

Increased humoral antibody response of foot-and-mouth disease virus vaccine in growing pigs pre-treated with poly- γ -glutamic acid

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This study was conducted to determine if humoral antibody response of foot-and-mouth disease (FMD) vaccine improved in 8-week-old growing pigs born to well-vaccinated sows pre-treated with 60 mg of poly- γ -glutamic acid (γ -PGA) three days before vaccination. Antibody against FMD virus serotype O was measured 0, 2, 4 and 6 weeks post-vaccination, using a PrioCHECK FMDV type O ELISA kit. The results showed that positive antibody reactions against FMDV serotype O antigen among a component of the vaccine significantly increased in response to pre-injection with γ -PGA.

Keywords: foot-and-mouth disease virus vaccine, humoral immune response, poly- γ -glutamic acid

Foot-and-mouth disease (FMD) in cloven-hoofed domestic animals such as cattle, sheep, goats and pigs causes devastating economic losses because of the highly transmissible, acute nature of the disease. A severe outbreak of FMD leaves a terrible negative impression on national and international livestock animal industries as well as consumers [5]. To date, at least four major FMD outbreaks have occurred in cattle or pig populations in Korea in 2000, 2002, 2010–2011 [5] and 2014–2015. Since the FMD outbreak of 2010–2011, a nationwide blanket vaccination policy has been enforced for ruminants and pigs as a sustainable preventive measure, and a routine vaccination program is still a pivotal control tool against FMD virus infection, although this program is somewhat controversial. The FMD vaccine adapted in Korea consists of inactivated viruses with double oil-based emulsion (DOE) and includes structural proteins of FMD viruses (FMDVs) (O₁ Manisa + A Malaysia + Asia 1 Shamir serotypes). This typical combination effectively reduced FMD incidence during 2011, and has been successfully used for FMD vaccination in Korea [5]. However, a single dose injection of this trivalent FMD vaccine in pigs has a few disadvantages, including poor humoral immune induction and a short period of immune duration compared to that of induced by natural FMDV infection [1,2]. These kind of concerns has been raised after using commercially available

FMDV type O ELISA kits. In addition, a non-immunogenic material, poly- γ - glutamic acid (γ -PGA), which is produced using *Bacillus* species, is a biodegradable and unusual anionic homopolyamide. γ -PGA is a versatile material that has been widely applied owing to its unique properties [4]. However, efficacy of this substance needs to be elucidated as an immune modulator of FMDV vaccine adjuvant with double oil-based emulsions.

In Korea, a single dose immunization of trivalent FMDV vaccine has been preferably adopted for 8 to 12 week old pigs because of the labor and costs involved, even though two immunizations of the vaccine are recommended for long-lasting antibody response [5]. Therefore, this study was conducted to investigate whether humoral antibody response to FMDV serotype O antigen, a component of the vaccine, improved in 8-week-old growing pigs born to well-vaccinated sows that had been pre-treated with poly- γ -glutamic acid.

Three swine farms without any history of FMD outbreak were selected, and routine sow's vaccination programs were performed, including the trivalent FMDV vaccine recommended by the Korean government. Each swine farm of an all-in/all-out pig flow was managed as a two-site production system with a nursery and finishing unit. A total of 24 conventional 8-week-old pigs with an average body weight of 23 ± 1 kg that

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originated from a single healthy herd born to well-vaccinated sows were randomly divided into 2 groups of 12 pigs. Each pig was numbered by an ear tag. Pigs in group 1 remained as a non-treated control, while those in Group 2 pigs were injected intramuscularly behind the base of one ear with 60 mg of γ -PGA (kindly provided by Dong Bang, Korea) 3 days before FMDV vaccination. All pigs were subsequently vaccinated intramuscularly behind the base of the other ear vaccinated with 2 mL of an inactivated FMDV vaccine (Aftopor Merial FMD vaccine bottled by Daesung Microbiological, Korea). This vaccine formulated a double oil-based emulsion (DOE) adjuvant with at least six 50% protective doses (PD₅₀) of inactivated trivalent FMDVs (O₁ Manisa + A Malaysia + Asia 1 Shamir serotypes). Pigs were then provided with typical diets until the end of the experiment.

At least 5 mL of blood were collected from the jugular vein on the day of FMDV vaccination, then bi-weekly until the end of the study. The clotted blood samples were subsequently

transported on ice to the laboratory and the total FMDV serotype O-specific antibody production was measured. The anti-FMDV serotype O antibody level of each serum sample was determined using a PrioCHECK FMDV type O ELISA kit (Prionics, Switzerland). A protocol of blocking the enzyme-linked immunosorbent assay (ELISA) to detect antibodies against FMDV serotype O was employed according to the manufacturer's instructions as previously described [6]. Briefly, fresh serum was separated by centrifugation at $2,000 \times g$ for 10 min at 4°C. The collected serum samples and provided reference samples were then added to each well of a provided ELISA plate, after which they were incubated at 37°C for 60 min. After washing the plate, a conjugate of anti-FMDV type O monoclonal antibody was diluted and dispensed to all wells, after which the plates were incubated for another 60 min. Following this incubation, TMB ready-to use substrate was added and the reaction was allowed for 15 min at $22 \pm 3^\circ\text{C}$, then inhibited by the addition of the provided stop solution. Finally,

Table 1. Positive reactions of foot-and-mouth disease virus antibody ELISA

Farm	PI level	Group	0 (wk)	2 (wk)	4 (wk)	6 (wk)
PJ	≥ 50	Control	3/12	1/12	0/12	1/12
		Pre-treated	1/12	1/12	0/12	1/12
		Diff (P-C)	-2	0	0	0
	≥ 40	Control	3/12	2/12	2/12	2/12
		Pre-treated	2/12	2/12	2/12	3/12
		Diff (P-C)	-1	0	0	1
	≥ 30	Control	4/12	7/12	5/12	3/12
		Pre-treated	4/12	9/12	4/12	5/12
		Diff (P-C)	0	2	-1	2
TS	≥ 50	Control	2/12	4/12	3/12	2/12
		Pre-treated	1/12	3/11	4/11	4/11
		Diff (P-C)	-1	-1 (?)	1 (?)	2 (?)
	≥ 40	Control	3/12	9/12	5/12	5/12
		Pre-treated	2/12	8/11	5/11	7/11
		Diff (P-C)	-1	-1 (?)	0 (?)	2 (?)
	≥ 30	Control	4/12	11/12	7/12	10/12
		Pre-treated	3/12	11/11	9/11	8/11
		Diff (P-C)	-1	0 (?)	2 (?)	-2 (?)
HB	≥ 50	Control	0/12	5/12	1/12	2/12
		Pre-treated	0/12	7/12	2/12	4/12
		Diff (P-C)	0	2	1	2
	≥ 40	Control	0/12	7/12	1/12	5/12
		Pre-treated	0/12	11/12	9/12	9/12
		Diff (P-C)	0	4	8	4
	≥ 30	Control	0/12	10/12	7/12	8/12
		Pre-treated	0/12	11/12	10/12	11/12
		Diff (P-C)	0	1	3	3

Diff (P-C) indicates difference in positive reactions between pre-treated group and control group. Question mark indicates difference even though one pig was dead during the experiment. PI level indicates percentage inhibition level of anti-FMDV antibody measured using a PrioCHECK FMDV type O kit.

the optical density (OD) was measured at 450 nm and calculated as the percentage inhibition (PI) according to the manufacturer's protocols. PI values below 50% reflect an absence of anti-FMDV type O antibodies in the test serum, while PIs above 50% indicate the presence of anti-FMDV type O antibodies.

To evaluate whether humoral antibody response against FMDV serotype O antigen, a component of the vaccine, had been improved in 8-week-old growing pigs pre-treated with 60 mg of poly- γ -glutamic acid (γ -PGA) 3 days before the vaccination, two designated groups (12 pigs each group) from each of three commercial swine farms were compared. Differences in positive reactions between pre-treated group and controls were determined according to the PI levels: ≥ 50 , ≥ 40 , and ≥ 30 (Table 1). Based on the ≥ 50 PI value, positive reactions of pre-treated groups of TS and HB farms were higher than those of control groups 2 or 6 weeks after FMDV vaccination. Fig. 1. shows a comparative distribution of percentage inhibition (PI) values at 0, 2, 4 and 6 weeks after FMD vaccination in 8-week-old pigs pre-injected with γ -PGA 3 days before vaccination. In addition, based on a PI value ≥ 40 , positive reactions of pre-treated groups of TS and HB farms were higher than those of control groups 2, 4 and 6 weeks after FMDV vaccination. However, based on a PI value ≥ 30 , positive reactions of pre-treated groups of PJ, TS and HB farms were higher than those of control groups 2, 4 or 6 weeks after FMDV vaccination. Pre-treated groups from the HB farm were distinctly higher than those of other farms. Taken together, positive antibody reactions of the vaccine were significantly increased by pre-injection of γ -PGA.

To control FMD in Korea, a mass vaccination policy partially supported by the government has been employed for swine populations since September of 2011 [5]. This policy established a single shot of a trivalent FMD vaccine as a routine

FMD vaccination program for 8 to 12 week old pigs; however, the antibody induction remained below 50% PI. A previous study by Liao *et al.* [7] reported that, for a single dose FMDV vaccination in pigs born to well-vaccinated sows, the proper age of administration was approximately 8 weeks-old when a single dose vaccination policy in pigs was applied for pigs younger than 10 weeks. In that study, pigs were fully protected by pathogenic challenge and had a mean serum neutralization (SN) titer of $1.89 \pm 0.95 \log_{10} \text{SN}_{50}$ at 24 weeks [7]. SN titers against a pathogenic FMD virus may represent the actual protective immune levels of a component of the FMD vaccine. This study also showed much better results with a monovalent FMD vaccine, a commercial high potency double oil-based emulsion vaccine containing at least 6 PD_{50} per dose of inactivated O/TWN/97 FMD virus [7]. In the present study, even though the SN test was a practical tool, we had a limitation of SN assay to use a pathogenic FMD virus by the Government regulation.

To support the effects of γ -PGA on the humoral responses against FMDV, FMDV-specific IgG titers should be measured from the collected serum samples following immunization. Indeed, this is the most important step. However, the results of the present study will be further strengthened by conducting additional functional assays, such as neutralizing assays on cell culture models, to test the abilities of serum samples against FMDV infection from different immunization protocols. Ideally, a pig protection assay will be much more convincing. After different immunization treatments, it is necessary to apply a lethal dose of a pathogenic FMDV to pigs to test the protection rates and determine if pigs immunized with FMD vaccine plus γ -PGA have a higher protection rate than those of the control.

Korean swine producers have expressed concern that antibody induction of FMDV serotype O antigen in response to the currently employed trivalent FMD vaccine was not

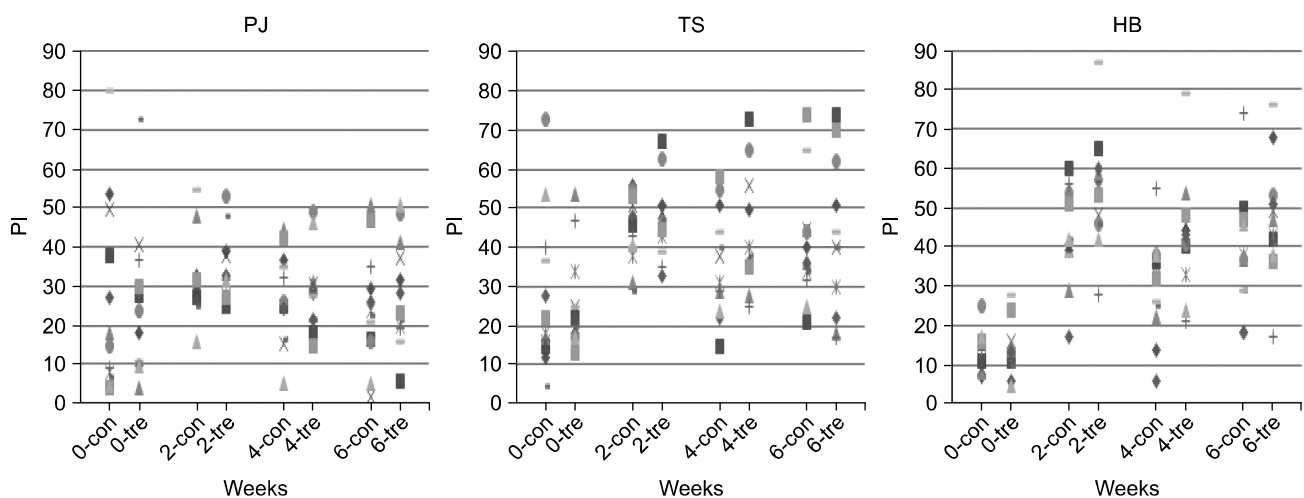


Fig. 1. Comparative distribution of percentage inhibition (PI) values at 0, 2, 4 and 6 weeks after foot-and-mouth disease vaccination in 8-week-old pigs pre-injected with γ -PGA at 3 days before vaccination. con, control; tre, treatment.

sufficient to reach positive reactions after one injection of the vaccine in a previous study of marketing pigs. Therefore, the swine producers have considered using an immune promoter. In addition, a recent report suggested that germanium biotite (GB), which is a novel alternative feed supplement, could play a role as an immunostimulator of boosting agent for improving the efficacy of FMDV vaccine in conventional 8-week old pigs. A commercially available high potency double oil-based vaccine contained at least 6 PD₅₀ per dose of inactivated O₁ Manisa FMD virus [6]. Thus, our study was conducted in 8-week-old growing pigs pre-treated with 60 mg of poly- γ -glutamic acid (γ -PGA) 3 days before vaccination. Throughout a pilot dose-dependent study, the pre-treatment dose was determined to be 60 mg of poly- γ -glutamic acid (γ -PGA) 3 days before vaccination (data not shown).

In this study, the FMDV vaccine used differed from that used in a previous study [6] that employed an antigenic formula of FMDV vaccine (O₁ Manisa + A Malaysia + Asia 1 Shamir serotypes), even though a single dose of trivalent FMDV vaccine to 8 week-old pigs (86.7% in type O, 88.0% in type A and 93.0% in type Asia 1) showed much higher sero-positive reactions than those of 12 week-old pigs vaccinated once (60.9% in type O, 62.8% in type A and 77.6% in Asia 1, respectively) [5]. This report supported our measurement of immune reactions against only FMDV serotype O antigen, suggesting that the trivalent FMD vaccine could induce an even antibody response regardless of serotype. In our study, the positive reactions of a single dose FMDV vaccination were not high enough to recommend a single injection for 8 week-old pigs based on PI levels ≥ 50 . Thus, we analyzed the results based on PI levels of ≥ 50 , ≥ 40 , and ≥ 30 . The results indicated that positive antibody reactions to the FMDV serotype O antigen of trivalent FMD vaccine were significantly increased by pre-injection with γ -PGA. In Korea, we have applied this trivalent FMDV vaccine over three years, but further experiments with γ -PGA are still needed to fully understand the effectiveness of a trivalent FMDV vaccine and solve the cell-mediated immunological response.

A cost-effective, recommendable FMD vaccination program is favorable for producers and one of the major factors required for a sustainable FMDV control campaign [7]. To support a fully sufficient protection for swine populations in FMDV endemic countries or areas, a routine immunization program with two doses of FMDV vaccine is generally recommended for pigs born to sows vaccinated before farrowing [2,3,5,8,9]. Therefore, a new approach of pre-injecting animals with γ -PGA 3 days before FMDV vaccination to improve the efficacy of FMD vaccines and generate more strong sustainable immune

reactions with a single dose immunization in 8-week-old pigs born to well-vaccinated sows.

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Conflict of Interest

There is no conflict of interest.

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