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# การศึกษาการตรวจภูมิคุ้มกันชนิดสารน้ำต่อโรค Porcine Reproductive and Respiratory Syndrome โดยใช้ชุดตรวจสำเร็จรูปอีไลซ่าชนิดต่างๆ ในสุกรขุน

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**บทคัดย่อ:** วัตถุประสงค์ในการศึกษาในครั้งนี้เพื่อศึกษาการตอบสนองทางภูมิคุ้มกันชนิดสารน้ำต่อโรค Porcine reproductive and respiratory syndrome (PRRS) ภายหลังได้รับวัคซีนเชื้อเป็นสายพันธุ์ยุโรปหรืออเมริกาเหนือในลูกสุกรที่มีระดับภูมิคุ้มกันจากแม่ในระดับสูงโดยใช้ชุดตรวจสำเร็จรูป Enzyme-linked immunosorbent assay (ELISA) ที่ใช้ตรวจรวมสายพันธุ์จากชุดตรวจ IDEXX PRRS X3 Ab Test (IDEXX®) เปรียบเทียบกับชุดตรวจแยกสายพันธุ์ระหว่าง LSIVet™ Porcine PRRS/EU-Serum (LSI-EU) ซึ่งใช้ตรวจสายพันธุ์ยุโรป และ LSIVet™ Porcine PRRS/US-Serum (LSI-US) ซึ่งใช้ตรวจสายพันธุ์อเมริกาเหนือ ผลการศึกษาพบว่าระดับภูมิคุ้มกันในกลุ่มสุกรที่ได้รับวัคซีนสายพันธุ์อเมริกาเหนือมีแนวโน้มคงตัวภายหลังได้รับวัคซีนในช่วง 6 สัปดาห์แรกและระดับภูมิคุ้มกันอยู่ในระดับสูงจนถึงน้ำหนักที่พร้อมส่งโรงฆ่า ในขณะที่สุกรกลุ่มที่ได้รับสายพันธุ์ยุโรปมีระดับภูมิคุ้มกันลดลงในช่วง 4 สัปดาห์แรกหลังได้รับวัคซีนและเพิ่มสูงขึ้นจนถึงน้ำหนักที่พร้อมส่งโรงฆ่า ข้อมูลนี้บ่งชี้ว่า วัคซีนสายพันธุ์อเมริกาเหนือมีแนวโน้มในการตอบสนองทางภูมิคุ้มกันได้ดีกว่าสายพันธุ์ยุโรปในสภาวะที่สุกรมีภูมิคุ้มกันที่ได้รับจากแม่, ตลอดจนช่วงระยะเวลาในการตอบสนองในการสร้างภูมิคุ้มกันใช้เวลาที่สั้นกว่า และระดับภูมิคุ้มกันที่คงที่ในระดับสูงจนถึงน้ำหนักที่ส่งโรงงฆ่า นอกจากนั้น การศึกษาครั้งนี้ได้ แสดงถึงความสัมพันธ์ของชุดตรวจสำเร็จรูปชนิด ELISA ที่ใช้แยกสายพันธุ์อเมริกาเหนือกับยุโรปเปรียบเทียบกับชนิดที่ไม่แยกสายพันธุ์ พบว่าค่าสัมประสิทธิ์ Kappa (K) มีค่าเท่ากับ 0.9097 และค่าสหสัมพันธ์ (Correlation coefficient) มีเท่ากับ 0.513 ซึ่งบ่งชี้ว่าชุดตรวจทั้งสองประเภทมีความสัมพันธ์กันในระดับสูงและปานกลางตามลำดับ ซึ่งสามารถใช้ทดแทนกันได้

**คำสำคัญ:** วัคซีนเชื้อเป็น Porcine reproductive and respiratory syndrome สายพันธุ์อเมริกาเหนือ สายพันธุ์ยุโรป ชุดตรวจสำเร็จรูป enzyme-linked immunosorbent assay

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# Longitudinal Study on Antibodies Detection against Porcine Reproductive and Respiratory Syndrome Virus in Vaccinated Fattening Pigs by Various Enzyme-linked Immunosorbent Assay Test Kits

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**Abstract:** This experiment aims to compare the efficacy of porcine reproductive and respiratory syndrome (PRRS) vaccine using North America and European genotypes attenuated vaccines including determine the correlation of enzyme-linked immunosorbent assay (ELISA) kit tests for antibody detection using LSIVet™ Porcine PRRS/EU-Serum (LSI-EU; LSI, France), LSIVet™ Porcine PRRS/US-Serum (LSI-US; LSI, France) and IDEXX PRRS X3 Ab Test (IDEXX®, USA). The antibody level of North America strain vaccinated group was rather steady during the first 6-weeks-post vaccination then increasing and steady until slaughtering. For European strain vaccinated group, was decrease up to 4 weeks post vaccination then increasing and steady until slaughtering. These data suggest that North American genotype vaccinated group have a tendency better than European genotype vaccinated group in term maintain the maternal immunity after vaccination, onset duration of antibody increasing and maintain high level of immunity until slaughtering. In addition, present study was compared the correlation between 2 groups of commercial ELISA test kits for PRRS antibody detection such as IDEXX that detects both NA and EU genotypes, and LSI's test kits that were developed to detect specifically either antibodies NA or EU strains of PRRS viruses. The correlation between LSI-EU and LSI-US compared with IDEXX, was showed in Kappa (**K**) value as 0.9097, interpreted as almost perfect agreement. And the degree of linear association between both LSIs and IDEXX were use a correlation coefficient as 0.513 ($p \leq 0.01$), indicated that both ELISA test kit was moderate correlation. The explanation for these results that both types of LSIVet™ ELISA test kit for PRRS antibody detection could be tested as same as IDEXX.

**Keywords:** Porcine reproductive and respiratory syndrome attenuated vaccine, North American strain, European strain, Enzyme-linked immunosorbent assay

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**Introduction**

Porcine reproductive and respiratory syndrome (PRRS) is caused by the PRRS virus (PRRSV), belong to genus *Arterivirus* in the family *Arteriviridae* in the order *Nidovirales* (Amonsin *et al.,* 2009; Cavanagh 1997). PRRS was first reported in North Carolina, the United States in 1987 (Keffaber, 1989). The causative agent was first isolated and was designated as VR-2332 (Collins *et al*., 1992). In Europe, the causative agent was first identified in 1990, the Netherlands and designated as Lelystad (Wensvoort *et al.*, 1991). PRRSV was classified by genetic, antigenic, and pathogenic differences into two distinct genotypes consisting of type 1 or European (EU) type and type 2 or North American (NA) type (Meng, 2000). To date, both types are circulating in swine population worldwide. In Thailand, PRRSV infection in swine herds has been reported as early as 1989 from the retrospective serological survey (Damrongwatanapokin *et al.*, 1996). NA type was first isolated in 1996 in Thailand after that EU type was found in Thai swine herds resulting in co-existence of both genotypes in Thai swine population (Damrongwatanapokin *et al.*, 1996; Thanawongnuwech *et al.*, 2004). Therefore, PRRS has become one of the most common diseases causing reproductive failure in gilts and sows population in Thailand (Oraveerakul *et al.*, 1995).

Surveillance and monitoring in pig farms are very important to understand the stage at which infection starts and at which PRRS becomes active. Several serological tests are available for PRRS diagnosis such as the immunofluorescent antibody test (IFA), the immunoperoxidase monolayer assay (IPMA), the serum neutralization (SN) test, and enzyme-linked immunosorbent assay (ELISA) (Alstine *et al*., 1993; Cho *et al*., 1996; Cho *et al*., 1993; Jusa *et al*., 1996; Matteu *et al*., 2006; Takikawa *et al*., 1997; Wellenberg, 2006; Yoon *et al*., 1992; Yoon *et al*., 1994).

The vaccination is the main approach for control and eradication of PRRSV infection in swine herds since no specific treatment and no antiviral drug are available for curing and control of PRRS. PRRS vaccines have been shown to be highly effective under experimental conditions (Gorcyca *et al*., 1995; Hesse *et al*., 1996a,b). However, several researchers reported that sometimes been less effective especially in the field (Mengeling *et al*., 1997; Zimmerman *et al*., 1997; Hurd *et al*., 2001). Although there is as yet no clear explanation for this difference, it is generally assumed that the two major variables are the relative virulence of strains to which vaccinated pigs are subsequently exposed and the degree of antigenic relatedness between vaccine and field

strains.

The objective of present study is comparison the efficiency between porcine reproductive and respiratory syndrome (PRRS) attenuated live vaccine using NA and EUgenotypes, and to determinethe correlation of antibody detection against PRRS with ELISA kit tests using LSIVet™ Porcine PRRS/EU-Serum (LSI-EU), LSIVet™ Porcine PRRS/US-Serum (LSI-US) which both kits could be used to identify the PRRS genotypes either European (EU) or North American (NA), and IDEXX PRRS X3 Ab Test (IDEXX, USA) which could detect both PRRS genotypes.

## Materials and methods

Thirty-two 2-weeks-old fattening piglets from a commercial pig farm, were equally allocated into 3 groups. All piglet was collected serum and vaccinated with North America strain attenuated vaccine (Ingelvac® PRRS™ MLV; Boehringer-Ingelheim Vetmedica Inc., St. Josept, Missouri, USA) or European strain attenuated vaccine (Porcilis® PRRS; Intervet Inc, The Netherlands) .) for the first and second group, respectively and another group as unvaccinated group. After that, each piglet was collected serum at 1, 2, 4, 6, 8, 12, 16 and 20 week after vaccination.

Enzyme-linked immunosorbent assay (ELISA) for porcine reproductive and respiratory syndrome (PRRS) antibody detection, was carried out using commercially kit of IDEXX PRRS X3 Ab test (IDEXX®, USA), LSIVet™ Porcine PRRS/EU-Serum (LSI-EU; LSI, France) and LSIVet™Porcine PRRS/US-Serum (LSI-US; LSI, France). The assay's procedure was followed the manufacture's instructions. LSI-EU and LSI-US were calculated based on the absorbance, named IRPC (relative index × 100) of sample with a cut off $>$ 20 determining seropositivity. For IDEXX, each sample was judged according to the sample-to-positive (S/P) ratio (based on the absorbance) with the S/P threshold values $>$ 0.4 (Ruenphet *et al*., 2014).

The strength of the agreement between the both types (IDEXX and LSI-EU or LSI-US) of ELISA test kits was determined by Kappa (**K**) according to Smith (Smith, 1995). Explanation of Kappa value is a chance-corrected measure of agreement between pairs of observers. It reflects the degree of agreement for a particular physical finding. In addition, statistic method involves correlation analysis where a correlation coefficient is reported representing the degree of linear association between 2 variables.

Interpretation of kappa was obtained and defined that less than 0 as less than chance agreement, 0.01–0.20 as slight agreement, 0.21– 0.40 as Fair agreement, 0.41–0.60 as moderate agreement, 0.61–0.80

as substantial agreement and 0.81–0.99 as almost perfect agreement, respectively. (Viera and Garrett, 2005).

In addition, statistic method involves correlation analysis where a correlation coefficient is reported representing the degree of linear association between 2 variables. Interpretation of correlation coefficient was obtained and defined that less than or equal 0.35 as low or weak correlation, 0.36-0.67 as moderate correlation, 0.68-0.89 as high correlation and more than 9.00 as very high correlation, respectively (Taylor, 1990).

## Results

Fig. 1 showed the average S/P ratio graph of ELISA value in each group that was determined by IDEXX test kit. All of piglets before vaccinated, were showed the S/P ratio as above 0.4. After vaccination, S/P ratio in unvaccinated group, was decrease to base line at 6-weeks-post vaccination. However, NA strain vaccinated group was rather steady during the first 6-weeks-post vaccination then increasing and steady until slaughtering. Finally, EU strain vaccinated group, was decrease up to 4-weeks post vaccination then increasing and steady until slaughtering.

Table 1 shown, the kappa value (**K**) of LSI-US and LSI-EU was compared with IDEXX as 0.9097 in all groups, while the unvaccinated group, NA and EU vaccinated group as 0.8338, 0.9362 and 0.9356, respectively.

In addition, the correlation coefficient between both LSI and IDEXX ELISA test kit were use a correlation coefficient as 0.513 ($p \leq 0.01$) (Fig. 2).

## Discussion

Generally, live attenuated commercial PRRSV vaccines have been available for prevention and controlling of PRRS problems. The present study was showed maternal immunity status in piglets before vaccination and immune response after PRRS vaccination including monitoring until sent to slaughterhouse using commercial ELISA test kit. Sows of piglets in present study, were vaccinated with PRRS vaccine twice a year and the antibody level of all piglets before vaccination at 2-weeks age was showed S/P ratio as >0.4 indicated that maternal immunity still maintained up to 6-weeks age especially in unvaccinated group. However, 4 of 8 piglets in unvaccinated group, was showed the clinical signs of acute PRRS-like symptoms such as loss of appetite, lethargy, obvious failure to thrive, labored or rapid breathing and respiratory distress during 4 to 8 week age. In addition, the antibody titer from all survived piglets was increasing until reaching slaughtering weight. This data

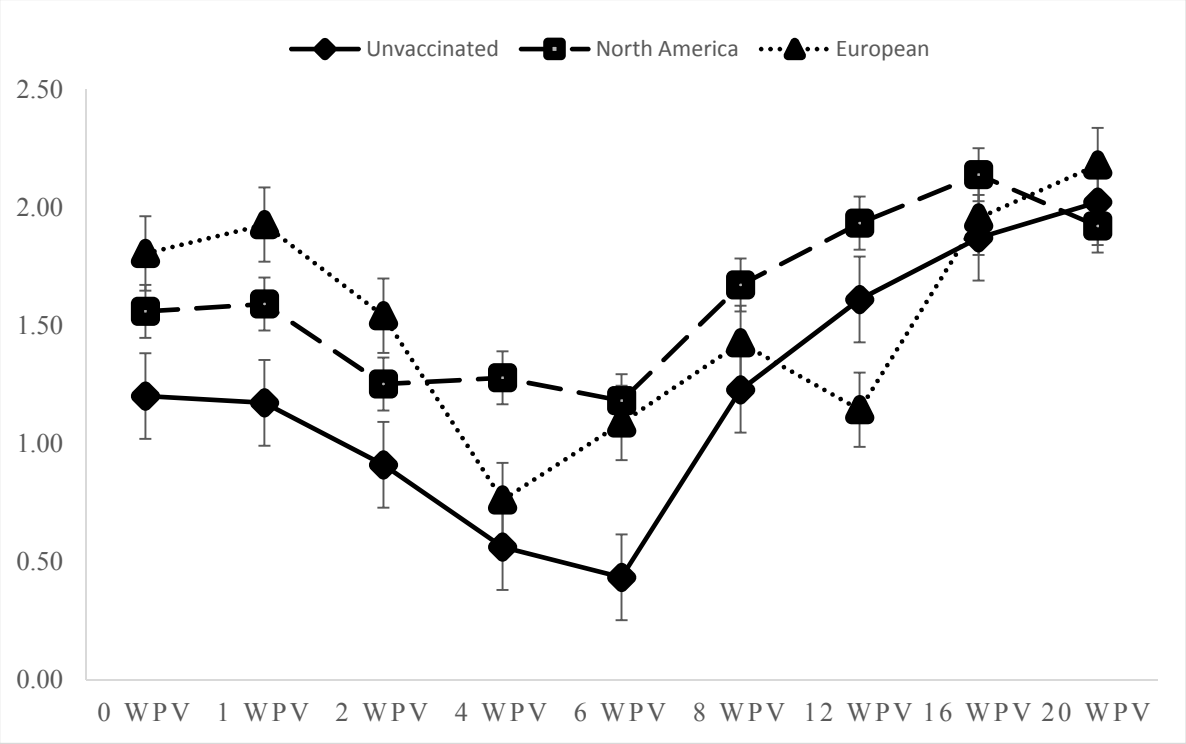


**Fig. 1** Graph was presented the mean and standard deviation of S/P ratio before and after vaccination by North America or European strain of live attenuated PRRS vaccine using IDEXX ELISA test kit.

**Table 1** Correlation between LSIVet™ Porcine PRRS/EU-Serum (LSI-EU) and LSIVet™ Porcine PRRS/US-Serum (LSI-US) compared with IDEXX PRRS X3 Ab Test (IDEXX)

| | | Control group | | US Vaccinated group | | EU Vaccinated group | | All group | |
|---|---|---|---|---|---|---|---|---|---|
| | | LSI US&EU | | LSI US&EU | | LSI US&EU | | LSI US&EU | |
| | | Positive | Negative | Positive | Negative | Positive | Negative | Positive | Negative |
| IDEXX | Positive | 38 | 10 | 66 | 6 | 65 | 5 | 169 | 21 |
| | Negative | 1 | 131 | 0 | 144 | 1 | 145 | 2 | 420 |
| Kappa (**K**) | | 0.8338 | | 0.9362 | | 0.9356 | | 0.9097 | |

suggested that these piglets might be infected with PRRSV which still circulated in the farm and infected in unvaccinated piglets during low level of antibody titer. Torrison *et al*. (1996) described PRRS virus shedding and spread between vaccinated pigs and non-vaccinated contact control. In this case might be caused virus from vaccinated group might be transmitted and infeted to unvaccinated group, that related with Botner *et al.* (1997) who descripted that some of the herds have experienced acute PRRS-like symptoms, and the NA genotype of PRRS vaccine has been isolated from fetuses or stillborn piglets.

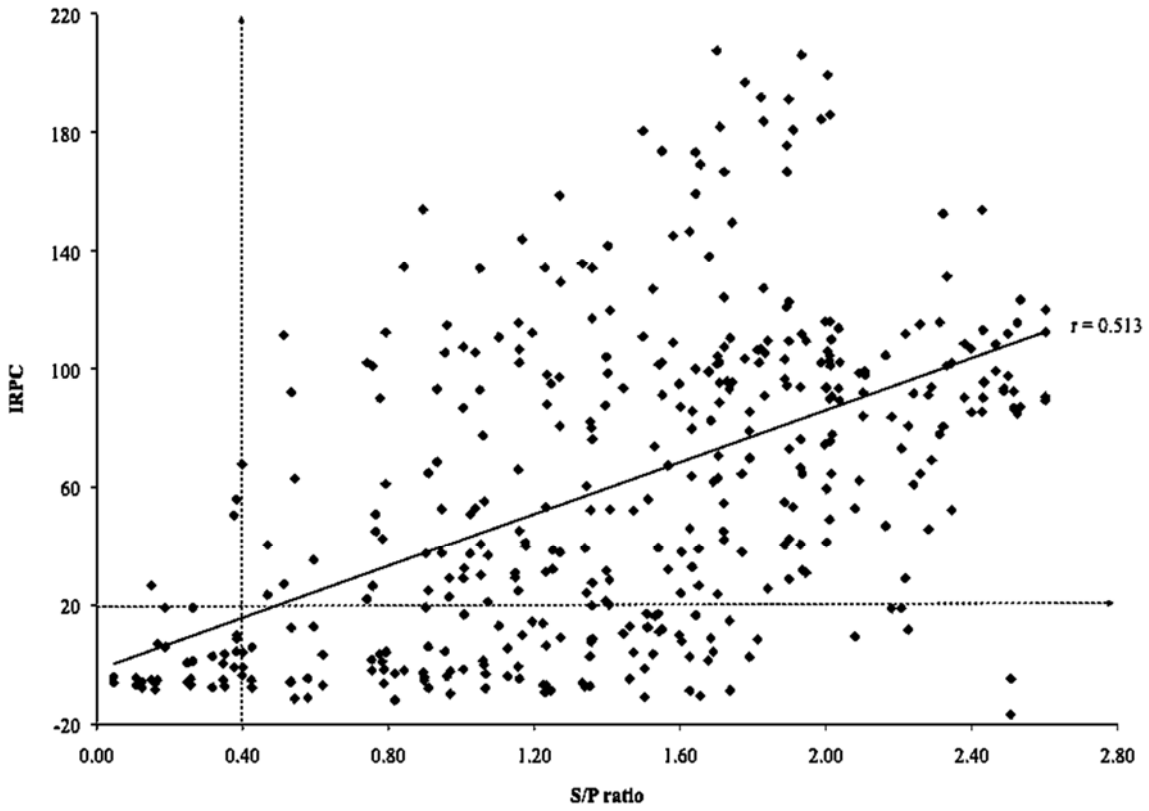


**Fig. 2** Correlation graph between LSIVet™ Porcine PRRS/EU-Serum (LSI-EU) and LSIVet™ Porcine PRRS/US-Serum (LSI-US) compared with IDEXX PRRS X3 Ab Test (IDEXX)

Nucleotide sequence data of PRRSV isolates showed that they originated from the vaccine strain.

The antibody level of the pigs in both vaccinated groups was rather decreasing after early vaccination (2 to 6 weeks post vaccination), after 8-weeks post vaccination the antibody level became increasing and steady until reaching slaughtering weight. Our results suggest that pigs in NA genotype vaccinated group have a tendency better than EU genotype vaccinated group, in term of maintaining the immunity after vaccination and onset duration of antibody increasing.

Generally, modified live attenuated PRRS vaccination is an effective way of inducing immunity and protecting herds from losses associated with infections by highly virulent strains of PRRSV (Benson *et al.*, 2000; Gorcyca *et al*., 1995). However, inactivated PRRS vaccines appear to have relatively low efficacy, but are associated with safety concerns (Botner *et al*., 1997; Nielsen *et al*., 2001). Several researchers reported that modified live vaccine (MLV) are contradictory reports on vaccine efficacy, with low efficacy assumed to be due to restricted cross protection between the genotypes and even

between strains that belong to a given genotype (Beilage, 1998; Labarque *et al*., 2003; Meng, 2000; Schagemann *et al*., 1999; van Woensel *et al*., 1998). However, when producers and veterinarians choose to use MLV vaccines, they should consider the potential risk of reversion to virulence.

In addition, present study was compared the correlation between 2 groups of commercial ELISA test kits for PRRS antibody detection such as IDEXX that detects both NA and EU genotypes, and LSI's test kits that were developed to detect specifically either antibodies NA or EU strains of PRRS viruses which the Kappa (**K**) value indicated that the degree of agreement as almost perfect agreement. The explanation for this result, could be used LSIs as same as IDEXX ELISA test kit. In addition, the degree of linear association between both LSIs and IDEXX ELISA test kit, were use a correlation coefficient as 0.513 ($p \leq 0.01$), indicated that both ELISA test kit was moderate correlation. The Kappa coefficient in present study, was showed the highly consistency reliability between LSIs and IDEXX test kit. It might be demonstrated that LSIs test kit had an accurate result compared to those from IDEXX test kit. However, correlation coefficient was showed the moderately validity between LSIs and IDEXX test kits. It might be indicated that LSIs test kit can be used to detect antibody of PRRS as well as IDEXX test kit.

In conclusion, both genotypes PRRS vaccines could be used for vaccination in commercial piglets, in term of maintaining the immunity after vaccination, onset duration of antibody increasing and maintaining high level of immunity until slaughtering. In addition, both types of LSIVet$^{TM}$ ELISA test kit for PRRS antibody detection could be tested as same as IDEXX.

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## References

Amonsin, A., Kedkovid, R., Puranaveja, S., Wongyanin, P., Suradhat, S. and Thanawongnuwech, R. 2009. Comparative analysis of complete

nucleotide sequence of porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Thailand (US and EU genotypes). *J. Virol.* 6: 143.

Alstine, W.G.V., Stevenson, G.W. and Kannitz, C.L. 1993. Diagnosis of porcine reproductive and respiratory syndrome. *Swine Health Prod*. 1: 24-28.

Beilage, E.G. 1998. Investigations on the use and efficacy of the vaccine Ingelvac® PRRS MLV in controlling the porcine reproductive and respiratory syndrome (PRRS). *Prakt. Tierarzt.* 79: 62-69.

Benson, J.E., Yaeger, M.J. and Lager, K.M. 2000. Effect of porcine reproductive and respiratory syndrome virus (PRRSV) exposure dose on fetal infection in vaccinated and nonvaccinated swine. *Swine Health Product.* 8: 155–160.

Botner, A., Strandbygaard, B., Sorensen, K.J., Have, P., Madsen, K.G., Madsen, E.S. and Alexandersen, S. 1997. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. *Vet. Record.* 141: 497-499.

Cavanagh, D. 1997. Nidovirales: a new oreder comparising Coronaviridae and Arteriviridae. *Arch. Virol.*, 42: 629-633.

Cho, S.J., Deregt, D. and Joo, H.S. 1996. An ELISA for porcine reproductive and respiratory syndrome: production of antigen of high quality. *Can. J. Vet. Res.* 60: 89-93.

Cho, S.H., Freese, W.R., Yoon, I.J., Trigo, A.V. and Joo, H.S. 1993. Seroprevalence of indirect fluorescent antibody to porcine reproductive and respiratory syndrome virus in selected swine herds. *J. Vet. Diag. Invest.* 5: 259-260.

Collins, J.E., Benfield, D.A., Christianson, W.T., Harris, L., Hennings, J.C., Shaw, D.P., Goyal, S.M., McCullough, S., Morrison, R.B., Joo, H.S. 1992. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. *J. Vet. Diagn. Invest*. 2: 117–126.

Damrongwatanapokin, S., Arsayuth, K., Kongkrong, C., Parchariyanon, S., Pinyochon, W. andTantaswasdi, U. 1996. Serological studies and isolation of porcine reproductive and respiratory syndrome (PRRS) virus in Thailand. *J. Thai. Vet. Med. Assoc.* 47: 19-30.

Gorcyca, D., Spronk, G., Morrison, R. and Polson, D. 1995. Field evaluation of a new MLV PRRS virus vaccine: applications for PRRS prevention and control in swine herds, Proceedings of the 26th Annual Meeting of the American Association of Swine

Practitioners, Omaha, Nebr. American Society of Swine Veterinarians, Perry, Iowa. pp. 401-411.

Gorcyca, D., Schlesinger, K., Chladek, D., Behan, W., Polson, D., Roof, M. and Doitchenoff, D. 1995. RespPRRS: a new tool for the prevention and control of PRRS in pigs. In: Proceedings of the 26th Annual Meeting of American Association of Swine Practitioners, Omaha, NE, USA, pp. 1–22.

Hesse, R.A., Couture, L.P., Lau, M.L., Dimmick, S.K. and Ellsworth, S.R. 1996a. Efficacy of Prime Pac PRRS in controlling PRRS reproductive disease: homologous challenge. Proceedings of the 27th Annual Meeting of American Association of Swine Practitioners, Quebec City, Que., Canada, pp. 103–105.

Hesse, R.A., Couture, L.P., Lau, M.L., Wunder, K.K. and Wasmoen, T.L. 1996b. Efficacy of Prime Pac PRRS in controlling PRRS reproductive disease: heterologous challenge. In: Proceedings of the 27th Annual Meeting of American Association of Swine Practitioners, Quebec City, Que., Canada, pp. 107–110.

Hurd, H.S., Bush, E.J., Losinger, W., Corso, B., Zimmerman, J.J., Wills, R., Swenson, S., Pyburn, D., Yeske, P. and Burkgren, T. 2001. Outbreaks of porcine reproductive failure: report on a collaborative field investigation. *J. Swine Health Prod*. 9: 103–108.

Jusa, E.R., Inaba, Y., Kouno, M., Hirose, O., Shibata, I., Kubota, M. and Yasuhara, H. 1996. Slow-reacting and complement-requiring neutralizing antibody in swine infected with porcine reproductive and respiratory syndrome (PRRS) virus. *J. Vet. Med. Sci.* 58: 749-753.

Keffaber, K.K. 1989. Reproductive failure of unknown etiology. *Am. Assoc. Swine Pract. Newsl.* 1: 1-19.

Labarque, G., Van Gucht, S., Van Reeth, K., Nauwynck, H. and Pensaert, M. 2003. Respiratory tract protection upon challenge of pigs vaccinated with attenuated porcine reproductive and respiratory syndrome virus vaccines. *Vet. Microbiol.* 95: 187-197.

Matteu, E., Pijoan, C., Coll, A., Casal, J. and Martin, M. 2006. Comparison of three ELISAs for the diagnosis of porcine reproductive and respiratory syndrome. *Vet. Rec.* 159: 717-718.

Meng, X.J. 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development. *Vet. Microbiol.* 74: 309-329.

Mengeling, W.L., Lager, K.L., Vorwald, A.C., Wesley, R.D. and Clouser, D.F. 1997. An update of research at the National

Animal Disease Center on current field strains of porcine reproductive and respiratory syndrome (PRRS) virus. In: Proceedings of the Allen D. Leman Swine Conference, Minneapolis, MN, USA, pp. 138–145.

Nielsen, H. S., Oleksiewicz, M.B., Forsberg, R., Stadejek, T., Botner, A. and Storgaard, T. 2001.Reversion of a live porcine reproductive and respiratory syndrome virus vaccine investigated by parallel mutations. *J. Gen.Virol.* 82: 1263-1272.

Oraveerakul, K., Punarriwatana, D., Leuengyosluechakul, S., Tantasuparuk, W. and Kunavongrit, A. 1995. The seroprevalence of porcine reproductive and respiratory syndrome (PRRS) virus among swine breeding farms in the central and north-eastern part of Thailand. *Thai J. Vet. Med.* 25: 233-240.

Ruenphet, S., Mitchaothai, J., Punyadarsaniya, D., Kumpolngam, P., Takahashi, K., Tsuchiya, A., Yanaka, T., Yoshida, M. and Takehara, K. 2014. Correlation of ELISA test kits for Porcine Reproductive and respiratory syndrome (PRRS) antibody detection using sera from unvaccinated pig farms in Thailand. *J. Mahanakorn Vet. Med.* 9(2): 159-171.

Schagemann, G., Ohlinger. V., Kump, A.W.S., Heggemann, R., Pesch, S. and Bischoff, C. 1999. Herd vaccination against porcine reproductive and respiratory syndrome (PRRS) - studies regarding the safety of application of Ingelvac PRRS MLV in pregnant sows. *Prakt. Tierarzt.* 80: 778.

Smith, R.D. 1995. Veterinary clinical epidemiology: a problem-oriented approach, 2nd ed. CRC Press, Inc., Boca Raton, Fla. pp. 149-150.

Takikawa, N., Kobayashi, S., Ide, S., Yamane, Y., Yanaka, Y., Higashihara, M. and Yamagishi, H. 1997. Early serodiagnosis of porcine reproductive and respiratory syndrome virus infection of pigs by detection of slow-reacting and complement requiring neutralizing antibody. *J. Vet. Med. Sci.* 59: 31-34.

Taylor, R. 1990. Interpretation of the correlation coefficient: A basic review. *J. Diag. Med. Sono.* 1: 35-39.

Thanawongnuwech, R., Amonsin, A., Tatsanakit, A. and Damrong-watanapokin, S. 2004. Genetics and geographical variation of porcine reproductive and respiratory syndrome virus (PRRSV) in Thailand. *Vet. Microbiol.* 101: 9- 21.

Torrison, J.L., Knoll, M. and Wiseman, B. 1996. Evidence of pig-to-pig transmission of a modified live vaccine, p. 89-91. In Proceedings of the 27th Annual Meeting of the American Association of Swine

Practitioners, Nashville, Tenn. American Society of Swine Veterinarians, Perry, Iowa.

van Woensel, P.A.M., Liefkens, K. and Demaret, S. 1998. Effect on viraemia of an American and a European serotype PRRSV vaccine after challenge with European wild type strains of the virus. *Vet. Rec.* 142: 510-512.

Viera, A.J. and Garrett, J.M. 2005. Understanding Interobserver Agreement: The Kappa Statistic. *Fam. Med.* 37(5): 360-363.

Wellenberg, G.J. 2006. Review: diagnostic methods for detection of Comparison of three ELISAs for the diagnosis of porcine reproductive and respiratory syndrome virus (PRRSV) infections. *Tijdschr. Diergeneeskd.* 131(16): 556-572.

Wensvoort, G., Terpstra, C., Pol, J.M.A., terLaak, E.A., Bloemraad, M., de Kluyver, E.P., Kragten, C., van Buiten, L., Den Besten, A., Wagenaar, F., Broekhuijsen, J.M., Moonen,P.L.J.M., Zeststra, T., de Boer, E.A., Tibben, H.J., de Jong, M.F., van Veld, P., Groenland, G.J.R., van Gennep, J.A., Voets, M., Verheijden, J.H.M. and Bramskamp, J. 1991. Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Vet. Quarter.* 13: 121–130.

Yoon, I.J., Joo, H.S., Christainson, W.T., Kim, H.S., Collins, J.E., Morrison, R.B. and Dial, G.D. 1992. An indirect fluorescent antibody test for detection of antibody to swine infertility and respiratory syndrome virus in swine sera. *J. Vet. Diag. Invest.* 4: 144-147.

Yoon, I.J., Joo, H.S., Goyal, S.M. and Molitor,.TW. 1994. A modified serum neutralization test for the detection of antibody to porcine reproductive and respiratory syndrome virus in swine sera. *J. Vet. Diag. Invest.* 6: 289-299.

Zimmerman, J., Epperson, W., Wills, R.W. and McKean, J.D. 1997. Results of the recent survey of the membership of the AASP for outbreaks of sow abortion and mortality. *Swine Health Prod*. 5(2): 74-75.