Research Articles

On-farm trial of modified live Classical swine fever vaccine induced neutralizing antibody titers: Comparison of intradermal and intramuscular vaccination methods

Kochakorn Direksin[1*] Sisavath Phommasichan[2] Suttisak Nopwinyoowong[3]

[1]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002 Thailand
[2]Department of Livestock and Fisheries, Faculty of Agriculture (Nabong campus), National University, Nabong, Vientiane, Lao PDR
[3]Department of Veterinary Pathobiology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002 Thailand

[*]Corresponding author, E-mail address: kochakrn@kku.ac.th

Abstract

The aim was to investigate on-farm application of an alternative Classical swine fever (CSF) vaccination, whether intra-dermal (ID) vaccination is suitable as an alternative for traditional intramuscular (IM) route in an organic pig farm. We compared antibody titers by using neutralizing peroxidase-linked monolayer assay (NPLA) between pigs vaccinated with lived CSF vaccine containing a normal or 5-fold reduced dose. The vaccine was government produced (Thailand), containing 10^2 PD_{50} (50% protective dose) of modified live Lapinized Chinese-strain CSF virus. A total of 18 nursery pigs were pair matched (according to litter, weight, and sex) and divided into 2 groups. Amount of 0.2 ml or 1 ml full-dose of vaccine was administered ID or IM, respectively. As compared with the 1ml IM vaccinated pigs, the pigs vaccinated ID with 0.2ml of the same vaccine had comparable antibody titers to IM group. Geometric mean titers (log10) at day 0, 14, 21, and 28 days post-vaccination of ID group were 5.44, 7.41, 11.76, and 38.05, and GMT of IM group were 4.67, 8.64, 12.70, and 32.00, respectively. We conclude that the ID route can be used as an alternative for IM application of CSF vaccine.

Keywords: *CSF, ID vaccination, neutralizing antibody, NPLA*

เปรียบเทียบแอนติบอดีจากการฉีดวัคซีนอหิวาต์สุกรเชื้อเป็นแบบเข้ากล้ามและเข้าในหนัง โดยทดสอบในฟาร์มสุกร

กชกร ดิเรกศิลป์[1*] Sisavath Phommasichan[2] สุทธิศักดิ์ นพวิญญูวงศ์[3]

[1]1ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น 40002 ประเทศไทย
[2]Department of Livestock and Fisheries, Faculty of Agriculture (Nabong campus), National University, Nabong, Vientiane, Lao PDR
[3]ภาควิชาพยาธิชีววิทยา คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น 40002 ประเทศไทย

[*]ผู้รับผิดชอบบทความ E-mail address: kochakrn@kku.ac.th

บทคัดย่อ

วัตถุประสงค์เพื่อทดสอบการประยุกต์ใช้การฉีดวัคซีนแบบในหนัง (ID) ใช้วัคซีนปริมาณ 0.2 มล. ว่าให้ผลในการกระตุ้นแอนติบอดีได้เท่าเทียมกับการฉีดเข้ากล้าม (IM) ใช้ 1 มล. แบบดั้งเดิม โดยทดสอบในฟาร์มสุกรอินทรีย์ ก่อนและหลังฉีดวัคซีนเจาะเก็บซีรัมเพื่อวิเคราะห์ระดับแอนติบอดีโดยวิธี NPLA วัคซีนอหิวาต์สุกรเป็นชนิดเชื้อเป็นสายพันธุ์ Chinese เพิ่มจำนวนในกระต่าย ผลิตในประเทศไทย ปริมาณความเข้มข้นของไวรัสเท่ากับ 10^2 PD_{50} (50% protective dose) ต่อหนึ่ง มล. สุกรทั้งหมด 18 ตัว แบ่ง 2 กลุ่มโดยคำนึงถึงครอก น้ำหนัก และเพศที่ใกล้เคียงกันที่สุด ระดับแอนติบอดี (GMT; log10) ในวันที่ 0, 14, 21 และ 28 หลังฉีดวัคซีน (dpv) ของ ID คือ 5.44, 7.41, 11.76 และ 38.05 ของ IM คือ 4.67, 8.64, 12.70 และ 32.00 ตามลำดับ ระดับ GMT ของทั้งสองกลุ่ม ไม่แตกต่างกัน ($p>0.05$) ในขณะที่ระดับไตเตอร์เพิ่มขึ้นอย่างชัดเจน ที่ 21 dpv จน 28 dpv สรุปได้ว่า ID สามารถใช้เป็นทางเลือกทดแทน IM ในการฉีดวัคซีนอหิวาต์สุกรแบบเชื้อเป็น

คำสำคัญ : *อหิวาต์สุกร ฉีดวัคซีนในหนัง แอนติบอดี NPLA*

Introduction

Classical swine fever (CSF) is notorious as a severe, highly contagious and economically significant viral disease of pigs. Only domestic pigs, wild boar and feral pigs are susceptible to CSF virus (Moennig and Greiser-Wilke, 2008). CSF is classified by the Office International des Épizooties (OIE) as a list A disease. It is present in Europe, Central and South America, and Asia, but absent from Australia, New Zealand, and North America (Blome et al., 2010). Re-emerging of CSF can be devastating. According to OIE (2009), in 1997-1998, an outbreak in the Netherlands involved more than 400 herds and cost $2.3 billion to eradicate (Martínez-López et al., 2011). The United Kingdom experienced an epidemic in 2000, and minor outbreaks were reported in Romania, Slovakia, Spain and Germany in 2001 (Karsten and Krieter, 2005; Boklund et al., 2009; Blome et al., 2010). Major reported CSF in Thailand in 2011, 2012, 2013, and 2014 were 4, 8, 22, and 8 outbreaks, respectively (Kamakawa et al., 2006; Kortekaas et al., 2011) (Joint FAO/OIE Workshop on Swine Disease Control in Asia Beijing, PR China, 18-20 November 2014).

Currently, CSF disease continues to occur in many countries across the world and especially in Asia. In countries where CSF is endemic, vaccines may be used to protect animals from clinical disease. Vaccination can reduce prevalence of infection during an eradication program (Kortekaas et al., 2011; Dürr et al., 2013; Zheng et al., 2013). Both modified live and subunit (marker) vaccines are manufactured, although availability varies with the countries. Traditional CSF vaccines are of three types; 1) inactivated CSF virus, 2) inactivated bovine diarrhea virus, and 3) live-attenuated CSF vaccines. Due to poor efficacy of the first two vaccines (Koenig et al., 2007; Kortekaas et al., 2011), only live-attenuated vaccine is now routinely used. A modern type of inactivated vaccine is developed by genetic engineering methods (Dong and Chen, 2007). Until now, only subunit marker vaccine based on recombinant Baculovirus-expressed E2 is commercially available (Bouma et al., 1999). Glycoprotein E2 subunit vaccine was capable of inducing protective immunity as well as distinctive antibody (ie., marker vaccine) (de Smit et al., 2001; Moormann et al., 2000; Sung et al., 2011; Renson et al., 2014; L?vai et al., 2015). However, E2 subunit vaccine could not prevent horizontal or vertical transmission (Moormann et al., 2000; Blome et al., 2014).

There are 3 strains of CSF modified live vaccines (MLV), Chinese or C strain, Japanese guinea-exaltation-negative or GPE-, and French Thiverval strains (Qiu et al., 2006). The MLV can induce a rapid and solid protection against a lethal challenge with CSF virus (Moennig and Becher, 2015). Blanket vaccination is routinely practiced in Thailand during CSF outbreaks (Suradhat et al., 2007). All registered vaccines are administered intramuscularly with 1 or 2 ml per dose. Giving reduced dosage of the vaccine while still activating sufficient immune response, vaccination cost can decrease tremendously. Reduction of vaccine doses might be accomplished by intra-dermal (ID) injection. Skin is populated with Dendritic and Langerhan cells, types of local Lymphocytes, which are efficient and potent antigen-presenting cells for induction of protective immunity, and can result in antigen dose-sparing (Weniger and Glenn, 2013; Tozuka et al. 2016). Many studies have shown that ID vaccination had greater or comparable immune responses such as hepatitis B (Chau et al., 2004; Barraclough et al., 2009; Roukens

et al., 2010; Bunupuradah et al., 2011), rabies (Ambrozaitis et al., 2006; Roukens et al., 2008; Shiota et al., 2008; Tanisaro et al., 2010; Yanagisawa et al., 2012; Lau and Hohl, 2013; Wongsaroj et al., 2013), Foot and mouth (Eblé et al., 2009), Pseudorabies (van Rooij et al., 1998), Porcine reproductive and respiratory syndrome (Martelli et al., 2007), and influenza vaccines (Auewarakul et al., 2007; Chuaychoo et al., 2010; Esposito et al., 2011; Ansaldi et al., 2012; Basileo et al., 2014; Arakane et al., 2015). It is also less painful than intramuscular injection and causes minimal tissue trauma. The ID injecting tool is easy to use (Jarrahian et al., 2012), and recently is becoming popularity especially in Thailand. The ID needle-free is convenient and injecting process is very quick. In our study, we manipulate ID manually in order to be ascertain that every pig get the vaccine antigen.

Materials and methods

Animals and experimental design

The trial was performed on an organic pig farm using 18 conventionally reared 6-weeks old piglets born of sows vaccinated with CSF vaccine once a year. The farm was a small-holder with 3-5 sows. The pigs were serologically negative to Porcine Circovirus, Porcine Reproductive and Respiratory Disease, Pseudorabies virus, and *Mycoplasma hyopneumoniae*. The only vaccine used on this farm is CSF. The pigs in each group thus represent youngsters of the same sow in order to reduce variation in their antibody titers. At the beginning, the pigs were dewormed, ear tagged, weighted, and bled. The pigs were pair wise divided into ID and IM groups, according to their weight, sex, and litter. At Day 0, pigs in ID group were injected intra-dermally (ID) at the back of their ears with 0.2 ml of CSF vaccine by using tuberculin syringes. Pigs in IM groups were vaccinated intramuscularly at the neck muscle with 1 ml dose.

The Lapinized Chinese-strain modified-lived vaccine used in this experiment is produced by the Department of Livestock Development, Thailand. It is a modified live vaccine containing 10^2 PD_{50} (50% protective dose) of the virus. The manufacturers recommended dose of the vaccine is one milliliter injecting intra-muscularly.

Tuberculin syringe connected with a $\frac{1}{2}$ inch 24 gauge needle was used to manually inject the antigen of CSF vaccine at the back of pig's ears. If the procedure was done correctly, a vesicle of 0.3-0.5 centimeter diameter would appear at the skin-site of inoculation. After vaccination, the pigs were inspected daily for clinical signs and rectal temperature. The location at which the vaccination was given was examined at 24, 48, and 72 hours for local hypersensitivity. Ten milliliters of blood samples were collected at 0, 14, 21, and 28 days post-vaccination (dpv). Serum samples were transferred to the tubes and centrifuged, collected the clear fluid portion, and stored at -20 ^{o}C until used.

Neutralizing Peroxidase-Linked Assay (NPLA) and statistical analysis

Neutralization test was performed in cell cultures using a constant-virus/varying-serum method. As CSF virus is non-cytopathic, any non-neutralized virus must be detected, after multiplication, by an indicator system. Neutralizing antibody titers (SN-titers) against CSF virus in serum samples were measured using the NPLA as described previously (OIE manual, 2008). End-point titers were calculated as the reciprocal of the final

serum dilution that neutralized 100 $TCID_{50}$ of CSF viruses in 50% of the wells.

The NPLA was carried out in flat-bottomed microtiter plates. Sera were first inactivated for 30 minutes at 56 °C. Dilutions of serum in growth medium (Eagle's MEM, 5% fetal bovine serum and antibiotics) were dispensed in 50 µl volumes into duplicate wells of a flat bottom microtiter plate. The fetal bovine serum must be free from both Bovine viral diarrhea virus and antibodies to it. A third well was included for each sample. This well contains serum only and was used as a serum control (for cytotoxicity and/or nonspecific staining). The amount of 50 µl of virus suspension, diluted in growth medium to contain approximately 100 $TCID_{50}$, was added to the wells, and mixed on a microplate shaker for 20 seconds. The plates were incubated in a CO_2 incubator for 1 hour at 37 °C. After mixing, 50 µl of growth medium containing 2 x 10^5 cells/ml were added. The cells were allowed to grow at 37 °C in 5% CO_2 to become confluent, usually within 3-4 days. The growth medium was discarded and the plates were rinsed once in 0.15 M NaCl. The plates were then drained by blotting on a paper towel. The plates were fixed with 80% acetone and incubated at 70-80 °C for 1 hour. Fifty µl of a hyperimmune porcine CSF antiserum, diluted in 0.5 M NaCl (containing 1% Tween 80 + 0.1% sodium azide, pH 7.6) was added to each well. The plates were then incubated at 37 °C for 15 minutes. The working dilution of the antiserum was determined by prior titration: and serum with an NPLA titer of 1/30,000 was used as recommended. The plates were washed five times in 0.15 M NaCl containing 1% Tween 80, pH 7.6. Then 50 µl of an anti-porcine or anti-murine (as appropriate) IgG-HRPO conjugate (diluted to its working concentration in 0.5 M NaCl with 1% Tween 80, pH 7.6) was added followed by incubation for 10 minutes at 37 °C. The plates were then washed five times in 0.15 M NaCl containing 1% Tween 80, pH 7.6). Chromogen-substrate solution was then added (50 µl to each well) and allowed to stand for 15-30 minutes at room temperature for staining. The test results were read visually. Infected cell sheets were completely or partially stained reddish brown. The cytoplasm of infected cells stained dark red. The monolayers were examined by low-power microscopy to determine the end-point of the titration. The following controls were included in the test: cell control, positive serum and back titration of test virus. The back-titration confirmed that virus has been used at a concentration of between 30 and 300 $TCID_{50/50}$ µl.

Differences in SN-titers post vaccination between ID and IM vaccinated groups were statistically analysed using the non-parametric Kruskal Wallis test (SPSS version 14.0). A non-parametric permutation test was used for pair-wise comparison between groups if the Kruskal-Wallis test gave a significant result. All significance levels were set at $p<0.05$.

Results

Clinical signs and rectal temperature

After vaccination, no systemic reactions were observed in any of the vaccinated pigs ie., no fever, no sign of depression, the pigs were alert and had good appetite (Table 1). In the IM vaccinated pigs, no local reactions could be seen at the site of injection. In the ID vaccinated pigs, the vesicle disappeared within 24 hours. Only red colored patch at the location of vaccination could be observed with approximately a one centimeter diameter which disappeared within 2 days.

Serological responses

On the day of vaccination (Day 0), all pigs had serum neutralizing antibody (SN) titers ranging from 1:2 to 1:16 (Table 1). After vaccination the SN titers of most pigs increased in both IM and ID groups. Some pigs had same or decreased titers. When SN titers were converted into geometric mean titers (GMT; Log10), there was no significant difference of GMTs between ID and IM vaccinated pigs at 0, 14, 21, and 28 days post-vaccination (dpv) ($p>0.05$) (Table 2). The GMTs of the IM vaccinated pigs were generally higher than those of the ID vaccinated pigs. Pigs vaccinated by ID and IM methods showed increased GMTs at 14 dpv which increased significantly at 21 dpv (Table 3).

Antibody titers of pigs vaccinated IM at the recommended dose rate of one ml were comparable to titers of the pigs vaccinated ID with 0.2ml of the same vaccine. Geometric mean titers (log10) at day 0, 14, 21, and 28 days post-vaccination of ID group were 0.67, 0.85, 1.10, and 1.51 and of IM group were 0.77, 0.87, 1.08, and 1.58, respectively. We conclude that the ID route can be used as an alternative for IM application of CSF vaccine.

Table 1. Allocation of pigs and rectal temperature and serum neutralizing results after CSF modified lived vaccination

Pig Pig No.	Sex	Weight (Kg)	Vaccination Route of vaccination	Rectal Temperature (^{0}C)	Serum neutralizing antibody titer SN antibody titers at days post vaccination (dpv)			
		Day 0		1dpv	0dpv	14dpv	21dpv	28dpv
1	Male	5	ID	38.6	1:2	1:2	1:8	1:64
2	Female	12	ID	39.4	1:4	1:32	1:64	1:128
3	Male	7	ID	39.0	1:4	1:4	1:8	ND
4	Female	9	ID	39.4	1:8	1:16	1:16	ND
5	Female	9	ID	38.8	1:8	1:4	1:8	ND
6	Female	7	ID	39.4	1:4	1:4	1:4	ND
7	Female	5	ID	39.4	1:8	1:4	1:8	1:16
8	Male	7	ID	39.0	1:16	1:16	1:16	1:16
9	Male	9	ID	39.0	1:8	1:16	1:16	ND
10	Male	5	IM	38.3	1:2	1:4	1:4	1:32
11	Female	10	IM	39.5	1:8	1:16	1:32	ND
12	Male	8	IM	38.6	1:8	1:8	1:16	1:32
13	Female	9	IM	39.2	1:4	1:4	1:4	ND
14	Female	9	IM	39.5	1:8	1:16	1:16	ND
15	Female	7	IM	39.7	1:8	1:32	1:128	1:128
16	Male	7	IM	38.0	1:2	1:4	1:8	1:64
17	Male	6	IM	38.8	1:8	1:4	1:4	1:4
18	Male	12	IM	39.8	1:2	1:16	1:16	ND

ND = not determined, no sample due to the pigs were sold out

ID = intradermal

IM = intramuscular

Table 2. Comparison of geometric mean titers (GMT; log10) against Classical swine fever virus between ID and IM groups

Days post-vaccination	Intra- dermal vaccination	SEM	Intra- muscular vaccination	SEM	p-value
Day 0	5.44[a]	1.40	4.67[a]	0.98	0.71
Day 14	7.41[a,b]	3.31	8.64[a,b]	3.15	0.69
Day 21	11.76[c]	6.12	12.70[c]	13.18	0.91
Day 28	38.05[d]	62.53	32.00[d]	21.21	0.69
Mean	9.57	4.58	9.93	5.51	0.83

[a,b,c,d] Letters indicate significant ($p<0.05$)

Table 3. Geometric mean titers (GMT; Log10) against CSF virus of all pigs vaccinated intradermal and intramuscular routes before and after vaccination

Day post-vaccination	Geometric mean titers (GMT)	Standard error of mean
Day 0	5.04[a]	0.84
Day 14	8.00[a,b]	2.22
Day 21	12.22[b]	7.13
Day 28	34.56[c]	15.59

[a,b,c] Letters indicate significant ($p<0.05$)

Discussion

At the start of vaccination (Day 0), all pig were approximately 42-46 days old. Majority of the pigs had SN titers of 1:2 to 1:4 which is the proper time for CSF vaccination. Maternal antibodies at these levels will not impede immune respond to vaccination. In previous study, maternal SN titers of greater than 1:64 inhibited efficacy of vaccination (Suradhat and Damrong-watanapokin, 2003). It was relatively surprising that pigs with titers of 1:8 at Day 0, their SN titers slightly dropped at 14 dpv. This phenomenon occurred in one IM pig, and 2 ID vaccinated pigs. However, SN titers of these 2 ID vaccinated pigs then re-increased above their baseline on 21 dpv afterward. The inclination of antibody responses in these pigs with existing MDA seemed to be slow. Unlike the vaccination in MDA-free or naïve pigs which protection and antibody production could be detected as early as 7 dpv (Lévai et al., 2015). There were 1 IM and 2 ID vaccinated pigs still had the same levels of SN titers from Day 0 to 28 dpv. Pigs in ID groups with SN titers of 1:4 and 1:16 at Day 0, had the same titers after vaccination. Titer of one of the low titer-IM pig remained 1:4 until 28 dpv. These vaccine non-responders were found in both IM and ID pigs, and accounted for 16.67% (3/18 pigs). This finding is different from previous study that stated that ID vaccination route could increase immune response in low-responders (Sugimura et al., 2008; Basileo et al., 2014; Chan et al., 2014). This was not true in our experiment.

A recent work proposed that postnatal persistent CSF infected pigs could occur (Munoz-Gonzalez et al., 2015). Persistently infected pigs will not exhibit antibody response to vaccination while at the same time have virus replication. However, antibody of the pig was not absent in our experiment. It is, therefore, just a matter of time or MDA interferes antibody respond to vaccination. In general recommendation, modified live CSF vaccine is given to young or nursery pigs only one shot. In some vaccinated pigs, although SN antibody was absent, the role of cell mediated immunity had been implicated as evident by clinical protection after challenge (Graham et al., 2010; Graham et al., 2012).

From our results, there must be an explanation on how can ID route induce sufficient immune responses even with a 1/5 fold reduced dose. There must be a special way of immune induction. Antigen presenting cells (APC) are highly populated at the skin such as Dendritic and Langerhans cells. These cells are specialized in antigen presentation. An enhancement of cytotoxic T lymphocytes (CTL), helper T cells or humoral responses were clearly demonstrated in mice (Alvarez et al., 2013). The dermis is also rich in micro-vascular systems that enable interaction between the cells of the immune system through network of regional lymph nodes.

Pigs vaccinated ID and IM had rising trends of GMTs at 14 dpv which increased gradually from 21 dpv to 28 dpv ($p<0.01$). It can be concluded that ID and IM vaccination induce comparable SN titers to CSF virus. SN titers of greater than 1:32 are acceptable protective levels for CSF (Suradhat and Damrongwatanapokin, 2003). In the present study maximum SN titers of 1:128 were seen as a result of ID and IM CSF vaccination. As compared with the 1ml IM vaccinated pigs, the pigs vaccinated ID with 0.2ml of the same vaccine had comparable antibody titers to IM group. We concluded that the ID route can be used as an alternative route for IM application of Lapinized Chinese-strain CSF vaccine. The cost of vaccine can be saved tremendously and can also increase a number of available of vaccine

doses, especially during CSF outbreaks where vaccine doses are demanding. However, it would be more convenient to use ID injecting tools rather than manually injecting of the vaccine, since it is laborious and time consuming. The novel devices which guarantee less invasive, reproducible and easier injection will lead to a practical and widely use of intradermal vaccination strategy. A thorough investigation on using this technique in a larger number of pigs would give more confident result.

Acknowledgements

The study was financially supported by the USAID. We thank Professor Dr. Anil Taku for proof reading on English preparation of the manuscript.

References

Alvarez B, Poderoso T, Alonso F, Ezquerra A, Domínguez J, Revilla C. Antigen targeting to apc: From mice to veterinary species. Developmental & Comparative Immunology 2013;41:153-163.

Ambrozaitis A, Laiškonis A, Balčiuniene L, Banzhoff A, Malerczyk C. Rabies post-exposure prophylaxis vaccination with purified chick embryo cell vaccine (pcecv) and purified vero cell rabies vaccine (pvrv) in a four-site intradermal schedule (4-0-2-0-1-1): An immunogenic, cost-effective and practical regimen. Vaccine 2006;24:4116-4121.

Ansaldi F, Canepa P, Ceravolo A, Valle L, de Florentiis D, Oomen R, Vogel FR, Denis M, Samson SI, Icardi G. Intanza® 15 mcg intradermal influenza vaccine elicits cross-reactive antibody responses against heterologous a (h3n2) influenza viruses. Vaccine 2012;30:2908-2913.

Arakane R, Annaka R, Takahama A, Ishida K, Yoshiike M, Nakayama T, Takeshita F. Superior immunogenicity profile of the new intradermal influenza vaccine compared to the standard subcutaneous vaccine in subjects 65 years and older: A randomized controlled phase iii study. Vaccine 2015;33:6650-6658.

Auewarakul P, Kositanont U, Sornsathapornkul P, Tothong P, Kanyok R, Thongcharoen P. Antibody responses after dose-sparing intradermal influenza vaccination. Vaccine 2007;25:659-663.

Barraclough KA, Wiggins KJ, Hawley CM, van Eps CL, Mudge DW, Johnson DW, Whitby M, Carpenter S, Playford EG. Intradermal versus intramuscular hepatitis b vaccination in hemodialysis patients: A prospective open-label randomized controlled trial in nonresponders to primary vaccination. American Journal of Kidney Diseases 2009; 54:95-103.

Basileo M, Iorio AM, Bartolini G, Bianchini C, Menculini G, Tozzi P, Camilloni B. Comparative study of immunogenicity of split, intradermal and mf59-adjuvanted influenza vaccines in elderly institutionalized subjects. Procedia in Vaccinology 2014;8:18-23.

Blome S, Gabriel C, Schmeiser S, Meyer D, Meindl-B?hmer A, Koenen F, Beer M. Efficacy of marker vaccine candidate cp7_e2alf against challenge with classical swine fever virus isolates of different genotypes. Veterinary Microbiology 2014;169:8-17.

Blome S, Grotha I, Moennig V, Greiser-Wilke I. Classical swine fever virus in south-eastern europe___retrospective analysis of the disease situation and molecular epidemiology. Veterinary Microbiology 2010;146:276-284.

Boklund A, Toft N, Alban L, Uttenthal Å. Comparing the epidemiological and economic effects of control strategies against classical swine fever in denmark. Preventive Veterinary Medicine 2009;90:180-193.

Bouma A, De Smit AJ, De Kluijver EP, Terpstra C, Moormann RJM. Efficacy and stability of a subunit vaccine based on glycoprotein e2 of classical swine fever virus. Veterinary Microbiology 1999;66: 101-114.

Bunupuradah T, Ananworanich J, Pancharoen C, Petoumenos K, Prasitsuebsai W, Wongngam W, Ubolyam S, Sriheara C, Lange J, Phanuphak P, Puthanakit T. Randomized study of intradermal compared to intramuscular hepatitis b vaccination in hiv-infected children without severe immuno-suppression. Vaccine 2011;29:2962-2967.

Chan T-C, Hung IF-N, Chan K-H, Li CP-Y, Li PT-W, Luk JK-H, Chu L-W, Chan FH-W. Immunogenicity and safety of intradermal trivalent influenza vaccination in nursing home older adults: A randomized controlled trial. Journal of the American Medical Directors Association 2014;15:607.e605-607.e612.

Chau KF, Cheng YL, Tsang DNC, Choi KS, Wong KM, Chak WL, Chan YH, Li CS. Efficacy and side effects of intradermal hepatitis b vaccination in capd patients: A comparison with the intramuscular vaccination. American Journal of Kidney Diseases 2004;43:910-917.

Chuaychoo B, Wongsurakiat P, Nana A, Kositanont U, Maranetra KN. The immunogenicity of intradermal influenza vaccination in copd patients. Vaccine 2010;28:4045-4051.

de Smit AJ, Bouma A, de Kluijver EP, Terpstra C, Moormann RJM. Duration of the protection of an e2 subunit marker vaccine against classical swine fever after a single vaccination. Veterinary Microbiology 2001;78:307-317.

Dong X-N, Chen Y-H. Marker vaccine strategies and candidate csfv marker vaccines. Vaccine 2007; 25:205-230.

Dürr S, zu Dohna H, Di Labio E, Carpenter TE, Doherr MG. Evaluation of control and surveillance strategies for classical swine fever using a simulation model. Preventive Veterinary Medicine 2013; 108:73-84.

Eblé PL, Weerdmeester K, van Hemert-Kluitenberg F, Dekker A. Intradermal vaccination of pigs against fmd with 1/10 dose results in comparable vaccine efficacy as intramuscular vaccination with a full dose. Vaccine 2009;27:1272-1278.

Esposito S, Daleno C, Picciolli I, Tagliaferri L, Scala A, Prunotto G, Montinaro V, Galeone C, Principi N. Immunogenicity and safety of intradermal influenza vaccine in children. Vaccine 2011;29:7606-7610.

Graham SP, Everett HE, Johns HL, Haines FJ, Rocca SAL, Khatri M, Wright IK, Drew T, Crooke HR. Characterisation of virus-specific peripheral blood cell cytokine responses following vaccination or infection with classical swine fever viruses. Veterinary Microbiology 2010;142:34-40.

Graham SP, Haines FJ, Johns HL, Sosan O, La Rocca SA, Lamp B, R?menapf T, Everett HE, Crooke HR. Characterisation of vaccine-induced, broadly cross-reactive ifn-γ secreting t cell responses that correlate with rapid protection against classical swine fever virus. Vaccine 2012;30:2742-2748.

Jarrahian C, Zehrung D, Saxon E, Griswold E, Klaff L. Clinical performance and safety of the id adapter, a prototype intradermal delivery technology for vaccines, drugs, and diagnostic tests. Procedia in Vaccinology 2012;6:125-133.

Kamakawa A, Thu HTV, Yamada S. Epidemiological survey of viral diseases of pigs in the mekong delta of vietnam between 1999 and 2003. Veterinary Microbiology 2006;118:47-56.

Karsten S, Krieter J: Epidemiology of classical swine fever and models to analyse virus spread. A review. Deutsche Tierarztliche Wochenschrift 2005;112: 180-188.

Koenig P, Hoffmann B, Depner KR, Reimann I, Teifke JP, Beer M. Detection of classical swine fever vaccine virus in blood and tissue samples of pigs vaccinated either with a conventional c-strain vaccine or a modified live marker vaccine. Veterinary Microbiology 2007;120:343-351.

Kortekaas J, Ketelaar J, Vloet RPM, Loeffen WL. Protective efficacy of a classical swine fever virus c-strain deletion mutant and ability to differentiate infected from vaccinated animals. Veterinary Microbiology 2011;147:11-18.

Lau CL, Hohl N. Immunogenicity of a modified intradermal pre-exposure rabies vaccination schedule using a purified chick embryo cell vaccine: An observational study. Travel Medicine and Infectious Disease 2013;11:427-430.

Lévai R, Barna T, Fábián K, Blome S, Belák K, Bálint Á, Koenen F, Kulcsar G, Farsang A. Pre-registration efficacy study of a novel marker vaccine against classical swine fever on maternally derived antibody negative (mda-) target animals. Biologicals 2015;43:92-99.

Martelli P, Cordioli P, Alborali LG, Gozio S, De Angelis E, Ferrari L, Lombardi G, Borghetti P. Protection and immune response in pigs intradermally vaccinated against porcine reproductive and respiratory syndrome (prrs) and subsequently exposed to a heterologous european (italian cluster) field strain. Vaccine 2007;25:3400-3408.

Martínez-López B, Ivorra B, Ramos AM, Sánchez-Vizcaíno JM. A novel spatial and stochastic model to evaluate the within- and between-farm transmission of classical swine fever virus. I. General concepts and description of the model. Veterinary Microbiology 2011;147:300-309.

Moennig V, Becher P. Pestivirus control programs: How far have we come and where are we going? Animal health research reviews / Conference of Research Workers in Animal Diseases 2015;16: 83-87.

Moennig V, Greiser-Wilke I. Classical swine fever virus; in Regenmortel BWJMHVV (ed): Encyclopedia of virology (third edition). Oxford, Academic Press, 2008, pp 525-532.

Moormann RJM, Bouma A, Kramps JA, Terpstra C, De Smit HJ. Development of a classical swine fever subunit marker vaccine and companion diagnostic test. Veterinary Microbiology 2000;73:209-219.

Munoz-Gonzalez S, Ruggli N, Rosell R, Perez LJ, Frias-Leuporeau MT, Fraile L, Montoya M, Cordoba L, Domingo M, Ehrensperger F, Summerfield A, Ganges L. Postnatal persistent infection with classical swine fever virus and its immunological implications. PloS one 2015;10:e0125692.

Qiu H-j, Shen R-x, Tong G-z. The lapinized chinese strain vaccine against classical swine fever virus: A retrospective review spanning half a century. Agricultural Sciences in China 2006;5:1-14.

Renson P, Le Dimna M, Gabriel C, Levai R, Blome S, Kulcsar G, Koenen F, Le Potier MF. Cytokine and immunoglobulin isotype profiles during cp7_e2alf vaccination against a challenge with the highly virulent koslov strain of classical swine fever virus. Research in Veterinary Science 2014;96:389-395.

Roukens AH, Vossen AC, Boland GJ, Verduyn W, van Dissel JT, Visser LG. Intradermal hepatitis b vaccination in non-responders after topical application of imiquimod (aldara®). Vaccine 2010;28:4288-4293.

Roukens AH, Vossen AC, van Dissel JT, Visser LG. Reduced dose pre-exposure primary and booster intradermal rabies vaccination with a purified chick embryo cell vaccine (pcecv) is immunogenic and safe in adults. Vaccine 2008;26:3438-3442.

Shiota S, Khawplod P, Ahmed K, Mifune K, Nishizono A. A pilot study on intradermal vaccination of japanese rabies vaccine for pre-exposure immunization. Vaccine 2008;26:6441-6444.

Sugimura T, Ito Y, Tananari Y, Ozaki Y, Maeno Y, Yamaoka T, Kudo Y. Improved antibody responses in infants less than 1 year old using intradermal influenza vaccination. Vaccine 2008;26:2700-2705.

Sung J-H, Kang M-L, Lee W-J, Shin M-K, Lim S-I, Kim B-H, Song J-Y, Yoo HS. Improved sero-monitoring assay for classical swine fever (csf) using the recombinant e2 protein of a recent korean isolate. Research in Veterinary Science 2011;90:329-335.

Suradhat S, Damrongwatanapokin S. The influence of maternal immunity on the efficacy of a classical swine fever vaccine against classical swine fever virus, genogroup 2.2, infection. Veterinary Microbiology 2003;92:187-194.

Suradhat S, Damrongwatanapokin S, Thanawongnuwech R. Factors critical for successful vaccination against classical swine fever in endemic areas. Veterinary Microbiology 2007;119:1-9.

Tanisaro T, Tantawichien T, Tiranathanagul K, Susantitaphong P, Chirananthavat T, Praditpornsilpa K, Sitprija V, Eiam-Ong S. Neutralizing antibody response after intradermal rabies vaccination in hemodialysis patients. Vaccine 2010;28:2385-2387.

Tozuka M, Oka T, Jounai N, Egawa G, Ishii KJ, Kabashima K, Takeshita F. Efficient antigen delivery to the draining lymph nodes is a key component in the immunogenic pathway of the intradermal vaccine. Journal of Dermatological Science 2016; 82(1): 38-45.

van Rooij EMA, Haagmans BL, de Visser YE, de Bruin MGM, Boersma W, Bianchi ATJ. Effect of vaccination route and composition of DNA vaccine on the induction of protective immunity against pseudorabies infection in pigs. Veterinary Immunology and Immunopathology 1998;66: 113-126.

Weniger BG, Glenn GM: Cutaneous vaccination. Antigen delivery into or onto the skin. Vaccine 2013;31: 3389-3391.

Wongsaroj P, Udomchaisakul P, Tepsumethanon S, Khawplod P, Tantawichien T. Rabies neutralizing antibody after 2 intradermal doses on days 0 and 21 for pre-exposure prophylaxis. Vaccine 2013; 31:1748-1751.

Yanagisawa N, Suganuma A, Takayama N, Nakayama E, Mannen K. Immunogenicity of intradermal vaccination of japanese rabies vaccine for preexposure immunization following who recommendation. Journal of Infection and Chemotherapy 2012; 18:66-68.

Zheng FY, Qiu CQ, Jia HJ, Chen GH, Zeng S, He XB, Fang YX, Lin GZ, Jing ZZ. Infection of mouse bone marrow-derived immature dendritic cells with classical swine fever virus c-strain promotes cells maturation and lymphocyte proliferation. Res Vet Sci 2013;95:1268-1270.