

# Seroprevalence of economically important viral pathogens in swine populations of Trinidad and Tobago, West Indies

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Received: 12 October 2016 / Accepted: 25 April 2017 / Published online: 18 May 2017  
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**Abstract** The objective of this study was to evaluate the seroprevalence and identify the strains of swine influenza virus (SwIV), as well as the seroprevalence of porcine parvovirus (PPV), transmissible gastroenteritis virus (TGEV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine respiratory coronavirus (PRCV), porcine circovirus type 2 (PCV-2), and classical swine fever virus (CSFV) in pigs in Trinidad and Tobago (T&T). Blood samples (309) were randomly collected from pigs at farms throughout T&T. Serum samples were tested for the presence of antibodies to the aforementioned viruses using commercial ELISA kits, and the circulating strains of SwIV were identified by the hemagglutination inhibition test (HIT). Antibodies against SwIV were detected in 114 out of the 309 samples (37%). Out of a total of 26 farms, 14 tested positive for SwIV antibodies. HI testing revealed high titers against the A/sw/Minnesota/593/99 H3N2 strain and the pH1N1 2009 pandemic strain. Antibodies against PPV were detected in 87 out of the 309 samples (28%), with 11 out of 26 farms testing positive for PPV antibodies. Antibodies against PCV-2 were detected in 205 out of the 309 samples tested (66%), with 25 out of the 26 farms testing positive for PCV-2 antibodies. No antibodies were

detected in any of the tested pigs to PRRSV, TGEV, PRCV, or CSFV.

**Keywords** Seroprevalence · Trinidad and Tobago · Porcine parvovirus · Porcine circovirus type 2 · Swine influenza A virus

## Introduction

Monitoring and surveillance for viruses that affect swine are crucial in the prevention and control of diseases that can cause severe economic losses to the swine populations of Trinidad and Tobago (T&T) and the wider Caribbean region. To date, there have been limited or no published studies investigating the prevalence of viruses circulating in T&T's swine populations. In late 2015, according to the World Animal Health Information System (WAHIS) database, Columbia reported to have experienced classical swine fever (CSF) limited to one or more zones and porcine reproductive and respiratory syndrome (PRRS) was reported to be present in Columbia and Suriname. Viruses such as classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and swine influenza virus (SwIV) have been reported to be circulating in Venezuelan swine herds (Kwiecien 2013). The close proximity (7 km) of Trinidad to Venezuela, and the known illegal trade of animals and animal products between the two countries, renders Trinidad a high-risk location for the introduction of these viruses.

The objectives of this study were to assess the prevalence of selected viruses, namely porcine parvovirus (PPV), transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus (PRCV), CSFV, PCV-2, PRRSV, and SwIV in the pig populations of T&T and to identify the strains of SwIV that are circulating in T&T pigs. Understanding the

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background levels of these viruses in swine populations in T&T will enable the development of science-based risk assessments, thus aiding the successful prevention, management, and control of future viral outbreaks within T&T and the Caribbean region. This will improve the health status and productivity of swine and pork production in T&T.

## Materials and methods

### Experimental design

The sample size ( $n = 309$ ) was estimated by using an anticipated true herd-level prevalence of 10%, tolerance of 7.5%, a 90% confidence level, and herd-level specificity and sensitivity of 70 and 90% respectively. This sample size was achieved as a modification of the Cannon and Roe (1982) formula for livestock disease surveys as modified by Humphry et al. 2004.

### Sampling

Sampling was conducted on pig farms throughout T&T. Sampling was carried out from October 2013 to February 2015, and samples were taken from 5 large pig farms and 21 small pig farms. Three of the small farms had animals slaughtered at public abattoirs. A map showing the location of the farms and abattoirs sampled is shown in Fig. 1. Small farms were classified as operations with 5–50 pigs and large

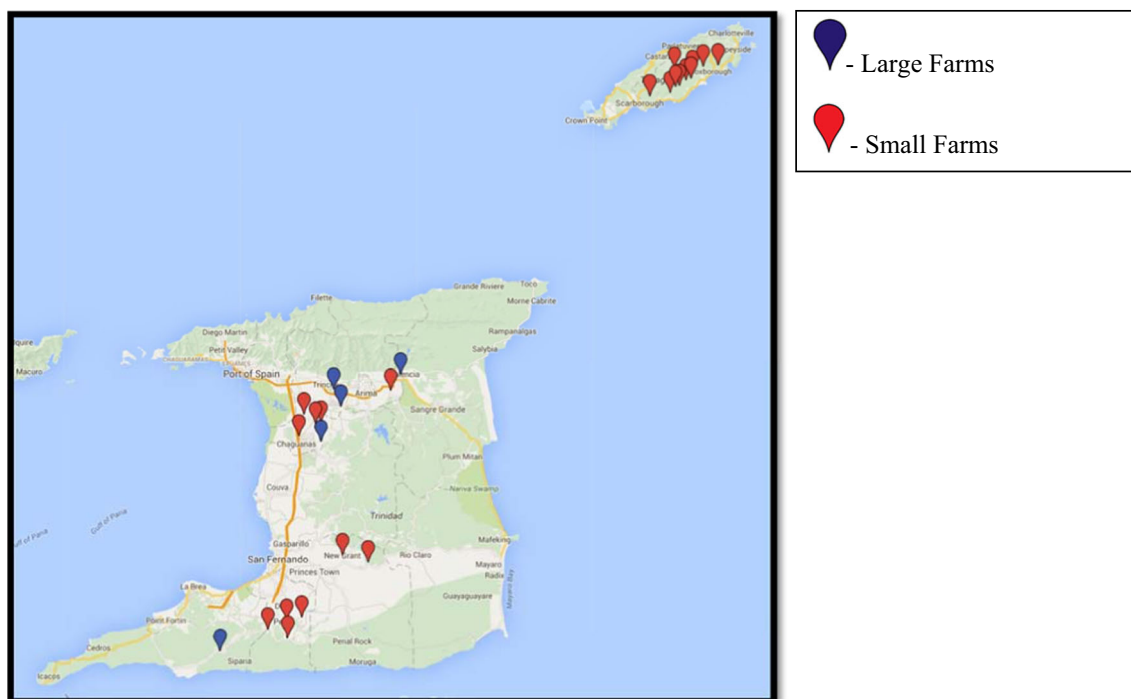
farms as operations with 100–500 pigs. None of the pigs sampled were vaccinated for any of the viruses under investigation.

Two large farms were sampled on slaughter days. Twenty-five pigs were randomly selected and these samples were used. On the 3 large farms where slaughtering was not being carried out, a randomization process was used. Each finisher, breeding sow, and boar were designated a number, the numbers were then placed into a bag, and 25 numbers were drawn at random. These 25 pigs were sampled for antibody testing. In total, 125 samples were collected from 5 large farms.

Ten samples were taken at random from each of the 3 small farms that slaughtered their pigs in public abattoirs. These 10 samples were taken at slaughter. The remaining 18 small farms were visited, and 10 samples were taken at random. On farms with fewer than 10 pigs, all of the pigs on the farm were sampled. In total, 184 samples were collected from 21 small farms.

### Porcine samples

Blood was taken from the right anterior vena cava of pigs using 18G 1.5-in. needles for nursery pigs and 3-in. needles for adults. Samples were collected in red-topped (no anticoagulant) tubes and centrifuged in a Beckman model TJ-6 centrifuge at 2500 rpm for 10 min to separate serum. The samples were collected from finisher pigs, and where possible, samples from older animals were taken.



**Fig. 1** Map of Trinidad and Tobago showing the location of the sampling sites of large farms and small farms

## Enzyme-linked immunosorbent assays (ELISAs) used in the study

Swine influenza A (SwIV): ID.vet ID Screen Influenza A Antibody Competition Multi-Species. Grabels, France

Porcine parvovirus (PPV): LSIVet™ Porcine Parvovirus—Serum. Lissieu, France

Porcine circovirus type 2 (PCV-2): BioChek PCV-2 Antibody Test Kit—The Netherlands

Porcine reproductive and respiratory syndrome virus (PRRSV): LSIVet™ Porcine PRRS/US—Serum. Lissieu, France

Transmissible gastroenteritis virus/porcine respiratory coronavirus (TGEV/PRCV): SVANOVIR TGEV/PRCV-Ab—Boehringer Ingelheim Svanova, Uppsala, Sweden

Classical swine fever virus (CSFV): Biochek CSFV-E2 Antibody Test Kit—Holland

## Hemagglutination inhibition testing (HIT)

Serum antibodies to influenza were detected by the HI test according to standard methods (Organization for Animal

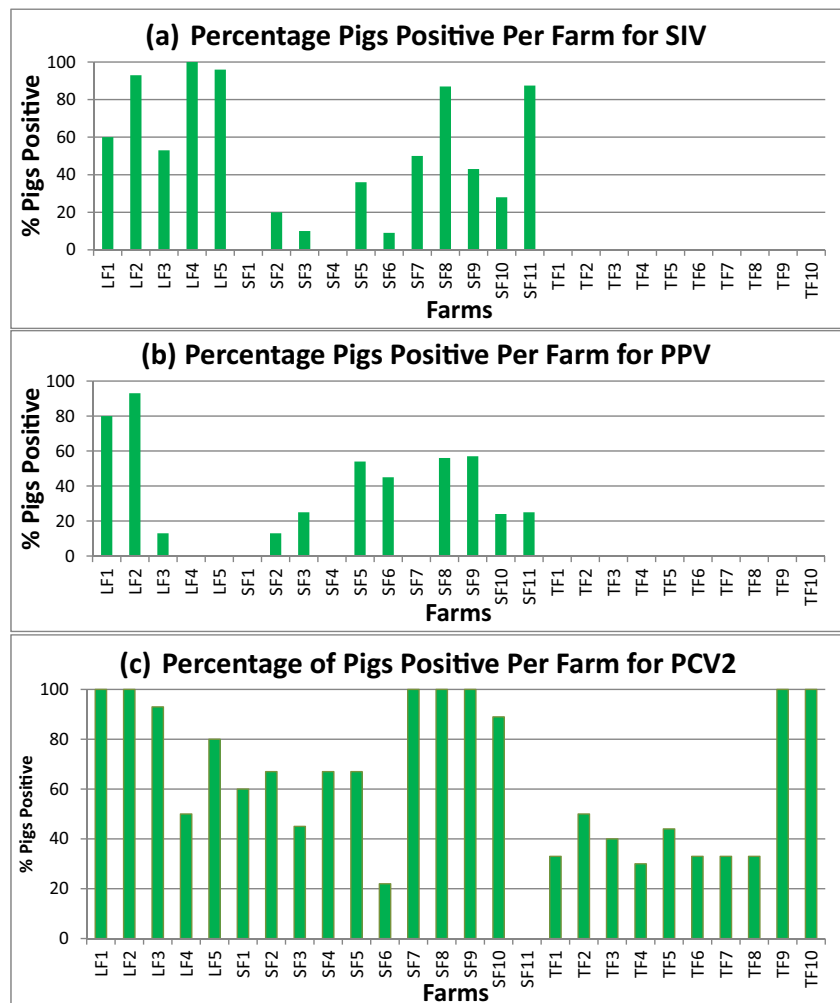
Health 2016). To reduce non-specific reactions, the sera were treated with 100 U/ml of receptor-destroying enzyme at 37 °C for 1 h, inactivated at 56 °C for 30 min, and adsorbed with 30% (v/v) chicken red blood cells (CRBCs) overnight at 4 °C. Following treatment, the starting dilution of serum was 1/5, and doubling dilutions were prepared, prior to the addition of an equal volume of both four hemagglutinating units of virus and 1% CRBCs. Following incubation at 4 °C for 30 min, the plates were examined for hemagglutination of CRBCs. HI titers were recorded as the reciprocal of the highest initial dilution of serum which completely inhibited hemagglutination.

## Results

### Swine influenza (SwIV)

ELISA testing revealed antibodies to SwIV in 37% (114 out of 309) of the sampled pigs and on 53% (14 out of 26) of the farms sampled in Trinidad. The percentage of pigs testing positive for antibodies to SwIV on each farm ranged from 4

**Fig. 2** Percentage of antibody-positive pigs for (a) swine influenza A virus (SwIV), (b) porcine parvovirus (PPV), and (c) porcine circovirus type 2 (PCV-2) on pig farms in T&T. *LF* large farm (Trinidad), *SF* small farm (Trinidad), *TF* Tobago farm



to 100% (Fig. 2a). The pigs sampled in Tobago were all negative for SwIV antibodies.

HI testing on a selection of 12 of the ELISA positive samples revealed high titers against the A/sw/Minnesota/593/99 H3N2 strain of SwIV as well as the pH1N1 2009 pandemic strain (Table 1). One of the pigs also showed a high titer against the A/Perth/16/09 H3N2 strain.

### Porcine parvovirus (PPV)

Antibodies to PPV were detected in 28% (87 out of 309) of sampled pigs and on 42% (11 out of 26) of the farms sampled, including 3 large farms in Central and Eastern Trinidad. Approximately 80% of PPV positives corresponded to an HI titer of 10,240 to 20,480 according to the test kit insert (LSIVet™ Porcine Parvovirus—Serum, Lissieu, France), suggesting that field infections of PPV were occurring in these swine. The percentage of pigs testing positive for antibodies to PPV on each farm ranged from 13 to 93% (Fig. 2b). The pigs sampled in Tobago were all negative for PPV antibodies.

### Porcine circovirus-2 (PCV-2)

Antibodies to PCV-2 were detected in 66% (205 out of 309) of sampled pigs from T&T and on 96% (25 out of 26) of the farms sampled on both islands, including the 5 major farms in Trinidad. Of the total number of pigs sampled from Trinidad, 43% (113 out of 264) tested positive for PCV-2 antibodies which were found on 87.5% (14 out of 16) of Trinidad farms. Of the total number of pigs sampled from

Tobago, 43% (19 out of 44) were positive for antibodies to PCV-2. Of the 10 farms sampled from Tobago, all tested positive for PCV-2 antibodies. Approximately 80% of the PCV-2 positives corresponded to an antibody titer of 3000 or higher according to the test kit insert (BioChek PCV-2 Antibody Test Kit—Holland) suggesting that field infections of PCV-2 were occurring in these swine. The percentage of pigs testing positive for antibodies to PCV-2 on each farm ranged from 30 to 100% (Fig. 2c).

Ten farms were positive for antibodies to SwIV, PPV, and PCV2, and three farms were positive for antibodies to both SwIV and PCV2, but not PPV. One small farm (SF11) was positive for antibodies to SwIV and PPV but not PCV2, and two small farms (SF1 and SF4) as well as all of the Tobago farms were positive for only PCV2. No antibodies were detected in any of the sampled pigs against PRRSV, TGEV/PRCV, or CSFV.

### Discussion

Out of approximately 35,000 domestic pigs in T&T, 309 (~1%) were sampled and tested for the presence of antibodies to the viruses under investigation. The sample size ( $n = 309$ ) was estimated by using an anticipated true herd-level prevalence of 10%, tolerance of 7.5%, a 90% confidence level, and herd-level specificity and sensitivity of 70 and 90% respectively (Cannon and Roe 1982). The amount of pigs sampled in this study was constrained by issues of free access to farms. Many farmers were reluctant to allow their pigs to be sampled due to the invasive nature of the blood sampling procedure and the resulting high levels of stress caused to the animals. The more common approach to determining sample size in this type of study uses the formula developed by Cannon and Roe (1982) where  $P$  is the anticipated population proportion which is assumed at 70% and  $Z$  is 1.96 which is the approximate value of the 97.5 percentile point of a normal distribution curve used to construct approximate 95% confidence intervals. This value is found using a  $Z_{\alpha/2}$  table where the confidence level used is 95% and  $\alpha$  is 0.05. Using the Canon and Roe formula, the sample size  $n$  was calculated to be 322 samples; however, to reduce the number of pigs to be sampled further, the confidence can be relaxed (decreased) and the tolerance, increased (Humphry et al. 2004) allowing for a sample size of 309.

SwIV can cause severe respiratory signs in pigs, with morbidity rates sometimes reaching 100%. The primary economic impact is related to retarded weight gain resulting in an increase in the number of days to reach market weight (Organization for Animal Health 2009). In the UK, the financial loss resulting from reduced weight gain in finishing pigs alone due to SwIV has been estimated at approximately £7 per pig (Morilla et al. 2008). In this study, antibodies against

**Table 1** Antibody titers measured by hemagglutination inhibition test (HIT) against six common strains of SwIV

Sample ID	Virus					
	A/Perth/16/09 H3N2	A/Eng/427/88 H3N2	A/sw/Eng/163266/87 H3N2	A/sw/Eng/201635/92 H3N2	A/sw/Minnesota/593/99 H3N2	A/Eng/195/09 pH1N1
SF1-13	<10	<10	20	<10	40	640
LF1-12	<10	<10	20	10	80	640
LF2-13	320	<10	20	10	40	160
LF3-3	<10	<10	<10	<10	160	160
LF3-15	<10	<10	20	<10	80	80
LF5-6	20	<10	20	10	80	20
LF4-2	10	<10	<10	<10	40	80
LF4-3	10	<10	20	<10	40	80
LF4-4	40	<10	20	20	160	40
LF4-8	40	<10	10	20	80	20
LF4-9	20	<10	20	20	40	40
LF4-10	10	<10	10	<10	40	80

SwIV were found in 114 out of 309 (37%) serum samples taken from pigs. In a study carried out on swine in the USA, 27.7% of the sampled pigs were found to be seropositive for SwIV in 2000 (Olsen et al. 2000) and, according to a World Organization for Animal Health (OIE) Technical Disease Card on Swine Influenza (Organization for Animal Health 2009), approximately 25–33% of finishers and 45% of breeding pigs have antibodies to influenza A viruses in the USA. Other studies carried out in the region revealed a herd seroprevalence of 36.3 and 34.6% in Guatemalan pigs in 2010 and 2011 respectively (Gonzalez and Ana 2015). In Mexico (Lopez-Robles et al. 2014), 38% of pigs tested positive for SwIV antibodies in 2014 and 41.3% of Brazilian pigs were found to be positive for SwIV antibodies in a study carried out in 2012 (Rajao et al. 2013). The 37% seroprevalence found in pigs of T&T is therefore consistent with similar studies carried out in Central and North American countries and suggests that SwIV is endemic in the Trinidadian domestic swine population, but not Tobago.

In order to detect the likely strains of SwIV circulating in the pigs, a selection of 12 ELISA positive samples were further tested using HIT. A combination of H3N2 strains was selected to try to understand the relative incidence of H3N2 in pigs within the country. Due to significant antigenic diversity, strains were selected from both European pigs, which may be representative of global strains but also more recent human strains, given the frequency of transmission from humans to pigs which may result in the establishment of a stable lineage of virus. The pandemic strain pH1N1 2009 was also included, which is known to be present in pigs worldwide at present.

The H3N2 serology results (Table 1) show different reactivity patterns. A/sw/Minnesota/593/99 is most significant since this is the strain of H3N2 virus that has undergone reassortment in North American pigs through multiple generations over several years (Anderson et al. 2015). Interestingly, it is well known that many of the pig populations of Trinidad originated from Minnesota farms indicating a potential introduction pathway. High titers of this strain were observed in the samples tested which would be indicative of quite recent exposure/active circulation of such viruses. One of the samples showed quite a high titer against the A/Perth/16/09 H3N2 strain which is a contemporary human virus that does not have a stable lineage in pigs. As expected, reactivity to the European viruses was low as there is very little transfer of swine between Europe and Trinidad. The pandemic pH1N1 2009 strain showed strong reactivity in all of the samples tested which indicates probable circulation of virus or recent exposure.

In the case of the small farms sampled in this study, all of the owners lived either on the farm or within 5 km of their farm and several other houses were present in close proximity to the pig farms. This close proximity of people and pigs could

enhance both zoonotic and reverse zoonotic transmission of influenza viruses from humans to pigs, thus maintaining influenza virus circulation in the swine herds (Australian Pork Industry Biosecurity Programme, APIBP 2003). These factors are akin to those which defined traditional influenza epicenters in Southeast Asia (Ma et al. 2009). Personal hygiene, the granting of sick leave to pig handlers showing flu-like symptoms, as well as biosecurity, should therefore be encouraged on both small- and large-scale farms. Such measures should include risk assessment checks for visitors, the use of protective clothing, respiratory protection for people to reduce viral dissemination (zoonotic and reverse zoonotic), handwashing before and after handling animals, restriction on sharing of equipment and tools between farms, and controls relating to the movement of animals and vehicles in and out of the farm. Training of workers on pig farms to recognize influenza-like symptoms in humans and pigs should also be carried out (Adeola et al. 2015).

Porcine parvovirus (PPV) causes severe economically devastating reproductive symptoms in breeding sows. It is known to cause abortion and is associated with stillbirths, mummifications, embryonic death, and infertility (SMEDI). PPV infection is one of the most common and important causes of infectious infertility in swine. PPV is ubiquitous and worldwide in its distribution; it is therefore an infection which needs to be carefully managed (Porcine Parvovirus, The Pig Site 2014). This study identified 87 out of the 309 serum samples (28%) as antibody positive for PPV. Interestingly, markedly different levels of seroprevalence for antibodies to PPV were observed across the different swine farms in T&T with seroprevalence levels on farms ranging from 12 to 92% (Fig. 2b). Antibodies were not observed in any of the pigs sampled on two of the largest farms in Trinidad, suggesting that the biosecurity and husbandry measures practiced on these two farms may be effective at stopping this introduction of PPV onto the farms. Interestingly, despite the relatively high seroprevalence for PPV observed in Trinidadian domestic pigs, there are limited reports of reproductive problems in the pigs. This could be due to farmers simply not reporting reproductive issues on their farms when they occur or alternatively could be due to a state of endemic stability on the affected farms, meaning that all the breeding sows are being infected with PPV prior to their first pregnancy. On the farms that contained serologically positive pigs, it was found that all the breeding sows that were sampled on these farms had antibodies to the virus prior to their first pregnancy, which would explain the lack of PPV-related reproductive clinical signs reported to the veterinary services. It is, however, very important to note that some pig farms in Trinidad, and all the pigs sampled on Tobago, tested negative for PPV antibodies. These PPV-negative farms should be particularly careful when bringing pigs into their farms, ensuring that they avoid the introduction of PPV-positive animals. These farmers should ensure that they test



all pigs for PPV antibodies and antigen prior to introduction, or alternatively, they should vaccinate their breeding sows prior to their first pregnancy. All pig farmers from PPV-negative farms should be advised of the importance of maintaining high levels of biosecurity in order to keep their farms free of PPV.

All of the samples taken from pigs on the island of Tobago ( $n = 45$ ) tested negative for antibodies to both SwIV and PPV. In light of this, Tobago pig farmers should be encouraged to maintain a closed system. Tobago has approximately 20 small backyard pig farms throughout the island, 10 of which were sampled. These Tobago pig farmers usually breed and rear their pigs on their farms and may occasionally buy replacement sows from neighboring farms in Tobago to reduce excessive inbreeding. Pig farmers from Tobago should therefore be advised to avoid importing pigs from Trinidad and should maintain their current husbandry practices in order to ensure that their pigs remain seronegative for SwIV and PPV. Any pigs, including boars, coming into the country should be quarantined and tested for the presence of these viruses. Farmers from Tobago should be educated on the serological situation of their pigs and be made aware of the risks they face in the event of their naïve pigs becoming exposed to these viruses. The farmers should also be educated on biosecurity measures to be implemented to ensure the prevention of PPV and SIV being introduced into their pig population.

Interestingly, Trinidad has at least three pig farms which, on occasions, have imported semen from the USA. This may possibly be the source of PPV infection in the country.

PCV-2 is one of the top three most economically important swine pathogens, behind PRRSV and *Mycoplasma pneumoniae* (Thacker 2013). If pigs are left unvaccinated for PCV-2, producers could see up to a US\$20 loss per pig (Thacker 2013). High seroprevalences (82.4%) of pigs for PCV-2 antibodies have been reported in Canada (Liu et al. 2002), the USA (80%) (Nawagitgul et al. 2002), Taiwan (83.5%) (Wang et al. 2004), Northern Ireland (>55%) (Walker et al. 2000), and Columbia (83.6%) (Monroy et al. 2014). In the present study, the overall seroprevalence for PCV-2 antibodies in T&T pigs was found to be 66%. There is, however, little to no evidence for PCV-2-associated diseases in T&T pigs. Given the high seroprevalence for PCV-2 observed in the T&T pigs, there is clearly a need to closely monitor pig herds for evidence of PCV-2-associated diseases. Routine surveillance for clinical signs and the regular examination of specimens from abattoirs should be adopted. Farmers should also be encouraged to adopt sound husbandry practices such as age segregation, good sanitation as well as measures to minimize stress and avoid overcrowding. The application of these measures is essential to avoid the development of diseases in pigs that are associated with PCV-2 infection such as post-weaning multisystemic wasting syndrome (PMWS).

Interestingly, some farms showed very high seroprevalence levels for certain viruses, whereas other farms showed low seroprevalence levels. It is possible that this was due to ongoing infection at the time of sampling. Alternatively, this could have been due to the different management practices and stocking densities on the farms. High population densities are known to facilitate the rapid spread of pathogens throughout livestock populations. A study in Belgium found that the number of pigs per pen was positively associated with swine influenza H3N2 seropositivity (Maes et al. 2000). Ewald et al. (1994) also found that a high pig density was a risk factor for herds to become infected with influenza H1N1 and H3N2 viruses and furthermore that a large number of pigs per pen creates physiological stress, which in turn can alter the immune system and predispose pigs to infection.

The reduced seroprevalence levels for SwIV observed on the small compared to the large farms may have been associated with the lower stocking densities, as well as the lower overall numbers of pigs, on the small farms. It is known that respiratory viruses are less efficiently maintained on small as opposed to large pig farms (Maes et al. 2000; Poljak et al. 2008; Simon-Grife et al. 2011). The two small farms (SF8 and SF11), on which over 80% of the pigs were seropositive for SwIV, were the two largest of the small farms sampled (with close to 30 pigs). The pigs on these farms were kept at a very high stocking density, which may have been the reason for the high seroprevalence for SwIV observed on those farms.

Economic losses resulting from viruses such as SIV and PCV2 are usually related to a decrease in average daily gain (ADG) and reduced feed conversion efficiency in affected pigs. These viruses may also result in increased carcass condemnation at slaughter and treatment costs for ill pigs (Van Alstine 2012). For SIV, seropositive and virus-positive pigs have been found to have significantly decreased growth performance compared to seronegative pigs, even though feed intake was not decreased. Reduced feed conversion efficiency led to lower average daily growth, additional feed requirements, and longer time needed to reach the 100 kg body weight (Er et al. 2014). Pigs vaccinated for PCV2 have been shown to deliver a sizable return on investment of up to approximately US\$20 per pig over unvaccinated pigs (Gillespie et al. 2009). Also, in the case of PPV, it has been suggested that the cost of an epidemic could result in losses of up to US\$50 per sow (Cutler and Gardner 1988). Losses of between US\$20 and \$50 per pig would be devastating to the pig industry in T&T and could result in the closure of affected farms.

This study highlights the importance of carrying out regular serological monitoring for economically important viruses of swine that are circulating in the region. Although antibodies to CSF, PRRS, TGE, and PRCV were not observed in pigs from T&T, it is important to continue monitoring for these viruses, as outbreaks of PRRS and CSF have recently been reported in South American countries neighboring Trinidad,

including Venezuela, Ecuador, Suriname, and Columbia (World Animal Health Information System, WAHIS Interface 2015). It is well known that domestic species, including pigs, are illegally imported into Trinidad from the South American mainland, especially Venezuela. Although the porcine coronaviruses (TGE and PRCV) have not previously been reported to be present in Trinidad, the presence of these viruses has been suspected for many years, possibly due to previous importation of pigs and pig semen from the USA and Canada. This study has revealed that these viruses are not present in domestic pigs in T&T.

In conclusion, this study shows that SwIV and PPV are present and circulating in Trinidadian domestic pig populations; however, these viruses were not observed to be present in pigs sampled from Tobago. Strains of SwIV confirmed as likely to be circulating in pigs on the island of Trinidad include a North American H3N2 strain and the pH1N1 2009 pandemic strain. PCV-2, however, was observed to be circulating in domestic pigs from the islands of both Trinidad and Tobago. Pigs on both islands of Trinidad and Tobago did not have antibodies to CSF, PRRS, TGE, and PRCV.

**Acknowledgements** We thank the pig farmers of Trinidad and Tobago for providing access to their pigs for sampling. We are grateful to the academic and technical staff of the UWI School of Veterinary Medicine especially Mr. Roger Malcolm and Dr. Marc Driscoll who were particularly instrumental in the sampling collection stages. We owe our gratitude to the Director of Veterinary Public Health, Dr. Saed Rahaman, and the Livestock and Livestock Products Board of Trinidad and Tobago. We also thank the UWI Trinidad and Tobago Research and Development Impact Fund (RDI Fund) for funding the study. We thank Natalie McGinn at the Animal and Plant Health Agency—Weybridge for technical support.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

**Ethical approval** All procedures performed in studies involving animals were in accordance with the ethical standards of the University of the West Indies (UWI) Research and Development Impact Fund and the UWI Ethics Committee.

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