

# Exploratory Investigation on the Occurrence, Spatial Distribution, and Risk Factors of Selected Zoonotic Enteropathogens in Davao City Backyard Farms

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## Abstract

The swine industry is the second largest economic contributor to Philippine agriculture and is dominated by backyard farms, which are plagued by outdated management practices and poor animal health support that promote the spread of pathogens. Zoonotic enteropathogens pose a public health threat, especially to backyard farmers who have daily close contact with the infected animals and their waste. Hence, there is a need to survey such pathogens. This exploratory study generated baseline information on enteropathogen occurrence in backyard farms of Davao City, Philippines; the spatial distribution of affected farms; and the risk factors for enteropathogen occurrence. Protozoans such as *Blastocystis* sp., *Balantidium coli*, *Entamoeba* sp., *Iodamoeba* sp., *Giardia* sp., and coccidia, while helminths such as hookworm and strongylids were identified by direct wet smear. Rotavirus A was detected by reverse transcription-nested polymerase chain reaction. Almost 73% of the farms harbored enteropathogens with mostly asymptomatic infections, and weaners and growers are major carriers. Geospatial analysis identified Barangay Bato in Toril District as a hotspot for the pathogens. Probit regression analysis revealed that use of treatments increased the likelihood of pathogen occurrence by 24%, possibly due to misapplication of medications such as anthelmintics. On the other hand, there was 40% reduced likelihood for farms that use traditional feeds, which can promote gut immunity. Therefore, high-fiber diet can be explored for broad-spectrum protection against enteropathogens. Promoting awareness on the benefits of traditional feeds and education on the proper use of medication are also recommended, especially for vulnerable farms in hotspot areas.

**Keywords:** backyard farms • enteropathogens • parasites • risk factors • rotavirus A

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## Introduction

THE SWINE INDUSTRY is the most important sector of Philippine livestock, with backyard farmers dominating the industry and relying on it for income and food security (Stanton, Emms, and Sia 2010). In particular, Davao Region ranks second next to Bicol Region in backyard swine production (PSA 2016). However, pig production in Southeast Asia is constrained by poor surveillance and control of infectious diseases (Huynh et al. 2007). Considering that backyard producers have limited access to farming information and support services (Stanton, Emms, and Sia 2010) and, unlike their large commercial counterparts, lack awareness on the benefits of disease control, their livestock are deemed vulnerable to infections.

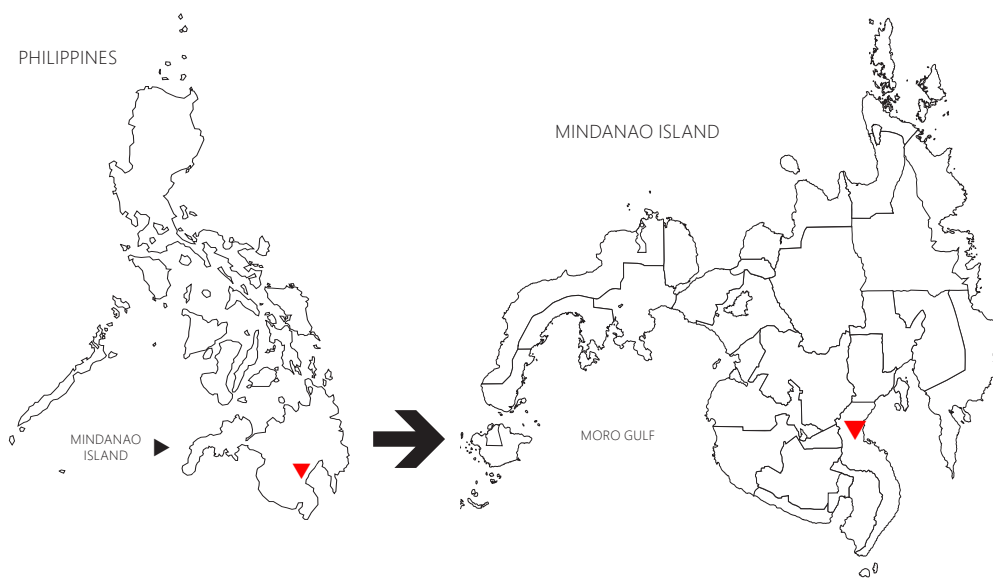
Diarrhea is one of the most common diseases affecting pigs, resulting in economic losses due to morbidity and mortality in suckling and weaned piglets (Ruiz et al. 2016). Various enteric pathogens, including bacteria (*Campylobacter* spp., *Clostridium perfringens*, *Escherichia coli*, *Salmonella* spp.), viruses (rotavirus A or RVA, transmissible gastroenteritis virus or TGEV, and porcine epidemic diarrhea virus or PEDV), and parasites are responsible for this disease (Ruiz et al. 2016). Aside from the economic burden, these

pathogens also have a zoonotic potential, i.e., they can be transmitted from pigs to humans. In fact, almost 60% of human infectious diseases are of zoonotic origin (Taylor et al. 2001), mostly from wildlife, but can also be associated with livestock especially in highly populated areas wherein close contact with animals promotes transmission (Jones et al. 2008; Klous et al. 2016). The intense interaction between animals and humans in a backyard farm setting thereby sets a predisposition towards zoonosis.

Rotavirus A (RVA) is the predominant etiological agent of gastroenteritis in pigs and humans (Vlasova et al. 2017), and there is growing evidence for its zoonotic transmission (Cook et al. 2004). This means that although human and porcine RVA infect specific hosts, cross-transmission to a different host may occur. Meanwhile, zoonotic infections have also been reported for other swine gastroenteritis-causing protozoans such as *Balantidium coli*, *Blastocystis* sp., *Entamoeba* spp., and *Giardia duodenalis* (Olson and Guselle 2000; Solaymani-Mohammadi and Petri 2006) and helminths such as *Ascaris suum*, *Oesophagostomum* spp., and *Trichuris suis* (McCarthy and Moore 2000; Nejsun et al. 2012; Thamsborg et al. 2017).

Enteric pathogens are commonly transmitted through animal wastes, potentially contaminating water and resulting to acute gastrointestinal diseases (Delgado et al. 1999; Catelo et al. 2001). Unfortunately, direct disposal of untreated wastes into rivers and streams is a common practice among poorly managed swine farms in the Philippines (Catelo et al. 2001). Groundwater contamination also poses a risk as the minute size of pathogens allows them to move through soil and eventually reach the water table (Fleming and Ford 2002).

The host-dependence of viruses and parasites makes them convenient targets for interventions to control spread compared to their ubiquitous counterpart, the bacteria. Yet despite the impending socioeconomic burden and public health threat of zoonotic enteric pathogens, there is no active surveillance in Philippine pig farms. Hence, this exploratory study was conducted to provide baseline data on zoonotic enteric viruses and parasites—i.e., RVA, protozoa, and



**FIGURE 1** Location of Davao City in Mindanao, Southern Philippines

helminths—and identify backyard swine farms within Davao City that act as reservoir of the pathogens. Enteropathogen occurrence in animal waste and the distribution of affected farms were evaluated, and risk factors for their circulation were determined to help develop appropriate and cost-effective interventions for pathogen control in these backyard farms.

## Materials and Methods

### Farm Selection

This is an exploratory cross-sectional study of smallholder farms from four districts of Davao City, Philippines (Bunawan, Calinan, Toril, and Tugbok)(Figure 1), which have the highest number of backyard swine farms based on interviews with the Bureau of Animal Industry Region XI, the Office of the City Veterinarian of Davao City, the Davao City Hog Backyard Raisers Association, and various veterinary technicians in Davao City. A database of backyard swine farm owners was generated in coordination with *barangays* (villages) in each district. Each farm was assigned with distinct ID numbers for anonymity.

With a population of 832 backyard farms gathered from the database, a 95% confidence interval, and 10% precision (Pourhoseingholi et al. 2013), the minimum number of farms to be sampled was calculated to be 87. A total of 101 farms were selected through stratified random sampling, wherein the number of farms to be selected per *barangay* was proportional to the overall percent distribution of farms for that *barangay* in relation to the total number of farms in the database. Each farm was selected using the assigned ID through a random number generator. The permission to conduct the survey and sampling was obtained from each *barangay*, and each participating farm was contacted beforehand. The criteria for inclusion were as follows: (1) herd size of 20 heads or less (piglets excluded) (PSA 2016), (2) nonsoil flooring of the pen to prevent soil contamination of fecal samples, and (3) no prior vaccination of piglets against rotavirus. In case of noncompliance with the criteria or refusal to participate, substitute farms were randomly drawn. Supplementary material 1 presents the breakdown of sampled farms for each district.

## Data Collection

A survey was done through questionnaires in order to acquire data on management practices, environmental conditions, and swine fecal information. The unit of analysis of the survey was at the household level. Officials or representatives from the local government accompanied the researchers during the fieldwork to monitor and assist in the activities.

## Fecal Sampling

A total of 101 fecal samples were collected between July and August 2016. The sampling was done in the morning or early afternoon, after the pigs have been fed, to ensure that freshly voided feces was collected. In all instances, only one on-ground feces was present in the pen. For farms with more than one pen, the sample was collected only from the pen with the most number of pigs since a higher probability of finding pathogens is expected in pens with more intense animal interactions. The total number of pigs per farm and number of pigs in the sampled pen were noted as well. The age group of the pigs in the pens was also recorded as nursing with sow, weaner, grower, gilt, finisher, board, or mixed (if heterogeneous ages), but the individual source of the feces could not be identified. Stool consistency was determined by classifying the sample as solid or hard (normal), semi-solid or soft (loose), and watery (diarrheic). Fecal samples were labeled accordingly with the farm ID and date of acquisition and were stored at  $-80^{\circ}\text{C}$ . For parasite detection, an aliquot of the stool samples were collected for direct fecal smear prior to refrigeration.

## RNA Extraction

Fecal suspensions (10% w/v) were prepared using DNA/RNA Shield (Zymo Research, California, USA) (Amimo et al. 2017). The QIAMP viral RNA kit (Qiagen, Hilden, Germany) was used to extract viral RNA from the suspension according to the manufacturer's protocol. The extracted RNA was stored at  $-80^{\circ}\text{C}$ .

## RVA Detection

The RNA was subjected to reverse transcription using the QuantiNova Reverse

Transcription kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The resulting cDNA sample was subjected to nested polymerase chain reaction (PCR) using RVA-specific primers that target the VP6 gene (Elschner et al. 2002; Truong et al. 2013) and the 2X Taq Master Mix (Vivantis, Subang Jaya, Malaysia) according to the manufacturer's instructions. Briefly, the first round of PCR was performed using 1X Taq mix,  $0.5\ \mu\text{M}$  each forward primer and reverse primer (i.e., 5'-AAGATGCTAGAGACAAAATTGT-3' and 5'-AATCAGATTGTGGTGCTATTCC-3'), and  $4\ \mu\text{L}$  of the cDNA for a  $10\ \mu\text{L}$  reaction volume with the following conditions: 2 min at  $94^{\circ}\text{C}$ ; 35 cycles of 30 s at  $94^{\circ}\text{C}$ , 30 s at  $51^{\circ}\text{C}$ , and 30 s at  $72^{\circ}\text{C}$ ; and 5 min at  $72^{\circ}\text{C}$ . The PCR product was used for a second round of PCR employing forward nested primer and reverse nested primer (i.e., 5'-GACAAAATTGTGCGAAGGCACATTATA-3' and 5'-TCGGTAGATTACCAATTCCTCCAG-3') using a similar protocol with the following cycling conditions: 2 min at  $94^{\circ}\text{C}$ ; 35 cycles of 30 s at  $94^{\circ}\text{C}$ , 30 s at  $54^{\circ}\text{C}$ , and 30 s at  $72^{\circ}\text{C}$ ; and 5 min at  $72^{\circ}\text{C}$ . The resulting PCR product was run in 1% agarose gel, stained with GelRed (Biotium, California, USA), and visualized using ultraviolet (UV) light transillumination.

## Parasite Identification

Parasites were identified through direct fecal smear. Fecal samples were randomly poked with a toothpick, which was smeared onto a drop of 0.85% saline solution on a glass slide. The slides were then examined under the microscope (Olympus, Japan) to identify protozoans and helminths. Image J software (National Institutes of Health, Maryland, USA) was used to add scalebars in the micrographs.

## Descriptive Statistics

The occurrence rate was determined for each pathogen and type of pathogen. The percent distribution of pathogens was also determined for the following categories: stool consistency, age group of the pigs from the sampled pen, and location.

## Hotspot Analysis

Hotspot analysis was employed using ArcGIS 10.0 (Esri, California, USA) to examine patterns of points, their spatial distribution in space, and the density of the points within the area using the approach recommended by Asmahani et al. (2010) and Aziz et al. (2012). The average nearest neighbor distance statistical test was used in analyzing the spatial pattern by measuring the distance between an identified centroid and the centroid location of its nearest neighbor. If the index returns a ratio of less than 1, then the points are considered clustered; and if it returns a ratio greater than 1, then the points are dispersed. Once the spatial distribution of points was determined, a mapping technique known as thematic point mapping was implemented to identify areas with potential hotspots (high z score and low p-value), coldspots (low negative z score and low p-value), and no spatial clustering (z score close to 0).

## Risk Factor Analysis

Farm-level occurrence of the enteropathogens was scored based on detection of at least one enteropathogen in the pen. Given that the values of detection (D<sub>xn</sub>) are dichotomous wherein detection of at least one enteropathogen is D<sub>xn</sub> = 1 and nondetection is D<sub>xn</sub> = 0, a probit model was utilized to estimate the effect of various factors on the likelihood of enteropathogen detection. Stata v.13 was used to estimate the corresponding coefficients and marginal effects of the probit model, i.e., the change in enteropathogen occurrence likelihood as the value of a risk factor is increased by one unit (Carter Hill et al. 2011). The marginal effect was calculated as the product of the estimated coefficient and probability density function, which were evaluated at the means (Greene 2008).

## Results and Discussion

### Enteropathogen Occurrence in Backyard Farms of Davao City

This exploratory investigation was undertaken to assess the level of enteropathogen occurrence in backyard swine farms of Davao City. A total of 101 fecal samples were collected from backyard

farms in four districts for farm-level detection of selected zoonotic enteric pathogens. Figure 2 shows a representative RT-nPCR detection of RVA. Meanwhile, six protozoan species (*Blastocystis* sp., *Balantidium coli*, *Entamoeba* sp., *Iodamoeba* sp., *Giardia* sp., and coccidia) and helminths (strongylid) egg were identified (Figure 3). The coccidia was hypothesized to be either *Isospora* sp. or *Eimeria* sp., which have morphologically similar oocysts (Figure 3C). On the other hand, hookworm/strongylid eggs were hypothesized to be *Globocephalus* sp., *Hyostromylus* sp., or *Oesophagostomum* sp. (Figure 3G) and *Strongyloides* sp. (Figure 3H) (Baticados and Baticados 2012; Thamsborg et al. 2017; Ybañez et al. 2017). The thin-shelled egg in Figure 3G has usually developed into the 16–32 cell stage when laid but needs a day or more to hatch, thus differentiation of *Oesophagostomum* sp. and *Hyostromylus* sp. would require fecal culture and identification of ensheated larvae (Greve 2012; Tyagi et al. 2015). Meanwhile, the egg of *Globocephalus* sp. is smaller in size though morphologically similar (Greve 2012; Tyagi et al. 2015). On the other hand, *Strongyloides* sp. eggs are already larvated upon release into the feces (Figure 3H) and hatch within a few hours (Greve 2012; Thamsborg et al. 2017). Nevertheless, correct species identification of these hookworm/strongylid eggs would require isolation of the adult worms from the infected swine. In general, morphological differences between species of coccidians, entamoeba, hookworm, and strongylids are difficult to recognize by direct fecal smear, making microscopy an insensitive

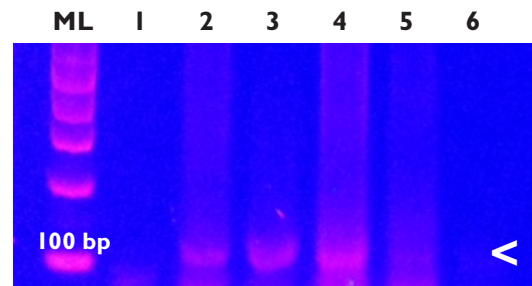
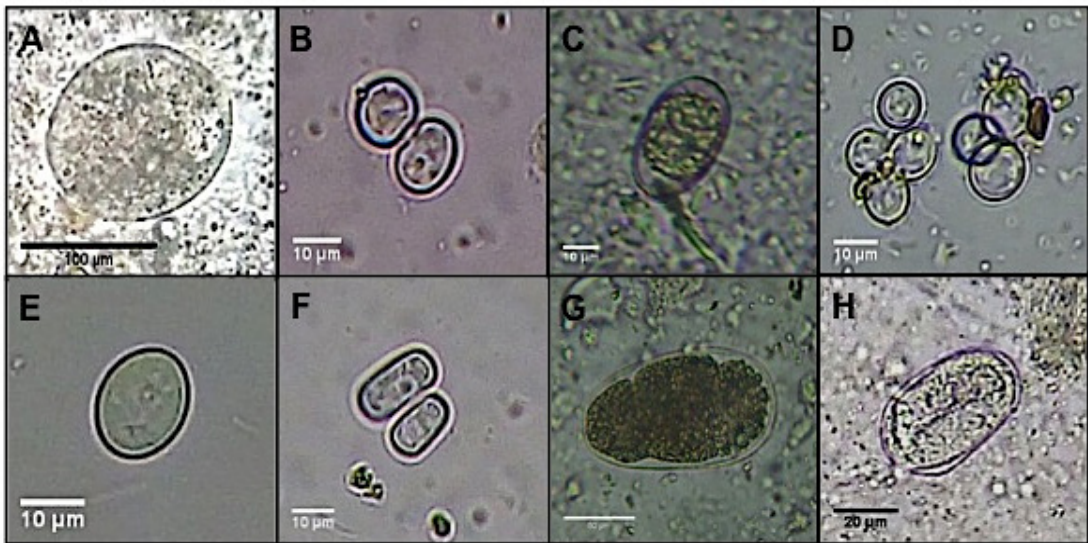


FIGURE 2 Agarose gel electrophoresis of rotavirus A VP6 RT-nPCR in pig stools. 1–6, samples obtained from backyard farms. ML, molecular ladder. Arrow indicates the expected 121-bp band.

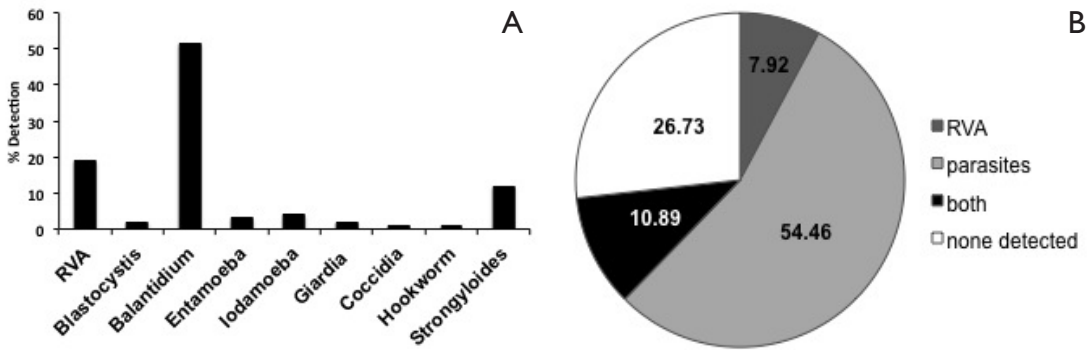


**FIGURE 3** (A) *Balantidium coli* trophozoite, (B) *Blastocystis* sp. cyst, (C) coccidian cyst, (D) *Entamoeba* sp. cyst, (E) *Giardia* sp. cyst, (F) *Iodamoeba* sp. cyst, (G) hookworm/strongylid ovum, and (H) *Strongyloides* sp. embryonated egg identified in freshly voided feces

method in identifying parasites up to species level. Nevertheless, further identification at the species level was not conducted in this study since farm-level occurrence was scored based on the presence of an enteropathogen regardless of its identity. As explained in the proceeding sections, the interest of this study is to find risk factors that apply to all enteropathogens, which could be a practical approach in formulating interventions with broad-scope targets. In future studies, the species identity of these parasites can be explored in the interest of developing pathogen-specific interventions. The direct fecal smear method was selected for detection because of the ease and rapidity of the technique, thereby providing a general and baseline overview on the level of parasite occurrence in farms. Interestingly, most of these pathogens have been reported to have zoonotic potential (McCarthy and Moore 2000; Olson and Guselle 2000; Solaymani-Mohammadi and Petri 2006; Youn 2009; Nejsum et al. 2012; Thamsborg et al. 2017), highlighting their potential spread in the environment through mishandling of animal waste and health threat to backyard handlers.

The most commonly detected enteropathogen was *Balantidium coli* (51%), followed by RVA (19%) and *Strongyloides* sp. (12%) while the

remaining parasites had a low occurrence that ranged between 1%–4% (Figure 4). *B. coli* and *Strongyloides* sp. also constituted the dominant groups of pathogens in commercial swine farms from Cebu, Philippines (Ybañez et al. 2017). *B. coli* is a commensal parasite of pigs, but its high occurrence must not be ignored as it may cause dysentery in humans similar to that of an amebic infection (Nilles-Bije and Rivera 2010). On the other hand, *Strongyloides* sp. is common among piglets due to a highly efficient vertical transmission. The occurrence rate reported in this study is remarkable since this parasite is relatively rare in other countries due to an intensive indoor rearing and high levels of hygiene. Hence, its presence among the surveyed farms indicates that piglets are at higher risk of developing diarrhea, weight loss, growth retardation, and sudden death when infected (Thamsborg et al. 2017). Although *Blastocystis* spp. infection occurred less frequently (probably due to an insensitive detection method), this is the first documentation of its presence among pigs in Davao City and is already considered to be an important emerging zoonotic pathogen (Rivera 2008). Similarly, this is the first report on porcine RVA in the Philippines. RVA is the major etiological agent of gastroenteritis in humans and animals (Vlasova et al. 2017). It



**FIGURE 4** Occurrence rate of selected zoonotic enteropathogens in backyard swine farms of Davao City, Philippines (A) as individual pathogens and (B) as RVA, parasite or both

is believed that there is constant but low-level introduction of animal rotaviruses into the human population (Cook et al. 2004), with specific porcine RVA strains having increased chances of transmission to humans, although the virus has yet to overcome other factors in order to adapt to its new host (Theuns et al. 2016). For example, the virus has to evade the defense systems in the new host and evolve to be the dominant and more viable strain in a quasispecies population (Bwogi et al. 2017). A contrasting farm-level prevalence of 74% has been reported for RVA in the nearby country of Vietnam (Pham et al. 2014), which may be attributed to sampling variations. The Vietnam study included large commercial farms aside from smallholder farms, where farming practices and conditions can vary, such as the highly dense pig populations that may be more favorable to RVA transmission (Dewey et al. 2003). Furthermore, sampling in this study was conducted towards the end of El Niño in 2016, and the extremely dry season may have affected viral circulation. A subsequent surveillance conducted by the authors has also demonstrated that porcine RVA peaks during the cooler months of the year (November to March), but the infection remains random and sporadic during the rest of the year (Murao et al. 2018). Hence, the timing of RVA surveillance should be considered in future studies.

Majority of the farms (55%) harbored parasites alone or in combination with RVA (11%) while a few (8%) had RVA only and 27% were negative for detection (Figure 4). Enteropathogen occurrence in these backyard farms is alarming considering that around 73% are potential reservoirs of

zoonotic agents. Ybañez et al. (2017) reported almost 80% prevalence of parasites in pooled stools from commercial farms in Cebu. Hence, this could be a conservative value considering that negative samples may carry parasites that were not detected by the less sensitive direct fecal smear technique. Nevertheless, these findings are relevant in providing a baseline assessment of enteropathogen occurrence in Davao City backyard farms. Consequently, surveillance research coupled with stratified random sampling is instrumental in identifying farms that, irrespective of their market value, are “important biologically in pathogen transmission and control” (Wilhelm et al. 2016).

### Stool and Pig Characteristics

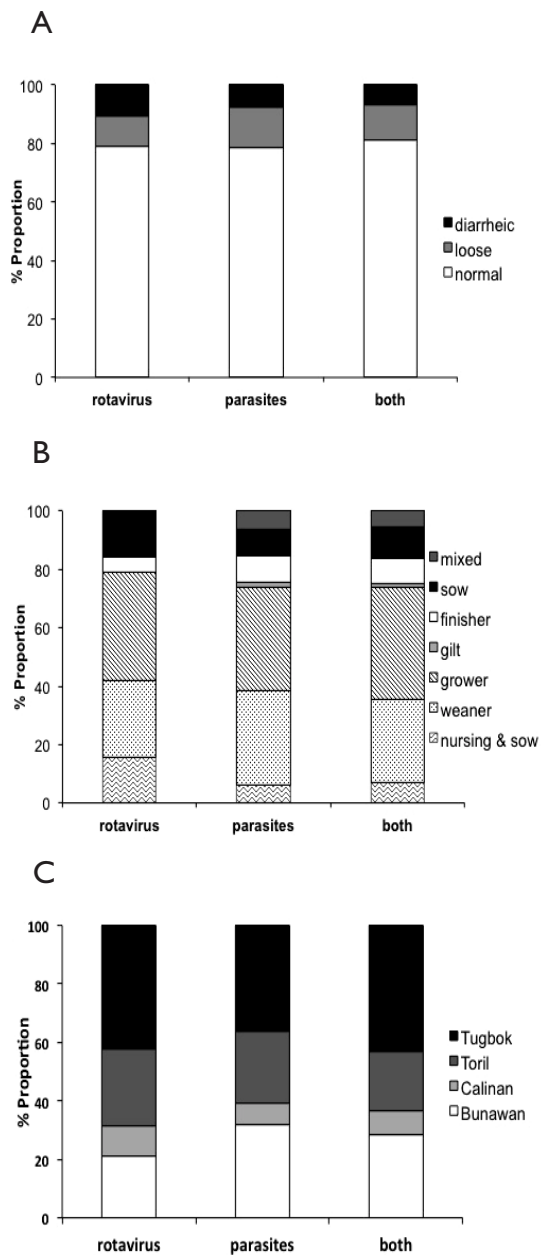
Around 78%–81% of the pathogen-infected stools were nondiarrheic (Figure 5A), implying asymptomatic circulation of these infectious agents. Silent circulation of zoonotic enteropathogens in backyard farms, if left undetected and unmanaged, can lead to persistent infection and spread and potential transmission to humans. These findings imply that undetected occurrence of such pathogens can be very challenging to control. Hence, interventions must include management approaches that are consistently practiced to help reduce the pressure of infection in these farms.

In terms of pig age class, pathogens were predominant in weaners and growers, which constituted 26%–32% and 35%–38% of infected pigs, respectively (Figure 5B). No boar was present in the sampled population. Weaners and growers are popularly raised in backyard farms for their

economic value, and there is constant turnover of weaners in particular, which are commonly sold to other backyard farms. Weaners could therefore play a critical role in farm-to-farm transmission of these pathogens. On the other hand, growers may be prone to reinfection in contaminated farms since they are raised for a longer period in these farms. In fact, a similar study on backyard and commercial farms from Brazil reported highest parasite detection in adults such as breeding boars, sows, and fatteners, and the authors attributed this to the longer stay of adult pigs in the farms, which predisposes them to reinfection (Barbosa et al. 2015).

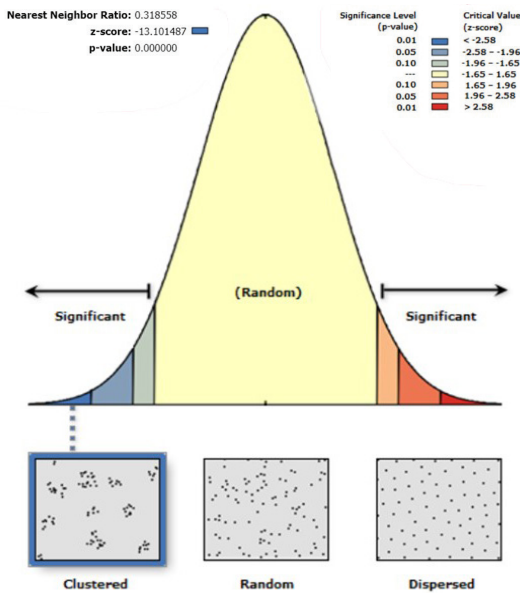
### Spatial Distribution of Affected Farms

The spatial distribution of the affected farms, i.e., farms with at least one enteropathogen, was analyzed. Majority of the enteropathogens detected were from Tugbok District (36%–43%) (Figure 5C) though this could also be attributed to the heavier sampling weight in the area (Supplemental material 1). The average nearest neighbor distance was applied to assess the clustering of affected farms. Our findings report that the nearest neighbor ratio is 0.318558 ( $p = 0.000$ ), indicating that affected farms exhibited a clustered pattern (Figure 6). Using hotspot analysis, the clustered areas were identified. More specifically, Barangay Bato in Toril District is a hotspot (orange spots) with 95% confidence, z scores of 2.0079 to 2.52268, and p-values of 0.011646 to 0.044645 (Figure 7). As a hotspot, Barangay Bato exhibited clustering of enteropathogen-positive farms with higher than average number of affected farms. On the other hand, Barangay Baracatan in Toril District and Barangay Tacunan in Tugbok District showed significant coldspots (dark and medium blue spots) at 95% confidence with z scores of  $-2.89655$  to  $-2.35305$  and p-values of 0.003773 to 0.01862 (Figure 7). Although enteropathogen-positive farms were also clustered in Barangay Baracatan and Tacunan, being a coldspot suggests that the number of affected farms is less than average. Areas in light orange, light blue, and beige did not exhibit significant clustering (z scores between  $-1.96$  to  $1.96$ ). The strong spatial cluster in Barangay Bato can be interpreted as increased occurrence or more intense transmission of



**FIGURE 5** Distribution of enteropathogen-positive stools in terms of (A) stool consistency, (B) pig age class, and (C) location





**FIGURE 6** Average nearest neighbor summary report of enteropathogens in backyard swine farms in four districts of Davao City, Philippines.

Given that the z-score of  $-13.10$ , there is a less than 1% likelihood that this clustered pattern could be the result of random chance.

enteropathogens compared to other areas (Lessler et al. 2017). This could be due to local practices such as exchange of farm animals and materials, lack of biosecurity measures, the presence of potentially contaminated water bodies, or the high density of farms in the area. Future studies should look into these locale-specific factors that heighten the occurrence or transmission among backyard farms. Furthermore, the stability of these hotspots should be monitored over time. Nevertheless, these findings call for intensified interventions that should be targeted in these areas. Although farms at greatest risk such as in Barangay Bato are of primary concern, targeting coldspots may also be useful especially when the infection has already spread from hotspot areas (Lessler et al. 2017).

### Backyard Farm Conditions and Practices

To have a better perspective of backyard farm conditions and practices, a questionnaire-based survey was conducted (Table 1). Majority of the farms raised very small herd sizes of one to five pigs

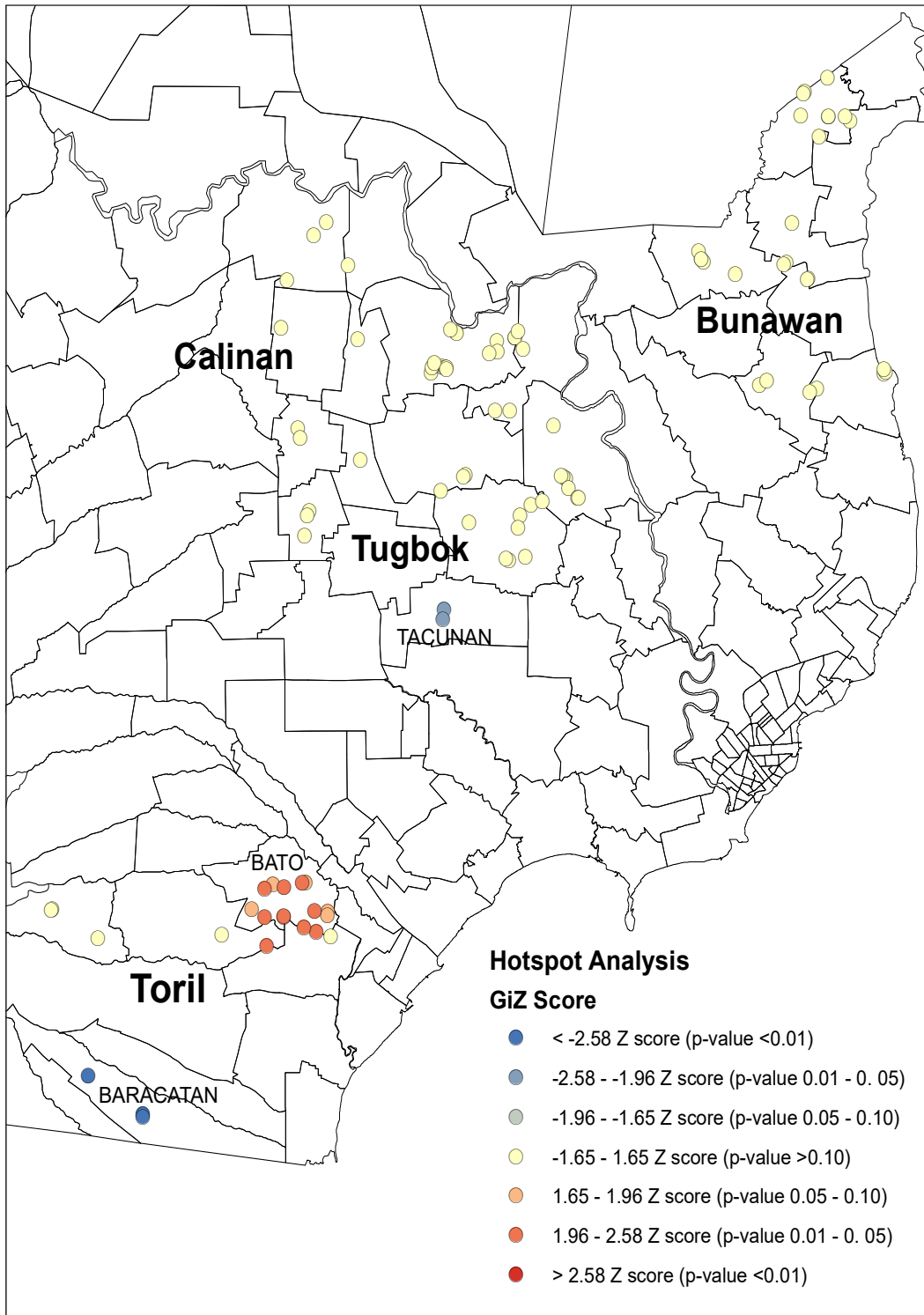
(64%), and the all-in-all-out approach for animal flow (83%) was popularly practiced as opposed to the continuous system which mixes pigs of different ages. According to Dewey et al. (2003), farms that have larger herd size and practice the all-in-all-out system are more likely to have RVA infection, probably due to the intense interaction between animals. Other enteropathogens may be affected in a similar manner.

Administration of treatments and nutritional practices were also surveyed. Around 82% of the farms administered various forms of treatment such as supplements (69.3%), antibiotics (43.6%), anthelmintics (34.65%), and vaccines (34.65%). Use of pure commercial feeds or mixed with traditional feeds such as forage and bran was most common (88%). Traditional feeds are rich in dietary fibers (Van Soest 1978) that are known to have immune-enhancing effects (Schley and Field 2002).

On the other hand, enteropathogens may be transmitted through contaminated food or water. Pigs were usually fed through shared troughs (74%), extending the possibility for cross-contamination via food. In the case of water, this was mainly sourced from the faucet (71%), which is deemed to be relatively clean but served also in troughs (67%) instead of drinking nipples, again posing a threat for cross-contamination.

Sanitation has been associated with reduced infections in pig farms (Martelli et al. 2017). Around half of the farms in this study practiced standard physical or mechanical cleaning while others employed additional cleaning methods such as disinfection and desiccant use. Most farms were equipped with a drainage system (71%), and half had a septic tank for waste disposal (47%). The rest of the farms directly disposed the waste on the ground or through composting.

The pen structure and environmental conditions of the farms were also assessed. An experiment demonstrated that calves release more *Cryptosporidium* oocysts when in confined housing compared to boxed stalls probably due to the stressful conditions of the former (Graef et al. 2018). To assess the housing conditions in this study, the type of housing was surveyed. The pig pens were made either of wooden or cemented wall, and one farm used metal walls. Most pens



**FIGURE 7** Hotspot analysis of enteropathogens in backyard swine farms in four districts of Davao City, Philippines

**TABLE 1** Farm characteristics and practices of backyard swine farms in Davao City, Philippines

Category	Variable	% distribution
Herd size	1 to 5 pigs	64.4
	> 5 pigs <sup>a</sup>	35.7
Animal flow	Continuous	16.8
	All-in-all-out <sup>a</sup>	83.2
Treatment	Administered	82.2
	Not administered <sup>a</sup>	17.8
Feeding material	Traditional feeds	11.9
	Commercial feeds or mixed <sup>a</sup>	88.1
Mode of feeding	Individual feeding	25.7
	Shared feeding <sup>a</sup>	74.3
Water source	Faucet	71.3
	Other water sources <sup>a</sup>	28.7
Mode of drinking	Nipple drinking	32.7
	Trough drinking <sup>a</sup>	67.3
Cleaning methods	Physical cleaning only <sup>a</sup>	49.5
	Plus other cleaning methods	50.5
Drainage	With drainage	71.3
	No drainage <sup>a</sup>	28.7
Waste disposal	Septic tank	46.5
	Other methods <sup>a</sup>	53.5
Pen wall material	Cement	52.5
	Wood or metal <sup>a</sup>	47.5
Roofing of pen	100% roofing	64.4
	< 100% roofing <sup>a</sup>	35.6
Fencing of pen	With fence	12.9
	No fence <sup>a</sup>	87.1
Vegetation enclosure	With vegetation barrier	96.0
	No vegetation barrier <sup>a</sup>	4.0
Presence of poultry aside from swine	Poultry only	50.5
	Mixed with other animals or none <sup>a</sup>	49.5
Presence of dog aside from swine	Dog only	39.6
	Mixed with other animals or none <sup>a</sup>	60.4

**NOTE:** <sup>a</sup> Reference category for risk factor analysis

were completely enclosed by a roof (64%), which is expected to keep the pigs healthy by keeping them protected from extreme weather conditions such as heat or rain. Most of the farms lacked a fence (87%), which can serve as a biosecurity measure, but were instead surrounded by vegetation barrier (96%). The presence of other animals was common in these farms, and these could be potential external sources of infection. Around half had poultry alone aside from pigs, but fewer farms (40%) had dogs alone aside from the pigs.

### Risk Factor Analysis for Enteropathogen Occurrence

To identify the factors associated with enteropathogen occurrence in these backyard farms, probit regression analysis was conducted using the variables listed in Table 1. The analysis was conducted for enteropathogens in general, i.e., farms that had at least one type of pathogen, in order to help identify risk factors that apply to a wide range of pathogens. This comprehensive approach is practical for backyard farmers who would rather invest on a single intervention that has a broad target instead of focusing only on a specific pathogen.

The reference categories for the probit model are indicated in Table 1. The statistical measures that evaluate the model are presented in Table 2. The p-value of the Wald  $\chi^2$  statistic for the probit model (0.0780) was statistically significant at  $\alpha = 0.1$ . Based on a cutoff value of 74% for the actual detection rate, the percentage of correct predictions for the model was 67.33%, with 67.57% sensitivity and 66.67% specificity (data not shown).

Table 2 further shows the variables tested, the estimated coefficients, and their corresponding marginal effect estimates. The calculated values of the variance inflation factors (VIF) for each of the independent variable were less than 10 (data not shown), indicating no degrading collinearity (Kennedy 2008). From the probit results, only two variables had significant association ( $p \leq 0.05$ ) with enteropathogen occurrence: treatment administration and traditional feeds, with the former having positive association while the latter had negative association.

Backyard farms that administer treatments such as vaccines, antibiotics, antihelminthics,

or supplements have an increased probability of enteropathogen occurrence by 24% ( $p = 0.023$ ) compared to farms that do not use treatments (Table 2). Contrary to the common notion that the use of vaccines, antibiotics, and anthelmintics prevent infections, administration of treatments in this study was associated with an increased probability of infection. This outcome could not have been driven by the use of vaccines or antibiotics since none of these types of medications targeted the pathogens being investigated. Furthermore, the effect of supplements could not be ascertained as the survey was conducted only once, hence consistent application of supplements was not documented. Continuous intake of proper supplements is necessary to provide a significant improvement in the nutrition and health of the pigs (De Vos et al. 2014). Instead, it is hypothesized that the positive-association of treatment use and enteropathogen occurrence may be due to misapplication of anthelmintics that could have driven the emergence of resistant parasites. A survey in Pakistan has revealed that livestock farmers had very limited knowledge on parasitic infections, and this was further exacerbated by incorrect use of anthelmintics in the manner of low dose and frequency (Saddiqi et al. 2012). Such practices can indeed lead to development of anthelmintic resistance. In fact, levamisole- and benzimidazole-resistant *Oesophagostomum* species have already been reported in pig farms (Gerwert et al. 2002). Interestingly, 13% of the farms in this study harbored helminths despite the use of anthelmintics by 34% of those surveyed (data not shown). Anthelmintic resistance was not directly assessed and details on anthelmintic practices by farmers were not surveyed in this study; hence, they are worth exploring in future studies to verify this hypothesis. Nevertheless, promoting awareness and education on parasitic pathogens and the proper use of medications can prove to be relevant and beneficial to these farms.

In contrast, farms that use pure traditional feeds had a lower likelihood of enteropathogen occurrence by 40% ( $p = 0.000$ ) compared to those that use commercial feeds (pure or mixed) (Table 2). Traditional feeds include forage such as bran, grains, beans, root crops, fruits, and vegetables, and leftovers including kitchen waste

**TABLE 2** Parameter and marginal effect estimates of variables under the probit model

Category	Variables	Estimates	p-values	Marginal effects	p-values
Farm characteristics	Herd size of 1–5 pigs	0.777	0.062	0.206	0.054
	Continuous animal flow in pens	0.271	0.550	0.072	0.549
Treatment and feeds	With treatment	0.918*	0.034	0.243*	0.023
	Traditional feeds	1.499*	0.002	−0.397*	0.000
	Individual feeding	−0.414	0.325	−0.11	0.318
Water	Faucet as water source	0.124	0.738	0.033	0.736
	Drinking with nipples	−0.175	0.666	−0.046	0.662
Sanitation and waste management	Physical plus other cleaning methods	0.300	0.343	0.079	0.345
	With drainage	−0.0006	0.999	−0.001	0.999
	With septic tank	−0.114	0.735	−0.03	0.733
Physical condition of pen	Cemented wall	0.058	0.900	0.015	0.899
	100% roofing	0.753	0.060	0.199	0.052
Environmental condition	Enclosed with fence	0.678	0.171	0.18	0.171
	With vegetation barrier	−0.96	0.179	−0.254	0.171
	With poultry only aside from swine	0.510	0.112	0.135	0.1
	With dog only aside from swine	0.321	0.340	0.0845	0.338
	Constant	−0.456	0.594		
n	101				
AIC	128.9204				
BIC	173.3774				
Wald $\chi^2$ (16)	24.56				
Prob > $\chi^2$	0.078				
Pseudo R <sup>2</sup>	0.1906				

**NOTE:** \*significant at  $p < 0.05$

and animal entrails (FAO 2009). Cereal bran, particularly corn bran, locally known as *tahop*, is a common noncommercial feed used among the surveyed farms. Brans are sources of less fermentable dietary fibers (Van Soest 1978), which have been shown to increase gut mucin excretion of many species, including pigs (Montagne et al. 2003). Mucin may serve as a highly specialized barrier against penetration by invading organisms such as restraining protozoan colonization by blocking surface Gal/GalNac-specific lectins of the parasite (Hicks et al. 2000) or deterring helminth infection by releasing modified mucin that will prevent the parasite from locating the host using signals from host surface mucus (Theodoropoulos et al. 2001). Although some farmers mixed commercial feeds with forage or

leftovers (Table 2), this may not have been enough in providing a significant amount of dietary fibers compared to a strict noncommercial feed diet. Unfortunately, only 12% of the backyard farms use pure traditional feeds (Table 2), indicating the need for awareness among farmers on the protective benefits of this type of nutrition.

## Conclusions and Recommendations

This was an exploratory study aimed at generating baseline information on zoonotic enteropathogens in backyard swine farms of Davao City, Philippines. Preliminary identification of parasites revealed the presence of zoonotic parasites such as protozoans and helminths along with RVA, although the

parasites constituted the predominant group. A high occurrence of enteropathogens (73% of the farms) in the absence of symptoms poses a threat to backyard handlers due to the intense interaction with the animals and potential contamination of the environment by animal waste without the farmers even being aware of it. Weaners and growers, the pig age groups predominantly raised in backyard farms, were found to be the major carriers of the pathogens. Barangay Bato was identified as a hotspot area for enteropathogens, which makes it an ideal location for targeted and intensified interventions. Such interventions must be formulated appropriately, and for this purpose, identification of risk factors that apply to a broad range of pathogens may be practical for the resource-limited backyard farmers. The use of treatments in these backyard farms increased the risk for enteropathogen occurrence. Although this is counterintuitive with the recognized benefits of treatments, it is hypothesized that misapplication of medications such as anthelmintics by the farmers is driving the emergence of drug resistance. On the other hand, traditional feeds, which are known to be rich in fiber for gut immunity, decreased the likelihood of enteropathogen occurrence; hence, it is highly recommended as a preventive strategy for pathogen control. Unfortunately, only 12% of the farmers used traditional feeds. Therefore, awareness and education on the proper application of medications and the protective benefits of traditional feeds should also be addressed.

The findings of this study provide useful information for zoonotic enteropathogen control in backyard farms of Davao City and can be a springboard for further research. For example, a larger population can be investigated in future studies, such as in the identified hotspot area of Barangay Bato. Furthermore, more sensitive diagnostic techniques can be used to provide a comprehensive picture of enteropathogen occurrence, as well as for specific identification of the parasites. The temporal aspect of enteropathogen occurrence should also be considered to check for seasonality of infections and stability of hotspots. Finally, the role of anthelmintic use on enteropathogen occurrence and emergence of anthelmintic resistance are certainly worth exploring in future studies.

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## Ethical Declaration

Direct handling or treatment of animals were not involved in this study.

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**Supplementary Material 1.** Stratified random sampling of backyard swine farms in Davao City, Philippines

District/ Barangay	Farms in database		Sampled farms	
	Number of farms	Percent of total database	Number of farms	Percent of total sampled
<b>Bunawan</b>				
Gatungan	42		5	
Ilang	26		3	
Mahayag	40		5	
Mudiang	36		4	
San Isidro	72		9	
Total	216	25.96	26	25.74
<b>Calinan</b>				
Dacudao	33		4	
Riverside	19		2	
Talomo River	7		2	
Total	59	7.09	8	7.92
<b>Tugbok</b>				
Bato	125		15	
Baracatan	28		3	
Bayabas	17		2	
Eden	21		3	
Total	191	22.96	23	22.77
<b>Tugbok</b>				
Biao Escuela	13		2	
Biao Guianga	21		2	
Los Amigos	21		3	
Matina Biao	67		8	
New Carmen	58		7	
New Valencia	20		2	
Tacunan	17		2	
Talandang	149		18	
Total	366	43.99	44	43.56
Grand total	832	100.00	101	100.00