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Serological evidence of antibodies to *Neospora caninum* in stray and owned Grenadian dogs

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Abstract. *Neospora caninum* causes abortion in cattle and neuromuscular disease in dogs, world wide. Cattle become infected by ingesting oocysts voided by dogs. The aim of this study was to estimate the seroprevalence of *Neospora caninum* in two populations of dogs (stray and owned) in Grenada, West Indies. Sera were collected from 625 dogs from all parishes in Grenada. Three hundred and sixty eight dogs were stray, while 257 dogs were owned. Sera were tested for the presence of antibodies against *N. caninum* using an indirect enzyme linked immunosorbant assay (ELISA) IDvet, France. Antibodies to *N. caninum* were found in 6 (1.6%) (95% confidence interval (CI): 0.32% to 2.88%) of the stray dogs and in 3 (1.2%, 95% CI: 0.13% to 2.53%) of the owned dogs. Seroprevalence did not differ significantly between the two populations (p=0.74) and between the males and females (p=1). These results suggest that the prevalence of *N. caninum* infection in dogs in Grenada is low.

INTRODUCTION

Neospora caninum is an intracellular protozoan parasite that mainly causes abortion, neonatal mortality in cattle and neuromuscular disorders in dogs. Dogs and coyotes are both definitive host and this parasite cycles between definitive host and herbivores intermediate hosts (cattle, sheep, goat, horse, bison and deer). There is no evidence for human infection. Dogs are capable of shedding oocysts in feces after eating tissues of infected animals and are thus important in the epidemiology of this parasite. The parasite can also be transmitted transplacentally in several hosts, being the major route of transmission in cattle (Dubey 2013). Neosporosis is one of the most common causes of bovine abortion and of great economic importance in intensely farmed cows. (Gozdzik *et al.*, 2011).

Seroprevalence of *N. caninum* in the dog has been reported from many countries of the world: Argentina (Basso *et al.*, 2001); New Zealand (Riechel *et al.*, 1998); Turkey (Coskun *et al.*, 2000); Brazil (Mineo *et al.*, 2001, Machado *et al.*, 2011); Italy (Capelli *et al.*, 2004, Paradies *et al.*, 2007); Chile (Patitucci *et al.*, 2001); Germany (Klein & Muller, 2001); Serbia (Kuruca *et al.*, 2013); Hungary (Hornok *et al.*, 2006); Switzerland (Sager *et al.*, 2006); Iran (Hosseininejad & Hosseini, 2011); Austria (Wanha *et al.*, 2005); Spain (Collantes- Fernandez *et al.*, 2008); Romania (Gavrea *et al.*, 2009); Poland (Gozdzik *et al.*, 2011); Senegal (Kamga-Waladjo *et al.*, 2013); India (Sharma *et al.*, 2008); Korea (Nguyen *et al.*, 2012); Costa Rica (Palovicini *et al.*, 2007). Dubey *et al.* (2007) reported isolation *N. caninum* from Argentina, Brazil, Germany, Australia, UK and USA.

There is paucity of information on neosporosis in dogs in the Caribbean countries. In the previous study in Grenada conducted 6 years ago, antibodies to *N. caninum* were determined by the indirect immunoflorescent antibody test (IFAT) revealing a seroprevalence in 2 of 107 dogs (1.8%) (Dubey *et al.*, 2008). The purpose of this study was to estimate the seroprevalence of *N. caninum* in dogs on larger sample size of owned and stray dogs.

MATERIALS AND METHODS

Blood samples were collected by venipuncture in owned (257) and stray dogs (368). Owned dogs were part of the "One Health One Medicine" fair organized by DVM students and faculty from the School of veterinary medicine St George's University (SGU) Grenada. Blood was collected from stray dogs captured by the Ministry of Health in conjunction with the Veterinary and Livestock division of the Ministry of Agriculture, Lands, Forestry, Fisheries and environment Government of Grenada under the stray dog control program. Two milliliters of blood was obtained from each animal. Blood samples were centrifuged at 1500g for 10 minutes, and the serum was collected and stored at -20ºC until assayed for antibodies to *N. caninum*. A commercial ELISA *N. caninum* kit from Pourquier laboratories (IDvet, France) was used for detection of *N. caninum* antibodies according to the manufacturer's instructions.

RESULTS

As shown in Table 1, antibodies to *N. caninum* were detected in 9 of 625 (1.47%) samples (6 from stray dogs and 3 from owned dogs). Out of the 6 stray dogs that were positive, 3 were male and 3 female. In the owned dog population, 2 were male and 1 female (Table 2). The seroprevalence of *N. caninum* in stray and owned dogs was not significantly different (p=0.74). Overall there was no significant difference in the exposure of *N. caninum* between male and female dogs (p=1).

DISCUSSION

The overall seroprevalence of of *N. caninum* in dogs in Grenada was 1.4% (95% CI: 0.48% to 2.32%). Compared to a previous study conducted on 107 dogs in Grenada (Dubey *et al.*, 2008), the prevalence remains similar over the last 6 years. Grenada is the only

Table 1. Seroprevalence of *N. caninum* in stray and owned dogs from Grenada.

Type of dog	Number tested	Number positive	Percent positive
Stray dogs	368	6	1.6
Owned dogs	257	3	1.2
Total	625	9	1.4

Table 2. Seroprevalence of *N. caninum* in dogs in Grenada according to gender

Sex	Stray dogs	Number positive	Percent positive	Owned dogs	Number positive	Percent positive
Male	148	3	2.0	137	2	0.7
Female	220	3	1.4	120	1	1.7

There is no statistical significance between gender and ownership status (p=1, Fisher's exact test).

Caribbean country known to have tested the prevalence of *N. caninum*. Therefore, it is difficult to contrast this study to similar geographical locations and island ecology dynamics. Prevalence comparable to our results was found in Austria 2.01% (Wanha *et al.*, 2005), Hungary 1.0% (Hornok *et al.*, 2006), Spain 2.9% (Collantes – Fernandes *et al.*, 2008). The Czech Republic 2.6% (Vaclavek *et al.*, 2003) and Sweden 0.5% (Bjorkman *et al.*, 1994). A higher seroprevalence have been reported in many countries: namely Serbia, (Kuruka *et al.*, 2013); Poland (Ploneczka & Mazurkiewicz, 2008), Katarzuna & Michal, 2008); Argentina (Basso *et al.*, 2001); India (Sharma *et al.*, 2008); and Spain (Regidor-Cerrilo *et al.*, 2010).

Current study was conducted to assess the seroprevalence of *N. canimum* in stray and owned dogs of Grenada. Although there was no significant difference between the two populations (stray and owned dogs; p=0.74), the results indicated slightly a higher seropositivity for *N. caninum* in stray dogs (1.6%; 95% CI: 0.32% to 2.88%) than in owned (1.2%: 95% CI: 0.13% to 2.53%). Exposure to *N. caninum* in owned and stray dogs has been investigated in several other countries. Their data suggest that farm/stray dogs have higher exposure and seropositivity than urban or owned dogs (Wanha *et al.*, 2005 in Austria; Basso *et al.*, 2001 in Argentina, Khanmohammadi & Fallah 2011, Haddadzadeh *et al.*, 2007 and Hosseininejad & Hosseini, 2011 in Iran, Hornok *et al.*, 2006 in Hungary, Katarzyna & Michal, 2008 in Poland, Regidor-Corilla *et al.*, 2010 in Spain, Antony and Williamson, 2003 in New Zeeland, Sanchez *et al.*, 2003 in Mexico, Ferroglio *et al.*, 2007 in Italy). Higher seroprevalence in stray and farm dogs indicates their accessibility to raw meat, abortion material from cattle/goat/sheep and oocyst contaminated food and water in comparison to owned dogs (Hosseininejad & Hosseini, 2011).

In our study there was no significant difference (p=1) between the gender of *N. caninum* antibody positive dogs (Table 2). Similar results have been reported by previous researchers (Ferroglio *et al.*, 2003; Wanha *et al.*, 2005; Hornok *et al.*, 2006; Sanchez *et al.*, 2007, Haddadzadeh *et al.*, 2007, Hosseininejad & Hosseini, 2011). However, some researchers found male dogs to be affected more than females (Klein & Muller, 2001; Khanmohammadi & Fallah, 2011, Katarzyna *et al.*, 2011) and they attributed for the higher serpositivity in males to be due to the preference of raising male dogs on farms and house holds. Yet still Wouda *et al.* (1999) suggested more seropositivity in female dogs.

The results of this study reveal a low seroprevalence (1.4%; 95% Ci: 0.48% to 2.32%) of *N. caninum* in the Grenadian dog population. Neosporosis is one of most common causes of bovine abortion. A recent survey of *N. caninum* antibodies in cattle in Grenada (unpublished data) indicated a low to moderate exposure (6.8%) to *N. caninum*. Further research related to oocyst shedding by seropositive dogs is proposed as this is considered to be a major risk factor in cattle production. Simultaneously cattle farmers need to be aware of the role of dogs in spreading this parasite to their livestock.

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