Needleless intradermal vaccination for foot-and-mouth disease induced granuloma-free effective protection in pigs

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ABSTRACT

Vaccination is one of the most effective ways of controlling and preventing foot-and-mouth disease (FMD) outbreaks. The effective prevention of this disease requires the use of high-quality vaccines to meet the criteria that enable customers to use them simply. The administration of FMD vaccines containing oil-based adjuvants in pigs can induce the formation of granuloma in the muscle of the vaccinated, which makes these vaccines a less preferable option. Therefore, it is important to establish an FMD vaccine and vaccine delivery tool that offers better immunity and safer application. This study compared the immune responses of intramuscular and needleless intradermal vaccination in pigs. When the same amount of an FMD virus (FMDV) antigen was administered to pigs, both the intradermally and intramuscularly vaccinated groups were protected completely against a challenge of the homologous FMDV, but the intramuscularly vaccinated group showed an overall higher level of neutralizing antibodies. Importantly, the formation of granuloma in muscle could be excluded in the intradermally vaccinated group. Of the oil-based adjuvants selected in this study, ISA 207 was effective in eliciting immunogenicity in intradermal vaccination. In conclusion, a new vaccine formula can be chosen for the delivery of intradermal route to exclude the possibility of local reactions in the muscle and generate protective immunity against an FMDV challenge.

Keywords: FMD; vaccine; needleless; intradermal route; protection

INTRODUCTION

Foot-and-mouth disease (FMD) is economically important and affects cloven-hoofed animals, including pigs, cattle, goats, swine or sheep, and wild animals. FMD viruses (FMDVs) can be divided into seven serotypes (Euroasiatic serotypes O, A, Asia1, and C; and South African territories [SAT] serotypes: SAT1, SAT2, and SAT3), among which no cross-serotype protection is expected [1].
Since 2011, it has been mandated that all susceptible livestock in Korea are to be vaccinated with an inactivated FMD vaccine [2,3]. Despite this, an FMD outbreak occurred in July 2014 [4-6]. An FMD vaccine is generally administered into the muscles of animals using a syringe and injection needle. Owing to the herd size, it is common that a number of pigs are vaccinated repeatedly using the same syringe needle. This process of administering FMD vaccines containing oil-based adjuvants in pigs can cause side effects and problems, such as the spread of other contagious diseases, local inflammation, the formation of granulomas, or edema in the area of injection [7]. In recent years, some of these problems have attracted particular attention in South Korea in terms of economic losses. This is because, the granulomatous tissue is formed mostly in the shoulder region of the pork, which is more expensive than other parts in South Korea and needs to be cut and discarded. A previous study reported that the economic losses to producers from discarding those deformed carcasses are substantial [8,9].

The intradermal (ID) delivery of immunogens is considered as an effective and efficient way of vaccination for humans and livestock; ID vaccination has the potential to save the production cost of FMD vaccines because it has been reported that a lower antigen payload is effective in generating protective immunity in pigs and cattle than the intramuscular (IM) route [10-12]. Moreover, it is a less painful method than IM vaccination and can reduce the formation of lesions at the time of vaccination [11,13]. ID vaccination can also decrease the time taken for vaccination by using a needle-free vaccine delivery system [14]. Regarding the vaccines for porcine reproductive and respiratory syndrome and Aujeszky’s disease in pigs, several studies on the application of ID vaccination have been conducted [14-16]. Countries that are subject to FMD vaccination should make efforts to establish the effective vaccination strategy that suits the conditions of their respective countries [8,17].

Therefore, this study evaluated vaccines with different adjuvants, which are deemed optimal for ID vaccination in a safe manner without an injection needle. In doing so, the differences in immune reactivity between the adjuvants were examined, and the immunity and protective efficacy of the two different delivery routes were compared.

**MATERIALS AND METHODS**

**Virus and cells, viral RNA extraction**

LF-BK cells were provided by the Plum Island Animal Disease Center (USA); this cell line was used for virus isolation. Baby hamster kidney (BHK)-21 (C-13) cell lines were provided by the American Type Culture Collection (CCL-10), and they were used for the virus passage. LF-BK and BHK-21 cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Cellgro, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% antibiotic-antimycotic (Gibco) was used for these experiments. O/SKR/Jincheon/02/2014 was isolated from the crust tissue of infected pigs reported in Jincheon, South Korea (SKR) in December 2014. The FMDV strains, O/SKR/Andong/2010 (AD 2010), O/SKR/Paju/2010 (PJ 2010), O/SKR/Jincheon/02/2014 (JC 2014), O/SKR/Cheonan/08/2014 (CA 2014), and O/SKR/Anseong/51/2015 (AS 2015) of the Southeast Asia (SEA) topotype, and O/SKR/Paju/2000 (PJ 2000), O/SKR/Chungju/2000 (CJ 2000), O/SKR/Anseong/2002 (AS 2002), O/UK/2007 (UK 2007), and O/Vietnam/GiaBinh/2013 (VN 2013), of the ME-SA topotype (PanAsia lineage) were used for the cross-virus neutralization test (VNT). Viral RNA was extracted using the Roche MagNA Pure 96 System (Roche Applied Science, Germany); 100 μL of the virus sample was used for viral RNA extraction and finally eluted to 50 μL.
Purification and quantification of the FMDV type O 146S antigen

For viral infection, the culture medium of BHK-21 cells was replaced with serum-free DMEM and the cells were inoculated with the virus, O/SKR/Jincheon/02/2014. The extracellular viruses were removed after 1 h of incubation at 37°C in an atmosphere containing 5% CO₂. Twenty-four hours after infection, the viruses were inactivated twice by treating with 0.003 N binary ethylenimine for 24 h in a shaking incubator and were concentrated with polyethylene glycol (PEG) 6000 (81260; Sigma-Aldrich, USA). The virus concentrate was layered on 15%–45% sucrose density gradients and centrifuged [18]. After ultracentrifugation, the bottom of the centrifuge tube was punctured and every 1 mL fractions were collected separately. The presence of FMDV particles in a sample of each fraction was determined using a lateral flow device (BioSign FMDV Ag; Princeton BioMeditech, USA). Before its use in the animal experiment, innocuity of the pre-PEG treatment supernatant was tested through a BHK-21 cell line and LF-BK cell line at least twice to check that no cytopathic effect (CPE) had occurred, thereby confirming the absence of live virus in the supernatant [19]. The 146S virus particles were quantified using the spectrophotometer at a wavelength of 259 nm. Additionally, the presence and integrity of the purified 146S virus particles were examined by transmission electron microscopy.

Animal experiment

The vaccine was made using the purified 146S antigen of O/SKR/Jincheon/02/2014 and Montanide ISA 201 VG (ISA 201; Seppic, France) or Montanide ISA 207 VG (ISA 207; Seppic), with varying antigen payloads, 2 μg/head or 10 μg/head. The vaccine volume of a single dose for ID and IM delivery was set to 0.2 mL and 1.0 mL, respectively (Table 1). The vaccines were delivered to the same neck region under or close to the right ear of the pig in two different ways. The syringe with 19G needle was used for IM vaccination, and the Pulse 50 (Pulse needle-free systems, Inc., USA), a needle free system, was used for ID vaccination.

Fourteen specific pathogen-free (SPF) pigs (White Yucatan, 6 weeks old; Optipharm, Korea) were divided to five groups for different inoculation schemes: group (G) 1 (#3, #9, #10) for ID route with 10 μg antigen formulated with ISA 201, G2 (#4, #11, #12) for ID route with 2 μg antigen formulated with ISA 201, G3 (#5, #13, #14) for ID route with 2 μg antigen formulated with ISA 207, G4 (#2, #6, #8) with IM route with 2 μg antigen formulated with ISA 201, G5 (#15, #16) for IM route with ISA 201 only. Twenty-eight days post-vaccination (dpv), all pigs were challenged with 100 μL of virus fluid with a virus titer of 10⁶ TCID₅₀/mL in the two sites on the left hind foot pad. The clinical signs were examined for 14 days post-challenge (dpc), and clinical samples were collected with a commercial sampling kit (Universal Viral Transport System, Cat. No. 220526 BD) daily from 0 to 10 dpc and at 14 dpc. The animals were treated in accordance with the ethical guidelines of the animal welfare committee of the Animal and Plant Quarantine Agency. The clinical score of each pig was calculated by summing up the values for FMD lesions or signs manifested in each category (maximum score = 16) according to the criteria defined by Alves et al. [20].

Table 1. Vaccination protocols for the enhanced immune response in SPF pigs with various administration routes and adjuvants

| Groups | No. of pigs | Individual No. of pigs | Antigen payload (μg/head) | Adjuvants | Administration route | Injected volume/dose (mL) |
|--------|-------------|-------------------------|--------------------------|-----------|----------------------|--------------------------|
| G1     | 3           | #3, #9, #10             | 10                       | ISA 201   | ID                   | 0.2                      |
| G2     | 3           | #4, #11, #12            | 2                        | ISA 201   | ID                   | 0.2                      |
| G3     | 3           | #5, #13, #14            | 2                        | ISA 207   | ID                   | 0.2                      |
| G4     | 3           | #2, #6, #8              | 2                        | ISA 201   | IM                   | 1.0                      |
| G5     | 2           | #15, #16                | None                     | ISA 201   | IM                   | 1.0                      |

SPF, specific pathogen-free; G, group; ID, intradermal; IM, intramuscular.
Quantification of the FMDV RNA in serum, nasal swab, and oral swab

The viral RNA was extracted using an automated nucleic acid purification system (MagNA Pure 96; Roche Applied Science). Quantification using the real-time reverse transcription-polymerase chain reaction (rRT-PCR) was performed on a CFX manager (Bio-Rad, USA) machine using an FMDV Real-time RT-PCR kit (Bioneer, Korea). The process involved the following: a 15 min RT step at 45°C, and a 5 min inactivation step at 95°C, linked to a 45-cycle PCR program (95°C for 5 sec and 55°C for 5 sec). Three types of oligonucleotide were used for the reaction: FMDV forward primer (5’ ACT GGG TTT TAC AAA CCT GTG A 3’), FMDV reverse primer (5’ GCG AGC CCT GCC ACG GA 3’), and FMDV probe (5’TCC TTT GCA CGC CGT GGG AC 3’). The probe was labeled with a 5’-reporter dye (6-FMA), a 3’ quencher (Iowa Black FQ), and an internal ZEN quencher; the primers and probe were synthesized by Integrated DNA Technologies, Inc. (USA). For absolute quantification of the viral RNAs in the samples, the \textit{in vitro} transcribed standard RNAs, which had been serially diluted by ten-fold, from $2 \times 10^1$ to $2 \times 10^5$ copies were placed in a 96 well plate together with the extracted RNAs from the clinical samples adjacently. The Cq values of the samples were transformed to the viral RNA copy number using the standard curve constructed using the Bio-Rad CFX manager version 3.1 program (Bio-Rad). All samples were tested in duplicate and the viral RNA copy numbers are expressed in a logarithmic scale.

Enzyme-linked immunosorbent assay (ELISA) for antibody detection

A PrioCHECK FMDV type O SP ELISA kit and NSP ELISA kit (Prionics AG, Switzerland) were used to detect the FMDV non-structural protein (NSP) antibodies or structural protein (SP) antibodies in the serum samples of pigs. The optical density (OD) of the samples is expressed as the percentage inhibition (PI) relative to the OD values of the negative control. Each sample was tested in duplicate.

VNT

The neutralizing antibody titer of the serum samples was estimated by micro- VNT using the OIE protocol (OIE, 2009). The sera were inactivated at 56°C for 30 min, serially diluted and mixed with the suspension fluid of ten different viruses of the same titer (100 TCID$_{50}$). The resulting mixtures were incubated on microtiter plates for 1 h, and LF-BK cells were then added to the plate and incubated for a period of 2–3 days [21]. The CPE was checked to determine the virus-neutralizing antibody titers [22]. Sera with neutralizing antibody titers of 1.2 or above were regarded as antibody positive.

Statistical analysis

SigmaPlot version 10.0 (Systat Software, USA) was used for drawing the graphs for the SP ELISA and NSP ELISA results. All the rRT-PCR results were analyzed in Bio-RAD CFX manager with the threshold value automated. The clinical score and FMD RNA results were used to draw the graphs in GraphPad Instat ver 3.05 (GraphPad software, USA). The same software was used in the analysis of variance test for the SP ELISA and neutralizing antibody titer.

RESULTS

Clinical score and virus detection in the immunized pigs

Two to three days after the challenge, all the pigs in G5, which had been inoculated with the adjuvant only, showed the typical clinical symptoms of FMD whereas none of the vaccination groups showed clinical symptoms (Fig. 1 and Table 2). The discharge and replication
of viruses was identified and measured by rRT-PCR using the sera and nasal swabs. IM vaccination in G4 prevented the occurrence of viremia in all of the pigs and decreased virus excretion to undetectable levels in pig #6 or very low levels in pigs #2 and #8 following a virus challenge. In the ID vaccination groups, some pigs showed the excretion of virus or viremia transiently for a single day with virus titers that were approximately 10–1,000 times higher than that of pig #2 in G4. On the other hand, viral replication shown in some pigs of the intradermally vaccinated groups (G1, G2, and G3) were considerably weaker than that in the pigs in G5 in terms of the titer and duration (Fig. 1). In the case of employing an ID vaccination method, the difference in antigen payloads in the vaccines made little differences in the strength of the immunity in terms of seroconversion (Fig. 2).

Humoral immunity in the pigs vaccinated with the inactivated antigen
Type O SP ELISA was performed using the blood samples collected at 0 to 14 dpc. At 28 dpv (0 dpc), a sharp increase in the PI values was observed in all vaccinated animals in G1, G2, G3 and G4. However, there is only one animal, #9 in G1, showing PI values higher than the cut-off value, 50 (Fig. 2). All pigs of G3 showed PI values that were close to the cut-off value evenly, compared to other groups of ID vaccination, G1 and G2, and were significantly higher than the PI values of the control group (p < 0.001) (Fig. 2). This suggests that, for ID vaccination, the use of adjuvant ISA 207 might be more stable in eliciting immunogenicity in pigs than the use of adjuvant ISA 201. VNT was performed using the blood samples collected at 28 dpv against the homologous virus isolate, O/SKR/Jincheon/02/2014 (JC 2014) and other heterologous virus isolates of the SEA topotype and of the PanAsia lineage of the ME-SA topotype (Figs. 3 and 4). The mean virus neutralizing (VN) log10 titers for JC 2014 were 1.61 (G1), 1.55 (G2), 1.91 (G3), and 2.21 (G4) (Fig. 3 and Table 2). All vaccinated animals, including the pig in G2 (#4) showing the lowest VN log10 titer less than 1.20, were protected from the homologous challenge. For the average VN log10 titers against the five different viruses of the SEA topotype, G1, G3, and G4 showed higher values than the control group (Fig. 3). In contrast, average VN log10 titers were very low across all the vaccinated groups against the viruses of the ME-SA topotype except for PJ 2000.

G1 and G2 showed differences in the mean VN log10 values at 28 dpv in proportion to the antigen payloads in the vaccines, particularly, against the viruses of AD 2010, PJ 2000, and PJ 2010. The ID vaccination group (G3) using the adjuvant ISA 207 produced VN log10 values comparable to ID vaccination group (G1) using ISA 201 with higher antigen payload, and this was true consistently against all the viruses except for AD 2010 and PJ 2010 (Figs. 3 and 4, Table 2). NSP ELISA showed that two pigs in the non-vaccinated group (G5) were seroconverted at approximately 9 dpc after showing clinical symptoms. In particular, without showing clinical symptoms, the pig #6 of G4 and pig #4 of G2 were seroconverted for NSP ELISA (Fig. 5), even though pig #6 of G4 had high neutralizing antibody titer at 0 dpc. No pigs in G1 and G3 showed the evidence of seroconversion for NSP ELISA (Fig. 5).

Observation of granuloma in muscle around ears
At first, the delivery of the commercial FMD vaccine to the pig skin using a needle free system (Pulse 50) was attempted on dead pigs to find the optimal settings for the equipment. The vaccine, which is white in color, could be delivered effectively through the skin layers, including the epidermis, dermis, and subcutaneous layer, and it did not pass through the fat tissue (Fig. 6). With the standardized method, the trial vaccines of G1, G2, and G3 were delivered to the pig skin in this study. The formation and duration of the nodule or scab on the vaccine-injected sites in G1, G2, and G3 were examined until 78 days after vaccination,
Fig. 1. Virus excretion and clinical score in SPF pigs immunized with the experimental vaccine according to the dose after the homologous virus (O/SKR/Jincheon/2014) challenge. (A) G1 (ISA 201/ID/10 µg), (B) G2 (ISA 201/ID/2 µg), (C) G3 (ISA 207/ID/2 µg), (D) G4 (ISA 201/IM 2 µg), (E) negative control (ISA 201/IM).

SPF, specific pathogen-free; G, group; ID, intradermal; IM, intramuscular.
Table 2. Summary of the clinical signs and laboratory tests in vaccinated and challenged SPF pigs

| Vaccinated groups (adjuvant/rout/µg) | Pig ID | VN titer against O/SKR/Jincheon/2014 at 28 dpv | Protective effects after challenge* | Virus detection by rRT-PCR for 10 days after challenge | NSP antibody detection for 14 days after challenge |
|--------------------------------------|--------|-----------------------------------------------|------------------------------------|-------------------------------------------------|--------------------------------------------------|
|                                      |        |                                              | Fever T > 40°C | Clinical score† | Nasal swabs | Saliva | Sera |                                      |
| G1 (ISA 201/ID/10 µg) #3             | 1.66 ± 0.21 | −       | 0 | −     | +     | +     | −     |                                      |
|                                       | #9      | 1.81 ± 0.00 | −   | 0   | +   | −     | +     | −     |
|                                       | #10     | 1.51 ± 0.00 | −   | 0   | +   | −     | +     | −     |
| G2 (ISA 201/ID/2 µg) #4              | < 1.20 ± 0.00 | −       | 0 | −     | −     | +     | −     |                                      |
|                                       | #11     | 1.88 ± 0.10 | −   | 0   | +   | −     | +     | −     |
|                                       | #12     | 1.73 ± 0.11 | −   | 0   | +   | −     | +     | −     |
| G3 (ISA 207/ID/2 µg) #5              | 2.11 ± 0.64 | −       | 0 | −     | −     | +     | −     |                                      |
|                                       | #13     | 1.65 ± 0.00 | −   | 0   | +   | −     | +     | −     |
|                                       | #14     | 1.42 ± 0.12 | −   | 0   | −   | −     | +     | −     |
| G4 (ISA 201/IM/2 µg) #2              | 1.81 ± 0.00 | −       | 0 | +     | −     | −     | −     |                                      |
|                                       | #6      | 2.03 ± 0.53 | −   | 0   | −   | −     | −     | +     |
|                                       | #8      | 2.11 ± 0.43 | −   | 0   | −   | −     | +     | −     |
| G5 (ISA 201/IM/None) #15             | < 1.20 ± 0.00 | +       | 10 | +    | +    | +    | +    |                                      |
|                                       | #16     | < 1.20 ± 0.00 | + | 10 | + | + | + |                                      |

SPF, specific pathogen-free; VN, virus neutralizing; rRT-PCR, real-time reverse transcription-polymerase chain reaction; NSP, non-structural protein; G, group; ID, intradermal; IM, intramuscular; dpv, days post-vaccination; dpc, days post-challenge; FMD, foot-and-mouth disease.

*Challenged with O/SKR/Jincheon/2014 at 28 dpv and examined until 14 dpc; †Clinical scores were based on the sum of each FMD lesion or sign (maximum score = 16) according to the method reported by Alves et al. [20] based on the addition of points.

Fig. 2. SP antibody responses in SPF pigs after vaccination and homologous virus (O/SKR/Jincheon/2014) challenge. The graph shows the type O SP ELISA antibody level. (A-E) PI value of SP ELISA in individuals in each group. (A) G1 (ISA 201/ID/10 µg), (B) G2 (ISA 201/ID/2 µg), (C) G3 (ISA 207/ID/2 µg), (D) G4 (ISA 201/IM/2 µg), (E) Negative control (ISA 201/IM/0 µg)

SP, structural protein; SPF, specific pathogen-free; ELISA, enzyme-linked immunosorbent assay; PI, percentage inhibition; G, group; ID, intradermal; IM, intramuscular; dpv, days post-challenge.
**Protection against FMD with needleless vaccination**

**Fig. 3.** VN log₁₀ titers in SFP pigs at 28 dpv in SEA topotype viruses. Bar diagram showing the mean VN titers (log₁₀) of SEA topotype viruses from Korea outbreaks. FMDV strains for VNT of the SEA topotype were O/SKR/Andong/2010 (AD 2010), O/SKR/Paju/2010 (PJ 2010), O/SKR/Jincheon/02/2014 (JC 2014), O/SKR/ Cheonan/08/2014 (CA 2014), and O/SKR/Anseong/51/2015 (AS 2015). G1 (ISA 201/ID/10 µg), G2 (ISA 201/ID/2 µg), G3 (ISA 207/ID/2 µg), G4 (ISA 201/IM/2 µg), G5 (negative control, ISA 201/IM/0 µg). The gray block presents a range of VN titers 1.42–1.65 log₁₀ because this is the cut-off range in VN titers for the vaccine efficacy test in South Korea. The median dotted line presents 1.53 log₁₀. VN titers for negative results of below 1.2 log₁₀ were fixed as 1.0 log₁₀ for statistical analysis.

VN, virus neutralizing; SPF, specific pathogen-free; dpv, days post-vaccination; SEA, Southeast Asia; FMDV, foot-and-mouth disease virus; VNT, virus neutralization test; G, group; ID, intradermal; IM, intramuscular.

* p < 0.05.

**Fig. 4.** Cross VN antibody in SFP pigs at 28 dpv in ME-SA topotype viruses. Bar diagram showing the mean VN titers (log₁₀) of ME-SA topotype viruses from Korea, the United Kingdom, and Vietnam outbreaks. FMDV strains for VNT of the ME-SA topotype (PanAsia lineage) were O/SKR/Paju/2000 (PJ 2000), O/SKR/Chungju/2000 (CJ 2000), O/SKR/Anseong/2002 (AS 2002), O/UK/2007 (UK 2007), and O/Vietnam/GiaBinh/2013 (VN 2013). G1 (ISA 201/ID/10 µg), G2 (ISA 201/ID/2 µg), G3 (ISA 207/ID/2 µg), G4 (ISA 201/IM/2 µg), G5 (negative control, ISA 201/IM/0 µg).

VN, virus neutralizing; SF, specific pathogen-free; dpv, days post-vaccination; SEA, Southeast Asia; FMDV, foot-and-mouth disease virus; VNT, virus neutralization test; G, group; ID, intradermal; IM, intramuscular.

* p < 0.05.
and nodules or scabs had formed in most of the pigs as a normal immune reaction but these had disappeared by 78 dpv. When the animals were euthanized at the end of the experiment, no granulomas were detected in any muscles under the neck or shoulder of the pigs vaccinated (Supplementary Figs. 1-3).

**DISCUSSION**

We tested new adjuvants for the ID route of vaccine delivery in pigs to circumvent the side effects of the conventional vaccination system, thereby ensuring the implementation of the national compulsory vaccination program. ID vaccination using a needle-free delivery system enables more effective, safer, and faster vaccinations on a larger number of animals than that using an injection needle [14,16,23]. Furthermore, the formation of granuloma or fibrosis in the muscle could be excluded in the needle-free ID vaccination herds. A previous report showed that ID FMD vaccination using the same antigen payload as that used for IM vaccination effectively protected the pigs from FMDVs [11]. The ID vaccination group (G1, G2, G3) had significantly higher PI values in the type O SP ELISA or VN titers than the control group. In particular, G3 adopting ISA 207 as an adjuvant appeared to show better protective immunity than G2 using ISA 201 as an adjuvant in terms of the level of neutralizing antibody titers and seroconversion rate in NSP ELISA. This highlights the importance of selecting an appropriate adjuvant for needle-free ID vaccination.
appropriate adjuvant for ID vaccination. The ID vaccination method using the needle-free delivery system involves the chances of an inaccurate injection in some animals because the pigs are moving and are difficult to restrain, which may result in the delivery of less dose than intended. Accordingly, the proper use of a needle-free system by training is very important and new devices, which are more robust and convenient, may need be developed to compensate for this downside.

The protective immunity shown in G3 could not be explained plainly by the humoral immunity compared to G4; it is reported that, when compared to IM vaccination, ID vaccination enables the protection of animals when their VNT antibody titers are low [11]. Whilst this effect is partly associated with the humoral immunity, ID vaccination is a major contributor to cell-mediated immune responses that induce the mobilization of inflammatory dendritic cells [11,24].

In conclusion, it was demonstrated that the ID route, which is actually a transdermal route in this study, is a very promising replacement for the current vaccination protocol employing the IM route for vaccine delivery, and a vaccine adjuvant that is more suitable for ID vaccination in pigs with the FMDV antigen was selected.

SUPPLEMENTARY MATERIALS

Supplementary Fig. 1
The ear skin sites injected with vaccine by intradermal route at 1–7 day post vaccination.

Click here to view

Supplementary Fig. 2
The ear skin sites injected with vaccine by intradermal route at 9–42 days post vaccination.

Click here to view
Supplementary Fig. 3
The ear skin sites injected with vaccine by intradermal route at 50 days post vaccination.

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