ORIGINAL ARTICLE

SEROLOGIC AND MOLECULAR DETECTION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN PLASMA OF DOMESTIC PIGS FROM SELECTED BACKYARD FARMS IN LUZON, PHILIPPINES

John Michael G. Bernardo, DVM[1], Emil Joseph S. Vergara, DVM, MSc[2], Gladys Maria V. Pangga, DVM, MSTH, MSc[1], Therese Marie A. Collantes, DVM, MS[1]

[1]Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, College, Laguna 4031, Philippines; [2]Institute for Infection and Immunity, St. George's University of London, Cranmer Terrace, London, United Kingdom

ABSTRACT

As a notifiable disease in the Philippines, porcine reproductive and respiratory syndrome (PRRS) surveillance and risk assessment is necessary at the farm and regional levels. This study primarily aimed to detect anti-PRRSv antibodies using indirect enzyme-linked immunosorbent assay (ELISA) and viral nucleic acids using conventional reverse transcriptase polymerase chain reaction (RT-PCR) in plasma of domestic pigs from selected backyard farms in selected provinces in Luzon, Philippines. A total of 382 individual and 117 pooled samples were subjected to ELISA and RT-PCR, respectively. Overall, ELISA results showed 5.50% seropositivity, with Bulacan (32.14%) and Cagayan (7.25%) having the highest positivity rates. Using RT-PCR, 5.98% of pooled samples, specifically from Batangas, Marinduque, and Cagayan, tested positive for PRRS viral nucleic acids. Using binary logistic regression, respiratory symptoms were more likely to be associated with PRRS positivity in ELISA and PCR ($p<0.05$). The results suggest persistent PRRS challenge in provinces with positive outcomes in the assays, warranting the need for improvement in PRRS monitoring in the Philippines.

Keywords: *ELISA, Luzon, plasma, PRRS, RT-PCR*

Philipp. J. Vet Med., 59(2): 133-142

INTRODUCTION

Porcine Reproductive and Respiratory Syndrome virus (PRRSv), the etiologic agent of PRRS, is a single-stranded, positive-sense, enveloped ribonucleic acid (RNA) virus which belongs to order Nidovirales and family *Arteriviridae*. The virus has been known to naturally infect domestic, wild, and feral pigs only. Two major genotypes with 60% homology have been identified namely: the VR-2332 or North American strain and the Lelystad or European strain (Dietze *et al.*, 2011). Nowadays, the introduction of each strain in other geographic origins makes the regional differences indistinct, and the movement of swine or semen is assumed to drive the presence of a strain in various locations (OIE, 2021).

Transmission of PRRSv occurs primarily via direct contact with infected pigs or via contact with contaminated feces, urine, and semen. Moreover, the aerosol route is assumed to induce chronic infection in farms or areas with high population density of pigs. Insects such as houseflies and mosquitoes can also serve as mechanical vectors of the virus (OIE, 2021). Transplacental transmission is also possible causing reproductive disease in breeding herds, resulting in stillbirth, mummified fetuses, or viremic piglets (Swine Health Information Center, 2021).

Infection with PRRSv can affect all ages and sexes of pigs and causes a variety of clinical

***FOR CORRESPONDENCE:**
(e-mail address: jgbernardo@up.edu.ph)

symptoms, mainly respiratory and reproductive signs (Beltran-Alcrudo & Lubroth, 2007). Symptomatic breeding herds may show reproductive syndrome such as abortions in the late gestation, fetal death, mummified fetus, stillbirths, giving birth to weak piglets or repeat breeding, while anorexia and transient fever are manifested by symptomatic boars and nonbreeding adult pigs. Subclinical infection may also occur in adult pigs. Respiratory symptoms, which are commonly exhibited by younger pigs, are characterized with dyspnea or "thumping", fever, anorexia, and listlessness (OIE, 2021). The agent can also continuously replicate in lymphoid tissues of pigs after the symptomatic stage causing chronic persistent infections (Zimmerman *et al.*, 2019).

Since clinical signs of PRRS are not pathognomonic, detecting the disease relies on laboratory testing (Henao-Diaz *et al.*, 2020). RT-PCR, virus isolation using porcine monocyte-derived macrophages, immunohisto-chemistry, and *in-situ* hybridization are used to identify the agent and confirm clinical cases. On one hand, ELISA, immunoperoxidase monolayer assay (IPMA), and immunofluorescence assay (IFA) are used to diagnose immune response against the agent (OIE, 2021). Among these techniques, RT-PCR, both conventional and real-time, and ELISA are recommended by the OIE (2021) for PRRS surveillance.

In 2006 and 2007, an emerging highly pathogenic strain of PRRSv, which was hypothesized to have evolved from VR-2332, caused swine mortalities and outbreaks in Vietnam and China. The same strain had been identified in PRRS farm outbreaks reported in Bulacan, Nueva Ecija, and Kalinga, Philippines from 2007 to 2010 (Nguyen, 2013). Animal disease monitoring in the Philippines further confirmed comorbidities with porcine circovirus 2, swine influenza, and classical swine fever (Dietze *et al.*, 2011). According to the Food and Agricultural Organization (FAO, 2011), about 20% of the Philippine swine production value was lost due to diseases. Specifically, pig industry experts estimated that economic losses due to PRRS in the Philippines may reach up to six billion pesos annually (Abao *et al.*, 2014). Hence, the Philippine Department of Agriculture (DA) through Administrative Order Number 1 in 2012 listed PRRS as one of the notifiable diseases which must be reported to the Bureau of Animal Industry (DA, 2018). Since 2006, majority of the PRRS isolates in Southeast Asia were from the North American strain (OIE, 2021).

To control PRRS in Southeast Asian countries, two of FAO's recommendations were to strengthen risk-based investigation for swine diseases and to perform risk assessment to understand PRRS epidemiology. Results of surveillance and analyses can provide awareness to swine farmers and other stakeholders on PRRS prevalence, risk, and impact (Dietze *et al.*, 2011). With early diagnosis and rapid confirmation, effective control and eradication strategies can also be prepared (Beltran-Alcrudo & Lubroth, 2007). Thus, this study aimed to detect anti-PRRSv antibodies using ELISA and viral nucleic acid using conventional RT-PCR in plasma of domestic pigs from backyard farms in selected provinces in Luzon, Philippines. Furthermore, associations between assay positivity and independent factors, namely clinical signs and life stages, were determined.

MATERIALS AND METHODS

Animal Care and Welfare

All procedures in this study, as reflected in IACUC Protocol Number 2018-0023, were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine in the University of the Philippines Los Baños based on the committee standards.

Domestic Pigs

Based on convenience sampling, 117 backyard swine farms from the provinces of Batangas, Bulacan, Cagayan, Camarines Sur, Isabela, Marinduque, Palawan, and Pangasinan in Luzon, Philippines were included in the study. A total of 382 domestic pigs (*Sus scrofa domesticus*) were sampled from March to November 2017. Two hundred thirty-six (236) pigs of any sex were purposively selected based on the following criteria: at least two weeks old, nongravid, lethargic, and with history or showed signs of respiratory and reproductive problems. Fifty-eight (58) apparently healthy pigs were also chosen in farms without animals which had history of, or exhibited, PRRS-related clinical signs at the time of collection. Meanwhile, the remaining 88 pigs lacked farm history. Sample collection was carried out with the consents of the owners.

Plasma samples

Blood samples were aseptically collected either from the external jugular vein, the cranial vena cava, or the lateral auricular vein. They were transferred in properly labeled vacutainers with ethylenediaminetetraacetic acid (EDTA).

Samples were kept in coolers (4˚C) during transport. Plasma isolation was performed through cold centrifugation at 10,000 revolutions per minute (rpm) for five minutes. Harvested samples were stored in sterile microcentrifuge tubes at -80˚C until use for ELISA and RNA extraction.

ELISA

The LSY-30010 Green Spring® ELISA kit (Shenzhen Lvshiyuan Biotechnology Co., Ltd., China) was used for the detection of anti-PRRSv immunoglobulin G (IgG). The serologic assay was carried out in accordance with the manufacturer's protocol (Shenzhen Lvshiyuan Biotechnology Co., Ltd., n.d). Optical density (OD) values at 450 nm and 630 nm were measured using the Multiskan™ Go Microplate Spectrophotometer (Thermo Fisher Scientific Corporation, Finland). All data were collected, and sample-to-positive (S/P) ratio was computed using Excel 2016 (Microsoft). A sample with an S/P ratio of at least 0.20 was classified as positive.

RNA extraction and conventional RT-PCR

Equal volumes of plasma samples from pigs sourced from one farm were pooled. A total of 117 samples were subjected to RNA extraction using the GF-1 Viral Nucleic Acid Extraction Kit (Vivantis® Technologies, Malaysia). Extraction step was carried out according to the manufacturer's instructions, and extracted RNA samples were stored in sterile PCR tubes at -80 ˚C.

Agarose gel electrophoresis

Polymerized 2% agarose gel was placed in the Scie-Plas® Electrophoresis Chamber (Progen Scientific©, United Kingdom) containing 1X Tris-Acetate-EDTA (TAE) as running buffer. Wells were filled with mixtures of loading dye (Vivantis®) and PCR product. Electrophoresis was run for 30 to 40 minutes at 110 volts. Gel staining with 0.025% ethidium bromide-1x TAE Buffer solution was conducted for 30 minutes. Band visualization was done under the UVP® High-Performance UV Transilluminator (Fisher Scientific, United States of America).

Data analysis

The geographic pattern of positive results in each assay was illustrated using the open-source software Quantum Geographic Information System (QGIS) version 3.14.0-Pi (QGIS Development Team). Statistical analyses were carried out using R software (version 4.2 by The R Foundation) and with the assumption that a pig is RT-PCR positive if it is included in a RT-PCR positive farm. Using the *irr* R package (Gamer *et al.*, 2012), the level of agreement between ELISA and RT-PCR results for individual pigs was determined using Cohen's kappa statistics (α=0.05).

The data on age of sampled pigs were grouped into: A (nursery, piglet, weaner), B (grower), C (fattener), and D (sow, gilt, boar). Clinical signs of each pig were also categorized based on the affected body system (i.e. respiratory, reproductive, digestive, and the like). Imputation of missing life stage and clinical symptoms was conducted using the *mode* method analysis (per farm basis or provincial level depending on estimation) and multivariate imputation by chained equations method (MICE) through the *mice* package, specifically with logistic regression method set at 100 iterations. Meanwhile, *glm* function was used to conduct binary logistic regression at 95% confidence level in order to estimate the association between assay positivity types (either positive in ELISA/PCR, PCR only, ELISA only) and independent factors – life stages and clinical signs.

RESULTS AND DISCUSSION

PRRS can cause high economic losses in affected countries and in the Philippines, it has been a notifiable animal disease since 2012 (DA, 2018; Thomann *et al.*, 2020). To control PRRS in the Southeast Asian Region, the FAO included risk-based investigation and risk assessment, which involve early disease diagnosis and monitoring, in their recommendations. These activities also result in better understanding of PRRS epidemiology such as prevalence, risk, and impact (Dietze *et al.*, 2011). In this study, laboratory detection of anti-PRRSv IgG and viral nucleic acids in plasma of sampled pigs from backyard farms in provinces of Luzon, Philippines was performed using ELISA and conventional RT-PCR, respectively. Statistical analyses were also carried out to determine the association between the positivity in the two assays and between positive results and independent variables.

ELISA results in Table 1 show that 5.50% of sampled pigs had a ntibodies against PRRSv. Bulacan and Cagayan had the highest seropositivity at 32.14% and 7.25%, respectively (Fig. 1). The total percentage of seropositive pigs in this study is lower in comparison with the available data in the Philippines. A study by Ducusin *et al.* (2015) revealed that 15.1% of tested pigs in an abattoir in Sariaya, Quezon was positive for PRRS antibodies, while another seroprevalence study in various

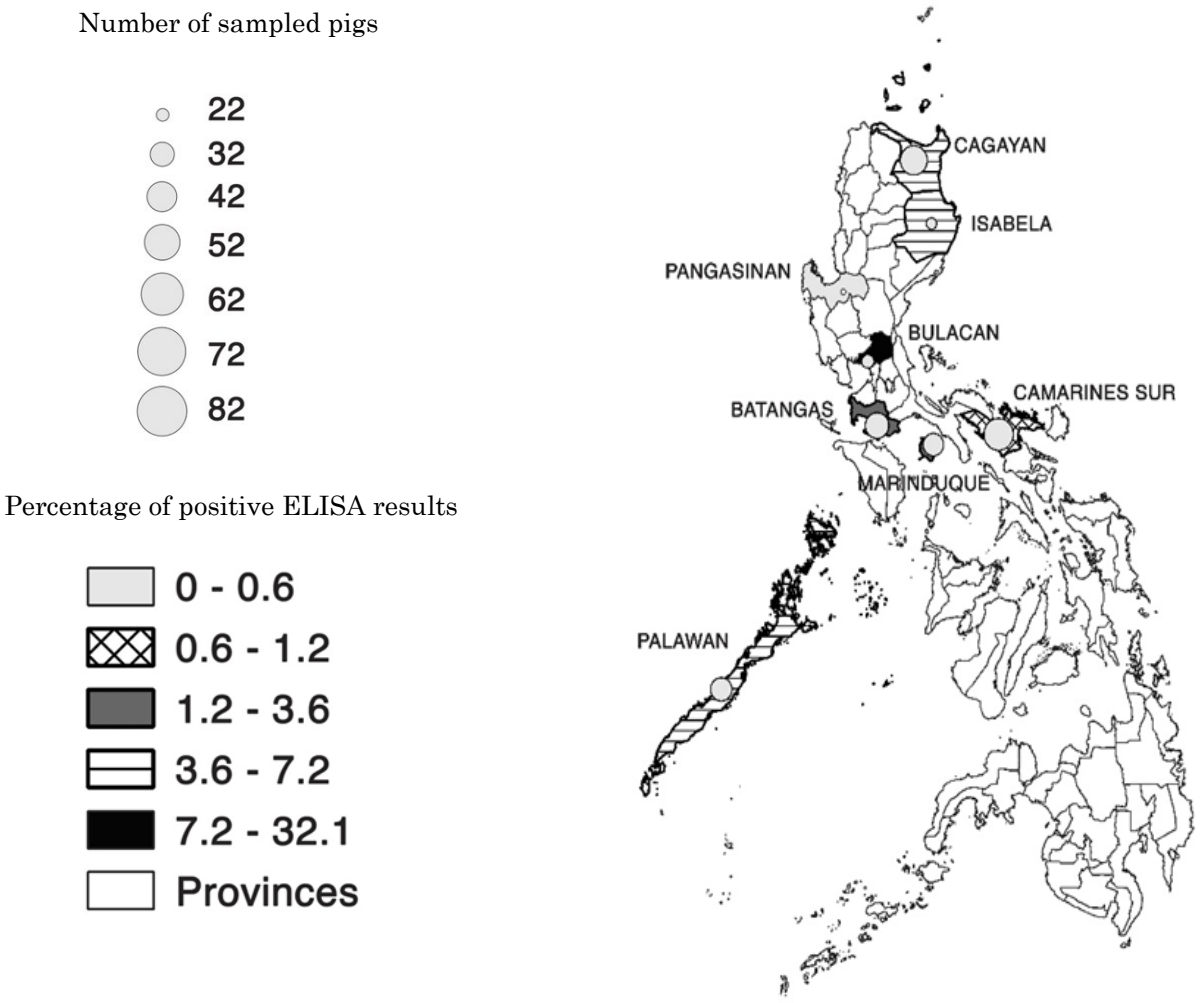

Figure 1. Geographic distribution of sampled pigs with PRRSv antibodies from backyard farms in Luzon, Philippines.

Table 1. Distribution of positive results for PRRS antibodies in ELISA and PRRS viral nucleic acids in RT-PCR in each province in Luzon, Philippines.

Province	**ELISA[1]**		**RT-PCR[2]**	
	Positive [n (%)]	**Total**	**Positive [n (%)]**	**Total**
Batangas	2 (3.57 %)	56	2 (22.22 %)	9
Bulacan	9 (32.14 %)	28	0	4
Cagayan	5 (7.24 %)	69	3 (9.68%)	31
Camarines Sur	1 (1.23 %)	81	0	32
Isabela	1 (3.84 %)	26	0	8
Marinduque	1 (3.33 %)	30	2 (12.5 %)	16
Palawan	2 (4.0 %)	50	0	12
Pangasinan	0	22	0	5
Total	21 (5.50 %)	382	7 (5.98 %)	117

[1]Plasma of each domestic pig
[2]Pooled plasma samples per backyard farm
$\kappa = -0.058$

farms in the Philippines by Maala *et al.* (2006) detected 59% seropositivity. Findings by Laranas (2007) (Unpublished Article) showed 63.3% seropositivity among unvaccinated pigs in a commercial farm in Pampanga

Seropositivity in pigs is often interpreted as previous exposure to an antigen and the ability of the animal to mount a humoral adaptive immune response. However, seropositivity may denote both past and recent infection, or exposure to a particular antigen. For instance, PRRSv maternal antibodies, which were derived from field infection or vaccination of the dam, can persist for up to four weeks in piglets (Kraft *et al.* 2019). This may account for the 4.69% (3/64) seropositivity in Group A (Table 2) of this study. In addition, vaccination can also increase the percentage of seropositive pigs. According to the study of Kittawornrat *et al.* (2013), vaccine-derived IgG was measurable 10 days post vaccination, and this can last for at least 120 days based on the findings of Dotti *et al.* (2011). Alexopoulos *et al.* (2005) has noted that modified live vaccine for PRRSv imitates the behavior of field strain. Aside from its capability to persist in the body of the host for several weeks or months, the vaccine virus can be channeled to immunologically naïve pigs (Mengeling *et al.*, 1996; Botner *et al.*, 1997). Semen and transplacental transmissions of vaccine-derived virus were also observed (Mengeling *et al.*, 1996; Christopher-Hennings *et al.*, 1997). However, interpretation based on the results of this study is limited due to unavailability of the PRRS vaccination record of the sampled farms.

On one hand, seronegative samples do not immediately guarantee PRRSv-naïve pigs. Antibodies against PRRSv begin to appear one week after field infection, and IgG titers peak at 21 to 35 days post-infection (PI) (Butler *et al.*, 2014). According to Nelson *et al.* (1994), anti-PRRSv IgG also disappears at 300 days PI. Aside from the absence of exposure to the virus, the delayed seroconversion and the diminishing antibody titers PI can also account to the number of seronegative pigs. Estimating the stage of infection is also Inaccurate in this serologic assay due to individual variations in humoral immunity (Christopher-Hennings *et al.*, 2002; Roberts, 2003).

Table 1 also shows that 5.98% of farms which were in Batangas, Marinduque, and Cagayan were RT-PCR positive (Fig. 2). This is lower compared to the RT-PCR findings of Parayao *et* al. (2021) which detected 24.67% positivity rate of PRRSv in blood samples coming from both backyard and commercial swine farms in Laguna. The molecular assay detects genetic material, indicating either an on-going infection or only the presence of viral nucleic acids in the sample (Christopher-Hennings *et al.,* 2002). A study by Rovira *et al.* (2007) showed that PRRSv nucleic acids can be detected in boar sera using RT -PCR as early as 24- and 72-hours PI at 5.60% and 77.80% positivity, respectively. Additionally, Henao-Diaz *et al.* (2020) outlined that RT-PCR may detect the agent in serum up to 98 days PI. PRRSv is also known to produce chronic persistent infections where the virus is cleared from the blood, but continuously replicates in lymphoid tissues. In this case, serum samples may be RT-PCR negative at 175 days PI, but may be positive in virus isolation in cell culture using lymphoid tissues (Henao-Diaz *et al.*, 2020). Nucleic acid-based tests like RT-PCR do not also distinguish the target gene of vaccinal virus incorporated in PRRS vaccines. A study by Kristensen *et al.* (2018) noted that 55 out of 56 pigs which were assigned to three vaccine groups became RT-PCR positive at least one day post-vaccination. Vaccination history of the sampled farms was not available hence, vaccine-derived viral nucleic acids cannot be further interpreted in this study. In conclusion, RT-PCR for PRRS diagnosis is useful for detecting acute viraemia and acute vaccine response but may exclude persistently infected pigs.

Using Cohen's kappa statistics, positivity in ELISA is not correlated with RT-PCR positive results ($\kappa = -0.058$). This finding agrees with the study of Henao-Diaz *et al.* (2020). Considering the disease transition stages of PRRS (acute viremia, antibody production, viral replication in lymphoid tissues, and final clearance), assay positivity would depend on the sample used for diagnosis, selected laboratory assay, and the period between sample collection and infection. According to their data, anti-PRRSv antibody in serum may be detected by ELISA from 14 days up to 175 days PI, while RT-PCR can detect PRRSv nucleic acids in serum from three days up to 98 days PI. Thus, the time between infection and sample collection may influence the differences in the assay outcome of an individual pig with PRRS (Henao-Diaz *et al.*, 2020).

Although the statistical analysis shows no association, Christopher-Hennings *et al.* (2002) suggested to interpret RT-PCR results with seronegative data to monitor PRRSv-free herd. IgG was undetectable using ELISA at 300 days PI (Nelson *et al.*, 1994). Likewise, Henao-Diaz *et al.* (2020) indicated that RT-PCR using serum sample at 175 days PI is unlikely to detect PRRSv nucleic

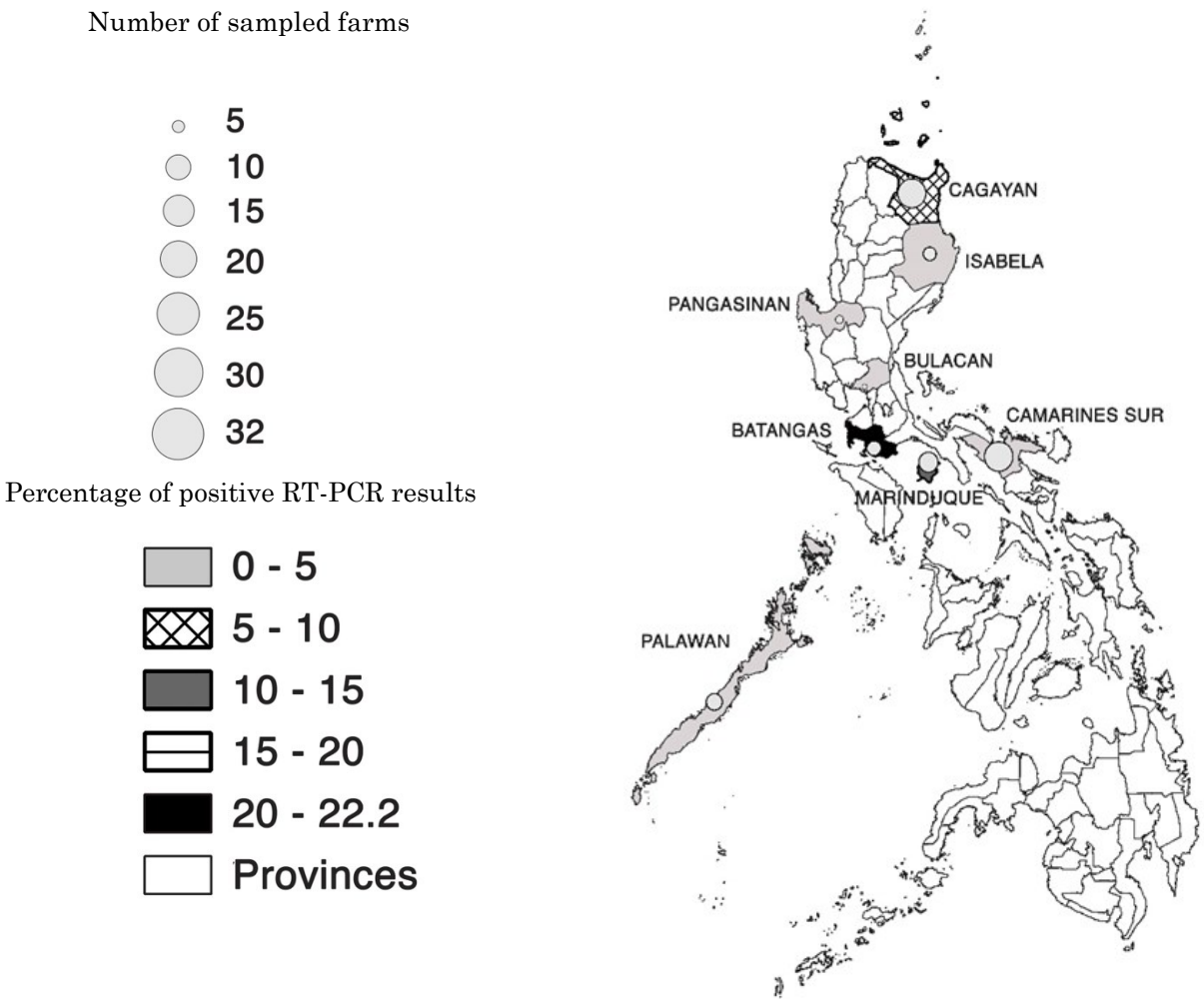

Figure 2. Geographic distribution of backyard farms in Luzon, Philippines which tested positive for PRRSv in RT-PCR.

Table 2. Distribution of positive results in ELISA and RT-PCR among age groups and groups of clinical signs.

	ELISA[1]	No. of pigs	RT-PCR[2]	No. of farms
Age group				
Group A	3	64	2	31
Group B	2	104	2	51
Group C	11	116	2	41
Group D	5	52	4	39
Clinical sign group				
Apparently healthy	1	58	0	32
Generalized	7	118	2	64
Respiratory	6	63	1	26
Reproductive	3	25	1	22
Integumentary	1	51	1	28
Musculoskeletal	0	4	0	3
Digestive	0	61	2	33
Others	0	10	0	8

[1]Plasma of each domestic pig
[2]Pooled plasma samples per backyard farm

acids. In virus isolation using lymphoid tissues, animals with persistent infections may still be positive at 175 days PI (Henao-Diaz *et al.*, 2020). Hence, the negative ELISA and RT-PCR results suggest that the sampled pigs were beyond their acute viremic and antibody production phases, but may still include those with replicating virus in lymphoid tissues.

Table 3 shows that assay positivity is not associated with any life stage. This finding is supported by current literature on PRRS, stating that all age groups are susceptible to the disease (Beltran-Alcrudo & Lubroth, 2007; Dietze *et al.*, 2011).

Among the clinical signs, having respiratory symptoms is more likely to produce a positive result in ELISA and RT-PCR compared to having no respiratory symptoms (p<0.05). Table 4 also adds that respiratory symptoms are more associated with seropositivity than the other clinical signs. This finding agrees with the paper of Dietze *et al.* (2011). Respiratory symptoms of PRRS which may be complicated by secondary bacterial or concurrent viral infection is common in piglets and grower pigs, leading to high mortality rate. At the same time, older pigs with PRRS may also manifest mild respiratory signs (Dietze *et al.*, 2011). Meanwhile, comparing respiratory symptoms among the other PRRS manifestations, the absence of association with RT -PCR positivity may be affected by the period that lasted from infection to sample collection (Henao-Diaz *et al.*, 2020).

Table 3. Crude and adjusted odd ratios of assay positivity among age groups.

	OR_{crude}			$OR_{adjusted}$		
	RT-PCR[1]	ELISA[2]	RT-PCR /ELISA	RT-PCR [1]	ELISA[2]	RT-PCR /ELISA
Group A	Ref	Ref	Ref	Ref	Ref	Ref
Group B	0.45 [0.06, 2.77]	0.53 [0.13, 2.08]	0.48 [0.15, 1.44]	0.70 [0.16, 2.84]	0.50 [0.06, 3.21]	0.59 [0.18, 1.84]
Group C	1.97 [0.59, 8.91]	1.07 [0.36, 3.54]	1.42 [0.62, 3.57]	1.05 [0.32, 3.86]	1.58 [0.44, 7.54]	1.27 [0.51, 3.45]
Group D	2.55 [0.60, 12.92]	1.52 [0.40, 5.75]	1.99 [0.73, 5.61]	2.28 [0.47, 10.92]	1.44 [0.22, 9.60]	2.14 [0.61, 7.48]

[1]Plasma of each domestic pig
[2]Pooled plasma samples per backyard farm

Musculoskeletal symptoms are also more likely to be associated with seropositivity based on Table 4 (p<0.05). To the author's knowledge, PRRS does not cause musculoskeletal signs such as limping and swollen legs as observed in this study. Aside from reproductive and respiratory diseases, PRRS has been known to manifest neurologic signs, skin lesions, and asymptomatic infections (Dietze *et al.*, 2011; OIE, 2021). Table 2 demonstrates that no sample with musculoskeletal symptoms tested positive in either ELISA or RT-PCR; hence, the significant association may only be due to the imputation of missing data.

The absence of association between reproductive symptoms and assay positivity in this study does not coincide with the published articles on PRRS. As previously mentioned, PRRSv-infected sows and breeding gilts commonly exhibit reproductive failure, leading to late term abortions, stillborn, mummification, and giving birth to weak piglets (OIE, 2021). The underrepresentation of breeding pigs under Group D (Table 2) may account for the lack of association between assay positivity and reproductive signs.

In summary, this study detected 5.50% seropositivity using ELISA among sampled pigs from all sampled provinces in Luzon except in Pangasinan, and this implies either previous exposure to or vaccination against PRRSv. Using RT-PCR, 5.98% of the backyard farms specifically from Batangas, Cagayan, and Marinduque had positive results which may indicate an active infection, presence of viral nucleic acids or vaccination. This study also showed no

Table 4. Crude and adjusted odd ratios of assay positivity among grouped clinical signs.

	OR_{crude}			$OR_{adjusted}$		
	RT-PCR[1]	ELISA[2]	RT-PCR /ELISA	RT-PCR[1]	ELISA[2]	RT-PCR /ELISA
Apparently healthy	1.02 [0.40, 2.50]	1.23 [0.52, 2.84]	1.13 [0.59, 2.12]	0.45 [0.12, 1.64]	0.69 [0.17, 2.72]	0.50 [0.19, 1.33]
Generalized	1.49 [0.64, 3.63]	1.76 [0.72, 4.76]	1.63 [0.87, 3.19]	0.92 [0.32, 2.65]	1.20 [0.40, 3.63]	1.01 [0.46, 2.23]
Respiratory	**4.15 [1.74, 10.97][3]**	**2.61 [1.07, 6.76][3]**	**3.57 [1.86, 7.06][3]**	**4.66 [1.43, 16.06][3]**	3.15 [0.90, 11.63]	**4.20 [1.72, 10.58][3]**
Reproductive	1.44 [0.40, 4.02]	1.57 [0.44, 4.45]	1.48 [0.61, 3.24]	0.90 [0.11, 5.87]	3.26 [0.57, 21.76]	1.57 [0.41, 6.04]
Integumentary	0.71 [0.20, 1.96]	1.17 [0.37, 3.09]	0.92 [0.40, 1.92]	0.59 [0.16, 2.04]	0.94 [0.26, 3.19]	0.72 [0.27, 1.78]
Musculoskeletal	**2.51 [1.04, 5.83][3]**	1.64 [0.61, 4.09]	**2.12 [1.08, 4.05][3]**	1.57 [0.07, 18.29]	1.75 [0.06, 21.73]	1.36 [0.13, 10.38]
Digestive	2.09 [0.91, 4.90]	0.81 [0.30, 2.01]	1.37 [0.73, 2.57]	2.39 [0.65, 8.23]	0.17 [0.00, 1.44]	1.08 [0.34, 3.01]
Others	2.31 [0.97, 5.37]	1.50 [0.55, 3.73]	1.95 [1.0, 3.72]	0.72 [0.02, 6.68]	0.69 [0.01, 7.92]	0.58 [0.04, 3.85]

[1]Plasma of each domestic pig
[2]Pooled plasma samples per backyard farm
[3]Significant based on binary logistic regression (95% level of confidence)

association between the results of the two assays but their simultaneous use can help in screening PRRSv-negative herds (Christopher-Hennings *et al.,* 2002; Henao-Diaz *et al.*, 2020). Although statistical analyses of this study only found significant association between assay positivity and respiratory signs, PRRS mainly causes respiratory disease in younger pigs and reproductive disease in breeding pigs. This study also supports the concept that all age groups of pigs are susceptible to PRRS (Dietze *et al.*, 2011; OIE, 2021). To have a complete understanding of PRRS epidemiology, the authors recommend gathering of complete farm history and comparable representation of each age group. Finally, the results of this study imply continuous PRRS problem in provinces with seropositive pigs and RT-PCR positive farms and provide additional data in PRRS surveillance in the Philippines.

ACKNOWLEDGEMENT

This research was supported by funding provided by the United States Public Law-480 Title I Program through the project entitled, "Profiling of economically important diseases of swine and cattle in the Philippines for enhanced disease management, surveillance and control". The authors would like to thank Dr. Ceferino P. Maala and Dr. Dennis V. Umali for their comments and suggestions for this study.

STATEMENT ON COMPETING INTEREST

The authors have no competing interests to declare.

AUTHOR'S CONTRIBUTION

TMAC proposed the project which got the funding from the United States Public Law-480 Title I Program. TMAC, JMGB, and EJSV took part in the sample collection and processing. Lastly, GMVP conducted the data analysis in this study

REFERENCES

Abao LNB, Kono H, Kubota S, Borejon JV, Gaerlan MZ, and Promentilla RR. 2014. PRRS outbreaks and farm hygiene management in the Philippines. *Japanese Journal of Farm Management* 52 (1-2): 131-136.

Alexopoulos C, Kritas SK, Kyriakis CS, Tzika E, and Kriakis SC. 2005. Sow performance in an endemically porcine reproductive and respiratory syndrome (PRRS)-infected farm after sow vaccination with an attenuated PRRS vaccine. *Veterinary Microbiology* 111: 151-157.

Beltran-Alcrudo D and Lubroth J. 2007. Porcine reproductive and respiratory syndrome (PRRS) regional awareness. *Focus on…*2: 1-5.

Botner A, Strandygaard B, Sorensen KJ, Have P, Madsen KG, Madsen S, and Alexandersen S. 1997. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified-live PRRS vaccine. *Veterinary Record* 41: 497-499.

Butler JE, Lager KM, Golde W, Faaberg KS, Sinkora M, Loving C, and Zhang YI. 2014. Porcine reproductive and respiratory syndrome (PRRS): an immune dysregulatory pandemic. *University of Iowa Immunology* 59: 81-108.

Christopher-Hennings J, Faaberg KS, Murtaugh MP, Nelson EA, Roof MB, Vaughn EM, YoonKJ, and Zimmerman JJ. 2002. Porcine reproductive and respiratory syndrome (PRRS) diagnostics: interpretation and lim itations. *Journal of Swine Health and Production* 10(5): 213-218.

Christopher-Hennings J, Nelson EA, Nelson JK, and Benfield DA. 1997. Effects of modified livevirus vaccine against porcine reproductive and respiratory syndrome in boars. *American Journal of Veterinary Research* 58: 40-45.

Department of Agriculture-Philippines. 2018. Administrative circular no. 3, series of 2018. https://www.da.gov.ph/wp-content uploads/2018/05/ac03_s2018.pdf. Accessed 27 July 2020.

Dietze K, Pinto J, Wainwright S, Hamilton C, and Khomenko S. 2011. Porcine reproductive andrespiratory syndrome (PRRS): virulence jumps and persistent circulation in Southeast Asia. *Focus on…* 5: 1-8.

Dotti S, Villa R, Sossi E, Guadagnini G, Salvini F, Ferrari M, and Amadori M. 2011. Comparativeevaluation of PRRS virus infection in vaccinated and naïve pigs. *Research in Veterinary Science* 90(2): 218-225.

Ducusin RJT, Padilla MA, Regondola JCI, Pajas AMGA, Domingo RD, Torres MIP, and Tan YS. 2015. Detection of antibodies against porcine reproductive and respiratory syn drome and porcine circovirus type 2 in smallholder swine farms from an abattoir in Sariaya, Quezon, Philippines. *Philippine Journal of Veterinary Medicine* 41(2): 153-159.

Food and Agricultural Organization (FAO). 2011. Swine industry profile of selected Southeast Asian countries. http://cdn.aphca.org/ dmdocuments/PAP_11_SE%20Asia%20Pig% 20Industry%20Profiles_TCP%20RAS% 203215.pdf. Accessed 29 April 2017. Gamer M, Lemon J, Fellows I, and Singh P. 2012. *Package 'irr'*. https://cran.r-project.org/web/ packages/irr/irr.pdf. Accessed 4 July 2022.

Henao-Diaz A, Ji J, Gimenez-Lirola L, Baum DH, and Zimmerman J. 2020. Understanding and interpreting PRRSV diagnostics in the contect of "disease transition stages". *Research in Veterinary Science* 131: 173-176.

Kittawornrat A, Engle M, Panyasing Y, Olsen C, Schwartz K, Rice A, Lizano S, Wang C, and Zimmerman J. 2013. Kinetics of the porcine reproductive and respiratory syndrome virus (PRRSV) humoral immune response in swine serum and oral fluids collected from individual boars. *BMC Veterinary Research* 9 (61): 1-11.

Kraft C, Hennies R, Dreckmann K, Noguera M, Rathkjen PH, Gassel M, and Gereke M. 2019. Evaluation of PRRSv specific, maternally derived and induced immune response in Ingelvac PRRSFLEX EU vaccinated piglets in the presence of maternally transferred immunity. *PLoS ONE* 14 (10): 1-21.

Kristensen CS, Kvisgaard LK, Pawlowski M, Holmgaard Carlsen S, Hjulsager CK, Heegard PMH, Botner A, Stadejek T, Haugegaard S, and Larsen LE. 2018. Efficacy and safety of simultaneous vaccination with two modified live virus vaccines against porcine reproductive and respiratory syndrome virus types 1 and 2 in pigs. *Vaccine* 36: 227-236.

Maala CUM, Cruz MS, Bulay AC, and Lising RT. 2006. An update on PRRS seroprevalence in the Philippines. *Proceedings of the 19th International Pig Veterinary Society Congress*, Denmark, pp. 28.

Mengeling WL, Lager KM, Vorwald AC, and Brockmeier SL. 1996. Comparison among strainsof porcine reproductive and respiratory syndrome virus for their ability to cause reproductive failure. *American Journal of Veterinary Research*. 59: 52-55.

Nelson EA, Christopher-Hennings J, and Benfield DA. 1994. Serum immune response to theproteins of porcine reproductive and respiratory syndrome (PRRS) virus. *Journal of Veterinary Diagnosis Investigation* 6 (4): 410-415.

Nguyen T. 2013. PRRS control in the region. http://www.oie.int/fileadmin/Home/eng/Publications_%26_Documentation/docs/pdf/TT/2013_ASI2_Nguyen.pdf. Accessed 27 March 2017.

Parayao AM, Kong S, Collantes TMA, Baldrias LR, and Dalmacio IF. 2021. Molecular detection and profiling of porcine reproductive and respiratory syndrome virus from abattoir-slaughtered pigs in Laguna, Philippines. *The Philippine Journal of Veterinary Medicine* 58 (2): 182-192.

QIAGEN. 2012. *QIAGEN ® One Step RT-PCR Handbook*. https://www.qiagen.com/us/resources/resourcedetail?id=57743726-84e1-423a-9d8f-a3fa89bbe7eb&lang=en. Accessed 17 July 2017.

Rovira A, Clement R, Christopher-Hennings J, Thompson B, Engle M, Reicks D, and Muñoz-Zanzi C. 2007. Evaluation of the sensitivity of reverse-transciption polymerase chain reaction to detect porcine reproductive and respiratory syndrome virus on individual and pooled samples from boars. *Journal of Veterinary Diagnosis Investigation* 19 (5): 502-509.

Shenzhen Lvshiyuan Biotechnology Co., Ltd. n.d. *LSY-30010 PRRSV Antibody ELISA Test Kit*. http://lsybt.com/en/ProDetail.aspx?c_id=51&id=591. Accessed 12 July 2017.

Swine Health Information Center. 2021. *Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus*. https/www.swinehealth.org/wp-content/uploads/2021/07/shic-factsheet-hp-prrsv-2021Jul13. Accessed 20 January 2022.

The World Health Organisation for Animal Health. 2021. *OIE Terrestrial Manual*. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.09.06_PRRS.pdf. Accessed 3 February 2022.

Thomann B, Rushton J, Schuepbach-Regula G, and Nathues H. 2020. Modelling economic effects of vaccination against porcine reproductive and respiratory syndrome: impact of vaccination, effectiveness, vaccine price, and vaccination coverage. *Frontiers in Veterinary Science* 7: 1-9.

Wills RW, Zimmerman JJ, Yoon KJ, Swenson SL, McGinley MJ, Hill HT, Platt KB, Christopher-Hennings J, and Nelson EA. 1997. Porcine reproductive and respiratory syndrome virus: a persistent infection. *Veterinary Microbiology* 55 (1-4): 231-240.

Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, and Stevenson GW. 2019. *Diseases of Swine*. 11th ed. Ames, Iowa: John Wiley & Sons, Inc.