

RESEARCH NOTE

DETECTION OF ANTIBODIES AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME AND PORCINE CIRCOVIRUS TYPE 2 IN SMALLHOLDER SWINE FARMS FROM AN ABATTOIR IN SARIAYA, QUEZON, PHILIPPINES

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ABSTRACT

To detect the presence of Porcine Reproductive and Respiratory Syndrome (PRRS) and Porcine Circovirus Type 2 (PCV2) antibodies from pigs raised in smallholder farms in Sariaya, Quezon, a total of 53 blood samples were collected from pigs in the Sariaya Abattoir, Quezon. Blood sera were harvested and then tested for the presence of PRRS and PCV2 antibodies using indirect enzyme-linked immunosorbent assay. This study revealed that out of 53 serum samples, 8 (15.1%) were seropositive for PRRS and 43 (81.1%) were seropositive for PCV2. Based on origin of serum samples, 5 (41.7%) out of 12 barangays studied in Sariaya, Quezon had PRRS-seropositive samples and 11 (91.7%) out of 12 barangays had PCV2-seropositive samples. The results suggest that pigs raised in smallholder farms in some villages in Sariaya, Quezon were infected with these two economically important diseases and can be a threat not only to smallholder farms but also to neighboring commercial farms.

Keywords: antibodies, ELISA, PCV2, PRRS, smallholder farm, swine, Quezon

INTRODUCTION

Porcine Reproductive and Respiratory Syndrome (PRRS) and Porcine Circovirus Type 2 (PCV2) are considered two of the top economically important diseases affecting the swine industry. These viral diseases affect all stages of swine, causing high morbidity and mortality, poor herd performance and increased production costs (Lukert, 1999; Dietze *et al.*, 2011).

In the classification of animal diseases in the Philippines, PRRS is considered a Disease of Farm Concern, *i.e.*, a disease which commonly affects farm animals and its prevention and control are of primary concern of the farm (Department of Agriculture, 2004). It is caused by the PRRS virus, which is a member of the family *Arteriviridae* (Benfield *et al.*, 1999). PRRS affects all stages of pig production. Respiratory signs characterized by dyspnea, tachypnea and deaths are usually seen in infected piglets and grower-finishers while reproductive signs characterized by acute illness with sluggishness and anorexia are seen in sows. Other clinical signs include infertility, agalactia, lowered farrowing rates, increase in late term abortions, and stillborn, mummified or weak live born piglets. Dermatological signs include reddish to blue discoloration and blotching of the

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skin (Dietze *et al.*, 2011). Subclinical infection usually occurs in finishing pigs, boars, gilts and sows.

On the other hand, PCV2 is considered as an Emerging Disease in the Philippines, *i.e.*, a communicable disease which has been recently detected to be present in the country but still confined in limited areas or farms (Department of Agriculture, 2004). The first report of its presence was in 2004 when pigs from four commercial farms showed clinical signs and lesions of PCV2 infection and subsequently PCV2 DNA was extracted (Maldonado *et al.*, 2004). PCV2 is under the genus *Circovirus* of the family *Circoviridae* (Lukert, 1999). It is associated with several swine diseases such as Postweaning Multisystemic Wasting Syndrome (PMWS), Porcine Dermatitis and Nephropathy Syndrome (PDNS), Porcine Respiratory Disease Complex (PRDC), congenital tremors, reproductive failure and enteritis (Lukert, 1999; Carroll, 2005). Clinical signs of PCV2 may vary from subclinical infection to severe infection. General clinical signs of PCV2 infections include rapid weight loss in early finishing with reduced water consumption, pneumonia, diarrhea, enlarged lymph nodes, high mortality, abortion and weak born pigs (Thacker, 2013). PMWS is characterized by progressive wasting of body condition and slow growth performance, causing 100% morbidity and 5-50% mortality in the herd (Carroll, 2005). PDNS severe infection is characterized by anorexia, depression, reluctance to move and sometimes presence of fever (Drolet *et al.*, 1999; Carroll, 2005). PRDC is caused by multifactorial etiologic agents including PCV2, PRRS virus, swine influenza virus (SIV) and *Mycoplasma hyopneumoniae*. Clinical signs exhibited include anorexia and lethargy in pneumonia cases (Harms *et al.*, 2000; Carroll, 2005). Reproductive failure caused by PCV2 is usually associated with late term abortions and increase in stillborn, mummified fetuses and nonviable piglets (O'Connor *et al.*, 2001; Park *et al.*, 2005; Carroll, 2005).

There are various ways to diagnose PRRS and PCV2. Diagnostic procedures include combination of herd medical history, clinical signs presented by the animals, gross lesions, and serology and virus detection such as Polymerase Chain Reaction (PCR), nucleotide or amino acid sequencing and Enzyme-Linked Immunosorbent Assay (ELISA). ELISA is one of the fastest and easiest methods used to detect viral antigens or antibodies (Murphy *et al.*, 1999; Crowther, 2009). The principle involves the binding of the virus and soluble viral antigens from the specimen to capture antibodies. After washing of unbound components, an enzyme-labeled antiviral antibody is added with an appropriate organic substrate for the particular enzyme and the readout is based on the color change of specimen. Quantitative data are gathered by serially diluting the antigen and spectrophotometry is used to measure the amount of enzyme-conjugated antibody bound to the captured antigen.

Since 2006, the Philippines has been continually hit by highly virulent strains of PRRS (Dietze *et al.*, 2011). Estacio (2012) and Tupaz (2013) conducted serological monitoring of PRRS and PCV2 on commercial farms, respectively. These farms have either at least 21 head of adults or at least 10 head of adults and 22 head of young animals (BAS, 2013). In line with this, the BAS (2013) reported that only 36% of total pig stocks in the Philippines came from commercial farms and that the rest are from non-commercial farms or smallholder farms. No studies have been conducted or reported to detect these diseases in smallholder farms in the Philippines. Serological studies for the presence of these two economically important diseases should also be done in smallholder farms because they have even a larger contribution of pig stocks than commercial farms. The result of the study may provide useful information for further studies of these diseases on smallholder setting. Moreover, the results of this study can help swine farmers - both

smallholder and commercial farms, and government veterinary offices design effective control, preventive and eradication programs for these two economically important swine diseases to help improve production performance and profit.

MATERIALS AND METHODS

The study was conducted in Sariaya Abattoir in Sariaya, Quezon. Among the towns in Quezon province, it has the highest population of swine. Just before slaughter, the records of the pigs were examined to determine their origin and if they were raised by smallholder farmers. Blood was collected by venipuncture just before the animals were slaughtered. In case a farmer submitted more than one animal for slaughter, only one of the pigs was chosen as representative. A total of 53 blood samples from 12 barangays of Sariaya, Quezon were collected and used in the study.

Five-milliliter blood samples were collected from each pig using a disposable syringe. The syringes were transported on ice to the diagnostic laboratory of the College of Veterinary Medicine, University of the Philippines Los Baños, College, Laguna. Sera were harvested after 6 hr and stored at -20°C until the samples were tested.

ELISA to detect antibodies against PRRS and PCV2 was performed using commercial test kits (CIVTEST SUIS PRRS E/S, Hipra, Spain and PCV2 Biochek, Smart Veterinary Diagnostics, Holland) following manufacturers' direction. For PRRS, serum with ≤ 20.0 IPRC value was read as seronegative while serum with > 20.0 IPRC value was seropositive for PRRS. For PCV2, serum with ≤ 1070 antibody titer (≤ 0.499 S/P ratio) was considered seronegative for PCV2 while serum with ≥ 1071 antibody titer (≥ 0.500 S/P ratio) was considered seropositive for PCV2.

RESULTS AND DISCUSSION

A total of 53 pigs in the municipal abattoir of Sariaya, Quezon were used to detect the presence of PRRS and PCV2 antibodies using indirect ELISA. The pigs were raised in smallholder farms from 12 barangays of Sariaya, namely: Antipolo, Balubal, Bucal, Canda, Castañas, Gibanga, Lutucan 1, Mamala 1, Montecillo, Pili, Talaan Aplaya and Tumbaga 1. In a parallel study involving knowledge, attitudes and practices (KAP) of smallholder swine farmers in Sariaya, none of the farmers reported vaccination of their pigs against PRRS and PCV2. It was, therefore, presumed that none of the slaughtered pigs was vaccinated against the above diseases.

Out of 53 serum samples, 8 (15.1%) serum samples were seropositive for PRRS and 43 (81.1%) were seropositive for PCV2. The specific barangays with seropositive results for PRRS and PCV2 are shown in the Figure. ELISA cannot differentiate antibodies to field isolates from vaccine-derived antibodies (Yoon *et al.*, 1993), but although the possibility exists that some of the pigs were vaccinated by any of the above diseases, this was unlikely because in a parallel study involving a survey on the KAP of smallholder swine farmers in Sariaya, none of them practiced vaccination against PRRS and PCV2. The presence of maternal antibodies is also unlikely because these do not persist for the lifetime of the animal. Maternal antibodies specific for PRRS virus have been demonstrated to persist in unvaccinated pigs up to 16 weeks of age (Van Alstine *et al.*, 1993), but the pigs in the present study were already finishers, *i.e.*, more than 25 weeks of age. These

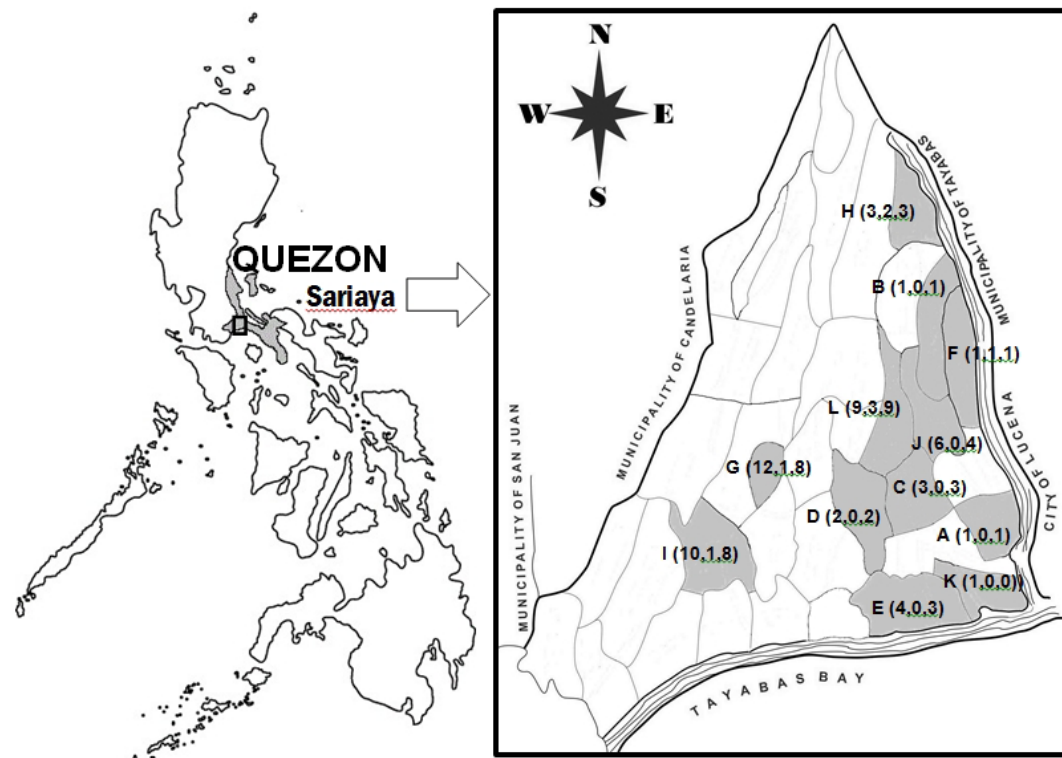


Figure. Map of Sariaya, Quezon showing the villages where the sampled pigs originated (shaded areas). A=Antipolo; B=Balubal; C=Bucal; D=Canda; E=Castañas; F=Gibanga; G=Lutucan 1; H=Mamala 1; I=Montecillo; J=Pili; K=Talaan Aplaya; L=Tumbaga 1. Numbers in parentheses from left to right indicate the total no. of samples, no. of samples seropositive for PRRS antibodies, and no. of samples seropositive for PCV2 antibodies.

results indicate that these pigs have been exposed or currently infected by PRRS and PCV2 virus subclinically because no clinical signs were observed during the conduct of the study. Conversely, the seronegative results have several interpretations: the pigs may not really be infected with virus; they may have been recently infected with virus but have not seroconverted yet; they were infected with the virus some time ago, but have since become seronegative; or the result was falsely negative due to a laboratory error (Collins *et al.*, 1996). As far as the study is concerned, the important information generated are the seropositive results for PRRS and PCV2.

Based on antibody titer, PRRS antibody titer ranged from 0 to 192. The PRRS antibody titer geometric mean of seropositive serum samples is 95.8. Out of 53 serum samples, 22 (41.5%) have 0 PRRS antibody titer, 23 (43.4%) have a trace PRRS and 8 (15.1%) serum samples have >20 PRRS antibody titer. On the other hand, PCV2 antibody titer ranged from 55 (0.0339 S/P ratio) to 8848 (3.4086 S/P ratio). The PCV2 antibody titer geometric mean of seropositive serum samples was 4724.8. Out of 53 serum samples, 10 (18.9%) serum samples have trace PCV2 antibodies and 43 (81.1%) have positive PCV2 antibodies. Absence of PRRS and PCV2 antibodies may suggest that these pigs did not

encounter PRRS and PCV2 field challenge. Trace PRRS and PCV2 antibodies refer to presence of antibodies in the serum at a low amount. This result suggests that these pigs with trace antibodies may have been previously infected by these diseases. Lastly, these results may also mean that these pigs had a concurrent PRRS or PCV2 infection. High antibody titer can be associated with viral field infection. The ELISA results suggest that the slaughtered pigs had subclinical infections.

Based on the origin (barangay) of the serum samples, 5 (41.7%) barangays have PRRS-seropositive results and 11 (91.7%) have PCV2-seropositive results. These results revealed that PRRS and PCV2 were present and possibly continuously infecting pigs in smallholder farms from different barangays in Sariaya, Quezon. This can contribute to the increase in the incidence rate of PRRS and PCV2 infection in the vicinity. In this context, Dietze *et al.* (2011) stated that the spread of highly infectious diseases may be facilitated by middle men who purchase finisher pigs from smallholder raisers then sell them to traders who, in turn, sell them to the abattoir or even other provinces.

There are studies conducted in the Philippines regarding serological profiling of PRRS and PCV2 in pigs but they focused mainly on commercial farm set-up. In a study by Cruz *et al.* (2005) on 167 swine farms in Luzon, 163 or 98% of the farms tested were seropositive for PRRS. Estacio (2012) noted that out of 10 piglets and 10 replacement gilts, 3 piglets were PRRS-seropositive from the start of the experiment and 7 were PRRS-seroconverted (negative to positive) during experiment while in replacement gilts, 9 out of 10 gilts were seroconverted from negative to positive while one pig remained seronegative. In a study by Laranas (2007) in Pampanga, she noted that 63.3% of pigs were positive for PRRS in a commercial swine farm where vaccination was not practiced. On the other hand, Maldonado *et al.* (2004) demonstrated the presence of PCV2 in four commercial swine farms in the Philippines through clinical, pathological and *in situ* hybridization. Tupaz (2013) reported that affected sows can produce PCV2 positive piglets. Based on the results of the present study, the presence of PRRS and PCV2 antibodies is evidence that the pigs sampled had been exposed to the above pathogens. These diseases can be transmitted through direct contact from affected pigs and even transplacental transmission. In addition, as noted by Benfield *et al.* (1999), PRRS tends to circulate indefinitely once it entered the farm. These diseases may have been circulating and affecting pigs raised in smallholder farms but due to lack of studies done, these diseases were not reported.

It is expected that transmission and occurrence rates of the viral diseases studied are higher in smallholder farms compared to commercial farms. Commercial farms have generally better herd health programs such as vaccination and biosecurity measures, and good husbandry management practices which could help lessen incidence of these diseases. On the other hand, smallholder farms have poor biosecurity measures and husbandry management. In a study of smallholder swine farms in Laguna, Ravallo (2008) noted that poor biosecurity measures predisposed animals to respiratory problems. Poor biosecurity practices include uncontrolled human traffic, poor animal handling, lack of sanitation program and opportunities for infectious organism. Smallholder farms usually raise pigs either free range or at the back of their houses. They do not usually vaccinate their pigs because of added cost. Smallholder farms often resort to overcrowding because they tend to stock more pigs in small spaces. Horizontal transmission of disease from infected to non-infected is likely to occur in smallholder farms because all stages of pigs from piglets to finisher are housed together due to lack of space. Poor husbandry practices also contribute to faster transmission of diseases and may house carrier animals. Ravallo (2008) noted that 83% of smallholder farmers acquire their stocks from other swine farms,

and this could contribute to the spread of diseases.

In conclusion, the results of this study suggest that pigs from smallholder farms slaughtered in Sariaya Abattoir were either exposed or subclinically infected by PRRS and PCV2. These results also show that these pigs from smallholder farms are potential carriers of these diseases and may affect other smallholder farms and even commercial farms. It is recommended to educate the smallholder farmers about the PRRS and PCV2 diseases and teach them the ways of preventing and controlling these diseases such as providing good nutrition and supplements to boost pig's immune system and good management practices such as strict hygiene and comfortable housing.

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