMD WRL, the r_1 value between the O1 Manisa strain and the O Jincheon strain was 0.10 ~ 0.30, and the r_1 value between the O 3039 strain and the O Jincheon strain was 0.42 ~ 0.73.

3.2. Vaccine efficacy study of the O1 Manisa + O 3039 vaccine

3.2.1. Clinical signs

All vaccinated animals in group A and unvaccinated control animals were not protected against challenge. Of 5 pigs in group B, only 1 pig was protected. All vaccinated pigs in group C were protected. In unvaccinated control animals, lesions were observed starting at 3 days postchallenge (dpc), and individual clinical scores ranged from 7 to 8 at 6 dpc. However, in vaccinated and challenged animals, the onset days of clinical signs in some unprotected pigs were delayed, and clinical scores in most unprotected pigs were lower than those in unvaccinated and challenged animals (Fig. 1A).

3.2.2. Detection of FMDV by qRT-PCR

Three pigs among 5 in groups A and B sporadically showed viremia lasting 1 or 2 days, and virus copy numbers ranged from $10^{0.2}$ to $10^{3.5}$. There was no viremia detected in any animal in group C. In the unvaccinated control animals (group D), virus antigens were detected at 1 or 2 dpc and lasted for 1 or 3 days, and virus copy numbers ranged from $10^{3.1}$ to $10^{3.9}$. No positive result was detected in nasal swabs in the efficacy test of the O1 Manisa + O 3039 vaccine (Fig. 1A).

3.2.3. Serological responses in efficacy tests

The SP ELISA antibody levels of all unvaccinated animals were seroconverted at 5 dpc. In the vaccinated groups, the SP ELISA antibody levels became seroconverted at 7 dpv and showed complete seroconversion approximately 7 days later (Fig. 2A).

The NSP ELISA antibody levels of all animals were negative reaction before challenge with the O Jincheon strain. But after challenge, the NSP ELISA levels in some animals showed increasing patterns. Eventually, the mean NSP ELISA antibody level of unvaccinated group became seropositive at 10 dpc (Fig. 2C).

The mean VNT titer to the heterologous strain, O Jincheon strain, in unvaccinated group became seropositive at 5 dpc, while the mean VNT titers to the O Jincheon strain in vaccinated groups mostly became seropositive at 14 dpv (Fig. 3A; Supplementary table 1). The mean VNT titers to the homologous strain, O 3039 virus, in unvaccinated animals became seropositive at 5 dpc, while the mean VNT titer to the O 3039 virus in group A became seropositive at 7 dpv and other vaccinated groups showed seropositivity at 14 dpv (Fig. 3C; Supplementary table 2).

3.3. Vaccine efficacy study of the O 3039 vaccine

3.3.1. Clinical signs

All animals in group F were protected against challenge, whereas 2 among 4 pigs in group E and all unvaccinated control animals were not protected. Among 2 unprotected pigs in group

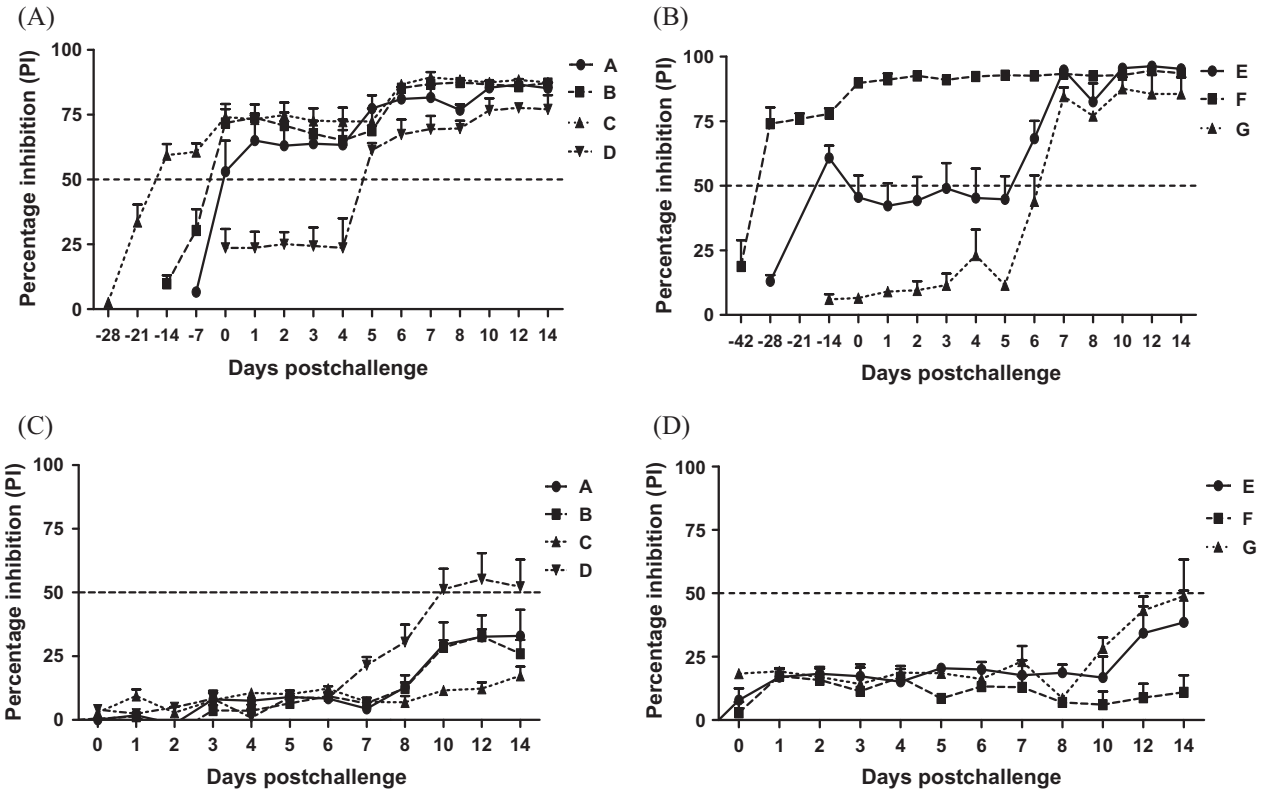

Fig. 2. Percentage inhibition of antibodies specific for SP and NSP in pigs vaccinated with the O1 Manisa + O 3039 vaccine or the O 3039 vaccine. Pigs of group A, B and C were vaccinated with the O1 Manisa + O 3039 vaccine and pigs of group E and F were vaccinated with the O 3039 vaccine. Pigs of group D and G were not vaccinated as the control groups. After vaccination, all pigs were challenged with FMDV O/Jincheon/SKR/2014 (O Jincheon) strain. Antibody were detected by using a PrioCHECK FMDV SP ELISA kit and NSP ELISA kit. (A) SP ELISA titers to type O antigen (A: 7 dpv, B: 14 dpv, C: 28 dpv, D: unvaccinated), (B) SP ELISA titers to type O antigen (E: 28 dpv, F: 42(prime) dpv, G: unvaccinated), (C) NSP ELISA titers (A: 7 dpv, B: 14 dpv, C: 28 dpv, D: unvaccinated), (D) NSP ELISA titers (E: 28 dpv, F: 42(prime) dpv, G: unvaccinated). Error bars represent SEM.

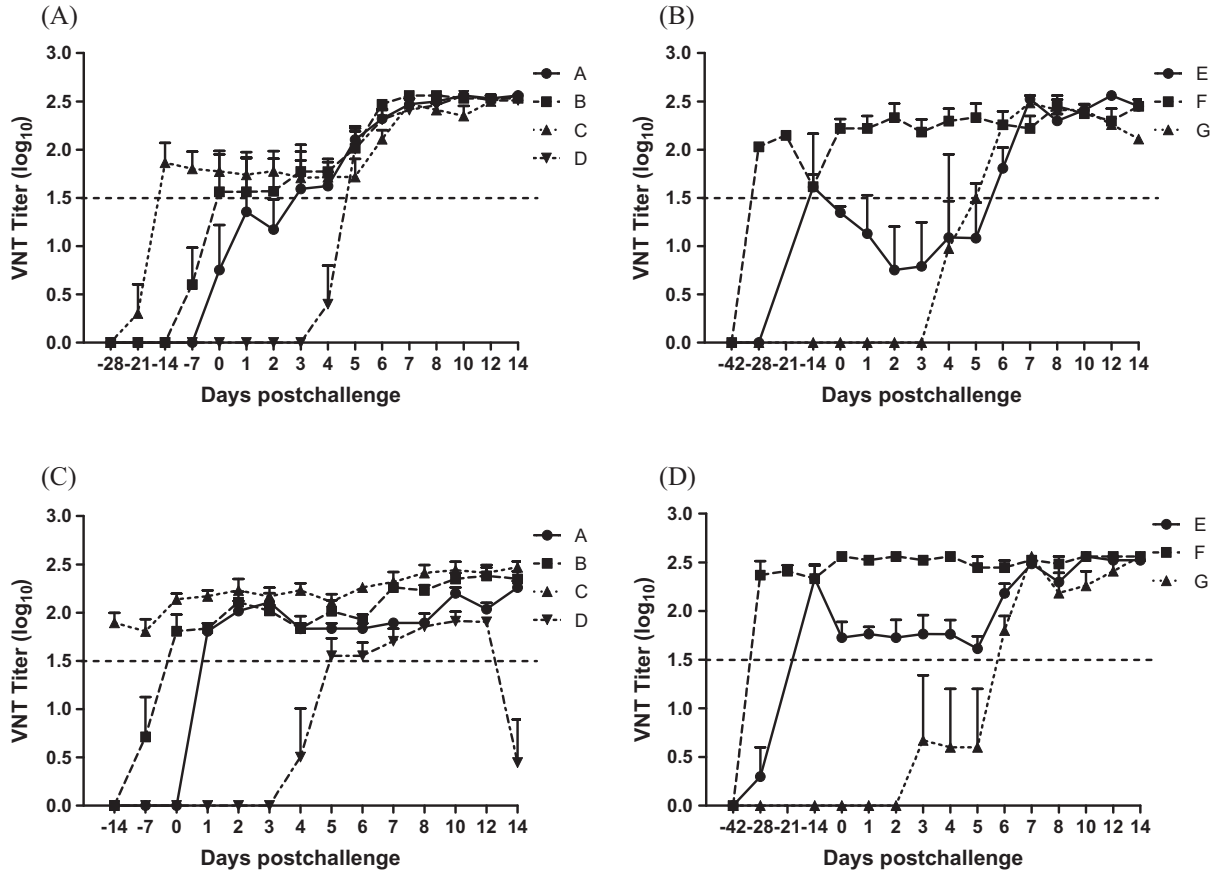

Fig. 3. Serum titers of virus neutralization antibody against FMD viruses in pigs administered with the O1 Manisa + O 3039 vaccine or the O 3039 vaccine. Pigs of group A, B and C were vaccinated with the O1 Manisa + O 3039 vaccine, and pigs of group E and F were vaccinated with the O 3039 vaccine. Pigs of group D and G were not vaccinated as the control groups. After vaccination, all pigs were challenged with the O Jincheon strain. (A) VNT titer to the O Jincheon strain (A: 7 dpv, B: 14 dpv, C: 28 dpv, D: unvaccinated), (B) VNT titer to the O Jincheon strain (E: 28 dpv, F: 42(prime) dpv, G: unvaccinated), (C) VNT titer to the O 3039 strain (A: 7 dpv, B: 14 dpv, C: 28 dpv, D: unvaccinated), (D) VNT titer to the O 3039 strain (E: 28 dpv, F: 42(prime) dpv, G: unvaccinated). Error bars represent SEM.

E, 1 pig showed clinical signs 4 days after clinical signs were first observed in the other pig (Fig. 1B).

3.3.2. Detection of FMDV by qRT-PCR

Only 1 pig among 4 in group E had a positive result from only nasal swabs on 1 dpc. There was no positive result from blood and nasal swabs in group F. In the unvaccinated control animals (group G), one pig showed positive results from sera and nasal swabs, and the other showed positive results from only nasal swabs (Fig. 1B).

3.3.3. Serological responses in efficacy tests

SP ELISA antibody levels of all unvaccinated animals were seroconverted at 7 dpc. In group E, the SP ELISA antibody levels became seropositive at 14 dpv, decreased somewhat and were completely seroconverted at 7 dpc. In group F, the SP ELISA antibody levels were completely seroconverted at 14 dpv (Fig. 2B).

After challenge with the O Jincheon strain, the NSP ELISA levels of some animals in unvaccinated group and group E (28 dpv vaccinated group) became seroconversion at 12 dpc. But, the mean NSP ELISA antibody levels of all groups were below the seropositive level until the end of this experiment (Fig. 2D).

The mean VNT titer to the heterologous strain, O Jincheon strain, in unvaccinated group became seropositive at 6 dpc. However, the mean VNT titers to the O Jincheon strain in group E were confirmed to be positive at 14 dpv, decreased below 1:32 (1.51 $\log_{10}$) from 0 to 5 dpc and recovered at 6 dpc (Fig. 3B; Supplementary table 1). The mean VNT titer to the homologous strain, O 3039 virus, in unvaccinated group became seropositive at 6 dpc, while the mean VNT titers became seropositive at 14 dpv and maintained a high titer until 14 dpc in groups E and F (Fig. 3D; Supplementary table 2).

3.4. Serological responses in the vaccine field trial

In single-vaccinated pigs from 2 conventional pig farms (JC and GW), the mean pre-existing positive rates of SP ELISA and VNT were 62.5% (25/40) and 27.5% (11/40), respectively. Although vaccination was applied, the mean positive rates of SP ELISA and VNT in the single-vaccinated pigs gradually decreased or stagnated to 25.6% (10/39) and 28.2% (11/39), respectively, until 16 weeks later. In double-vaccinated pigs from same 2 conventional pig farms, the mean pre-existing positive rates of SP ELISA and VNT were 62.5% (25/40) and 20.0% (8/40), respectively, approximately the same levels of the single-vaccinated pig groups. However, steady immune responses were shown in the double-vaccinated pigs, in that mean positive rates of SP ELISA and VNT were 57.5% (23/40) and 72.5% (29/40) at 12 weeks after booster immunization, respectively (Table 2). In the results of the ELISA antibody levels in JC and GW farms, the statistical significances between the single-vaccinated and double-vaccinated groups were observed from 8 weeks after the first vaccination ($p < 0.001$ in JC and $p < 0.01$ in GW; Fig. 4A) to the end of the experiment ($p < 0.01$ in JC and $p < 0.05$ in GW; Fig. 4A). In the results of the VNT titers in JC and

Table 2
Changes of serological results in field trials with the O1 Manisa + O 3039 vaccine.

Group	Farm ID	Test	Round of vaccination	Weeks after vaccination (positive no/tested no, positive rate)				
				0	4	8	12	16
Conventional pig farm	JC	SP ELISA[a]	1	13/20[c] (65.0[d])	6/20 (30.0)	6/20 (30.0)	6/20 (30.0)	6/20 (30.0)
			2	14/20 (70.0)	8/20 (40.0)	18/20 (90.0)	15/20 (75.0)	13/20 (65.0)
		VNT[b]	1	7/20 (35.0)	5/20 (25.0)	0/19 (0.0)	2/17 (11.8)	5/20 (25.0)
			2	6/20 (30.0)	10/20 (50.0)	15/20 (75.0)	9/19 (47.3)	17/20 (85.0)
	GW	SP ELISA	1	12/20 (60.0)	10/20 (50.0)	7/20 (35.0)	6/20 (30.0)	4/19 (21.1)
			2	11/20 (55.0)	8/20 (40.0)	13/20 (65.0)	12/20 (60.0)	10/20 (50.0)
		VNT	1	4/20 (20.0)	8/20 (40.0)	10/20 (50.0)	8/16 (50.0)	6/19 (31.6)
			2	2/20 (10.0)	5/20 (25.0)	17/20 (85.0)	9/19 (47.0)	12/20 (60.0)
	IC[e]	SP ELISA	1	19/20 (95.0)	11/20 (55.0)	1/20 (5.0)	13/15 (86.7)	10/13 (76.9)
			2	17/20 (85.0)	14/20 (70.0)	10/20 (50.0)	17/18 (94.4)	12/17 (70.6)
		VNT	1	20/20 (100.0)	6/20 (30.0)	4/20 (20.0)	11/13 (84.6)	9/12 (75.0)
			2	16/20 (80.0)	4/20 (20.0)	10/20 (50.0)	13/18 (72.2)	14/17 (82.4)
NIAS[f]		SP ELISA	2	21/30 (70.0)	20/30 (66.7)	30/30 (100.0)		22/22 (100.0)
		VNT	2	12/30 (40.0)	13/30 (43.3)	26/30 (86.7)		22/22 (100.0)
Pig importing farm[g]		SP ELISA	2	0/29 (0.0)	18/29 (62.1)	27/27 (100.0)		
		VNT	2	0/29 (0.0)	18/29 (62.1)	25/27 (92.6)		

[a] Test results of O type SP ELISA kit (Prionics®).
[b] Test results of VNT against the homologous virus, O 3039 strain.
[c] No. of positive animal/ No. of tested animal.
[d] Positive rate of the serological results (%).
[e] The farm mistakenly had an additional round of vaccination 8 weeks after the prime vaccination, in accordance with the routine national vaccination program.
[f] The farm belonging to the National Institute of Animal Science (NIAS) in the South Korea.
[g] The farm that imports the pig from FMD-free countries.

GW farms, the statistical significances between two vaccinated groups were also observed at 8 weeks after the first vaccination ($p < 0.001$; Fig. 4B).

In single-vaccinated pigs from IC farm, the mean positive rates, which were 95.0% in SP ELISA and 100% in VNT before the vaccination, dropped into 5.0% and 20.0%, 8 weeks after the vaccination, respectively. And in double-vaccinated pigs from IC farm, the mean positive rates, which were 85.0% in SP ELISA and 80% in VNT before the vaccination, were slightly decreased to 50.0% (10/20), 4 weeks after the boost vaccination (Table 2). In the results of the ELISA antibody levels in IC farm, the statistical significances between two vaccinated groups were also observed at 8 weeks after the first vaccination ($p < 0.001$; Fig. 4A).

In NIAS farm, the mean positive rates, which were 70.0% in SP ELISA and 40% in VNT before the vaccination, increased into 100.0% and 86.7% 4 weeks after the boost vaccination, respectively, and maintained until the end of the experiment. And in the pigs of the pig importing farm, which were FMD seronegative, strong immune responses were observed both after the prime vaccination and after the boost vaccination (Table 2; Fig. 4).

4. Discussion and conclusions

In general, one of the considerations to select the FMD vaccine strain is the r_1 value between the vaccine strain and the field FMDV [15]. According to vaccine matching results reported by FMD WRL, the r_1 values of O1 Manisa and O 3039 with the O Jincheon strain were $0.10 \sim 0.30$ and $0.42 \sim 0.73$, respectively. If the r_1 value between the vaccine strain and the field virus is greater than 0.3, it is generally assumed that the vaccine containing the vaccine strain is likely to confer protection against challenge with the field virus [15]. In this study, however, the high-potency O 3039 monovalent vaccine did not confer proper immunity to prevent all vaccinated animals challenged with the O Jincheon strain at 28 dpv from displaying clinical signs, although the high-potency O1 Manisa + O 3039 vaccine provided all vaccinated animals full protection. It is likely that the increased antigen payload or the synergetic effect of cross protection of two antigens in the O1 Manisa + O 3039 vaccine might affect the efficacy of protection against the O Jincheon strain, but this explanation would be needed to be studied further in the future.

Our FMD vaccination and challenge studies showed that humoral immunity plays an important role in the generation of protective immunity against the challenge virus. Instead of VNT titers against the homologous vaccine strain, O 3039 (Supplementary table 2), individual VNT titers against the heterologous challenge strain, O Jincheon, were related to the dynamics of clinical symptoms in each animal. As for the efficacy results of A-1, A-2, E-2 and E-4 among vaccinated animals (Supplementary table 1), relatively low VNT titers against the O Jincheon strain at the challenge time, 0 and 1 dpc, resulted in failure of protection against the

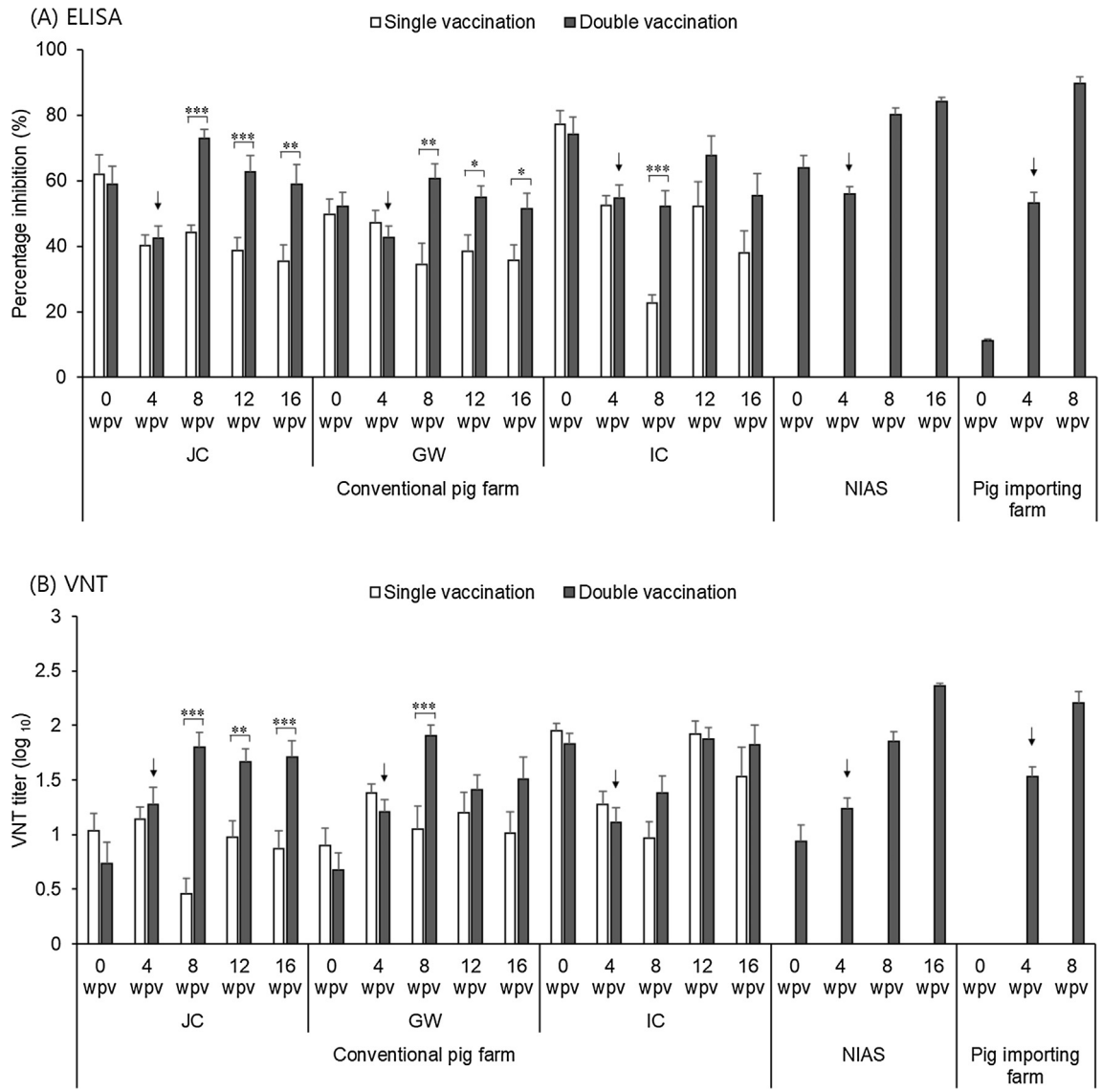

Fig. 4. ELISA (A) and VNT (B) antibody responses in single vaccinated groups and double vaccinated groups in field trials with the O1 Manisa + O 3039 vaccine. Each of 3 conventional pig farms had a single vaccinated group immunized once at 8 weeks old and a double vaccinated group immunized twice at 8 and 12 weeks old. However, the farm IC among 3 conventional pig farms had mistakenly another round of vaccination in both immunized groups after 16 weeks old. Both NIAS and pig importing farm had only one group which were vaccinated twice at 4-week intervals. The NIAS farm belongs to the National Institute of Animal Science (NIAS) in the South Korea. The pigs in the pig importing farm were imported from FMD-free countries. Black arrows indicate boost vaccination. Error bars represent standard error of the mean (SEM). wpv: weeks postvaccination. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

challenge virus, such as a shorter incubation time (3 to 5 dpc) and a higher clinical peak score of individuals (7 to 12). However, in clinically affected animals, such as the cases of A-3, A-4, A-5, B-1 and B-2 (Supplementary table 1), which had high VNT titers to the O Jincheon strain at the challenge time, symptoms tended to be displayed on later days, 5 to 8 dpc, and the highest clinical score of individuals were relatively low, ranging from 1 to 3 in very limited local lesions, such as the mouth or one leg of each pig.

Several studies of infection dynamics in various environments, such as vaccination, non-vaccination, within pens, or between pens, concluded that virus transmission between FMD-infected pigs and normal pigs could occur within groups of pigs even if pigs were immunized with FMD vaccine [16–19]. Judging from the relationship between VNT titers and clinical aspects in this study, the early onset clinical signs seemed to be directly contributed to by contact challenge, and pigs having late onset clinical signs could be secondarily contracted by primary infection from contact challenge. According to the PD_{50} test in the OIE manual [15], clinical signs should be counted except for clinical signs near the infection route. Because the challenge method was contact infection with a donor pig intradermally inoculated into the heel bulbs, the infection route to each challenged pig could not be specified. Therefore, it could be considered that animals having these kinds of mild clinical signs were protected against primary challenge. If so, the efficacy of the O1 Manisa + O 3039 vaccine could be more effective for protection against O Jincheon challenge than the efficacy results displayed in this study.

Maternally derived antibodies (MDA) from sows have been indicated as an important factor with considerable influence on serological performance in piglets applied with FMD vaccines in a field situation [20–22]. Therefore, field trials with administration of FMD vaccines were conducted to select the best vaccines among many FMD vaccines applicable in field. In this field trial study of the high-potency O1 Manisa + O 3039 vaccine, the positive rates

of ELISA and VNT in the single-vaccinated groups did not increase along with periods after vaccination, even though strong immune responses from the prime vaccinated animals were shown in the absence of MDA, like FMD seronegative pigs in the pig importing farm. But the positive rates of ELISA and VNT in the double-vaccinated groups in JC and GW farms showed an increasing pattern 4 weeks after boost vaccination. And the significant differences were observed in average ELISA antibody levels and VNT titers between the single-vaccinated and double-vaccinated groups in JC and GW farms. Even though the positive rate of both ELISA and VNT in double-vaccinated IC farm pigs were decreased to 50% at 4 weeks after boost vaccination, it could be probably due to the surprisingly high pre-existing positive rates which were more than 80% in both ELISA and VNT. Comparing with the poor positive rates of ELISA and VNT in single-vaccinated IC farm pigs, double vaccination would be a good solution to cope with the effects of high MDA. But due to the accidental one more round of vaccination, the results of two groups in IC farm could not be comparable after 16 weeks old. And among double-vaccinated groups, pigs in NIAS farm showed better serological performance than pigs in other conventional farms. Considering that NIAS is one of the government institutes that are responsible for studying scientific husbandry management of Korean livestock, it could be assumed that this serological performance would be attributed to the better management of husbandry or hygiene in the NIAS farm. From these results, we concluded that the effect of MDAs to FMDV could be overcome in the field when the O1 Manisa + O 3039 vaccine was applied with double vaccination.

FMD outbreaks in South Korea were caused by FMDV belonging to O/SEA/Mya-98 lineage from Nov 2010 to Aug 2014 and the high-potency FMD vaccines containing O1 Manisa was effective during the period [7]. However, despite the FMD outbreak caused by the same Mya-98 lineage in Dec 2014 and Korea's strong vaccination policies with high-potency trivalent FMD vaccine, O1 Manisa + A Malaysia 97 + Asia 1 Sharmir, the type O FMD outbreak resulted in 185 cases during 5 months since Dec 2014 [8]. Most of the infected farms were pig farms, except for 5 cattle farms. Therefore, the efficacy studies of the FMD vaccines were necessary to change the O type vaccine antigen. In this study, we estimated the efficacy of the O1 Manisa + O 3039 vaccine and the O 3039 vaccine against O Jincheon in a biologically secured facility and finally conducted a field trial with the O1 Manisa + O 3039 vaccine. Consequently, the O1 Manisa + O 3039 vaccine replaced the O1 Manisa + A Malaisa 97 + Asia 1 Shamir vaccine as an emergency vaccine to control the FMD situation in South Korea since May 2015. Even though incomplete protection 7 days and 14 days after vaccination with the O1 Manisa + O 3039 vaccine was concerning, it was thought that double vaccination with the high potency O1 Manisa + O 3039 vaccine would be sufficient to cover the FMD situation caused by the O Jincheon FMDV. It is also concluded that the combination of efficacy studies and field trials would be necessary to find and select better vaccines to control heterologous FMDV strains in the field.

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Conflict of interest

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2018.11.080.

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