



Serologic Profiles of Classical Swine Fever Vaccinated Backyard Pig Farms in Khon Kaen Province, Thailand

Kochakorn Direksin^{1*}, Arinee Chatchawancholtheera² and Sujira Pachariyanon³

¹Faculty of Veterinary Medicine, Khon Kaen University, Muang, Khon Kaen, THAILAND

²Faculty of Pathobiology, Khon Kaen University, Muang, Khon Kaen, THAILAND

³Animal Health Institute, Department of Livestock Development, Bangkok, THAILAND

*Corresponding author: K Direksin; Email: kochakrn@kku.ac.th

Received: 29 June, 2016

Accepted: 10 July, 2016

ABSTRACT

Small and marginal farmers with low livestock holdings do not strictly follow recommended vaccination schedules and procedures. The aim of this study was to follow antibody titers against Classical Swine Fever (CSF) virus before and after vaccination of cross-bred pigs from three farms in Khon Kaen. One dose of vaccine was given to the pigs at 41, 49, and 52 days age. It is a modified live vaccine containing 10^2 PD₅₀ (50% protective dose) of the CSF virus (government produced Lapinized Chinese-strain, Department of Livestock Disease, Thailand). Blood samples were collected at 0, 14, and 22 days post-vaccination (dpv). Neutralization test was performed in cell cultures using a constant-virus/varying-serum method. In Pigs born to sows that had been vaccinated once a year, geometric mean titers at 0 dpv of 41, 49, and 52 days old pigs were 2.05, 1.24, and 0.72, respectively. The lowest/highest maternal antibody titers were 1:32/1:512, 1:8/1:64, 1:2/1:16, the median titers were 1:32, 1:16, and 1:8, in farms 1, 2, and 3, respectively. At 14 dpv, SN antibody titres of pigs in farm 1 decreased but that of farm 3 increased. Consequently, SN antibody titers of pigs in farms 2 and 3 increased after vaccination and had GMT of 1.82 and 1.09 ($p < 0.05$) at 22 dpv, respectively. General vaccination guideline is not applicable to all farms. Higher SN titers at the day of first vaccination impeded antibody response (14 days old). However, the lowest titer (1:8) and the oldest pigs (52 days old) did not have the best SN titer after CSF vaccination. There should be laboratory confirmations of CSF antibody titers before and after vaccination in order to assure successful outcome of vaccination practice.

Keywords: Classical swine fever, vaccination, pig, backyard farm

Classical swine fever (CSF) is a 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). The disease 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. The United Kingdom experienced an epidemic in 2000, and minor outbreaks were reported in Romania, Slovakia, Spain and Germany in 2001 (Boklund *et al.*, 2009; Martínez-López *et al.*, 2011).

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 (Dürr *et al.*, 2013; Kortekaas *et al.*, 2011). In Thailand, despite eighty percent of commercial swine herds being vaccinated against CSF, official reports of CSF in Thailand in 2011, 2012, 2013, and 2014 were 4, 8, 22, and 8 outbreaks, respectively (Kamakawa *et al.*, 2006). It is believed that maternal derived antibody was a major issue of vaccine failure (Suradhat and Damrongwatanapokin 2003; Suradhat *et al.*, 2007).

General recommendation in Thailand for CSF vaccination programs in breeders are: 1) Gilts to be vaccinated once before breeding, 2) Sows to be vaccinated 2-3 weeks pre-farrowing or 2-3 weeks post-farrowing or 3 times a year, 3) Boars to be vaccinated every 6 months. The mentioned



programs provided good protective levels of antibody in 1-3 weeks old piglet (Suradhat and Damrongwatanapokin 2003). When there is no clinical CSF outbreak, one dose of vaccination is practiced in 7-8 weeks old pigs. In high-risk areas, 2 doses of vaccination are given to 5 weeks old pigs followed by booster 4 weeks later.

Small-holder or backyard farmers rarely follow this guideline. They will vaccinate sows and pigs whenever convenient. The disease situation is dynamic and there must be clear guidelines regarding vaccination schedule. Commercial farms usually have a good CSF monitoring system, while backyard pig farms do not. Pigs in small-holder farms may be great risks for transmitting CSF virus to intensive farms (Martínez-López *et al.*, 2013). Therefore, it is essential to routinely confirm antibody titers before and after vaccination in small-holder farms.

MATERIALS AND METHODS

The trial was performed on 3 backyard farms in Khon Kaen province using 17-20 pigs per farm. The same pigs were followed for their changes in antibody titers. The pigs were crossbred Largewhite-Landrace-Duroc. The ages of pigs in farms 1, 2, and 3, at first vaccination were 41, 49, 52 days, respectively. These piglets born to sows vaccinated with CSF vaccine once a year. At the beginning, the pigs were randomly selected and ear tagged. The pigs were injected intramuscularly with 1 ml of CSF vaccine at the zero-day post-vaccination (0 dpv). The vaccine used in this experiment is a government produced Lapinized Chinese-strain (Department of Livestock Disease, Thailand). It is a modified live vaccine containing 10^2 PD₅₀ (50% protective dose) of the CSF virus. Blood samples were collected at 0, 14, and 22 dpv. Serum samples were centrifuged, collected the clear fluid portion, and stored at 4°C until use.

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 TCID₅₀ of CSF viruses in 50% of the wells.

Differences in neutralizing (SN) antibody titers post-vaccination at 0, 14, 22 dpv were statistically analyzed 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 AND DISCUSSION

Piglets consume colostrum milk from CSF vaccinated sows will receive passive antibodies from their mother. That passive transfer immune is called maternally derived antibody (MDV). Before vaccination (0 dpv), MDA titers were varied greatly among pigs in each farm. In farm 1, there were 6 pigs with the lowest MDA titer of 1:16, and 6 pigs with the highest MDA of 1:521 (Fig. 1). There was a decreased antibody in 19 pigs at 14 days after vaccination with CSF lives vaccine. One pig (No. 20) still had the same level of SN titer. This result suggests that MDA interferes immune response to vaccination. In farm 2, there was only one pig (No. 9) had the lowest MDA of 1:4, and one (No. 11) with the highest MDA of 1:64 (Fig. 2). The pigs in farm 2 had a good immune response to vaccination. Sixteen pigs, with MDA titers ranges 1:4 to 1:32, exhibited boosting antibody responses at 22 days after vaccination, except one pig (No. 11), with MDA titer of 1:64, had slightly declined in the titer. In farm 3, there were 3 pigs had the lowest existing MDA of 1:2, and merely one (No. 14) pig had the highest MDA of 1:16 (Fig. 3). However, on this farm, pigs did not seem to respond well to vaccination as compared to farm 2. Only one pig (No. 7) had the highest SN titer of 1:128 at 22 dpv.

The lowest/highest MDA titers were 1:32/1:512, 1:8/1:64, 1:2/1:16, the median MDA titers were 1:32, 1:16, and 1:8, in farms 1, 2, and 3, respectively. Maternal antibody declined as the pigs get older and their antibody titers changed drastically even one week apart. Geometric mean titers at 0 dpv of 41, 49, and 52 days old pigs were 2.05, 1.24, and 0.72, respectively ($p < 0.05$) (Table 1).

After vaccination, overall SN antibody of pigs in farm 1 decreased, while those in farms 2 and 3 increased (Fig. 1, 2 and 3). It was unfortunate that we could not follow SN titers of pigs in farm 1 at 22 dpv and in farm 2 at 14 dpv. However, after one dose of CSF vaccination, SN antibody titers of pigs in farms 2 and 3 increased thereafter at 22

dpv. At 22 dpv, pigs in farm 2 and 3 had GMT of 1.82 and 1.09, respectively. In general, all pigs in 3 farms had increased antibody responses to vaccination (Fig. 4).

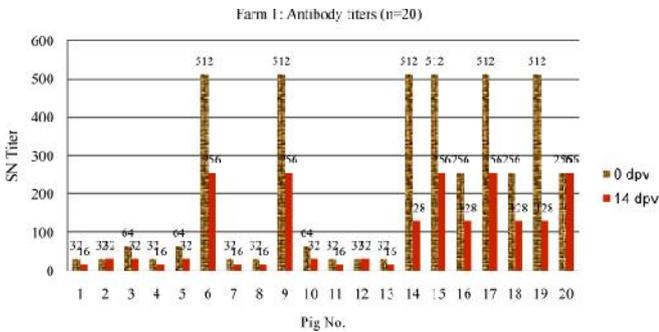


Fig. 1: SN titers of individual pigs (No.1-20) of farm 1 before (0 dpv) and after (14 dpv) CSF vaccination. SN titers were relatively high before vaccination, and dropped after vaccination. There was a declined antibody at 14 days in 19 pigs after vaccination with CSF lives vaccine, but one pig (No. 20) still had the same level of SN titer.

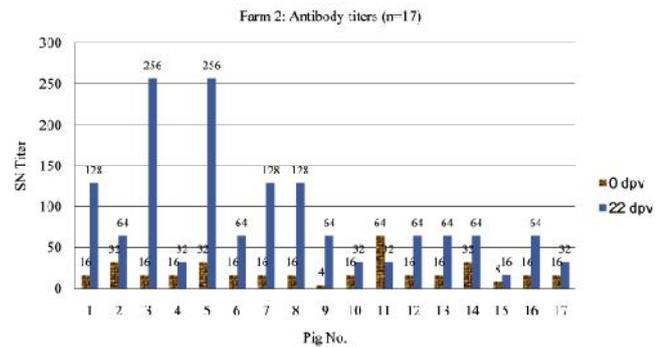


Fig. 2: SN titer of an individual pig of farm 2 before (0 dpv) and after (22 dpv) CSF vaccination. The pigs in farm 2 had a good immune response to vaccination. Sixteen pigs exhibited boosting antibody responses at 22 days after vaccination, except one pig (No. 11) had slightly decline in the titer.

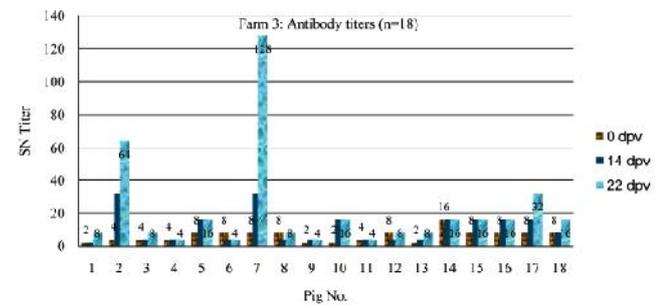


Fig. 3: SN titers of individual pig (No. 1-18) of farm 3 at 0, 14, and 22 dpv. There were 3 pigs had the lowest existing MDA of 1:2, and one pig had the highest MDA of 1:16. On this farm, pigs

did not seem to respond well to vaccination. Only one pig (No. 7) had the highest SN titer of 1:128 at 22 dpv.

Table 1: Serum neutralizing antibody against CSF virus; Geometric mean titer (GMT) of pigs at different ages, farms, and days post vaccination

Farm	Age of pig at first vaccination	0 Day post-vaccination	14 Days post-vaccination	22 Days post-vaccination
1 (20 pigs)	41 days	2.05 ^a	1.76 ^d	Not determined
2 (17 pigs)	49 days	1.24 ^b	Not determined	1.82 ^d
3 (18 pigs)	52 days	0.72 ^c	0.90 ^c	1.09 ^b

a,b,c Letters indicate significant difference (p<0.05)

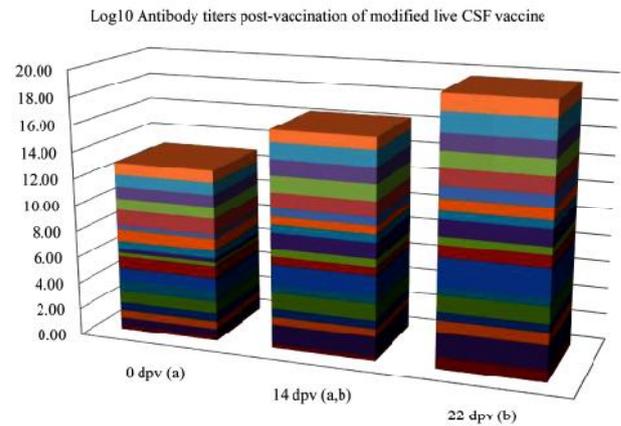


Fig. 4: Log10 SN titers of all pigs in 3 farms at different days post-vaccination. In general, all pigs had increased antibody responses to CSF vaccination.

General vaccination guidelines although helpful are not always followed uniformly on all farms. There should be laboratory confirmations of CSF antibody titers before and after vaccination in order to assure successful practice. From this study, immune response to one dose of CSF vaccine was relatively slower when given to younger pigs (41 days old). However, it is not quite clear when compared between 49 and 52 days old pigs, why the oldest 52 days old pigs with MDA titer of 1:2 did not have the best SN titer response when compared to 49 days old with SN titer of 1:16 at the day of vaccination. It is quite certain that maternal derived SN titer of 1:32 or greater interfered



with vaccine immune responses but SN titer of 1:16 did not. Our results were similar to a previous study (Suradhat and Damrongwatanapokin, 2003) who reported factors such as the influence of age at primary vaccination and status of maternal immunity on CSF vaccination outcome. From our results, pigs in farm 2 (49 days old) had a better immune response to the vaccine than 52 days old pigs in farm 3. Therefore, it is not always true when considering pig ages or MDA titers at the day of first vaccination. We should be careful on the injecting of the vaccine. The accuracy in the amount of each dose, the stability, or temperature keeping of lived vaccine virus.

CSF vaccine should be given to pigs at the age of 49-52 days old. We recommend that in the case of a great risk, early CSF vaccination can be done but should consider second boosting dose. Monitoring of vaccination programs and surveillance of titers post vaccination helps in disease prevention. As monitoring is difficult in farming communities with low livestock holdings, guidelines regarding vaccination against CSF are needed to be rigorously enforced for successful disease control program.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest in the execution and reporting of the work in this study. The relationship with persons and organization did not in any way influence the reporting of the findings.

REFERENCES

Blome, S., Grotha, I., Moennig, V. and Greiser-Wilke, I. 2010. Classical swine fever virus in South-Eastern Europe—Retrospective analysis of the disease situation and molecular epidemiology. *Vet. Microbiol.*, **146**: 276-284.

- Boklund, A., Toft, N., Alban, L. and Uttenthal, Å. 2009. Comparing the epidemiological and economic effects of control strategies against classical swine fever in Denmark. *Prev. Vet. Med.*, **90**: 180-193.
- Dürr, S., zu Dohna, H., Di Labio, E., Carpenter, T.E. and Doherr, M.G. 2013. Evaluation of control and surveillance strategies for classical swine fever using a simulation model. *Prev. Vet. Med.*, **108**: 73-84.
- Kamakawa, A., Thu, H.T.V. and Yamada, S. 2006. Epidemiological survey of viral diseases of pigs in the Mekong delta of Vietnam between 1999 and 2003. *Vet. Microbiol.*, **118**: 47-56.
- Kortekaas, J., Ketelaar, J., Vloet, R.P.M. and Loeffen, W.L. 2011. Protective efficacy of a Classical swine fever virus C-strain deletion mutant and ability to differentiate infected from vaccinated animals. *Vet. Microbiol.*, **147**: 11-18.
- Martínez-López, B., Ivorra, B., Ramos, A.M., Fernández-Carrión, E., Alexandrov, T. and Sánchez-Vizcaíno, J.M. 2013. Evaluation of the risk of classical swine fever (CSF) spread from backyard pigs to other domestic pigs by using the spatial stochastic disease spread model Be-FAST: The example of Bulgaria. *Vet. Microbiol.*, **165**: 79-85.
- Martínez-López, B., Ivorra, B., Ramos, A.M. and Sánchez-Vizcaíno, J.M. 2011. 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. *Vet. Microbiol.*, **147**: 300-309.
- Moennig, V. and Greiser-Wilke, I. 2008. Classical Swine Fever Virus, In: Regenmortel, B.W.J.M.H.V.V. (Ed.) *Encyclopedia of Virology (Third Edition)*. pp. 525-532. Academic Press, Oxford, UK..
- Suradhat, S. and Damrongwatanapokin, S. 2003. The influence of maternal immunity on the efficacy of a classical swine fever vaccine against classical swine fever virus, genogroup 2.2, infection. *Vet. Microbiol.*, **92**: 187-194.
- Suradhat, S., Damrongwatanapokin, S. and Thanawongnuwech, R. 2007. Factors critical for successful vaccination against classical swine fever in endemic areas. *Vet. Microbiol.*, **119**: 1-9.