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# Different porcine reproductive and respiratory syndrome (PRRS) vaccine regimes and its effect on pig immunity status at Southeast Asia pig farms

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**Abstract.** Porcine reproductive and respiratory syndrome (PRRS) is a disease characterised by late-term reproductive failure in sows and gilts, and respiratory problems in piglets and growing pigs. In this study, 240 sera were collected from four farms that had been practicing different PRRS vaccination regime for more than a year and vaccinations were done at 2 months before sampling. Fifteen sera samples from four age groups: sows, growers, weaners and piglets were collected from each farm and analysed using IDEXX PRRS X3 ELISA for PRRSV antibodies. Pooled serum samples were tested by using nested-PCR that enable the differentiation of Type I and Type II PRRSV. Out of 80 pooled serum samples, none were positive for PRRSV indicating all age groups were not viraemic after vaccination. Results by ELISA test showed all the farms were seropositive for PRRS. ELISA testing showed no significant difference between the farms except for Farm B which practised whole herd US MLV vaccination. Farm B showed significantly lower ($p<0.05$) S/P ratio in their piglet, grower and sow groups which suggest there was low virus circulation in herd. Farm A which practised US MLV on sow was the only farm found to have seronegative status in their weaners. Data indicates PRRS MLV vaccination will not cause viraemia post four weeks vaccination and whole herd MLV vaccination may help to reduce virus circulation in PRRS endemic farm.

## INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) continues to be clinically relevant and economically significant since it was first described and the causative agent, PRRS virus (PRRSV) was identified more than two decades ago (Neumann *et al.*, 2005). Classical clinical signs of the disease include late-term reproductive failure in sows and gilts, and respiratory problems in piglets and growing pigs. Most PRRSV isolates from South America and Asia are of type 2 and it is assumed these viruses were introduced through the movement of swine and semen.

Seroprevalence study carried out in Malaysia showed more than 90% of the farms involved were seropositive for PRRS and more than 80% of the pigs were seropositive (Jasbir *et al.*, 2008). Later, a genetic characterization study done in 2012 showed a seroprevalence of 89.2% with only American PRRS virus strains detected in selected pig farms in Malaysia (Vania & Ooi, 2012).

Highly pathogenic PRRS (HP-PRRS), which emerged in China in 2006, has spread to South-East Asian countries since 2007. The disease was characterized by high fever (40-42°C) in all age groups, abortions in sows and high mortality in suckling piglets, weaners and growers (Tian *et al.*, 2007). The first case of HP-PRRS in Thailand was detected in Phitsanulok province in early

2010. The production system that has been affected is mainly the backyard sector with low farm biosecurity (Nguyen, 2013). The disease became established first in countries with a larger commercial production units and high animal densities (Vietnam, Thailand) and subsequently affected countries with a less developed commercial sector (Cambodia and Laos) due to the absence of disease surveillance at community level, weakness of the Veterinary Services in dealing with outbreaks in a timely manner, lack of biosecurity in value chains and the absence of regulations and incentives to control pig diseases (Nguyen, 2013).

Because of the huge impact of PRRS in the swine industry, vaccination is a key component of PRRS disease control strategies. It is also the most economic strategy for all sizes of pig farms compared with other control strategies. There are two types of PRRSV commercial available vaccines which are Killed Virus (KV) or inactivated vaccines and Modified-Live Virus (MLV) or attenuated vaccines. Information about vaccination schedule of available commercial PRRSV vaccines in Malaysia are summarized in Table 1.

Inactivated PRRSV vaccines are used for the immunization of breeding herd. Their main advantage is safety, as the vaccine virus cannot transmit to other pigs and cannot revert to virulence (Papatsiros, 2012). Unfortunately, the efficacy of inactivated PRRS vaccines has been seriously questioned. Studies with commercial KV vaccines showed that the vaccination did not induce VN antibodies and did not sufficiently protect against viremia or prevent from the clinical signs of PRRS (Nilubol *et al.*, 2004).

On the other hand, MLV vaccines are used for the prevention and control of PRRS infection both in breeding stock and young piglets. Those vaccine prepared from attenuated virus are the most likely to provide the highest level of clinical protection compare to other conventional vaccines

Table 1. Currently available commercial porcine reproductive and respiratory syndrome (PRRS) vaccines in Malaysia

| Current name | Type | Virus strain | Manufacturer | Vaccination schedule |
|---|---|---|---|---|
| Amervac® PRRS | Attenuated | European | Hipra Laboratorios | Piglets: one dose (IM) at age of 4–5 wk |
| Ingelvac® PRRS MLV | Attenuated | North American | Boehringer Ingelheim | Gilts: 2 doses 30 days apart.<br>At least 30 days prior to entry.<br>Sows : All animals on site at the same time (Mass Vaccine)<br>1 dose, 4 times per year<br>Piglets: one dose at day 10-14 |
| Progressis® | Inactivated | European | Merial | Primary vaccination (gilts and sows): twice (IM), 3-4 wk interval at least 3 wk prior to mating.<br>Revaccination (booster) one dose (IM) at 60-70d of each gestation |
| Suipravac® PRRS | Inactivated | European | Hipra Laboratorios | Breeding stock Primary vaccination: entering the farm /Sows (pregnancy or lactation): Twice (IM), at 3-4 wk interval.<br>Revaccination (booster) |

Note. Data adapted from Papatsiros (2012) inAmerican Journal of Animal and Veterinary Sciences and Mengeling (2005) in Journal of Swine Health and Production.

available today. They have the potential to replicate extensively over a long period of time in the vaccinated pig and thus repeatedly expose the pig's immune system to the entire spectrum of viral antigens (Mengeling, 2005). This long interval of replication may be more important for PRRS immunity because neutralizing antibody against PRRSV is slow to develop. However, MLV vaccines usage remains debatable. The major concern is the safety and reversion of vaccine virus to virulence result from genetic mutations or recombination with field strains (Murtaugh *et al*., 2010).

According to Holtkamp *et al*, 2011, a herd classification system was developed for describing the PRRSV status of herds, based upon a set of definitions reflecting the biology and ecology of PRRSV. The herd classification system was established by a definitions committee formed by the American Association of Swine Veterinarians (AASV) and the United States Department of Agriculture PRRS-Coordinated Agricultural Project, and was approved by the AASV Board of Directors.

Breeding herds, with or without growing pigs on the same premises, are categorized as Positive Unstable (Category I), Positive Stable (Category II), Provisional Negative (Category III), or Negative (Category IV) on the basis of herd shedding and exposure status. Category II is further divided into two subcategories which are Positive Stable (II-A) and Positive Stable Undergoing Elimination (II-B). Growing-pig herds are categorized as Positive or Negative. Preferred testing methods to determine shedding include direct detection of the virus by PCR whereas exposure is determined by antibody testing: enzyme-linked immunosorbent assay (ELISA), immunofluorescent antibody (IFA), or immunoperoxidase monolayer assay (IPMA). ELISA is the preferred testing method.

Thus, this study aims to determine the viremic and serological status of piglets, weaners, growers and sows in farm after different types of PRRS vaccination in tropical pig farms.

## MATERIAL AND METHODS

### Animal and Sample Collection

A total of four farms were identified and the farms chosen had been practicing different PRRS vaccination regime for more than one year. Farm A used PRRS US strain MLV on sows; Farm B practiced PRRS US strain MLV vaccine on both sows and piglets; Farm C vaccinated sow with PRRS killed vaccine and Farm D vaccinated sow and piglet with EU strain MLV. All farms were single site except for Farm B which had separated weaning-finishing unit and farrowing unit. Blood was collected after one month PRRS vaccination. A total of 240 sera were collected from 4 farms respectively. Each farm have 60 sera samples from five different age groups (15 from five days old piglets, 15 from six weeks old weaners, 15 from 20 weeks old growers and 15 from sow). Pigs were sero-sampled with standard venepuncture using plain tube. All samples were collected under the supervision of veterinarians from the Faculty of Veterinary Medicine, UPM. The study was conducted following the guidelines as stated in the Code of Practice for Care and use of Animals for Scientific Purposes as stipulated by Universiti Putra Malaysia (UPM/IACUC/FYP-2016/FPV.036), complied with the current guidelines for the care and use of animals, and was approved by the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia.

### Serological test

Serum samples were tested using commercial IDEXX PRRS X3 ELISA assay (HerdChek; IDEXX Laboratories Inc, Switzerland). Samples were considered positive if the calculated sample to positive (S/P) ratio was 0.4 or greater. The test kit used the indirect ELISA format. Test results were expressed by calculating the sample-to-positive control (S/P) ratio for each sample using commercial software (IDEXX XCheck® software).

**RNA Extraction and Synthesis of cDNA**
The RNA was extracted from the serum samples using QIAamp® Viral RNA Kits (QIAGEN®, Germany) according to the manufacture protocol. RNA collected was converted into cDNA using QuantiNova™ Reverse Transcription Kit (Qiagen®, Germany).

**Nested-PCR**
The serum samples were assessed using a nested-PCR assay that will amplify 241 nucleotide or 337 nucleotide fragment of the ORF7 gene for European and North American strains respectively to detect presence of PRRSV. Primers suggested in published journals were used (Pesch, 2003) with nested-PCR to amplify PRRSV that were extracted from the serum. A total of three sets of primers were used. The first set of primers with sequence of PRRS-F: 5'-ATG GCC AGC CAG TCA ATC-3'; PRRS-R: 5'-TCG CCC TAA TTG AAT AGG TG-3' (Mardassi *et al.*, 1994) amplified a common site in ORF7 of both virus strains. After that, nPCR primers specific for North American genotype: NA-F: 5'-AGT CCA GAG GCA AGG GAC CG-3'; NA-R: 5'-TCA ATC AGT GCC ATT CAC CAC-3' and European genotype: EU-F: 5'-ATG ATA AAG TCC CAG CGC CAG-3'; EU-R: 5'-CTG TAT GAG CAA CCG GCA GCA T-3' were used to distinguish the two different strains. These sets of primers were chosen as this nested-PCR had proven its high sensitivity compared to other primers in detecting the virus (Truyen *et al.*, 2006). At the end of amplification, nPCR products of different sizes were produced for different strain.

HotStarTaq® Plus Master Mix Kit (Qiagen®, Germany) was used for the process. The RT-PCR cycle conditions were carried out as the protocol. In brief as follows: 95°C for 5 min to activate DNA polymerase and 30 cycles of 94°C for 30 seconds, 58°C for 45 seconds and 68°C for 45 seconds, followed by a final prolongation of 10 min at 72°C.

1 µL of RT-PCR product was used as template for the nested-PCR. Nested-PCR was carried out in a total of 20 µl containing 1 µl of template, 1 µl forward primer each, 0.5 µl reverse primer each, 10 µl of HotStarTaq Plus MasterMix, 2x (1x PCR buffer, 200 µM of each dNTP, 1 unit HotStarTaq Plus DNA Polymerase) and 7 µl RNase-free water. PCR cycle conditions were as follows: 95°C for 5 min and 30 cycles of 94°C for 1 minute, 58°C for 1 min and 72°C for 1 min, plus a final prolongation of 10 min at 72°C.

**Statistical Analysis**
The IBM® SPSS Statistics 20 statistical software was used. Comparisons of mean S/P ratio of pigs among the four farms for each age group were done using one way ANOVA with Tukey's post hoc test. Statistical significance is recorded at $p < 0.05$ and confidence interval of 95%.

## RESULTS

**Nested-PCR results**
PCR amplification was carried out on 240 serum samples collected from four farms. None of the 240 serum samples collected showed positive result for either American strain or European strains.

**ELISA results**
**Seroprevalence rate in different farms**
In total, 240 blood samples were screened for the presence of PRRSV antibodies. In all farms, antibodies against PRRSV were found and 191 (79.6%) of the pigs were seropositive for PRRS virus. The mean seroprevalence in Farm A, B, C and D on herd level were 73.3%, 58.3%, 96.7% and 90% respectively. Seroprevalence of each farm was shown in Table 2.

**Mean S/P ratio of the pigs according to different farms**
A one-way ANOVA was conducted to determine if mean S/P ratio based on age group was different for farms with different vaccination scheme. Data was mean ± standard error as tabulated in Table 3. There were several important points that should be taken into account when interpreting PRRS ELISA results. First, it measured exposure, not protection which means a high antibody titre detected only suggested that the animal had been previously exposed to the virus

Table 2. Seroprevalence of the pigs according to different farms, vaccination scheme and age group

| Farm | Used vaccination | | Seroprevalence % | | | |
|---|---|---|---|---|---|---|
| | Type PRRSV-vaccine | Used scheme | Piglets 5 days old | Weaners 6 weeks old | Growers 20 weeks old | Sow |
| A | US-MLV | S | 93.3 (14/15) | 13.3(2/15) | 93.3 (14/15) | 93.3 (14/15) |
| B | US-MLV | S+P | 46.7 (7/15) | 46.7 (7/15) | 86.7 (13/15) | 53.3 (8/15) |
| C | EU-KV | S | 100 (15/15) | 86.7 (13/15) | 100 (15/15) | 100 (15/15) |
| D | EU-MLV | S+P | 100 (15/15) | 66.7 (10/15) | 100 (15/15) | 93.3 (14/15) |
| Mean | | | 85 | 53.35 | 95 | 62.5 |

Table 3. Mean S/P ratio of the pigs according to different farms, vaccination scheme and age group

| Farm | Used Vaccination | | Piglets 5 days old Mean ± SE | Weaners 6 weeks old Mean ± SE | Growers 20 weeks old Mean ± SE | Sow Mean ± SE |
|---|---|---|---|---|---|---|
| | Type PRRSV-vaccine | Used scheme | | | | |
| A | US-MLV | S | $1.18^{a} \pm 0.13$ | $0.15^{a} \pm 0.03$ | $1.53^{a} \pm 0.10$ | $1.00^{a} \pm 0.11$ |
| B | US-MLV | S+P | $0.4^{b} \pm 0.07$ | $0.59^{b} \pm 0.15$ | $1.02^{b} \pm 0.16$ | $0.44^{c} \pm 0.08$ |
| C | EU-KV | S | $1.62^{a} \pm 0.19$ | $1.62^{c} \pm 0.13$ | $1.82^{a} \pm 0.10$ | $1.10^{ab} \pm 0.14$ |
| D | EU-MLV | S+P | $1.70^{a} \pm 0.10$ | $0.47^{ab} \pm 0.05$ | $1.55^{a} \pm 0.12$ | $1.29^{b} \pm 0.09$ |

$^{a,b,c}$ = Means with different superscript within columns differed significantly at $P<0.05$.

and not the degree of protection from the disease (Hennings *et al.*, 2002). Secondly, PRRS antibodies presence was not correlated to protection.

For the sow group, Farm B has significantly lower mean S/P ratio of 0.44 with p value less than 0.05 compared to Farm A, C and D. The mean S/P ratio of 0.4 for piglets in farm B was significantly different ($p<0.05$) from the rest of the farms. As for week 6 weaners, there was no significant difference between Farm B and D. Both Farm B and D which practiced vaccination on piglets at day 10-14 had a higher mean S/P ratio of 0.59 and 0.47 respectively, which it suggested exposure to vaccine virus. Farm C which did not practice vaccination on piglet has the highest mean S/P ratio of 1.62 and it was significantly different ($p<0.05$) from Farm A, B and D. It was suggestive of exposure to field virus. As for Farm A which has low mean S/P ratio of 0.246, it may be because of there was no vaccination of piglets and lack of exposure of weaners to field virus. Generally, all farms showed higher mean S/P ratio in grower group compared to other age group (Table 3). This may be because of when transferred to the finishing floor with other infected pigs, previously uninfected pigs will become infected and seroconvert. However, there is significantly lower mean S/P ratio of growers in Farm B ($p<0.05$). It suggests that there is a lower resident PRRSV circulation and thus a lower exposure to virus giving rise to a lower mean S/P ratio.

## DISCUSSION

The negative results from PCR were suggestive of no active infection in the pigs, hence no detectable viraemia status in the pigs. Although viraemia from PRRS virus able to be detected up to 92 days (Stadejek *et al.*, 2005), none of the pigs were positive in this study. In this study, PCR and ELISA were used as diagnostic methods. The actual virus circulation early infection can be determined by using PCR technique (Van Maanen *et al.*, 2006). Compared to serological tests, the

advantage of using PCR methods is that this test method is not influenced by the presence of either maternal antibodies, or antibodies induced by vaccination (Duinhof *et al.*, 2011).The PCR test on serum detects viral genetic material and is relied upon to define shedding status. A negative result indicates the absence of viremia, but negative PCR results do not necessarily rule out the possibility that the animal is infected or shedding (Christopher-Hennings *et al.*, 1995). However, monitoring serum from weaning age pigs by PCR can be used as an indirect measure to monitor vertical transmission as well as horizontal sow-to-piglet and piglet-to-piglet transmission in breeding herds (Cano *et al.*, 2009). Negative PCR tests on weaning-age pigs alone are not sufficient to establish a negative shedding status for a breeding herd. The ideal method for detecting shedding and transmission of virus in the breeding herd involves use of sentinel animals.

Besides, a variety of studies had shown that PRRSV viremia was often resolved before neutralizing antibodies were detected and PRRSV can be isolated from blood of pigs that have neutralizing antibodies (Murtaugh & Genzow, 2011). Serological tests for PRRSV normally detect serum antibody response after 14–21 days post-infection, and do not allow the distinction between infected and vaccinated animals (Batista, 2005).

Farm B have lower seropositive sow (53.3%) compare to other farms. The reason for the absence of amnestic immune responses is yet unknown. In this case, the mean S/P ratio of the sow group of Farm B was relatively lower as compare to others which was only 0.44. This antibody profile which was obtained for a properly vaccinated sow herd under a four dose per year strategy suggested that the farm have excellent immunity. The research team believed that farm B achieved the stabilization through mass vaccination, reduction of resident PRRSV circulation (Angulo, 2007). Thus, the low mean S/P ratio is likely due to lower exposure of pigs to virus. As a whole, Farm B had a lower mean S/P ratio across all age group. This may suggest the reduced circulation of PRRS virus in herd and therefore exposure status of pigs to virus was lower. Regular monitoring is needed to ensure that a new strain of PRRS virus has not entered the herd.

As for Farm A, the seroprevalence of the weaners was only 13.3% where 13 out of 15 six weeks old weaners tested were seronegative for PRRS. This may be due to a low virus circulation load in the weaner pen and the pigs were not exposed or seroconverted for PRRS since their farm did not practice vaccination on piglets, vaccinated herds were more likely to be serologically stable than non-vaccinated herds. Pigs negative for PRRSV were produced repeatedly from these relatively small, closed, serologically stable herds (<700 sows) with good biosecurity (Rajic *et al.*, 2001). When there was little PRRS virus in circulation among breeding adults in the herd, most piglets will be negative by six weeks of age as seen in Farm A where the S/P ratio of six weeks old weaners were generally lower than the cut off value of 0.4 in PRRS X3 ELISA test. (Rajic *et al.*, 2001)

On the other hand, Farm C showed consistently high seroprevalence percentage across age groups which was suggestive of a high PRRSV load circulation in their farm. As the farm only practiced killed vaccination on the sow herd, the high seropositive percentage in the growers and weaners group may due to the exposure of field PRRS virus circulated in the farm. The mean S/P ratio was always on the higher end, suggested there was still presence of high PRRS virus circulation or exposure in farm. Farm C practised killed vaccine on sows. Study had reported killed vaccine tested provided weak memory responses with sequential challenge without any obvious active immune responses in the vaccinated pigs (Kim *et al.*, 2011). This lack of cell mediated immunity may cause a higher risk of pigs being infected during weaning when maternal antibody had decayed. Thus, the shedding and circulation of virus in weaning and finishing unit remain high. The use of inactivated PRRSV vaccine should be administered on a regular basis for obtaining the maximum beneficial effect, as it has been observed that the higher the degree

of immunization of sows, the better the improvement of their health status and reproductive performance (Papatsiros, 2012).

Tentatively, all four farms participated in the study may be classified into Stage IIa (Positive stable breeding herds that are not undergoing PRRSV elimination) where there were an uncertain shedding status and positive exposure status. Farm B may be one step ahead compared to the other farms in terms of reducing virus shedding in farm. Steps had been taken to reduce virus shedding which are practice of whole-herd exposure to MLV vaccination. However, absence of clinical signs of PRRS in the breeding-herd population and a constant lack of detectable viremia in sampled weaners and growers for a minimum of 90 days is required for the confirmation of the PRRS herd staging. This classification also requires a minimum of four consecutive negative PCR herd tests in weaners sampled every 30 days or more frequently (Holtkamp *et al.*, 2011).

Stage II is important for managing PRRSV in farm production setting. The absence of viremia is important for farm in management of pig flow, potentials about improved reproductive and grower performance. For breeding herds that are trying to control the virus, being Positive Stable (II) will be the goal of farm. In terms of national elimination efforts, subdivision of Stage II into II-A and II-B is crucial to differentiate the risk of existing or future shedding of virus by animals in the farm (Holtkamp *et al.*, 2011).

## CONCLUSION

As a conclusion, the present study showed that application of PRRS MLV vaccine will not cause viraemia post four weeks vaccination. Thus, we know that the PRRS MLV vaccines used in Malaysia may not shed post 4 weeks of vaccination. In addition, MLV vaccination on sows and piglets may help to reduce the virus exposure to the pigs in the farm. Author can't conclude that there is no virus circulation in all the farms despite a negative PCR result as there is no sentinel animals used in the study. Tentatively, all four farms participated in the study may be classified into Stage IIa (Positive stable breeding herds that are not undergoing PRRSV elimination) where there were an uncertain shedding status and positive exposure status. Thus, the author strongly suggested a continuous time-point assessment of PRRSV shedding status & exposure status of weaners & breeding herd using ELISA and PCR for confirmation of PRRS herd classification.

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