Original Research

Prevalence of Antibodies against *Leptospira* spp. in Pigs from Grenada, West Indies

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Abstract

In this cross-sectional study, we sought to determine the seroprevalence of leptospirosis among pigs in Grenada. Eleven farms were randomly selected amongst Grenada's six parishes and blood was collected through venipuncture from a total of 368 pigs. Enzyme-linked immunosorbent assay tests (ELISA) were performed on serum to screen for antibodies to Leptospira spp. Overall, seroprevalence to Leptospira spp. was 23.4% (95% CL 19.3-27.9). The percentage of positive cases based on parish is as follows: St. Mark, 21.6%; St. Andrew, 21.5%%; St. George, 25.7%; St. Patrick, 23.6%; St. David, 31.3% and St. John, 9%. There was no significant difference ($p>0.05$, $\chi 2$) in seroprevalence between males (18.8%) and females (25%). Regular assessment of pigs in Grenada for Leptospira spp. and their role in transmission to humans and other animals is recommended for the sake of public health.

Key words: Antibodies, Grenada, Leptospirosis, Pigs

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Introduction

Leptospirosis is a neglected tropical disease that is infectious and zoonotic in nature. It is caused by the pathogenic spirochete, *Leptospira*, which is prevalent in warm, tropical regions worldwide, including Grenada (Sperling, 2016). Leptospirosis has been reported in livestock (bovine, sheep, goats, pigs) and wild animals including rodents. In infected animals *Leptospira* organisms multiply in kidney and are shed in the urine, causing contamination of water, food and soil. Transmission of *Leptospira* spp. amongst the animals and humans is by two routes. One route of infection is by ingestion of *Leptospira* contaminated water and food. The second route of transmission is through injured skin. Humans and animals become infected when broken skin comes in contact with contaminated soil or water (Faine, 1994). After ingestion or skin

transmission, bacteria invade the blood and are disseminated to multiple organs in the body causing hepatic failure, pulmonary hemorrhagic fever (specific to humans), meningitis, myocarditis, and renal failure.

Most livestock, particularly pigs, have subclinical disease. However, heavily infected swine herds may experience additional disease outcomes such as abortions, still births, and birth of weak piglets usually between 14 -60 days after infection (Ramos *et al.*, 2006). In Grenada and other Caribbean nations, farmers rely heavily on livestock production including swine industry. Leptospirosis in humans and animals is worldwide and a few studies have been conducted on the incidence of Leptospirosis in the Caribbean and South America. Schneider *et al.* (2013) reported alarming prevalence of leptospirosis in animals including swine (17-75%) in Central America. In the Caribbean, seropositivity for leptospirosis in swine has been reported from Jamaica, Mexico, Argentina and Trinidad (Petrakowski *et al.*, 2014). In Grenada, the first report of Leptospirosis was by Everard *et al.* in 1985. They reported 35% prevalence of leptospirosis in swine, indicating the presence of leptospirosis much before the time of the present study.

While brown rats (*Rattus norvegicus*) have been primarily implicated in the spread of leptospirosis, pigs have also been responsible and yet overlooked for their role in disease transmission to other animals, humans, as well as environmental contamination (Mwachui *et al.*, 2015). The aim of this study was to estimate the seroprevalence of antibodies against *Leptospira* spp. in farmed pigs in Grenada, after three decades of the first report.

Materials and Methods

Ethical Approval

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

Study Area

Grenada is the southernmost country in the Caribbean Sea with an area of 348.5 Km^2. It is separated into six parishes: St. Patrick, St. Mark, St. Andrew, St. John, St. George and St. David. With a tropical climate, it is most suitable for pig production.

Sample Collection and Processing

After approval of IACUC, 11 pig farms were randomly selected from all parishes of the country. Two farms from each parish and one from St. John were selected for the sample collection. 5 to 10 ml blood from 30-40 pigs from each farm was collected in red top tube via venipuncture from the cranial vena cava giving a sample size of 368 pigs. Each pig was assigned a number and the gender and parish were recorded. The

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blood samples were centrifuged for 15 minutes at 1500g at room temperature and serum was separated and stored in a freezer at – 80^0 C until tested.

Enzyme-Linked Immunosorbent Assay (ELISA) Test

Porcine Leptospira antibody ELISA from DRG instruments, GMBH, Germany was used for the test. The test was performed according to manufacturer's instructions.

Statistical Analysis

Data were analyzed using a chi-squared (X^2) analysis and stratified by gender of the pig and Parish of the farm in Microsoft Excel 2017 software Statistical significance was set at p=0.05.

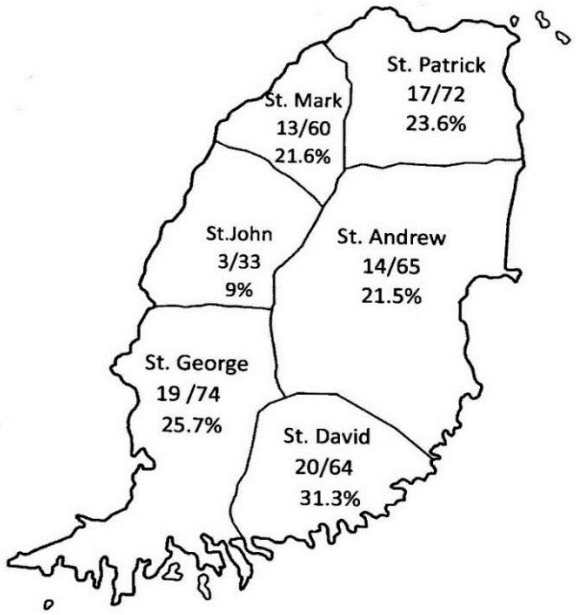

Fig. 1: Map of Grenada, W.I., demonstrating positive cases based on parish

Results

The overall prevalence 23.4% (95% Cl:19.14-28.03) of *Leptospira* antibodies was found in pig population in Grenada. The result of seropositivity for *Leptospira* spp. has been presented in Table 1 and Fig. 1. No statistical significance in seroprevalence was observed between parishes except St. John where a low prevalence of 9% was recorded. Prevalence of antibodies for *Leptospira* spp. according to sex and parishes has been included in Table 1.

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Table 1: Seroprevalence of antibodies to *Leptospira* spp. in pigs from Grenada according to parish and gender

Parish	Tested	Positive (%)	Male		Female	
			Tested	Positive (%)	Tested	Positive (%)
St. Georges	74	19 (25.7)	14	2 (14.3)	60	17 (28.3)
St. David	64	20 (31.3)	16	4 (25.0)	48	16 (33.3)
St. Andrew	65	14 (21.5)	20	4 (20.0)	45	10 (22.2)
St. Patrick	72	17 (23.6)	23	5 (21.7)	49	12(24.4)
St. Mark	60	13 (21.6)	8	3 (16.7)	42	10 (23.8)
St. John	33	3 (9.0)	10	1 (10.0)	23	2 (8.7)
Total	368	86 (23.4)	101	19 (18.8)	267	67 (25.0)

Discussion

In the present study, prevalence of serum antibodies for *Leptospira* in farm pigs from Grenada was 23.4%. The seroprevalence in all parishes was similar except St. John. This disparity is not well explained since the climate, rain fall and terrain for the entire country is similar. Future research involving more number of pigs from St. John may answer this difference. The only report on prevalence of serum antibodies in swine in Grenada was about 30 years ago by Everard *et al.* (1985), who found a slightly higher prevalence (35%) compared to the present result. Azevedo *et al.* (2008) reported a similar prevalence (33.6%) in Brazilian pigs. Lower prevalence of Leptospirosis in swine has been found in other countries of the world such as Vietnam, where Lee *et al.* (2017) found 8.1% and India, Bojiraj *et al.* (2017) reported 15.8% seropositivity in swine. Prevalence of *Leptospira* in swine population in countries of Latin America and the Caribbean has been reported in Trinidad, Mexico, and Argentina (Petrakovsky *et al.*, 2014). The variation in seropositivity of *Leptospira* in different countries is not well explained. However, the variation is indicated because of the difference in rain fall, flooding, humid climate and leptospirosis outbreaks in different countries of the world (WHO, 2008; Costa *et al.*, 2012).

In present study, seroprevalence among female pigs (25%) was found to be slightly higher than among male pigs (18.8%), but the difference was not significant. Our observation on sex difference is concurrent to Lee *et al.*, (2017). The farms used in this study had a predominance of female pigs which creates room for bias as far as sex and association with disease.

Conclusion

Compared to other countries, Grenada is a small developing nation making it easy for animal and human surroundings to intermix. A moderate prevalence of antibodies for *Leptospira* spp. (23.4%) recorded in the present study pose potential public health problems. Without proper animal husbandry and sanitation protocols enforced, disease transmission to pigs and then to farmers, abattoir workers and other animal handlers remains a high risk and should not be overlooked. It is important that researchers in and around

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Grenada consistently monitor trends of pathogenic *Leptospira* spp. in animals wherein close human interaction is anticipated.

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Conflict of Interest

The authors declare there is no conflict of interest.

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