Very Low Seroprevalence of Porcine Reproductive and Respiratory Syndrome among Backyard Pigs in Leyte Province and Factors associated with S/P Ratios

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Abstract

Porcine Reproductive and Respiratory Syndrome (PRRS) is a viral disease that causes significant production and economic losses to swine raisers. To estimate the seroprevalence of PRRS in pigs from the backyard and small-hold farms in the province of Leyte, Philippines, a total of 384 pigs were sampled at random from 11 localities and their sera were tested for PRRS antibody using indirect enzyme-linked immunoassay. Univariable and multivariable regression analyses were performed to determine the factors associated with the S/P ratios. Results revealed that the true seroprevalence for PRRS in backyard pigs was 0.28% (0.0001 to 0.0155, 95% CI) and the true herd-level seroprevalence was 1.02% (0.0005 to 0.1588, 95% CI). Factors significantly associated with the S/P ratios were: Large White (breed) (adjusted β *= 0.22,* $p = 0.0014$*), the presence of goats (adjusted* $\beta = -0.63$, $p < 0.0001$*) in farm vicinity, disposing wastes to bodies of water (adjusted* $\beta = 0.27$, $p < 0.0001$*) and separating sick animals (adjusted* $\beta = 0.34$, $p < 0.0001$*). The very low seroprevalence in the backyard and small-hold pig farms may indicate a low prevalence of PRRS in the province. Practices in backyard farms like disposing of pig wastes to water bodies and separating or moving sick animals were present and may promote the spread of the virus and pose higher risks when future disease outbreaks occur. It is recommended that the government impose proper waste management on backyard swine farms to prevent the spread of PRRS and other economically important swine diseases.*

Keywords*:* ELISA, Leyte, PRRS, seroprevalence, swine farms

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease that can cause significant production and economic losses. In the United States, yearly estimated losses due to PRRS virus (PRRSv) were US$664 million making the disease to be the costliest viral pathogen of the modern pig industry (Neumann *et al*., 2005; Johnson *et al*., 2004; Chand *et al*., 2012; Montaner-Tarbes *et al*., 2019). Worldwide, PRRS is considered a notifiable disease as listed by World Organization for Animal Health (OIE) (2018). In the Philippines, it is listed as one of the priority livestock diseases and categorized as a disease of farm concern as it could cause severe economic loss to farmers; their prevention and control are matters of greatest importance (Department of Agriculture [DA], 2004). In 2007, an outbreak of atypical PRRS occurred in Central Luzon, Philippines causing high mortality and morbidity with case fatality rates reaching almost 40% and the prevalence of PRRS ranging from 3.24 to 10.76%, which resulted in a great economic loss (Cudal, 2009; Baltazar, 2009; Dumenden, 2009). The domestic PRRS-related economic losses in the Philippines were estimated to run up to six billion pesos (US$138 million) per year (Abao *et al*., 2014).

PRRS is caused by an enveloped, RNA *Arterivirus*. This virus has two genotypes – Type I (European genotype) and Type II (North American genotype). Type I is further divided into three subtypes: Pan-European subtype 1 and Eastern European subtypes 2 and 3 (Stadejek *et al*., 2008; Dietze *et al*., 2011; Chae, 2021). Despite the differences in genotype, PRRSv produces common signs including reproductive loss or failure in breeding animals, post-weaning pneumonia and increased mortality in growing pigs. Other signs are inappetence, fever, discoloration of ears, lethargy and respiratory signs. In general, it affects breeding animals characterized by reproductive failure and respiratory disease in pigs of all ages (Dietze *et al*., 2011; Rahe and Murtaugh, 2017).

The Eastern Visayas region continues to be near the bottom in per region distribution of swine production contributing only 282,410 heads (2.22%) of the total stock of the country (Philippine Statistics Authority [PSA], 2018). The province of Leyte accounts for more than half of the swine population in the region. Swine diseases in the province with high mortality and morbidity are left undiagnosed and the presence of PRRSv is yet to be justified because of lacking scientific evidence. If antibodies against PRRSv are identified in

the unvaccinated animal population, the existence of natural infection can be demonstrated.

This study aimed to determine the seroprevalence of PRRS in Leyte and determine the factors associated with enzyme-linked immunoassay (ELISA) results. The results of this study can help swine farmers and government veterinary offices in designing effective control, preventive and eradication programs to help improve animal production.

2. Methodology

2.1 Study Site

Leyte is a province in the northern three-quarters of Leyte island, Philippines; it accounts for more than half of the swine population in the Eastern Visayas region. The study site was sampled from October 2018 to December 2018.

2.2 Sample Size Determination

The sample size was determined using StatCalc function in Epi Info 7.2.2 software (Centers for Disease Control and Prevention [CDC], 2022). To compute the sample size, necessary data required by the software were inputted: minimum estimated seroprevalence set at 50%, the margin of error at 5%, the pig population of the province at 170,041 heads (PSA, 2017) and the confidence interval at 95%. The total number of samples needed to estimate the seroprevalence was 384.

2.3 Study and Sampling Design

A cross-sectional study was used to assess the seroprevalence of PRRS. The backyard and small-hold pig population was the target population since they constituted about 95.35% (269,264/282,410) of the swine industry in the province (PSA, 2018). A multi-stage sampling was used. In the first stage of sampling, 11 localities were purposively chosen as the principal sampling units. Purposive sampling was the sampling method used because of several considerations: distance from the processing and storage laboratory, peace and order, cost of travel, the limited resources and personnel. The computed

sample size (384) was then proportionally allocated to the chosen localities based on the number of their households as shown in Table 1.

Table 1. Distribution of number of samples among selected localities with their respective barangays

Locality	No. of barangays[a]	No. of household[b]	Selected barangays*	No. of samples collected	Total no. of samples per locality
1. Baybay City	92	23,475	Bunga Candadam San Isidro Bubon May patag Palhi	13 13 12 7 5 14	64
2. Inopacan	20	4,579	Cabulisan Conalum	2 10	12
3. Hindang	20	4,769	Ma-asin	13	13
4. Hilongos	51	12,877	Tuguipa Talisay Cantandog Liberty	2 5 14 14	35
5. Bato	32	7,813	San Agustin Tagaytay	10 11	21
6. Matalom	30	7,013	Esperanza Elevado	7 12	19
7. Albuera	16	9,182	Mahayahay Salvacion	9 16	25
8. Ormoc	110	41,996	Dolores Libertad Labrador Nasunogan Liloan Mabini	19 19 20 19 19 19	115
9. Merida	22	6,715	Masumbang	20	20
10. Isabel	24	10,075	Mahayag	26	26
11. Palompon	50	12,818	Rizal Taberna Tinago San Miguel	20 6 5 3	34
Total		141,312			384

[a]PSA (2019); [b] National Statistics Office (2012)

*The number of randomly selected barangays is ~5% of the total number of barangays in each locality.

Proportional allocation was done by dividing the total number of households in a locality by the total number of households in the 11 localities; the quotient

of which was then multiplied by the computed sample size (384). More samples were collected in the localities with more households as there were more backyard farms found in these areas.

Five percent of the total number of barangays in every chosen locality were randomly selected for sampling. Farms within these barangays were then selected based on the accessibility of their location, pig production and willingness of the farm owners to cooperate. With the lack of data on pig production in every barangay, the farms with the highest production, as recommended by the barangay officials, were first sampled, followed by other farms with lesser pig populations. Finally, a representative number of pigs (25% of the swine population) from each of the farms was randomly sampled. A total of 384 blood samples were collected from apparently healthy pigs tested for PRRSv antibodies. Data on the farmer (e.g., age, sex, years of experience in swine raising, etc.), farm characteristics (e.g., type of production, other livestock animals raised, water source, etc.) and farm management (e.g., feeding and housing management, health management and biosecurity, etc.) were collected through pretested questionnaire. A total of 104 farmers were able to participate in the study. The questionnaire was constructed in English and subsequently translated into Cebuano during the interview by the first author.

2.4 Sample Collection, Transport and Processing

Blood samples were taken from pigs on each farm and tested for the presence of antibodies directed against PRRSV using ELISA. Blood (5 mL) was collected from each sample. Blood collection was done aseptically via the anterior vena cava (piglets) or the jugular veins (older pigs) using a 23G needle and 10-mL vacutainer tube containing no anticoagulant. Samples collected before serology were placed in a cool box, which was then carried to the Veterinary Microbiology Laboratory of the College of Veterinary Medicine, Visayas State University, Baybay City. Sera were collected from the blood samples in the laboratory and were stored in a freezer (Arctiko ULUF 450-2M®, Arctiko, Denmark) at -20 °C. All frozen sera were transported using a cool storage box to the Molecular Microbiology Laboratory of the College of Veterinary Medicine, University of the Philippines Los Baños (UPLB), Laguna. The frozen sera were thawed at room temperature only on the day of the serologic analysis.

2.5 ELISA

The PRRS serological status of the farms was detected using an indirect ELISA commercial kit (GreenSpring® PRRSV, Shenzhen Zhiyuan Technology Co., Ltd., China). Procedures followed the manufacturer's instructions. Optical density (OD) values were determined by measuring using a photometer (Multiskan™ FC Microplate Photometer, Thermo Scientific, United States) at 450/630 nm. A sample-to-positive (S/P) ratio of equal to or greater than 0.20 was considered positive in this study as indicated by the manufacturer.

2.6 Data Management and Analysis

Data from the questionnaire were compiled and managed in a spreadsheet program (Microsoft Corporation, 2018) together with the ELISA results. Data were exported to Epi Info 7.2.2 statistical software for analysis (CDC, 2022). Continuous covariates were categorized into two groups based on > median and ≤ median values. Descriptive statistics were generated from the farmers' demography and management practices. Frequency distributions and measures of central tendency were computed. Individual and farm seroprevalences at a 95% confidence interval were then calculated. True seroprevalences were computed using the estimator of true prevalence with an imperfect test function of Epitools online software (Sergeant, 2018) considering the sensitivity and specificity of the ELISA kit at 94 and 100%, respectively (Catalog No. LSY-30010, Shenzhen Zhiyuan Technology Co., Ltd., China).

To evaluate factors associated with the S/P ratios, all variables were initially screened by running a univariable linear regression analysis with logged S/P ratio results (dependent variable) at a 95% confidence interval (Elzo *et al*., 2006; Eisenberg *et al*., 2015). Variables with a P-value ≤ 0.05 were considered in the multivariable linear regression analysis. This was done using the forward stepwise approach with a P-value of 0.01 as the limit. Rejected variables were added separately into the final model to ensure that no significant variables were omitted. To prevent collinearity among the variables, the least significant variable correlated to another variable was dropped (Dohoo *et al*., 2014).

2.7 Ethical Considerations

The study protocol was approved by the International Animal Care and Use Committee of the UPLB in October 2018. For the interview, verbal informed

consent was obtained from all the respondents. The information gathered during the interviews was handled in accordance with Republic Act No. 10173 (National Privacy Commission, 2016).

3. Results and Discussion

Out of 384 healthy unvaccinated pigs tested, only one sample had a titer considered positive for antibodies against PRRSv. This gave a seroprevalence of 0.28% with an interval of 0.0001 to 0.0155 containing the true seroprevalence with a probability of 0.95. Only one farm from Palompon (with red dot in Figure 1) tested positive for antibodies against PRRSv out of 104 farms or an apparent herd-level seroprevalence of 1.02% (Figure 1). Out of three samples from that farm, only one tested positive.

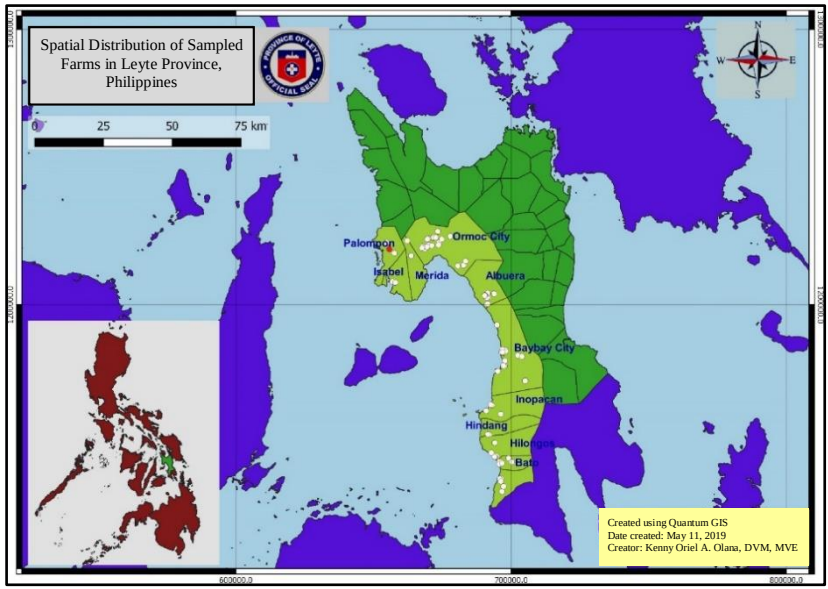

Figure 1. Spatial distribution of sampled farms in Leyte province, Philippines

In the Philippines, from 2012 up to the present, only two studies on the seroprevalence of PRRS were documented. These studies include that of Boloron and Doysabas (2015) in Bukidnon province (Mindanao island) and Ducusin *et al*. (2015) in Quezon province (Luzon island). The seroprevalence found in this study is much lower than that of Ducusin *et al*. (2015) and

Boloron and Doysabas (2015) from which 15.1 and 9.5% were derived, respectively. Both studies used the CIVTEST suis PRRS E/S PLUS (Laboratorios HIPRA SA, Spain) ELISA test kit with sensitivity and specificity of 91.5 and 96.45%, respectively (Sattler *et al*., 2014). In comparison, the GreenSpring® PRRSV antibody test kit in this study has an analytical specificity and sensitivity of 100 and 94%, respectively. However, its epidemiologic sensitivity and specificity have yet to be validated. In the study of Ducusin *et al*. (2015), only 53 samples were analyzed but derived a 15.1% (8/53) seroprevalence. This may be explained by the fact that seropositivity to PRRS could be higher in Luzon because it had a higher swine population density than other regions; it was also where the past PRRS outbreaks occurred (Cruz *et al*., 2005; Abao *et al*., 2014; World Organization for Animal Health [OIE] World Animal Health Information System, 2014). Boloron and Doysabas (2015) collected and analyzed 200 samples from Bukidnon and derived a seroprevalence of 9.5% (19/200). So far, there are no reports of PRRS outbreaks in Bukidnon and there are no studies on the prevalence of the PRRS virus using molecular methods. Low seropositivity in sampled pigs still has to be validated by further studies.

This study differentiates from the previous studies as it involved more samples that were adequate to demonstrate disease freedom in swine populations using ELISA commercial test kit to detect prevalence as small as 1% in the geographic area. Seropositivity may indicate viral exposure from natural infection or through vaccination. Non-exposure to the virus through natural infection may have been attributed to the very low seroprevalence found in this study as the sampled farms were all unvaccinated with the PRRS vaccine. PRRSv is very susceptible to adverse conditions. The virus can only survive for less than a day in fomites at normal environmental temperature (25-27 °C) in which normal clean-up procedures with disinfection and drying would kill the virus (Morrow and Roberts, 2001). The majority of the respondents implemented regular cleaning (344) (89.58%) of their farm with the use of water and disinfectant or detergent. However, most of them had no footbath (368) (95.83%) at farm entrances – a part of the operational biosecurity to prevent the introduction of any disease in the farm. To date, there are no reports of PRRS outbreaks in the province. However, further surveillance must be conducted to validate this information since there are no studies published related to the herd-level seroprevalence of PRRS in the Philippines in the past decade. In the study site, the lack of awareness of the backyard farmers on PRRS may account for not practicing vaccination (Olana *et al*., 2020).

To evaluate the factors having an influence on ELISA results, regression analyses were done using the log transformed S/P ratios of optical densities derived from 430/650 nm wavelengths as the outcome variable. Table 2 shows the variables significantly associated with the ELISA results including the breed of pig – Large White ($\beta = 0.224$, $p = 0.0014$), the presence of domestic animals particularly goats ($\beta = -0.630$, $p < 0.0001$), separating sick animals ($\beta = 0.336$, $p < 0.0001$) and disposing of wastes to bodies of water ($\beta = 0.269$, $p < 0.0001$).

Table 2. Multivariable linear regression on factors affecting S/P ratios

Variable	Coefficient	95% confidence limits		Std. error	F-test	P-value
Large hite	0.224	0.088	0.361	0.069	10.4312	0.0014
Presence of goats	-0.630	-0.879	-0.381	0.127	24.7449	<0.0001
Separating sick pigs	0.336	0.189	0.484	0.076	20.1156	<0.0001
Disposing wastes to bodies of water	0.269	0.135	0.403	0.068	15.6363	<0.0001
Constant $R^2 = 0.23$ P-value = <0.0001	-2.628	-2.768	-2.488	0.071	1363.137	0.000000

Disease susceptibility usually varies among species of animal, breed and/or sex. Certain diseases such as PRRS affect all types of pigs whether of varying breeds, ages, or sex. It can even affect all types of production herds from intensive to extensive, large or small, and/or backyard or commercial herds (Velasova *et al*., 2012; Tummaruk *et al*., 2013; Wiratsudakul *et al*., 2013). Large White (234, 60.94%) is the most common breed seen in the sampled farms and was used as a mix to produce hybrid pigs. Large White as the breed of pigs showed a significant, positive, direct relationship with S/P ratios with a coefficient of 0.22. However, its predisposition, susceptibility, or resistance to PRRSv was not yet fully understood. Breed susceptibility differences to PRRS were studied by some authors (Halbur *et al*., 1998; Meng *et al*., 2008; Reiner *et al*., 2010); however, they are exclusive of Large White pigs. Meng *et al*. (2018) differentiated the susceptibility of commercial breed (Landrace) and native breeds in China based on PRRSv proliferation dynamics. Results showed that Dingyuan pigs were the most susceptible to PRRSv infection, while Jiangquhai pigs were the least susceptible. In another study by Halbur *et al*. (1998), the susceptibility of Duroc, Hampshire and Meishan breeds to

highly virulent PRRSv was compared. Severe macroscopic lung lesion scores were significantly higher in Hampshire pigs (43.0±3.7) than in Duroc (29.43±3.3) or Meishan (25.0±3.5) pigs. Although Meishan pigs had significantly less PRRSV antigen detected in the lungs, myocarditis and encephalitis were observed more significantly in these pigs. Duroc pigs had significantly lower normalized serum antibody titers to PRRSv infection (Halbur *et al*., 1998). In the study of Reiner *et al*. (2010), Wiesenauer miniature pigs were more efficient in antibody production than Pietrain pigs; however, viral replication was to be higher (3.3%) in these pigs than in Pietrain.

Domestic animals may serve as carriers of PRRSv (Zimmerman *et al*., 1997). Only 8.85% of the farms had goats near their pig pens; however, the presence of goats showed a significant inverse relationship with the S/P ratio (β = -0.630), which indicated that when there were goats in the vicinity of pig pens, the S/P ratio tended to decrease by 0.63. This needs further investigation as to whether this inverse relationship has biological plausibility. Even so, the authors removed confounding variables and variables with multiple collinearities. Nonetheless, 76.56% (294) of farms had other domestic animals in the vicinity of pig pens. Chickens predominate among domestic animals (233) (60.68%), followed by dogs (135, 35.16%), which could transmit PRRSv within the farm (Zimmerman *et al*., 1997).

When animals get sick, 65.63% (252) of the raisers separate the sick ones from the healthy pigs, with 15.63% (60) separating at least by a 10-m distance. Separating sick pigs was a common practice among swine farmers, but it was advised by Rathkjen and Dall (2017) to not move diseased pigs as they are often immunocompromised and have comorbidities that increase their likelihood of carrying PRRSv. This may be the reason why separating sick animals was positively associated ($\beta = 0.336$, $p < 0.0001$) with the S/P ratio. The viral load of these animals was also likely to be higher, which increases the risk of spreading infection. Hence, the sick pigs should be remained in their place to limit the viral spread, and moribund animals should be euthanized.

The frequency of disposing of pig wastes in bodies of water was positively associated with the S/P ratio. This is explained by the ability of PRRSv to survive longer in wet environments (Dee *et al*., 2002). Transmission is highly probable and could be more extensive in such conducive conditions. The final model was highly significant ($p < 0.0001$) showing the associations that may

be related to possible risk factors in the occurrence of PRRS. However, these results should be interpreted with caution since the R^2 value of the model was only 0.23, which indicated that the variation of the logged S/P ratio was explained by only 23% of the variation of these predictors. A large component (87%) of the variation of the S/P ratio was explicated by other influencing factors which are unknown. Risk factors of PRRS outbreaks identified in recent studies were increments of swine density and herd size (Arruda *et al*., 2017), type of production system (Arruda *et al*., 2016), and developed and topographical regions (Mahesh *et al*., 2015). According to Nathues *et al*. (2018), the following farm practices must be avoided to maintain farm stability against PRRSv: a suckling period $\leq$ 21 days, a low distance between the cadaver collection site and the actual sow barn, $\geq$ 2 pig herds in a 1,000-m radius, presence of external employees, a time interval between the purchase of gilts of $\leq$ 9 weeks and a one- or two-week farrowing rhythm.

4. Conclusion and Recommendation

In conclusion, the very low seroprevalence in backyard and smallholder pig farms in Leyte may indicate the low prevalence of PRRSv since there are no documented reports of PRRS outbreaks in the area. Targeted surveillance studies on the prevalence of PRRSv involving molecular methods are recommended to detect PRRSv and identify the risk factors associated. Nevertheless, in the backyard setting, practices such as disposing of pig wastes to water bodies and separating or moving sick animals still exist, which may promote disease transmission and pose higher risks when future outbreaks occur. Educating the backyard farmers along with the strict implementation of the local government on proper waste management is an indispensable preventive measure against the spread of PRRS and other economically important swine diseases. To the best of the authors' knowledge, this is the first study on PRRS in the whole Eastern Visayas region, particularly in the province of Leyte.

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