

# Seroprevalence of *Neospora caninum* in Sheep and Goats from Grenada, West Indies

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## Abstract

*Neospora caninum* (*N. caninum*), an intracellular protozoan parasite, causes abortion and neonatal mortality in many herbivores including sheep and goats. The aim of this study was to estimate the Seroprevalence of *N. caninum* in sheep and goats in Grenada, West Indies. Sera were collected from 138 sheep and 138 goats from all six parishes of Grenada and Carriacou, a small island within Grenada. Animals were identified by gender. Sera were tested for antibodies for *N. caninum* using an indirect Enzyme Linked Immunosorbant Assay (ELISA) multispecies Kit from Pourquire Laboratoire (IDvet, France). Antibodies to *N. caninum* were detected in 18 of 138 sheep (13%, 95% confidence interval (CI): 7.39% to 18.61%) and 8 of 138 goats (5.8%, 95% CI: 1.9% to 9.7%). Although seropositivity was high in female sheep and in male goats, there was no statistical difference in seropositivity of males and females in both species of animals ( $p > 0.05$ ,  $\chi^2$ ). The results of this study revealed that sheep and goats in Grenada were exposed to *N. caninum* and dogs might be considered as a source of infection. There was a need to educate the farmers, possessing small ruminants, about the transmission cycle of *N. caninum* and methods of prevention, to minimize the exposure of their flocks. This is the first report on seroprevalence of *N. caninum* in Grenada, West Indies.

## Keywords

Grenada, *Neosporacanium*, Seroprevalence, Sheep/Goats

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## 1. Introduction

*Neospora caninum* is an intracellular protozoan parasite. Dogs and coyotes are definitive hosts which shed infective oocyst in feces. Infected dogs suffer from neuromuscular disorders [1]. Intermediate hosts, mainly herbivores (cattle, sheep, goat, horse, bison and deer), get infected by ingestion of oocysts. The parasite can be transmitted transplacentally in several hosts including cattle, in which it is a major route of transmission. The parasite causes economic losses in intermediate hosts through abortion and neonatal mortality. It is a major cause of abortion in cattle, in many countries. Pregnant sheep are highly susceptible to experimental infection by *N. caninum* [2].

Seroprevalence of *N. caninum* in sheep and goats has been reported from a few countries of the world. In sheep it has been reported from Slovakia, [3], Brazil [4] [5], and New South Wales [6]. In goats it has been reported from Poland [7], Brazil [8] and Romania in dairy goats [9]. As far as the authors are aware, there is no published research on *N. caninum* in sheep and goats from the Caribbean region including Grenada.

The aim of this study is to report for the first time the seroprevalence of *N. caninum* in sheep and goats from Grenada, West Indies.

## 2. Material and Methods

### 2.1. Ethical Approval

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

### 2.2. Collection of Samples

Grenada is the southern most of the Windward Islands of Eastern Caribbean. Its area is approximately 344 Km<sup>2</sup>. It is divided in 6 parishes. Blood was collected from sheep and goats from all 6 parishes of Grenada and Carriacou. Carriacou is largest island of Grenadines, approximately 34 Km<sup>2</sup> with dependency on Grenada. To cover the representative samples, blood was collected from sheep and goats from all six parishes of Grenada and Carriacou. Blood was centrifuged at 3000 g for 5 minutes at 25°C and sera were stored at -20°C until tested.

A total of 138 blood samples from goats, and same number of samples from sheep, were collected for assay. It included 35 blood samples from goats and 35 from sheep, from Carriacou. Both species of animals were identified by gender.

### 2.3. Methodology

A commercial ELISA *N. caninum* multispecies kit from Pourquier Laboratories (IDvet, France) was used, for detection of *N. caninum* antibodies, according to manufacturer's instructions. According to manufacturer *N. caninum* multispecies kit has 100% sensitivity and 100% specificity.

### 2.4. Statistical Analysis

A Chi-square test ( $\chi^2$ ) of significance for comparison of seroprevalence of *N. caninum* in sheep and goats was used (<http://www.graphpad.com/quickcalcs/contingency>).

## 3. Results

As shown in **Table 1**, antibodies to *N. caninum* were detected in 18 of 138 sheep (13%, 95% confidence interval (CI): 7.39% to 18.61%) and 8 of 138 goats (5.8%, 95% CI: 1.9% to 9.7%). Chi-square value for comparison of seroprevalence in sheep and goats is as follows: Chi-squared equals 3.439 with 1 degree of freedom. The two tailed *P* value corresponding to chi-squared value is equal to 0.0637.

**Table 2** shows gender wise positivity in both species of animals. Out of 18 sheep that were positive, 2 were male (5.88%) and 16 (15.3%) females. In goats, 5 males of 45 (11.1%) tested were positive, while only 3 females of 93 tested were positive (3.2%).

Seroprevalence of *N. caninum* according to parish is shown in **Table 3**.

**Table 1.** Seroprevalence of *Neospora caninum* in sheep and goats from Grenada (West Indies).

Species of animal	Number of tested	Number of positive	Percent positive
Sheep	138	18	13.0%
Goats	138	8	5.8%

**Table 2.** Seroprevalence of *Neospora caninum* in sheep and goats from Grenada (West Indies) according to gender.

Sex	Sheep			Goats		
	No. tested	No. positive	Percent positive	No. tested	No. positive	Percent positive
Male	34	2	5.88	45	5	11.1
Female	104	16	15.3	93	3	3.2

**Table 3.** Proportion of sheep and goats seropositive to *Neospora caninum* according to parish in Grenada, WI.

Parish	Sheep	Goats	Sheep	Goats
	No. positive/No. tested	No. positive/No. tested	Percentage positive	Percentage positive
St. George	1/11	0/24	9%	0%
St. David	4/20	1/18	20%	5.5%
St. Andrew	5/37	0/27	13.5%	0%
St. Patrick	0/5	2/15	0%	13.3%
St. Mark	0/15	0/10	0%	0%
St. John	4/15	0/9	26.7%	0%
Carriacou	4/35	5/35	11.8%	14.2%

#### 4. Discussion

In the present study, the prevalence of antibodies for *N. caninum* in sheep is 13%, 95% confidence interval (CI): 7.39% to 18.61%), which is comparable to multiple Brazilian ovine *N. caninum* seroprevalence studies: 12.8% [10] and 9.2% [4] 10.3% in Switzerland [11] 16.8% from Greece [12] and 12.0% in Czech Republic [13]. Our results were much higher compared to *N. caninum* prevalence reported from the United Kingdom 2.2% [6], 3.7% from Slovakia [3], 1.8% from Brazil [5] and 2.0% from Italy [14].

We found seroprevalence of *N. caninum* in goats at 5.8%, which was similar with Anastasia *et al.* [12] who recorded 6.9% prevalence in goats in Jordan. High seropositivity compared to our results had been reported in Brazil at 15% [8] and in Poland at 9% [7]. In other studies, researchers found lower seroprevalence of *N. caninum* in goats compared to our results. Anameria *et al.* [9] in Romania found 2.3% seropositivity, Eduardo *et al.* [15] reported 3.3% goats with antibodies in Northeast region of Brazil; Abo-shehada and Abu-Halaweh [16] reported 2.0% seropositivity in goats, in Northern Jordan.

We reported higher prevalence of *N. caninum* antibodies in sheep (13%) compared to goats (5.8%) in Grenada. However, the difference was not statistically significant ( $p > 0.05$ ,  $\chi^2$ ). Anastasia *et al.* [12] also reported similar results while comparing the seroprevalence of *N. caninum* in these two species of animals.

In our study, there was no significant difference between the gender of sheep and goats for *N. caninum* antibody (Table 2). In sheep 15.3% females and 5.88% males had antibodies for *N. caninum* (Chi-square = 0.153,  $p$ -value 0.70), while in goats 11.1% males and 3.2% females had antibodies for *N. caninum* (Chi-Square = 0.004,  $p$ -value = 0.95). Eduardo *et al.* [15] also found no association between sex of goats and prevalence of anti-*N. caninum* antibodies. Similar studies comparing seroprevalence of *N. caninum* with gender was not available for sheep.

A few studies indicated the correlation between the presence of dogs on the farm and seroprevalence for *N. caninum* in small ruminants [16]. Herbert *et al.* [5] and Gustavo *et al.* [17] suggested positive correlation be-

tween the presence of dogs on the farm and seroprevalence of antibodies to *N. caninum* in sheep and goats. However, Figliuolo *et al.* [4] reported no association between dogs and prevalence of *N. caninum* antibodies in sheep.

There was paucity of information on neosporosis in dogs in the Caribbean region. A study conducted 6 years ago, on a small number of dogs, in Grenada revealed 1.8% seropositivity using indirect immunofluorescent antibody test (IFAT) [18]. Recent study by Sharma *et al.* [19] using ELISA revealed low seroprevalence (1.4%) of *N. caninum* in Grenadian dogs. Since dogs were positive for *N. caninum* antibodies and the results of this study revealed antibodies to *N. caninum* in of sheep and goats, therefore in Grenada, dogs might be considered as a source of infection to small ruminants. Further studies were warranted to assess this hypothesis.

*N. caninum* was considered as a major pathogen for cattle and dogs. It occasionally caused clinical infections in horses, sheep, goats and deer [1]. There was a poor documentation of reproductive disorders in Grenada, by farmers, in their sheep and goats. There was an urgent need for campaign to educate farmers about keeping a good record of reproductive disorders in their sheep and goat flocks in order to assess the role of *N. caninum* in clinical cases of reproductive disorders. Farmers might further be made aware to maintain hygienic conditions without presence of dogs on the small ruminant flocks.

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## Competing Interests

The authors declare that there is no competing interest.

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