Journal of Environmental Science and Engineering B 12 (2023) 96-103
doi:10.17265/2162-5263/2023.02.005

Histopathological Characterization of Lungs from Slaughtered Swine in Selected Abattoirs in Laguna, Philippines and Its Relation to Serological Profiling for Porcine Pleuropneumonia (PPP), Porcine Reproductive and Respiratory Syndrome (PRRS) and Enzootic Pneumonia (EP)

Sokun Khoeun[1], Joseph S. Masangkay[2], Billy P. Divina[2] and Joseph P. Olarve[3]

1. Faculty of Agriculture, University of Svay Rieng, Svay Rieng 200705, Cambodia

2. Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine, University of the Philippines, Los Baños 4031, Philippines

3. Department of Basic Veterinary Science, College of Veterinary Medicine, University of the Philippines, Los Baños 4031, Philippines

Abstract: Porcine Respiratory Disease Complex (PRDC) is one of the most important health concerns in swine production due to consumer demand for high quality pork from healthy animals. This study was conducted to characterize and compare the gross microscopic lesions and serological profiles of swine pneumonic lungs from selected slaughterhouses in Laguna, Philippines. Blood and lung samples were collected from the municipalities of Santa Cruz, San Pablo, Los Baños, and Cabuyao. Enzyme-Linked Immunosorbent Assay (ELISA) test was conducted for the blood samples and the histopathological changes in the lung tissues were observed microscopically. The results showed that the presence of Porcine Pleuropneumoniae (PPP), Enzootic Pneumoniae (EP) and Porcine Reproductive and Respiratory Syndrome (PRRS) virus were detected using ELISA. In Los Baños, Sta. Cruz and San Pablo the most prevalent disease was EP at 26.1%, 13.0% and 17.4% respectively. While in Cabuyao the diseases found were PRRS and EP, both at 8.7%. Based on the histopathological examination, it was found out that the most occurring types of lesions were hemorrhage, congestion and lymphoid hyperplasia of BALT (Bronchial Associated Lymphoid Tissue). However, the histopathological findings were found to be not significantly associated with the disease present in the sampled swine.

Key words: Pneumonia, PRDC, ELISA.

1. Introduction

The swine production in the Philippines is the largest among the livestock and poultry industry. It plays a vital role by providing 60% of the meat consumed by the Filipinos [1]. As of 1 July 2020, the total swine inventory was estimated at 11.74 million heads indicating an annual decline of 7.6 percent as it recorded a stock of 12.70 million heads in the same period of 2019. Population of swine in both backyard and commercial farms posted decreases of 2.7 percent and 15.9 percent, respectively. Of the total swine inventory, 66.5 percent were raised in backyard farms while the remaining 33.5 percent were from commercial farms [2]. Pork accounts for around 60% of all meat produced and consumed in the Philippines. The industry is large and highly diverse, with a wide range of

Corresponding author: Sokun Khoeun, MSVM, research fields: respiratory disease complex, veterinary pathology.

production systems from large-scale commercial to low input subsistence, but is dominated by smallholder farmers. The designation of the Philippines as free of the Foot-and-Mouth Disease (FMD) provides the opportunity to export pigs and pork to hitherto closed markets such as Singapore [3].

Respiratory disease in pigs is arguably the most important health concern of swine producers today. According to Thacker [4] and Hansen *et al.,* [5], porcine respiratory disease complex (PRDC) is used to describe a multifactorial and complex disease of finishing pigs from 14 to 22 weeks of age caused by a combination of infectious viral or bacterial pathogens, environmental stressors, differences in production systems, and various management practices. As cited by Hansen *et al.,* [5], morbidity ranges from 10% to 40% and mortality from 2% to 20% according to Harding and Halbur [6] and Harms *et al.* [7]. The term "PRDC" has been widely used by pig veterinarians and producers to describe the complex characteristics of respiratory symptoms and poor growth in growing and finishing pigs [8]. Primary agents in pigs include viral and bacterial agents. Two of the three primary bacterial agents are the *Myocplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* while the most common opportunistic agent is *Pasteurella multocida* [9].

Pig husbandry practices have rapidly changed over the last two decades and have resulted in a decline in the total number of pig farms and the perception of an increase in stocking density worldwide. Consequently, PRDC has increased in the global pig population, leading to huge economic losses due to retarded growth performance, increased antimicrobial use and extra costs for control measures such as vaccination. PRDC remains a major constraint to profitability because the vast majority of PRDC occurs in growing-finishing pigs [8]. In recent years, the interest of consumers in quality assurance along the pork production chain has greatly heightened. Thus, there is a strong increase in the demand for pork obtained from healthy animals not subjected to treatment during the fattening period [10].

2. Material and Methods

2.1 Sample Sources and Selection

The samples in this study were taken from four (4) pig slaughterhouses located in the municipalities of Santa Cruz, San Pablo, Los Baños, and Cabuyao in Laguna province. The slaughterhouses in Santa Cruz and San Pablo are classified as "A", while those in Los Baños and Cabuyao are "AA". As guided by Executive Order Number 137 in 1993, and the National Meat Inspection Code of the Philippines (RA 9296) of 2004, "AAA" slaughterhouses are those with facilities and operational procedures appropriate to slaughter livestock and fowls for sale in any market, domestic or international; "AA" slaughterhouses have facilities and operational procedures sufficiently adequate where the livestock and fowls slaughtered therein are suitable for sale in any market domestic or international; and "A" slaughterhouses have facilities and procedures of minimum adequacy where the livestock and fowls slaughtered therein are suitable for distribution and sale only within the city or municipality where the slaughterhouse is located [11].

Purposive sampling was done wherein a total of 92 pneumonic lungs and blood samples from each pig were collected. A total of 23 pigs were sampled from each slaughterhouse. The collection period took two days per abattoir in each municipality.

2.1.1 Blood Samples

All blood samples were collected from swine during slaughter while the butchers were bleeding the animals as part of the slaughter operation. Fifteen (15) mL sterile test tubes were used to collect and store blood samples from the slaughterhouses until they reached the laboratory for serum extraction. All test tubes were properly labeled with the animals' identification.

2.1.2 Lung Sample

Following the opening of the thoracic cavity of slaughtered pigs, lungs were examined for gross lesions that indicated right cardiac lobe (Top) chronic CVP (Cranio-Ventral Pneumonia), swollen lung lesions in

cardiac lobe (top), enlarged lungs due to lack of pulmonary collapse and hemorrhagic necrosis, necro-hemorrhagic areas of consolidation in the lung. The lung samples were kept in 10% formalin solution for one week. Standard H&E (Hematoxylin and Eosin) method of preparing histopathological section was used.

2.2 Histological Slide Preparation and Examination

2.2.1 Histopathology Slide Preparation

The lung tissue samples were stored in 10% formalin for one week. Histopathology slides were prepared following standard paraffin technique and eventually stained with H&E stain [12].

2.2.2 Examination of Slides

Standard method of histopathological examination of slides using compound optical microscope was done to determine the different histopathological lesions [13].

2.2.3 Serum Extraction Procedure

To facilitate serum extraction, each tube of blood sample was held at room temperature in the test tube rack for 30 min. Blood samples were then centrifuged (Jouan BR4i, Labcare, UK) at 5,000 rpm for 11 min at 8 ℃. The serum was then transferred by pipetting from the test tube to the microcentrifuge tube and labeled properly. All serum samples were stored at ≤ -80 ℃ (Thermo Fisher Scientific LLC, US). ELISA (Enzyme-Linked Immunosorbent Assay) kit could be performed in Appendix B [14].

2.2.4 Serological Analysis

The ELISA kit IDEXX APP-ApxIV, IDEXX M. hyo and IDEXX PRRS X3 (IDEXX company), Manufacturer IDEXX Switzerland AG Stationsstrasse 12 CH-3097 Liebefeld-Bern Switzerland were used to determine the antibodies in the blood of the sample pigs and APP (*Actinobacillus pleuropneumoniae*), and PRRS (Porcine Reproductive and Respiratory Syndrome). With profits on the line, diagnostic testing is no longer discretionary; it is necessary. The IDEXX APP-ApxIV Ab Test lets you detect APP before it harms the herd and producer profits, with 99.8% specificity, 80.8% sensitivity and a PPV (Positive Predictive Value) of 99.6% on the individual animal level [15].

Mycoplasma hyponeumoniae Detection Kit for the sensitive and specific detection of *Mycoplasma hyopneumoniae* in porcine samples was used which could detect low sensitivity and/or specificity [16].

All blood samples were collected from swine during slaughter while the butchers were bleeding the animals as part of the slaughter operation. Samples were placed in 15 mL test tube. All test tubes were properly labeled with the animal's identification and probable source location. After collection, all blood samples were kept in styrofoam box with ice packs to ensure that they were kept in cool condition during transport from the slaughterhouses to the laboratory. The whole blood samples were kept in cold temperature prior to serum extraction.

2.2.5 Analysis of Data

Data gathered from four (4) slaughterhouses were analyzed through descriptive statistics using the SPSS (Solutions Statistical Package for the Social Sciences) program. A Chi-square test determined the correlation between the pathological findings and antibody (Ab) levels of the major swine respiratory disease.

To test if there is an association found between the detected diseases using ELISA result and the diagnosis of histopathology variable, *p*-value using Fisher's Exact Test was used and the computed Phi coefficient was interpreted using Table 1.

Table 1 The index of absolute value ranges.

Absolute value ranges	Strength of association	Interpretation
Below 0.10	Weak	The ELISA result is weakly associated with the diagnosis
0.10-0.30	Moderate	The ELISA result is moderately associated with the diagnosis
Above 0.30	Strong	The ELISA result is strongly associated with the diagnosis

3. Results and Discussion

The samples in this study were taken from four (4) slaughterhouses located at the municipalities of Santa Cruz and San Pablo (A) and in Los Baños and Cabuyao (AA) in Laguna province.

Purposive sampling was done during the experiment wherein a total of 92 pneumonic lungs and blood from the same animals were collected as samples. Each slaughterhouse contributed 23 pigs. Data gathered were analyzed using SAS (Statistical Analysis System) v.9.4 to determine if there was an association between the detected diseases in the blood samples using ELISA (Enzyme-Linked Immunosorbent Assay Test) and the histopathologic changes in the lung tissues collected.

3.1 ELISA Results

Using the ELISA test to determine the antibodies in the blood of the sampled pigs, the presence of the following was detected: APP (*Actinobacillus pleuropneumoniae*), MHY (*Mycoplasma hyopneumoniae*), PRRS, with some suspected cases of pathogens (SUS). These were correlated with the types of histopathological findings observed such as: lymphoid hyperplasia of BALT (lh_balt), inflammatory cell (infcell), hemorrhage (hemor), congestion (conge), absce (abscess), edema (edema), atelectasis (atelec), thin alveoli (thinalv), thick alveoli (thickalv), consolidation (conso), hyperemia (hyper), emphysema (emphy), purulent exudate (puru), fibr (fibrin), serous exudate (serous), catarrhal exudate (catarr), hemorrhagic, and lymphocytic infiltration (lymph).

3.2 Microscopic Characterization of Lesions

Fig. 1 shows the sample animals' histopathological changes in the lungs. Results reveal some cases of lymphoid tissue hyperplasia, inflammation of the cells indicative of acute pneumonia, red blood cells indicating hemorrhage or congestion, fluid-filled alveola indicative of edematous lungs, collapse in the lungs, thin epithelium of the lungs, or thick lung epithelium with multiple layer of alveolar cells, over-inflated alveoli in some parts of the lung suggestive of emphysema, alveolar spaces indicative of hemorrhagic exudates, and tissues indicative of lymphocytic exudates.

Table 2 shows that the presence of PPP (Porcine Pleuropneumonia) was detected in slaughterhouses in Los Baños (13%) and in San Pablo (4.3%). There was none detected in Santa Cruz and Cabuyao slaughterhouses. For the PRRS, all slaughterhouses were found positive, namely 8.7% (2) in the Los Baños slaughterhouse, 4.3% (1) in Santa Cruz, 8.7% (2) in San Pablo, and 8.7% in Cabuyao. The enzootic pleuropneumonia registered the highest among the diseases detected among all four slaughterhouses. There were six cases (26.1%) in Los Baños, three (13%) in Santa Cruz, four (17.4%) in San Pablo, and two (8.7%) in Cabuyao. Los Baños slaughterhouse was detected with the highest presence of diseases (47.8%) or 11 cases among the four slaughterhouses, while Santa Cruz and Cabuyao had the least (17.4%) presence of disease according to the ELISA Test.

For the slaughterhouse in Los Baños, the prevalence of EP (26.1%) was more prominent compared to PPP (13%) and PRRS (8.7%). In relation to the serologic tests, the sampled lungs were detected with the following conditions: lymphoid hyperplasia, hemorrhage, congestion, and thin alveoli. However, there were no presences of abscess, atelectasis, hyperemia, purulent exudate, fibrin, serous exudate, catarrhal exudate, and lymphocytic infiltration in the lungs from the Los Baños slaughterhouse (Table 3). Moreover, there were also inflammatory cells for the PPP, thick alveoli for the PPP and PRRS, consolidation for the EP, emphysema for the PPP and EP, and hemorrhage for the EP.

In Santa Cruz slaughterhouse, there was no PPP positive among the sampled lungs. There were about 4.3% PRRS detected and 13% EP positive. In this slaughterhouse, there was predominantly zero presence of abscess, edema, atelectasis, thick alveoli, consolidation,

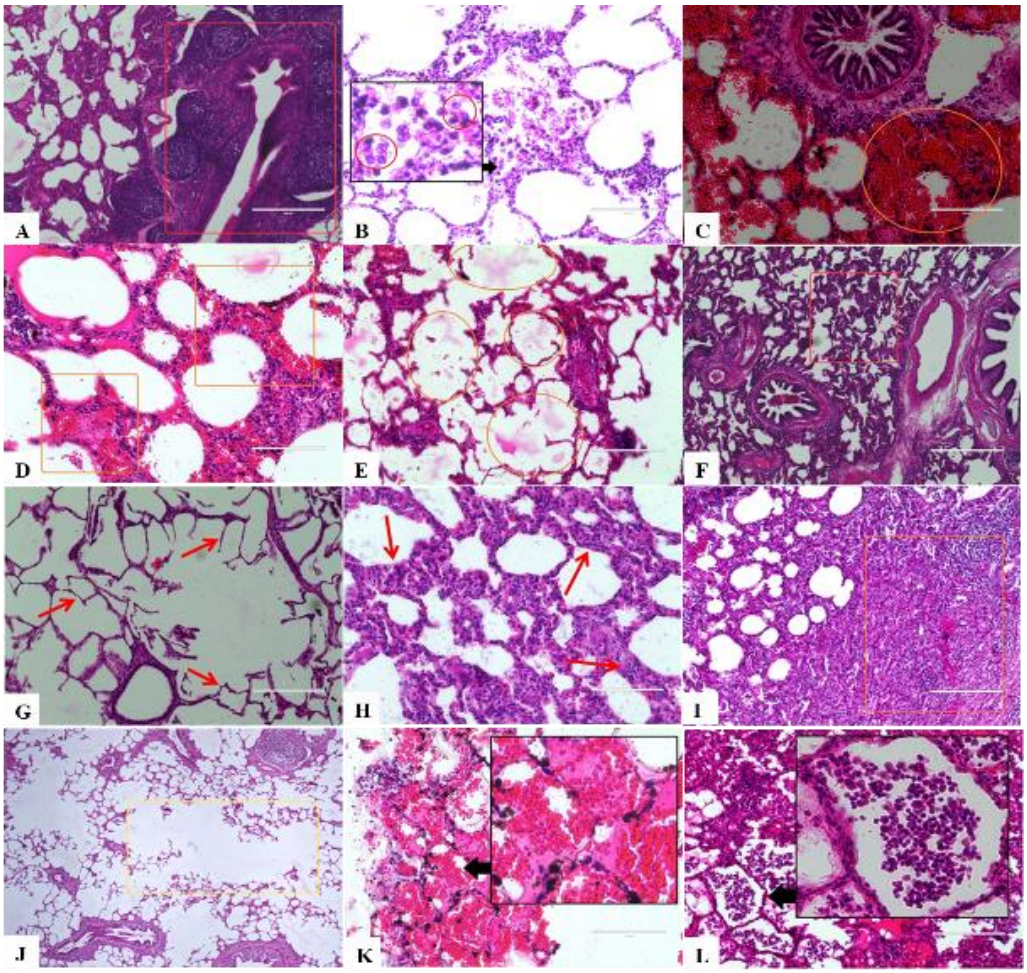

Fig. 1 Histological changes found in the lungs of pigs. (A) Bronchus-associated lymphoid tissue hyperplasia (red box) manifested by peribronchial lymphoid cuffing. H&E. stain. (10×). (B) Presence of inflammatory cells (neutrophils, alveolar macrophages-encircled) in the alveolar spaces of the lung tissue indicative of acute pneumonia. H&E. stain. (100×). (C) Alveolar spaces filled with red blood cells (encircled) indicating hemorrhage. H&E. stain. (40×). (D) Blood capillaries of the alveolar epithelium are engorged with red blood cells (box) indicating congestion. H&E. stain. (100×). (E) Fluid filled alveolus (encircled) indicating pulmonary edema. H&E. stain. (40×). (F) Collapse of alveoli (box) indicating atelectasis. H&E. stain. (40×). (G) Apparently normal lung showing alveolar wall with single layer of cell. H&E. stain. (100×). (H) Alveolar epithelium of the lung appears to be thick (arrows) with multiple layers of cell. H&E. stain. (100×). (I) Consolidation (box) of the lung was showing plenty of cells completely occupying the lung parenchyma with no functional alveolar unit. H&E. stain. (40×). (J) Alveoli appear to be over-inflated (box) in different parts of the lung section suggesting emphysema. H&E. stain. (10×). (K) Alveolar spaces filled with red blood cells, transudates, and few neutrophils (box), indicating hemorrhagic exudate. H&E. stain. (100×). (L) Alveolar spaces filled with lymphocytes (box) and some neutrophils and epithelial cells, indicating lymphocytic exudate. H&E. stain. (100×).

Table 2 Number (%) of seropositive.

Disease	Number (%) Seropositive							
	Los Baños "AA" (n = 23)		Santa Cruz "A" (n = 23)		San Pablo "A" (n = 23)		Cabuyao "AA" (n = 23)	
APP	3	13.0%	0	0%	1	4.3%	0	0%
PRRS	2	8.7%	1	4.3%	2	8.7%	2	8.7%
EP	6	26.1%	3	13.0%	4	17.4%	2	8.7%
Total	11	47.8%	4	17.4%	7	30.4%	4	17.4%

Table 3 Number of lungs positive for histopathologic lesions in relation to seropositivity for APP, PRRS and EP.

Histopthology	Abattoir, name of disease and number (%) of lungs positive											
	Los Baños "AA" (n = 23)			Santa Cruz "A" (n = 23)			San Pablo"A" (n = 23)			Cabuyao "AA" (n = 23)		
	PPP (x = 13.0%)	PRRS (x = 8.7%)	EP (x = 26.1%)	PPP (x = 0%)	PRRS (x = 4.3%)	EP (x = 13.0%)	PPP (x = 4.3%)	PRRS (x = 8.7%)	EP (x = 17.4%)	PPP (x = 0%)	PRRS (x = 8.7%)	EP (x = 8.7%)
Lh-balt	1	1	1	0	1	1	1	1	1	0	1	1
Infcell	1	0	0	0	1	1	0	0	0	0	0	1
Haemor	1	1	1	0	1	1	1	1	1	0	1	1
Conge	1	1	1	0	1	1	1	1	1	0	1	1
Abcess	0	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0
Atelec	0	0	0	0	0	0	0	0	0	0	0	0
Theinalv	1	1	1	0	1	1	1	1	1	0	1	1
Thickalv	1	1	0	0	0	0	0	0	1	0	0	1
Conso	0	0	1	0	0	0	0	0	0	0	0	1
Hyper	0	0	0	0	0	0	0	0	0	0	0	0
Emphy	1	0	1	0	0	0	0	0	1	0	1	0
Exudate	0	0	1	0	0	1	1	1	1	0	1	1
Puru	0	0	0	0	0	1	0	0	0	0	1	1
Fibrin	0	0	0	0	0	0	0	0	0	0	0	0
Serous	0	0	0	0	0	0	0	0	0	0	0	0
Catarrhal	0	0	0	0	0	0	0	0	0	0	0	0
Hemorr	0	0	1	0	0	0	1	1	1	0	1	1
Lymph	0	0	0	0	0	0	0	0	0	0	0	0

n: Total number of samples, *x*: Percentage of positive samples.

hyperemia, emphysema, serous exudate, catarrhal exudate, hemorrhage, and lymphocytic infiltration. There was presence of lymphoid hyperplasia, inflammatory cells, hemorrhage, congestion, thin alveoli, and purulent exudate for PRRS and EP except for PPP disease (Table 3).

The EP (17.4%) showed the highest presence of disease among samples of lungs in San Pablo City slaughterhouse. The PRRS in San Pablo was positive at 8.7% while the APP was 4.3%. The samples were also positive for the following: lymphoid hyperplasia, hemorrhage, congestion, and thin alveoli, exudate. The samples were detected without the presence of the following: abscess, edema, atelectasis, consolidation, hyperemia, purulent exudate, fibrin, serous exudate, catarrhal exudate, and lymphocytic infiltration. Likewise, there was also minor presence of thick alveoli and emphysema in the EP (Table 3).

For the Cabuyao slaughterhouse, no PPP was detected among the samples and both PRRS and EP were 8.7% positive. There following were not detected, abscess, edema, atelectasis, hyperemia, fibrin, serous exudate, catarrhal exudate and lymphocytic infiltration. There were inflammatory cells for the EP, and emphysema for the PRRS.

Overall, the presence of histopathological lesions was not directly associated with the three diseases identified such as *Actinobacillus pleuropneumoniae*, PRRS, and enzootic pleuropneumoniae.

Among the four slaughterhouses in general, the histopathologic lesions identified with each disease were: Lh-balt, Infcell, Hemor, Conge, Atelec, Thinalv, Emphy for the PPP; and Conge, Thinalv, Thickalv. PRRS showed Thinalv, Conso, Emphy, and Exudate (Hemor and Puru) for EP.

The above data were subjected to Fisher's Exact Test and Phi Coefficient wherein the p value of 1.0000 is greater than $\alpha = 5\%$.

Table 4 Histopathological lesions vs. ELISA.

Number	Lesions	PPP	Mhy	PRRS	SUS
1	Lh-balt	11%	39%	32%	3%
2	Infcell	4%	10%	8%	0%
3	Haemor	14%	45%	37%	4%
4	Conge	13%	43%	34%	3%
5	Abcess	14%	45%	37%	4%
6	Edema	0%	0%	37%	4%
7	Atelec	14%	45%	37%	4%
8	Theinalv	13%	36%	37%	3%
9	Thickalv	4%	10%	37%	1%
10	Conso	0%	2%	37%	0%
11	Hyper	14%	45%	37%	4%
12	Emphy	5%	21%	37%	2%
13	Exudate	3%	13%	37%	1%
14	Puru	1%	4%	37%	0%
15	Fibrin	0%	0%	37%	4%
16	Serous	14%	45%	37%	4%
17	Catarrhal	14%	45%	37%	4%
18	Hemorr	2%	9%	37%	1%
19	LYMPH	0%	2%	37%	0%

Table 4 details the summary of the histopathological lesions of the lung samples as against the presence of four diseases, namely PPP, *Mycoplasma hyopneumoniae* (Mhy), PRRS, and other suspected diseases (SUS). The presence of APP among the samples ranges from 0 to 14%, while Mhy ranges from 0 to 45%, the PRRS-positive was almost constant at 37% but with fluctuating values from 8%, 32% and 34%. The suspected diseases had a very low range from 0 to 4%.

In addition, values from *Mycoplasma hyopneumoniae* were the highest among the four diseases, followed by PRRS, then PPP, and then SUS.

4. Conclusions

Based on the results of the study, the researcher concludes that:

(1) Gross lesions of pneumonic lungs could be associated with the presence of adhesions, rib imprints and texture of lung;

(2) Majority of the diseases resulting from ELISA kit were *Mycoplasma hyopneumoniae* and PRRS detected in lung samples collected from different slaughterhouses;

(3) *Mycoplasma hyopneumoniae* was the most predominant type of pneumonic lesion and it was most frequently observed in the middle of both lungs in the form of suppurative bronchopneumonia.

(4) As a general conclusion, the histopathological findings were found to be not significantly associated with the three diseases present in the sample pigs.

5. Recommendations

(1) A regular monitoring that will run for at least one whole year should be done to monitor the trend of the prevalence of subclinical cases in other slaughterhouses in other provinces or regions in the Philippines.

(2) Evaluation of the present management practices of both backyard and commercial farms must be considered in order to reduce the severity of pneumonic lesions;

(3) It is strongly suggested to encourage the farmers to use vaccination to protect animals from respiratory diseases to reduce economic losses.

(4) A comprehensive pathogen profiling of pneumonic lungs that detail all the bacteria, viruses,

and parasites is highly suggested to detect at once the various infections in the lungs of the pigs vis-à-vis their causative agents.

(5) There should be a study on serotyping of the different porcine pleuropneumoniae (PPP), Porcine Reproductive and Respiratory Syndrome (PRRS) and Mycoplasma pneumonia in Laguna, Philippines.

Acknowledgement

The authors would like to thank the Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine, UPLB (University of the Philippines Los Baños) for providing necessary facilities to make this project possible, SEARCA for the funding, and committee chair and members for the unwavering support and insight of this study.

References

[1] PCAARRD. 2016. Livestock Research Division, DOST-PCAARRD S&T Media Service. "Philippines Pork to the World." Accessed February 16, 2023. https://www.pcaarrd.dost.gov.ph/index.php/quick-information-dispatch-qid-articles/philippine-pork-to-the-world.

[2] Philippine Statistics Authority. 2020. Accessed February 16, 2023. file:///C:/Users/Dell/Downloads/Documents/Special-Release-Swine-Situation-Report-April-to-June-2020-signed.pdf.

[3] Australian Centre for International Agricultural Research. 2012. "Philippines soon to be declared totally foot-and-mouth disease-free." Accessed February 16, 2023. https://www.officialgazette.gov.ph/2011/03/17/philippines-soon-to-be-declared-totally-foot-and-mouth-disease-free/.

[4] Thacker, E. 2001. "Immunology of the Porcine Respiratory Disease Complex." Accessed August 1, 2017. https://www.ncbi.nlm.nih.gov/pubmed/11692508.

[5] Hansen, M. S., Pors, S. E., Jensen, H. E., Bille-Hansen, V., Bisgaard, M., Flachs, E. M. and Nielsen, O. L. 2010. "An Investigation of the Pathology and Pathogens Associated with Porcine Respiratory Disease Complex in Denmark." *Journal of Comparative Pathology* 143: 120-31. Accessed March 21, 2016. https://www.ncbi.nlm.nih.gov/pubmed/20181357.

[6] Harding, J. C., and Halbur, P. G. 2002. "PMWS or a Group of PCV2-Associated Syndromes: Ever-Growing Concerns." In *Proceedings of the 17th International Pig Veterinary Society Congress*, pp. 19-31.

[7] Harms, A. P., Halbur, P. G., and Sorden, S. D. 2002. "Three Cases of Porcine Respiratory Disease Complex Associated with Porcine Circovirus Type 2 Infection." Accessed August 01, 2017. https://www.aasv.org/shap/issues/v10n1/v10n1p27.html.

[8] Chae, C. 2015. "Porcine Respiratory Disease Complex: Interaction of Vaccination and Porcine Circovirus Type 2, Porcine Reproductive and Respiratory Syndrome Virus, and Mycoplasma Hyopneumoniae." *The Veterinary Journal* 212: 1-6. Accessed March 21, 2016. https://www.ncbi.nlm.nih.gov/pubmed/27256017[10.

[9] Brockmeier, S. L., Halbur, P. G., and Thacker, E. 2002. "Porcine Respiratory Disease Complex." In *Polymicrobial Diseases*, edited by Brogden, K. A., and Guthmiller, J. M. Washington (DC): ASM Press, Chapter 13. Accessed March 21, 2016. https://www.ncbi.nlm.nih.gov/books/NBK2481/.

[10] Hoeltig, D., Hennig-Pauka, I., Thies, K., Rehm, T., Beyerbach, M., Strutzberg-Minder, K., et al. 2009. "A Novel Respiratory Health Score (RHS) Supports a Role of Acute Lung Damage and Pig Breed in the course of an *Actinobacillus pleuropneumoniae* Infection." *BMC Veterinary Research* 5: 14.

[11] Philippines Law. 1993. "Providing for the Implementing Rules and Regulations Governing the Devolution of Certain Powers and Functions of the National Meat Inspection Commission to the Local Government Units Pursuant to Republic Act No. 7160, Otherwise Government Code Of 1991." Accessed May 4, 2018. https://www.lawphil.net/executive/execord/eo1993/eo_137_1993.html.

[12] Zhou, X., and Moore, B. 2017. "Lung Section Staining and Microscopy." Accessed May 3, 2018. https://www.urmc.rochester.edu/medialibraries/.../core.../histology/.../handeprotocol.p.

[13] Jirawattanapong, P., Stockhofe-Zurwieden, N., Leengoed, L. V., Wisselink, H., Raymkers, R., Cruijsen, T., et al. 2009. "Pleuritis in Slaughter Pigs; Relations between Lung Lesions and Bacteriology in 10 Herds with High Pleuritis." *Research in Veterinary Science* 88: 11-5.

[14] Kong, S. 2017. "Prevalence of Reston Ebolavirus in Swine from Slaughterhouses of Laguna, Philippines Using Real-Time RT-PCR Detection." M.Sc. thesis, University of the Philippines Los Banos, Laguna.

[15] IDEXX APP-Apxiv Ab Test. 2011. "Reliable Screening It's an Economic Necessity." Accessed May 3, 2018. www.idexx.es/pdf/es_es/livestock-poultry/app-apx-iv-ab-ss-eng.pdf.

[16] Thermofisher. 1992. "Mycoplasma Hyopneumoniae Detection Kit." Accessed May 3, 2018. https://tools.thermofisher.com/content/sfs/manuals/X7838.pdf.