

Article

First Serosurvey of Porcine Circovirus Type 3 in Central Luzon, Philippines

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Abstract

Porcine circovirus type 3 (PCV3) is an emerging circovirus detected worldwide in pigs with and without clinical disease, including reproductive and systemic disorders. However, baseline information on PCV3 exposure in Philippine swine populations remains limited. A cross-sectional serosurvey was conducted in selected provinces of Central Luzon, Philippines, from May to June 2025. A total of 337 serum samples from slaughter pigs were tested using an indirect enzyme-linked immunosorbent assay based on recombinant PCV3 open reading frame 2 (ORF2) capsid antigen. Samples with sample-to-positive (S/P) ratios ≥ 0.5 were classified as seropositive. Overall, 143 of 337 pigs were seropositive, corresponding to an apparent seroprevalence of 42.4% (95% confidence interval [CI]: 37.1–47.9%). Province-level apparent seroprevalence was 46.1% in Bulacan, 38.0% in Nueva Ecija, and 42.2% in Pampanga, with no significant difference among provinces ($p = 0.470$). However, seroprevalence differed significantly between slaughterhouses in Bulacan (66.1% in Malolos versus 27.3% in Meycauayan; $p < 0.0001$). These findings provide the first serological evidence of PCV3 exposure in slaughter pigs from selected provinces of Central Luzon. Because molecular testing was not performed, the results should be interpreted as evidence of an antibody response to PCV3 rather than confirmation of active infection. Expanded surveillance integrating serology, qPCR, sequencing, farm-level epidemiological data, and clinicopathological investigations is warranted.

Keywords: porcine circovirus type 3 (PCV3); serosurvey; indirect ELISA; Central Luzon; Philippines



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1. Introduction

Porcine circoviruses are small, non-enveloped viruses with covalently closed circular single-stranded DNA genomes classified within the family Circoviridae [1]. Among the four recognized porcine circoviruses, PCV2 is a well-established swine pathogen associated with porcine circovirus-associated disease and major economic losses in pig production [2]. In the Philippines, PCV2 has already been documented in pigs: antibodies against PCV2 were detected in smallholder pigs sampled from an abattoir in Quezon, and a later study confirmed PCV2 infection in grower-finisher pigs [3,4].

PCV3 was first reported in 2016 in pigs with multisystemic inflammation, reproductive failure, and porcine dermatitis and nephropathy syndrome-like disease [5,6]. PCV3 has been detected in domestic pigs and wild suids worldwide, in both clinically affected and apparently healthy animals [7–10].

Although PCV3 has been associated with several clinical conditions, viral detection alone is insufficient to establish disease causation [9,11–13]. Serological surveillance is useful for estimating population-level exposure, particularly in settings where PCV3 has not been well documented. Several PCV3 antibody assays have been developed using recombinant capsid protein or virus-like-particle approaches [14,15]. Detection of PCV3-specific IgG indicates an antibody response but does not establish active infection or PCV3-associated disease [16].

Across Asia, PCV3 has been detected in China, Thailand, Malaysia, India, and other swine-producing settings [17–21]. However, baseline information on PCV3 exposure in Philippine swine populations remains unavailable. Therefore, this study aimed to estimate the apparent seroprevalence of PCV3 antibodies among slaughter pigs from selected slaughterhouses in Bulacan, Nueva Ecija, and Pampanga, Central Luzon, Philippines, and to examine provincial and slaughterhouse-level differences. To our knowledge, this represents the first PCV3 serosurvey in the Philippines and provides baseline information for future molecular and epidemiological investigations.

2. Materials and Methods

2.1. Study Design and Study Area

A cross-sectional serosurvey was conducted in Bulacan, Nueva Ecija, and Pampanga, Central Luzon, Philippines from May to June 2025 (Figure 1).

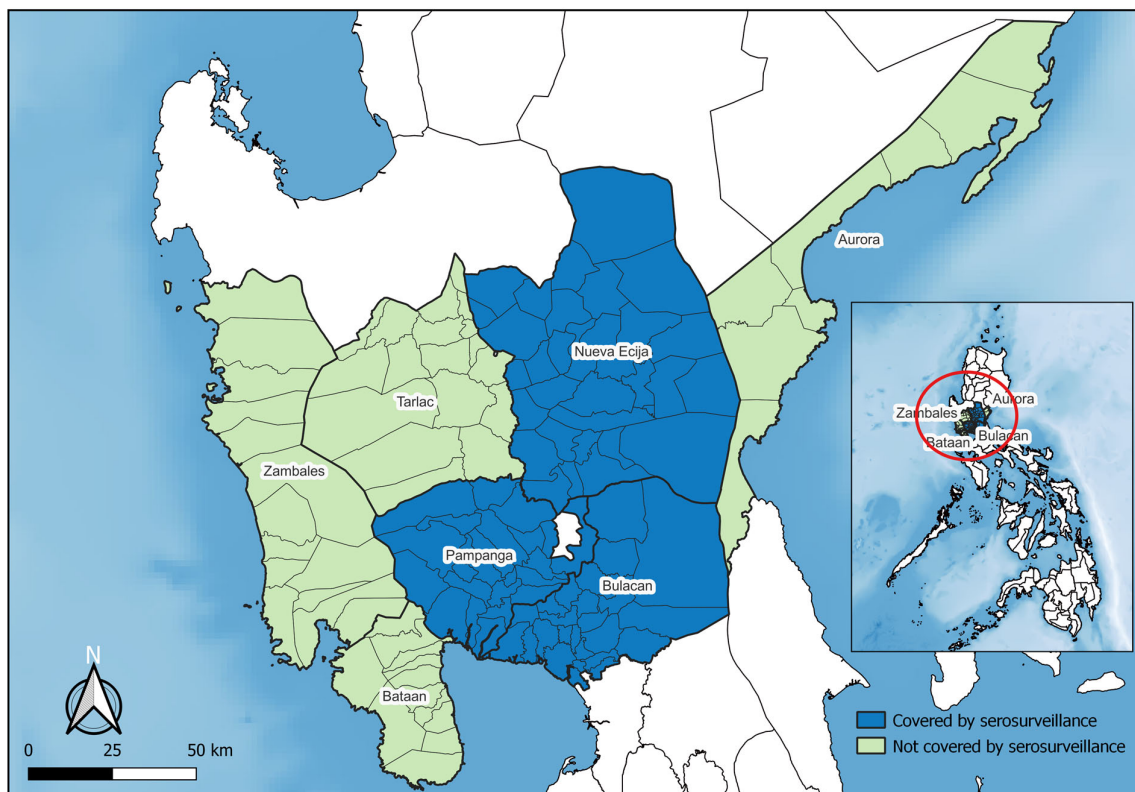


Figure 1. Map of Central Luzon highlighting the provinces of Bulacan, Pampanga, and Nueva Ecija (blue shade), the three provinces included in the serosurvey. The inset map (red circle) shows the location of Central Luzon within the Philippines. Mapping was performed in QGIS version 3.22.10.

2.2. Sampling Sites and Study Population

Serum samples were obtained from 337 slaughter pigs presented at seven participating slaughterhouses. In Bulacan, samples were collected from Malolos and Meycauayan slaughterhouses ($n = 128$). In Nueva Ecija, samples were collected from slaughterhouses in San Jose City, Science City of Muñoz, and Cabanatuan City ($n = 100$). In Pampanga, samples were collected from Angeles City and Magalang slaughterhouses ($n = 109$). The number of pigs sampled at each province is provided in Table 1.

Table 1. Province-level PCV3 seroprevalence among slaughter pigs in Central Luzon, Philippines.

Province	Negative	Positive	Total	Seroprevalence (%)	95% CI (%)
Bulacan	69	59	128	46.1	37.2–55.1
Nueva Ecija	62	38	100	38.0	28.5–48.3
Pampanga	63	46	109	42.2	32.8–52.0
Overall	194	143	337	42.4	37.1–47.9

Within each slaughterhouse visit, pigs were selected from animals presented for routine slaughter, using systematic selection along the slaughter line where daily throughput allowed. Inclusion criteria were routine slaughter eligibility, sufficient sample volume, and availability of minimum animal-level information. Samples with insufficient volume, unsuitable serum quality, or invalid ELISA results were excluded. Sex, age or production category, farm type, and reported place of origin were recorded when available. Breed and source-herd clinical histories were not consistently available.

2.3. Sample Collection and Handling

Approximately 5 mL of blood was collected by jugular venipuncture from selected slaughter pigs before slaughter using sterile red-top Vacutainer® tubes (3-1 America, Seattle, WA, USA). Samples were transported under cold-chain conditions (2–8 °C) for serum separation by centrifugation. Serum was stored at −20 °C until analysis.

2.4. Serological Detection of PCV3 Antibodies

PCV3-specific antibodies were detected using a previously described indirect enzyme-linked immunosorbent assay targeting recombinant PCV3 ORF2 capsid antigen [22]. The assay was performed according to the previously published protocol [22]; antigen production and assay optimization were not part of the present study. Briefly, antigen-coated 96-well plates were incubated at 37 °C for 1 h with diluted serum samples, while positive and negative control sera were included in duplicate on each plate. Following incubation, plates were washed four times with 300 µL/well of phosphate-buffered saline containing 0.1% Tween 20 (PBS-T). Subsequently, 100 µL of horseradish peroxidase (HRP)-conjugated goat anti-pig IgG (Bethyl Laboratories Inc., Montgomery, TX, USA) was added to each well, and the plates were incubated at 37 °C for an additional 1 h. After a second washing step, 100 µL of tetramethylbenzidine (TMB)-hydrogen peroxide substrate solution (Surmodics IVD, Inc., Eden Prairie, MN, USA) was dispensed into each well, and the plates were incubated for 5 min at room temperature in the dark. The enzymatic reaction was terminated by adding 100 µL of stop solution (Surmodics IVD, Inc.) to each well. Optical density (OD) was measured at 450 nm using an Agilent BioTek 800 TS microplate absorbance reader (BioTek Instruments, Inc., Winooski, VT, USA). Antibody responses were expressed as S/P ratios using the following formula: $S/P \text{ ratio} = (OD_{\text{sample}} - \text{mean } OD_{\text{negative control}}) / (\text{mean } OD_{\text{positive control}} - \text{mean } OD_{\text{negative control}})$. Samples with S/P ratios ≥ 0.5 were classified as seropositive. This conservative cutoff was selected to prioritize diagnostic specificity [14,22].

2.5. Data Analysis

Apparent seroprevalence was calculated as the proportion of seropositive samples among all tested samples. Exact binomial 95% confidence intervals (CIs) were calculated for overall and province-level seroprevalence estimates. Differences in seropositivity among provinces and among slaughterhouse sources within provinces were assessed using the chi-square test; Fisher's exact test was used when expected cell counts were small. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using R version 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria). True prevalence was not estimated because reliable diagnostic sensitivity and specificity estimates for the target population were unavailable. Seropositivity was summarized descriptively according to slaughterhouse and available animal-level characteristics, and these analyses were interpreted as exploratory rather than formal risk-factor analyses.

3. Results

3.1. Overall and Province-Level Seroprevalence

Across the three provinces, 143 of 337 serum samples were seropositive for PCV3 antibodies, resulting in an overall apparent seroprevalence of 42.4% (95% CI: 37.1–47.9%). Province-level apparent seroprevalence ranged from 38.0% in Nueva Ecija to 46.1% in Bulacan and did not differ significantly among provinces (chi-square = 1.51, degrees of freedom = 2, $p = 0.470$). Province-level estimates are summarized in Table 1.

3.2. Slaughterhouse-Level Seroprevalence

In Bulacan, apparent seroprevalence was higher in Malolos (66.1%) than in Meycauayan (27.3%) ($p < 0.0001$). No significant slaughterhouse-level differences were observed in Nueva Ecija ($p = 0.660$) or Pampanga ($p = 0.967$) (Table 2).

Table 2. Slaughterhouse-level PCV3 seroprevalence among sampled pigs in Central Luzon.

Province	Slaughterhouse Location	Negative	Positive	Total	Seroprevalence (%)	Within-Province p -Value
Bulacan	Malolos	21	41	62	66.1	<0.0001
	Meycauayan	48	18	66	27.3	
Nueva Ecija	San Jose City	17	13	30	43.3	0.660
	Science City of Muñoz	17	13	30	43.3	
	Cabanatuan City	28	12	40	30.0	
Pampanga	Angeles City	34	25	59	42.4	0.967
	Magalang	29	21	50	42.0	

3.3. Province-Specific Epidemiological Patterns

In Bulacan, seropositivity also differed significantly by reported place of origin, with higher values among pigs originating from Pandi and San Ildefonso than in the reference group. No significant differences were observed by sex or age group.

No significant differences were identified across the evaluated animal-level characteristics in Nueva Ecija or Pampanga. In Pampanga, seropositivity was numerically higher among sows than fattening pigs and among backyard-origin than commercial-origin pigs, although these differences were not statistically significant. The underlying animal-level data supporting these exploratory summaries are provided in the Supplementary Materials (Table S1).

3.4. S/P-Ratio Distribution

Across the three provinces, most S/P ratios were distributed below or above the predefined positivity cutoff (0.5), with relatively few observations near the cutoff (Figure 2).

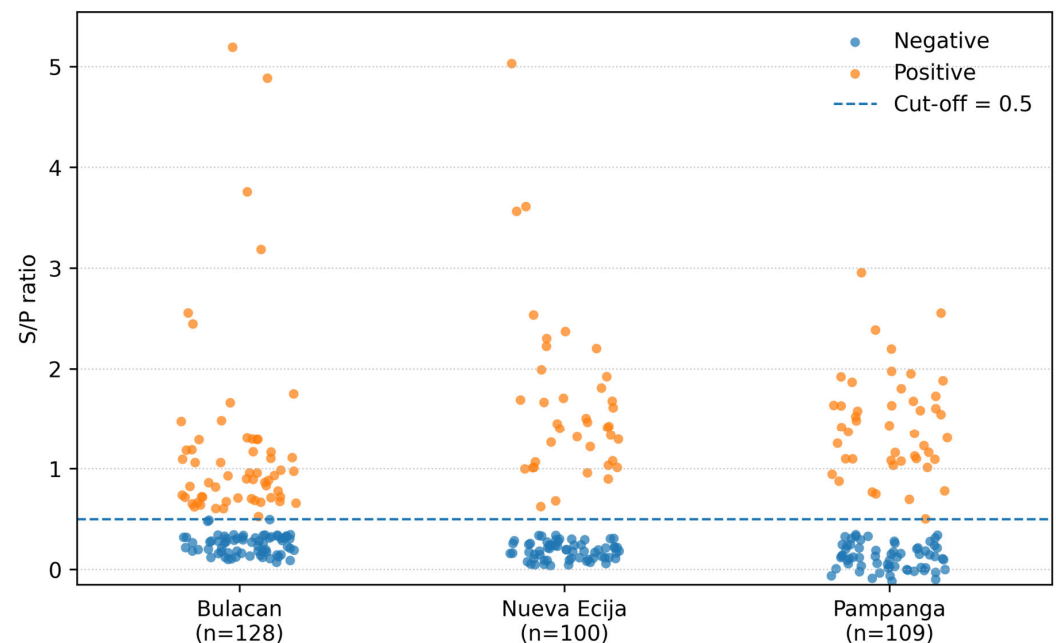


Figure 2. Distribution of PCV3 IgG ELISA sample-to-positive (S/P) ratios among serum samples collected from slaughter pigs in three provinces of Central Luzon, Philippines. Each point represents one serum sample. The dashed horizontal line indicates the ELISA positivity cutoff value of 0.5; samples with S/P ratios ≥ 0.5 were classified as seropositive.

4. Discussion

To our knowledge, this study provides the first serological evidence of PCV3 exposure in slaughter pigs from the Philippines. The overall apparent seroprevalence of 42.4% indicates that a substantial proportion of sampled pigs had detectable antibodies against PCV3. However, detection of PCV3-specific IgG indicates an antibody response and does not establish active infection, viral shedding, or PCV3-associated disease. The detection of PCV3 antibodies in all three provinces is consistent with the global distribution of PCV3 reported in previous studies and reviews [7–10,17–21,23–25]. The apparent seroprevalence observed in Central Luzon was similar to the 42.87% seroprevalence reported in clinically healthy pigs from multiple provinces in China [17] and falls within the broad range reported in other Chinese serological studies, including region-specific estimates of 20–84% in Hunan Province [14]. Retrospective work from Mexico also demonstrated PCV3 DNA and antibody detection in archived samples collected over multiple years [26], while studies from Colombia and Argentina have documented PCV3 circulation and genetic diversity in the Americas [24,25]. Although these reports demonstrate the broad geographic distribution of PCV3, differences in sampling populations, assays, and diagnostic approaches limit direct comparisons. In particular, molecular detection frequencies should not be compared directly with seroprevalence estimates.

The origin and routes of PCV3 circulation in the Philippines remain unknown. Animal movement, breeding-stock exchange, and regional connectivity represent possible pathways for viral introduction or dissemination. Phylogeographic analyses have suggested that PCV3 has moved across Asia, Europe, and the Americas, with Southeast Asian countries implicated in some inferred migration routes [27]. However, these pathways cannot

be evaluated without molecular detection and sequence-based comparisons of Philippine PCV3 sequences.

PCV3 has also been detected in wild boars in Europe and Asia [28,29]. Although wildlife was not investigated in this study, potential domestic pig–wild suid interfaces deserve consideration in future surveillance studies, particularly where free-ranging production systems occur.

No significant differences in apparent seroprevalence were observed among provinces, although this finding does not establish equivalent exposure across the region. In contrast, the significant difference between the Malolos and Meycauayan slaughterhouses in Bulacan suggests local heterogeneity, potentially reflecting differences in the geographic origin or composition of pigs entering each slaughterhouse. The additional association with reported place of origin supports further investigation of animal-source networks. However, slaughterhouse-based sampling and incomplete farm-level information preclude identification of the underlying determinants of these differences.

The numerically higher seropositivity observed among sows in Pampanga was not statistically significant and should be interpreted with caution. PCV3 has been associated with reproductive disorders and detected in reproductive tissues, aborted fetuses, stillborn piglets, and semen [12,22,24,30]. However, the absence of source-herd clinical and reproductive records prevents assessment of potential associations between seropositivity and reproductive disease in the present study. Higher antibody detection in sows could also reflect cumulative exposure with age. Future investigations should include breeding herds, reproductive histories, and molecular testing of clinically relevant samples.

The recombinant capsid-based indirect ELISA provided a practical approach for detecting PCV3-specific IgG antibodies [22]. Capsid- and virus-like particle-based serological assays have been described previously [14,15]. Although relatively few samples had S/P ratios near the predefined cutoff, this distribution does not independently establish diagnostic sensitivity or specificity. Furthermore, IgG detection cannot determine the timing of infection or demonstrate active viral replication. Because reliable diagnostic sensitivity and specificity estimates for the target population were unavailable, the results were reported as apparent rather than true seroprevalence. Complementary serological methods and molecular testing would strengthen future epidemiological investigations.

Although PCV2 vaccination is established in swine production, cross-protection against PCV3 has not been demonstrated [31–33]. Consequently, improved surveillance and diagnostic capacity remain important for assessing PCV3 exposure and its potential clinical significance under field conditions.

This study has several limitations. Sampling was restricted to selected slaughterhouses, limiting representativeness and preventing extrapolation to the broader Philippine swine population. Incomplete farm-level, management, and clinical information precluded formal risk-factor analysis and assessment of PCV3-associated disease. Molecular and pathological investigations were not performed, preventing confirmation of active infection or characterization of circulating strains. The sampling design also did not permit assessment of temporal or seasonal patterns.

Overall, these findings establish a preliminary serological baseline for PCV3 exposure among slaughter pigs from selected provinces of Central Luzon, Philippines. These findings support expanded surveillance integrating serology, qPCR, sequencing, farm-level epidemiological data, and lesion-based diagnostics to characterize PCV3 circulation and clarify its clinical significance in Philippine swine populations.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pathogens15111101/s1>, Table S1: animal-level metadata and ELISA S/P ratios; Data Dictionary, description of variables included in Table S1.

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Institutional Review Board Statement: The province-level studies were reviewed by the Central Luzon State University Ethics Review Committee (ERC) under ERC codes 2025-505, 2025-504, and 2025-506 and approved on 21 May 2025.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study can be downloaded at Supplementary Materials.

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Conflicts of Interest: The authors declare no conflicts of interest.

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