SERO-SURVEILLANCE OF HOG CHOLERA IN BACKYARD PIGGERY FARMS IN NUEVA ECIJA AND PAMPANGA, PHILIPPINES

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ABSTRACT

A sero-surveillance study on hog cholera was conducted in the provinces of Nueva Ecija and Pampanga in the Philippines from June to November 2011 utilizing ELISA. Eight to nine samples of blood were gathered from each barangay making up 40-45 representative samples from each municipality and a total of 210 for the entire province. The absence of detectable levels of antibodies in the sera of pigs was the sole basis for evaluating the prevalence of hog cholera. Results of the study show that hog cholera was prevalent in pigs with and without a previous record of hog cholera vaccination. In the province of Nueva Ecija, the prevalence of hog cholera was 50.66% among vaccinated pigs and 31.67% in unvaccinated pigs. In Pampanga, hog cholera had a prevalence rate of 52.60% and 60.71% among vaccinated and unvaccinated pigs, respectively. Data demonstrated that unexpected vaccination failure and non-vaccination of pigs are the main factors that contribute to the prevalence of hog cholera in pigs raised under backyard conditions in the two provinces. This information will be necessary for many animal health practitioners whose advocacy is on the promotion of hog cholera control and prevention.

Keywords: backyard piggery farms, ELISA, hog cholera, sero-surveillance

INTRODUCTION

The Philippine swine industry is a large, continuously growing livestock industry. In terms of volume and value, it is seen as a strong arm of the agricultural sector in ensuring food security for the Filipinos (Argañosa *et al.,* 2000). The per capita consumption of pork among Filipinos is estimated at 13.4 kg (Berro, 2011), which shows that pork is preferred by many Filipinos. The swine population has reached 11,960,000 at the time of the study where 7,750,000 of which come from backyard farms while 4,210,000 from commercial farms (BAS, 2011).

Although it is a developed livestock industry, swine raising is hampered with many problems such as unabated spread of diseases, limited availability and high cost of biologicals, antibiotics and other medications. Management practices, inadequate veterinary facilities, insufficient diagnostic laboratories and lack of a

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nationwide disease surveillance and monitoring systems are reported as contributory factors to production losses (PCAARRD, 1996).

Hog cholera is a highly contagious disease categorized as List A by the Office International des Epizootic. It is endemic in the Philippines and reports on the outbreak of the disease occurred in 14 provinces (BAI, 2002) with a prevalence rate of 30 to 40% based on the confirmatory diagnosis of positive swine serum samples examined by the Philippine Animal Health Center (PAHC) (Diagnostic Services, PAHC, BAI DA Report, 2012). Pampanga and Nueva Ecija were included among those 14 provinces with a hog cholera outbreak making a large part of the swine population reared under backyard operation - 62,627 or 47.49% of 131,864 and 67,838 or 59.92% of 113,212 of total swine populations in Nueva Ecija and Pampanga, respectively at stake (Bureau of Agricultural Statistics, 2011). As hog cholera remained a menace among hog producing areas of the provinces of Nueva Ecija and Pampanga, various speculations on its possible occurrence became widespread even without the affirmation of the animal health authorities like the Bureau of Animal Industry (BAI) and PAHC.

Vaccination activities made in line with hog cholera control program of the BAI were subsequently undertaken in many parts of the country for a span of eight years (Bureau of Agricultural Statistics, 2011) after reports on the outbreak of the disease in the 14 provinces (BAI, 2002) were disseminated. Vaccination against hog cholera has been coordinated and undertaken in the provinces of Nueva Ecija and Pampanga but information that describes the extent of protection accorded to backyard-raised swine after vaccination is limited. This sero-surveillance study was carried out from June to November 2011 in the two provinces to gather information on the status of hog cholera after control and eradication programs have been carried out by animal health representatives from various sectors of the government.

MATERIALS AND METHODS

Sampling

Records of the Veterinary offices of the provinces of Nueva Ecija and Pampanga were reviewed to identify five municipalities in each province with the highest number of swine population operating under backyard production scheme. A survey was made in each of the five municipalities where information on five barangays with the highest number of swine population was generated. Requests and questionnaires that sought information on the name of the animal owner, age of animals sampled and history of vaccination were made before the collection of blood. Backyard piggery farms in the five barangays were assigned with numbers (from 1 to 45). Random selection of farms involved taking those farms represented by numbers divisible by five as sites for blood collection. A total of 8-9 blood samples were taken from each barangay making up 40-45 representative samples from each municipality and a total of 210 for the entire province. Increasing the number of farms and pigs as donors of blood in one municipality was optionally adapted as a measure to compensate for any deficiency in the number of samples in another municipality within a province.

Collection of blood samples

Blood samples were collected from the ear vein of apparently healthy backyard-raised pigs, 3-month-old and above, of both sexes, with the use of non-heparinized vacutainer tubes. Sera were collected from blood samples that were allowed to stand for 2-3 hr at room temperature (RT). Sera were dispensed in sterile vials and stored frozen at -80ºC before analysis. Serum samples were labeled bearing the name of the owner, age of the animal and vaccination record of the animals.

Enzyme-linked immunosorbent assay (ELISA) of antibodies to hog cholera

The test utilized a form of an indirect ELISA kit provided by the PAHC where CSFV or Hog cholera antigen was coated on microtitre plates. Fifty µl of the sample diluent was added to each well before plating the serum samples, the positive and negative control (50 µl) in duplicate wells with reference to the lay-out on a Herd Chek Worksheet. The contents of the wells were mixed gently on a microtitre plate shaker before incubation at RT for 2 hr. The wells were washed three times (Wash solution, 300 µl/well) before adding 100 µl of the anti-CSFV-horseradish peroxidase conjugate. The plates were incubated again at RT for 30 min and washed three times as before. The substrate solution (100 µl/well) was added to each well before incubation of plates at RT for 10 min in a dark chamber. After 10 min, the Stop solution (100 µl/well) was added to prevent further reaction. A pale yellow color which formed inside the wells 10 min after addition of the Stop solution indicated a sero-positive sample while samples with a dark straw color were sero-negative to hog cholera antibody titers. The absorbance of the serum samples was read in an ELISA reader at a dual wavelength of 450 and 620 nm.

The mean optical densities of duplicated serum samples, positive and negative control were recorded. The difference of the mean optical density of the negative control and the optical density of the tested serum sample over the optical density of the negative control x 100% was used to compute for the blocking percentage (%) of each serum sample. The presence of hog cholera antibody titers in serum samples tested was validated by a blocking % greater than or equal to 40 after computation. The absence of hog cholera antibody titers was indicated by blocking % equal to or less than 39.

Evaluation for sero-prevalence of hog cholera

The prevalence of hog cholera among vaccinated and unvaccinated animals was evaluated separately. The prevalence of hog cholera among unvaccinated pigs was derived as a ratio of the number of serum samples from unvaccinated pigs that were negative to antibodies against hog cholera after ELISA over the total number of serum samples from unvaccinated pig donors tested x 100%.

The prevalence of hog cholera among vaccinated pigs was also computed as the ratio of the number of serum samples from vaccinated pigs that were negative to antibodies against hog cholera after ELISA over the total number of serum samples from vaccinated pig donors tested x 100%.

Statistical analysis

The differences on the mean blocking % of sero-positive and sero-negative samples from vaccinated pigs to antibodies against hog cholera were compared statistically using Student's t-test.

RESULT AND DISCUSSION

Prevalence of hog cholera in Nueva Ecija

Table 1 provides a summary of the origin of the 210 serum samples collected from Nueva Ecija. Out of these samples, 150 were taken from pigs with a previous record of vaccination while 60 came from pigs with no history of vaccination.

Table 1. Origin of backyard-raised pig serum donors from the province of Nueva Ecija.

Municipalities	Total number of serum collected	Number of serum from vaccinated pigs	Number of serum from unvaccinated pigs
Muñoz	49	39	10
San Jose	42	37	5
Gapan	35	24	11
Talavera	42	27	15
Jaen	42	23	19
Total	210	150	60

Comparative data on the computed blocking % of serum samples after ELISA demonstrate that out of 150 samples analyzed, 74 were sero-positive while 76 were sero-negative (Table 2). The detection of antibody titers in 74 sero-positive samples was substantiated by significantly higher ($P<0.01$) mean blocking % (71.72±14.05) compared to the mean blocking % (10.92±8.99) of 76 sero-negative samples.

Data in Table 3 show the prevalence of hog cholera among vaccinated pigs in Nueva Ecija. Results reveal that more than half of the total number of sera examined (50.66%) by ELISA lack the antibodies against the disease. These data demonstrate that prevalence of hog cholera is largely contributed by the inability of vaccinated animals to produce and exhibit detectable levels of antibodies in the serum. The non-detection of antibodies in the serum of vaccinated animals may indicate unexpected breaks or failure in vaccination that can hamper normal antibody production.

A summary of the prevalence of hog cholera among unvaccinated pigs in Nueva Ecija is shown in Table 4. Results show that 19 out of the 60 serum samples

Table 2. ELISA-derived blocking % of hog cholera sero-positive and sero-negative samples from vaccinated backyard-raised pigs in Nueva Ecija.

Parameters	Serum samples with Ab titer	Serum samples with no Ab titer
Blocking %	71.72% (±14.05)[a]	10.92 % (±8.99)[b]
Range	41 to 94%	-1 to 39%
Number of serum tested	74	76

Values represent mean blocking % (±standard deviation) of sera from vaccinated backyard-raised pigs after ELISA. Blocking % greater than or equal to 40% indicates antibody titers to hog cholera while blocking % equal to or less than 39% indicates absence of a protective antibody titer. Ab (Antibody).
[a]Significant differences, $P<0.01$.
[b]Total number of serum evaluated = 150.

Table 3. Prevalence of hog cholera among vaccinated pigs in five municipalities of Nueva Ecija.

Municipalities	Number of serum samples from vaccinated pigs	Number of serum samples negative to antibodies against hog cholera	Prevalence Rate (%)
Muñoz	39	12	8.0
San Jose	37	20	13.33
Gapan	24	16	10.67
Talavera	27	17	11.33
Jaen	23	11	7.33
Total	150	76	50.66

examined were sero-negative to hog cholera (31.67%). These data explain that non-administration or non-giving of a regular hog cholera vaccination is contributory to the prevalence of the disease in pigs.

Analysis of 60 samples from unvaccinated pigs revealed that eight were positive to antibodies against hog cholera. This information suggests that hog cholera exists in the area and that natural exposure-mediated production of antibodies like those detected in the eight sero-positive samples may only take place in a small proportion of the population.

Table 4. Prevalence of hog cholera among unvaccinated pigs from five municipalities of Nueva Ecija.

Municipalities	Number of unvaccinated pigs	Number of samples sero-positive to antibodies against hog cholera	Number of samples sero-negative to antibodies against hog cholera	Prevalence rate (%)
Muñoz	10	0	4	6.66
San Jose	5	1	1	1.66
Gapan	11	0	4	6.66
Talavera	15	3	3	5.0
Jaen	19	4	7	11.67
Total	60	8	19	31.67

Prevalence of hog cholera in Pampanga

Table 5 shows a total of 210 serum samples of pigs from Pampanga. Out of that number, 154 came from pigs with history of vaccination while 56 represented pigs which did not receive a prior vaccination against hog cholera .

In the analysis of the 154 serum samples from vaccinated pigs with the use of ELISA, 73 (47.40%) were found positive to hog cholera antibodies while 81 (52.59%) were negative to the antibodies (Table 6). These findings were confirmed by the significantly higher ($P<0.01$) mean blocking % of the 73 serum samples from vaccinated pigs compared to the blocking % of 81 sero-negative samples.

Table 5. Origin of backyard-raised pig serum donors from the province of Pampanga.

Municipalities	Total number of serum collected	Number of serum from vaccinated pigs	Number of serum from unvaccinated pigs
Magalang	42	20	22
Mabalacat	42	38	4
Porac	42	42	0
San Fernando	42	33	9
Arayat	42	21	21
Total	210	154	56

Data on the prevalence of hog cholera among vaccinated pigs in Pampanga are shown in Table 7. Results demonstrate that out of 154 serum samples from vaccinated pigs, 81 (52.60%) did not demonstrate antibody titers to hog cholera. This figure further provides explanation on the contribution and weight of vaccination failure on the prevalence of hog cholera in a relatively large number of pigs not protected by a previous vaccination.

Table 6. ELISA-derived blocking % of hog cholera sero-positive and sero-negative samples from vaccinated backyard-raised pigs in Pampanga.

Parameters	Serum samples with Ab titer	Serum samples with no Ab titer
Blocking %	73.96 (±11.18)[a]	11.99 (±9.17)[b]
Range (%)	52 to 93	-1 to 39
Number of serum tested	73	81

Values represent mean blocking % (±standard deviation) of sera from vaccinated backyard-raised pigs after ELISA. Blocking % greater than or equal to 40% indicates antibody titers to hog cholera while blocking % equal to or less than 39% indicates absence of a protective antibody titer. Ab (Antibody).

[a]Significant differences, $P<0.01$.

[b]Total number of serum evaluated - 154.

Table 7. Prevalence of hog cholera among vaccinated pigs in five municipalities of Pampanga.

Municipalities	Number of serum samples from vaccinated pigs	Number of serum samples negative to antibodies against hog cholera	Prevalence Rate (%)
Magalang	20	12	7.79
Mabalacat	38	26	16.88
Porac	42	16	10.38
San Fernando	33	21	13.63
Arayat	21	6	3.89
Total	154	81	52.60

Table 8 summarizes the prevalence of hog cholera among unvaccinated pigs in Pampanga. Results demonstrate that out of 56 samples examined, 34 (60.71%) were sero-negative to hog cholera antibodies. Data shed light on the impact of not giving vaccinations against hog cholera on the prevalence of the disease in susceptible animals. Detection of antibodies in 16 serum samples of unvaccinated pigs provides explanation on the possible exposure of animals to a low grade or subclinical condition of hog cholera that triggered detectable levels of antibody titers. This finding illustrates that conferment of immunity possibly mediated by a natural exposure to disease may be effective but only to a limited number of animals in the herd.

Table 8. Prevalence of hog cholera among unvaccinated pigs from five municipalities of Pampanga.

Municipalities	Number of unvaccinated pigs	Number of samples sero-positive to antibodies against hog cholera	Number of samples sero-negative to antibodies against hog cholera	Prevalence rate (%)
Magalang	22	4	5	8.93
Mabalacat	4	0	4	7.14
Porac	0	0	5	8.92
San Fernando	9	5	9	16.00
Arayat	21	7	11	19.64
Total	56	16	34	60.71

The application of an indirect ELISA has been used to determine the sero-prevalence of hog cholera in five municipalities of Nueva Ecija and Pampanga utilizing pig farms with the highest number of swine population that operate under backyard production schemes. The prevalence of hog cholera was evaluated based on the absence of protective antibody titers in the sera of pigs. The prevalence of the disease was exhibited by the high rates of sero-negative samples in vaccinated pigs from Nueva Ecija (50.66%) and Pampanga (52.60%). The absence of detectable antibodies in the sera of pigs with a previous record of vaccination indicates susceptibility of animals to the disease as protection has not been accorded by a previous vaccination. These data strengthen the claim of Geerts *et al*. (1995) who described infectious patterns and persistence of hog cholera in infected farms in the Philippines in spite of the administration of vaccination programs. The inability of animals to mount an immunity post-vaccination is often interpreted as a vaccination failure. This is an impending cause for alarm because 51 to 53% of vaccinated pigs remain vulnerable to the disease with the apparent inability to

acquire protection post-vaccination. Results herein presented provide evidence on hog cholera vaccination failure in the local setting. Vaccination failure is seen by experts as a common factor that limits the success of every vaccination program. Several assertions have been raised on the relationship between vaccination failure and hog cholera vaccines given in the field. Various concerns were brought in because of minimal scientific data that support efficiency of hog cholera vaccine, inadequate knowledge on the antigenic property of the vaccine and failure to identify specific part of the immune system that mediate immune reactions (Funk, 1996). The inability of an antigen to stimulate an immunologic function has been considered as a reason for vaccination failure (Aiello, 2003).

Vaccination failure has been reported to go along with immuno-suppression, parasitism, malnourishment in animals, stress, extreme cold, heat, fatigue, poor administration of vaccines, improper storage, chemical sterilization of syringes and excessive use of alcohol on the skin. Other factors such as administration of a vaccine at a time when an animal is already incubating the disease, interference of maternal antibodies for CSF, administration of abused vaccines and poor vaccination practices were all cited as contributory factors to vaccination failure (Too, 2002). A lack of understanding on the immune status of the herd, mechanism of immunological patterns, pathogenesis of hog cholera and its epidemiology have also been cited as causes of hog cholera vaccination failure. To deal with these problems, several factors that were critical for successful hog cholera vaccination in the field have been explored by other investigators (Suradhat *et al*., 2007).

Non-vaccination of animals against hog cholera apparently does not induce antibody production in pigs. This is another cause for apprehension as there is a possibility that 31-60% of unvaccinated pigs like those observed in pigs from Nueva Ecija and Pampanga can acquire the infection under natural conditions. In these conditions, pigs without immunity can survive for long period not necessarily manifesting clinical signs and serve as source of infection for other pigs in the farm, thereby increasing the prevalence of subacute and chronic forms of hog cholera. The mechanisms that mediate the spread of hog cholera among animals and in between farms (Patton and Greiser-Wilke, 2003) and disease transmission through direct and indirect methods in endemic areas (Suradhat *et al*., 2007) have been described.

One significant finding in this study was the detection of antibodies in a limited number of pigs with no history of vaccination. This observation suggests that unvaccinated animals may develop immunity in response to a subclinical form of hog cholera in the environment. Although protective antibody titers can be mounted in some animals, efficient conferment of immunity to a relatively bigger herd size may not be viable.

The foregoing data indicate the significance of a sero-surveillance study in monitoring hog cholera status among backyard-raised pigs. Dissemination of the above data will be important for the incessant attempt of farmers, practitioners and clinicians to control and prevent hog cholera in the region.

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