

Current Status of *M. hyopneumoniae* in Pigs in Grenada, West Indies

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Abstract

Mycoplasma hyopneumoniae, cause of enzootic pneumonia is known for serious economic losses in pigs. The disease is prevalent all over the world. There is no published report on *Mycoplasma hyopneumoniae* incidence in Grenadian pigs. The aim of this study was to estimate seroprevalence of antibodies for *Mycoplasma hyopneumoniae* in non vaccinated pigs in Grenada. Sera were collected randomly from 459 pigs of all ages from all six parishes of Grenada. Sera were tested for antibodies to *Mycoplasma hyopneumoniae* using an indirect Enzyme Linked Immunosorbant Assay (ELISA) kit. Antibodies to *Mycoplasma hyopneumoniae* were found in 8.71% (95% CI: 0.0644 to 0.1167) pigs. The greatest percent of positives (62.5%) (95% CI: 0.4699 to 0.7582) were in youngest group <1 year, followed by 15% (95% CI: 0.0668 to 0.2946) in < 1 - 2 year > 22.5% (95% CI: 0.1211 to 0.3771) in >2 year. Positive females were overrepresented compared to males by 3:1. This is the first report on the seroprevalence of *Mycoplasma hyopneumoniae* in pigs from Grenada, West Indies.

Keywords

Grenada, *Mycoplasma hyopneumoniae*, Pigs, Seroprevalence

1. Introduction

Mycoplasma hyopneumoniae is the causative agent of Porcine Enzootic Pneumonia, and is a primary contributor to the porcine respiratory disease complex [1]. Enzootic pneumonia in pigs is a highly contagious and chronic disease [2]. *M. hyopneumoniae* is a gram positive, small (400 - 1200 nm) bacteria that lacks a cell wall [1] [3]. Pure infection of *M. hyopneumoniae* does not produce serious disease, however, it produces chronic disease when concurrently infected with bacteria and other viruses. The increase in duration and severity of the disease

has been reported with concurrent infection with Porcine Respiratory and Reproductive Syndrome Virus (PRRSV), porcine circovirus type 2 (PCV2) and *Pasteurella multocida* [4] [5]. Lesions with mycoplasma pneumonia are consolidation of antero-ventral part of lungs. Since similar lesions may be also observed with other pathogens, culture of mycoplasma, PCR and serology along with study of lesions are suggested for the diagnosis. Serology is the most common tool used to determine the presence or absence of *M. hyopneumoniae* within a herd [1]. ELISA [6] and complement fixation test [7] were the most commonly used serological tests for *M. hyopneumoniae* [1]. Transmission of *M. hyopneumoniae* occurs either by vertical route from sow to the off spring by direct nose to nose contact [8] or aerosol transmission among pigs [9] [10] [11] [12]. *M. hyopneumoniae* is primarily a problem of grow-finish pigs, although disease is reported in all age groups [13] [14]. This pathogen is considered a major economic concern within the swine industry because it causes a significant reduction in the weight of growing pigs [15]. Porcine enzootic pneumonia is endemic worldwide and *M. hyopneumoniae* is present in almost every pig herd [16]. As far as authors are aware there is no published report on the presence of *M. hyopneumoniae* in Grenada and other Caribbean nation. This paper looks at the current status of *M. hyopneumoniae* within the pig farming industry in Grenada, West Indies with an aim to establish a current baseline of the prevalence of this pathogen.

2. Materials and Methods

2.1. Ethical Approval

The project was approved by St. George's University Institutional Animal Care and Use Committee (IACUC).

2.2. Country of Research

Grenada is a tri-island state located between the Atlantic Ocean and the Caribbean sea, roughly 200 miles south of Trinidad and Tobago. The main island is separated into 6 parishes: St. Patrick, St. Mark, St. Andrew, St. John St. George and St. David. St George's University is located at the southernmost tip of the island in the second largest parish St George.

2.3. Sample Size Determination and Selection of Farms

Sample size of pigs was determined using formula of Glenn 2002 [17]. The formula is $N = t^2(p)(1 - p)/d^2$. Where $t = 1.96$, $P =$ estimated prevalence and $d =$ desired level of precision. For calculation of sample size we used $p = 50\%$ and desired level of precision = 5%. An estimated sample size of 384 was determined. Depending on number of farms in various parish, a total of 18 farms were selected (5 each in St George and St. Andrew, 2 each in St. David, St. Mark, St. John and St. Patrick). Pig farms included in the study were selected randomly in all six parishes of the country. A written consent was obtained from the farm

owner for inclusion in the study. Owner of the farm was informed about the objectives and benefit of the study, before obtaining the consent.

2.4. Collection of Samples

Blood samples from 459 pigs were collected randomly over a 2-year period (2014-2015) from selected swine farms throughout the 6 parishes in Grenada. A 3 - 5 ml. blood was collected by aseptic technique using anterior vena cava puncture, and a 20 gauge 1.5 inch needle. The blood was allowed to clot for 20 minutes at room temperature and transported to the Pathobiology diagnostic laboratory, School of Veterinary Medicine, St. George's University in cool box over ice. Blood was centrifuged at 1500 - 2000 g for 10 - 15 minutes. The serum was removed and placed into plastic cryovial 1.0 cc tubes and frozen at a -20°C until testing. During blood collection herd particulars for age, sex, body condition, breeding problems and other clinical information were recorded. Blood was collected from 3 age groups: <1 year, 1 - 2 year and >2 year.

2.5. Serosurveillance

Serum samples from swine were tested for antibodies against *Mycoplasma hyopneumoniae*. A herd and farm will be considered *M. hyopneumoniae* positive when at least one of the sampled pig tests positive. The seropositivity within each farm was calculated as the ratio between the number of pigs testing positive and the total number of sampled pigs on that farm. These numbers were then re-calculated within a parish to allow for a parish-to-parish comparison, enabling determination of the areas that have higher concentrations of *M. hyopneumoniae*. The samples were also compared by sex and age.

The serologic testing of all samples was carried out using the commercial *M. hyopneumoniae* ELISA (HerdChek; IDEXX Laboratories Inc, Westbrook, Maine, USA). These kits use an indirect method to detect serum antibodies. ELISA test was performed according to manufacturer's instructions.

2.6. Statistical Analysis

The data were analyzed by the statistical methods: Fisher's exact test, using a graphpad statistical software (<http://www.graphpad.com/quickcalcs/contingency2>).

3. Results

Seropositive pigs were found in all six parishes of Grenada. Out of 459 pigs 40 (8.71%; 95% CI: 0.0644 to 0.1167) were found seropositive. Results by parish and sex are presented in **Table 1** and **Figure 1**. St. Patrick (17.39%; CI: 0.1008 to 0.2814) shows a highest number positive samples followed by St. Andrews (11.57%; CI: 0.0690 to 0.1860). St. Johns had the lowest positive samples (1.72%). Fisher's exact test applied between value in parishes revealed the results. There were significant difference in seropositivity between St. Patrick and St. George (p value 0.0069), between St Andrew and St John (p value 0.0230) and between

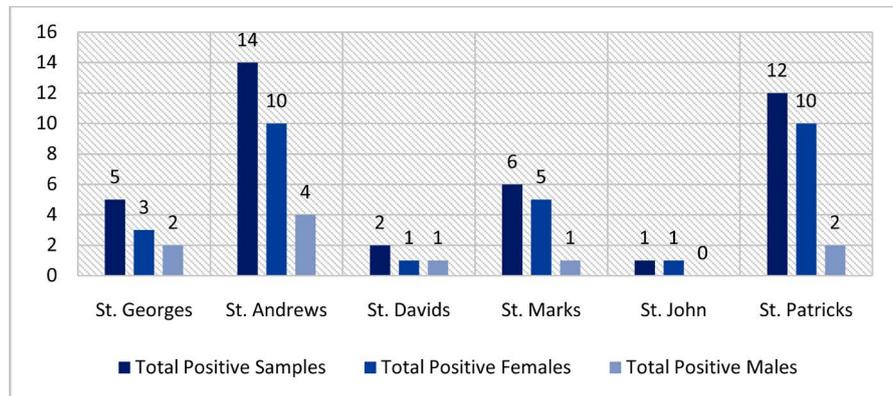


Figure 1. Totals by parish and sex; all parishes.

Table 1. Parish/region wise distribution of pigs positive for antibodies against *M. hypneumoniae*.

Parish/region	Total samples tested	Numbers positive	Percentage positive (%)
St. Andrews	121	14	11.57
St. George	113	5	4.42
St. David	33	2	6.06
St. Mark	65	6	9.23
St. John	58	1	1.72
St. Patrick	69	12	17.39
Total	459	40	8.71

Table 2. Age wise distribution of samples positive for antibodies against *M. hypneumoniae*.

Age groups	Total Pig samples positive (n = 40)	Percentage positive (%)
6 month-1 yr	25	62.5
1 yr to 2 yrs	6	15
>2 yrs	9	22.5

St. Patrick and St John (p value 0.0032).

Amongst the 3 age group of evaluated pigs, the youngest group (< 1 year) had the highest positive pigs (62.5%). Older groups represented 15% (1 - 2 year) and 22.5% (>2 year). The results are presented in **Table 2**. Statistical analysis applying Fisher' exact test revealed no statistical significance between pigs of <1 year and 1 - 2 year age (p value 0.0765) and between <1 year and >2 year (p value 0.2501), however, the difference of positive cases was statistically significant in pigs of age 1 - 2 year and >2 year. Seropositive pigs by age and sex are presented in **Figure 2**, age and seropositivity in scatter graph (**Figure 3**) and graph plot (**Figure 4**).

4. Discussion

A total number of 459 samples were tested from pigs for *M. hypopneumoniae*, out

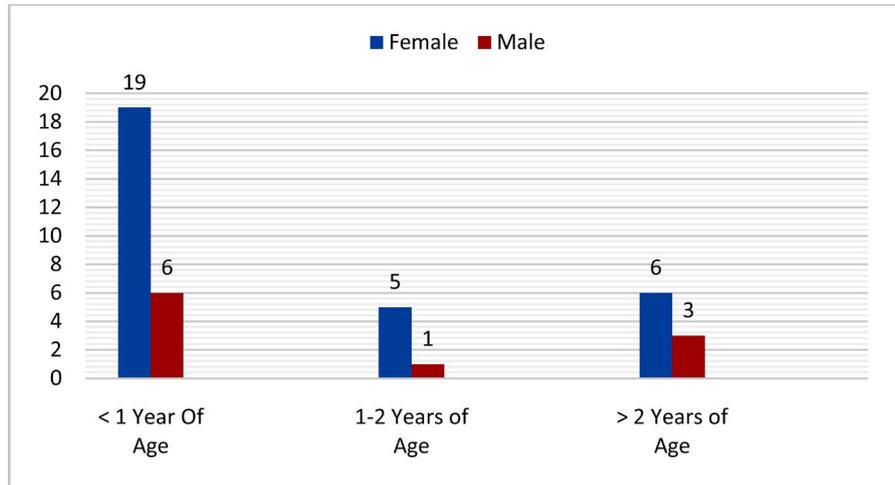


Figure 2. Positive results by age and sex.

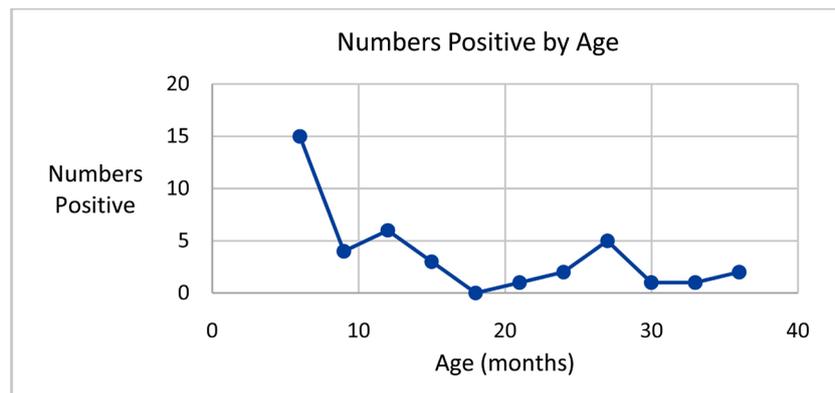


Figure 3. Scatter graph demonstrating Numbers Positive by Age based on ELISA results.

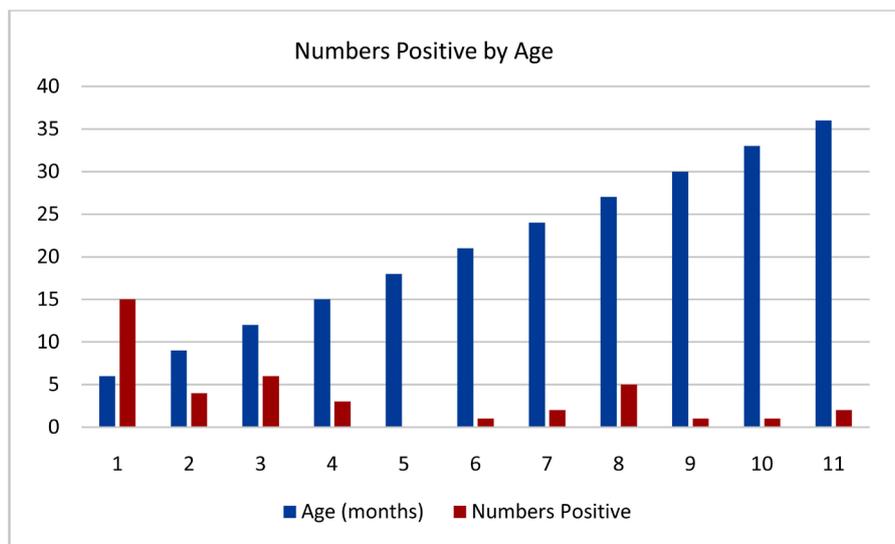


Figure 4. Box plot demonstrating Numbers Positive by Age based on ELISA results.

of these samples 40 were found to be positive, giving a 8.71% prevalence for this pathogen in Grenada. The youngest group of pigs (<1 year) had high (62.5%)

seroprevalence. Our findings are in agreement with previous researchers who also reported more seropositivity in younger age pigs [9] [18] [19]. In another study Grosse *et al.* [20] found older sows in some herds in Germany more often seropositive than younger ones. Previous researchers reported variation in the incidence of the disease in different countries of the world. The incidence varied from 2.6% in Switzerland [21] to 65% in sow herds in North-West Germany [22]. The differences in incidence of *M. hyopneumoniae* in various part of the world may be because of samples and the methodology used. The researchers used PCR on nasal swabs [18] [23], various serological tests [9] [22], bacterial cultures of lung tissue and combination of tests. Nathues *et al.* [19] used a combination of PCR on nasal swabs and ELISA for antibodies in serum. Makhanon *et al.* [24] compared the results of culture and PCR in various tissues of slaughtered pigs for the detection of different species of Mycoplasma. In the present study we used ELISA to detect antibodies in serum.

During our Seroprevalence survey for antibodies for *Mycoplasma hyopneumoniae* females are overrepresented compared to males by 3 to 1 (30 positive females/10 positive males) in all evaluated age groups. Females under the age of 1 year are more strongly affected. Our findings are in agreement with Nathues *et al.* [19] who also reported gender difference in the incidence of *Mycoplasma hyopneumoniae* in suckling pigs. They found 56.1% incidence in females compared to 43.9% in males. Further studies on gender variation in the incidence of *M. hyopneumoniae* may explain the higher prevalence in females.

The variation in the incidence of seropositivity observed in different parishes of the country is not well understood since the risk factors do not differ from parish to parish. Further research is warranted to explain the current findings.

5. Conclusion

This paper presents the first report on the surveillance of *Mycoplasma hyopneumoniae* in pigs of Grenada. Since pigs in Grenada are not vaccinated with *Mycoplasma hyopneumoniae* vaccine, our results have shown that *M. hyopneumoniae* has been introduced and is circulating at a low level in the porcine population in Grenada.

6. Recommendation

There is a strong need for regular surveillance for *Mycoplasma hyopneumoniae* in pigs in Grenada. Focus may be also on the role of coinfections in the incidence of *M. hyopneumoniae* infection. Grenadian pig farmers should be educated on excellent management and housing of sows and piglets to prevent transmission of *M. hyopneumoniae* infection in young age pigs.

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Competing Interest

The authors declare that there is no competing interest.

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