Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/September-2014/6.pdf

RESEARCH ARTICLE
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Prevalence of *Toxoplasma gondii* antibodies in stray and owned dogs of Grenada, West Indies

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Received: 21-05-2014, **Revised:** 18-07-2014, **Accepted:** 25-07-2014, **Published online:** 07-09-2014

doi: 10.14202/vetworld.2014.661-664. **How to cite this article:** Sharma RN, Ordas G, Tiwari K, Chikweto A, Bhaiyat MI, Allie CD, Paterson T (2014) Prevalence of *Toxoplasma gondii* antibodies in stray and owned dogs of Grenada, West Indies, *Veterinary World* 7(9): 661-664.

Abstract

Aim: This serological survey was undertaken to estimate the prevalence of *Toxoplasma gondii* in two populations of dogs (stray and owned dogs) in Grenada. Dogs get infected with oocysts voided from cats, definitive hosts of *T. gondii*. In dogs, *T. gondii* causes subclinical to clinical disease. Earlier studies conducted in Grenada on a small population of owned dogs showed evidence of exposure to *T. gondii*.

Materials and Methods: Antibodies to *T. gondii* were determined in serum samples from 625 dogs (368 stray and 257 owned dogs) from around all six parishes in Grenada, West Indies, using an indirect enzyme linked immunosorbent assay.

Results: Antibodies to *T. gondii* were found in 123 (33.4%; 95% confidence interval [CI]: 28.58-38.22%) of stray dogs and in 64 (25%; 95% CI: 19.71-30.29%) of the owned dogs. Seropositivity was higher in stray dogs than in pet dogs (p=0.026). Whereas, there was no sex predisposition to seropositivity in owned dogs (p=1.0), female stray dogs showed a higher prevalence than male stray dogs (p=0.04).

Conclusion: These results support previous findings that *T. gondii* is prevalent in Grenada. In this study, overall seropositivity for *T. gondii* in dogs in Grenada is lower than noted in 2008, but is still higher in stray dogs than in owned dogs.

Keywords: dog, ELISA, Grenada, *T. gondii.*

Introduction

Toxoplasma gondii is a coccidian parasite that is widely prevalent in all warm blooded animals including humans worldwide [1]. *T. gondii* is an important zoonotically as it causes congenital defects or abortion and fatal disease in immunocompromised humans. Humans and other animals can become infected by ingesting tissue cysts from undercooked meat or from food or drink contaminated with oocysts shed in cat feces. Dogs play an important role in the mechanical transmission of oocysts to humans. Dogs roll in cat feces and other smelly substances, thus contaminating their fur, serving as a source of infection to young children [2]. Humans also become infected by consuming undercooked dog meat where dogs are used for food [3].

Not all *T. gondii* infected dogs show clinical signs. However, *T. gondii* causes respiratory, alimentary and neurological signs and muscular abnormalities occasionally in conjunction with viral infection and stress [3,4]. To the best of our knowledge, there is no published report on the prevalence of *T. gondii* in dogs in the Caribbean countries except Grenada. The prevalence of *T. gondii* antibodies has been reported in dogs, pigs, sheep, goats, and cattle in Grenada, West Indies [5,6]. In 2008, a study using the modified agglutination test (MAT) on 107 dogs revealed 48% positivity for *T. gondii* in Grenada [5] In Grenada, large numbers of stray dogs are found roaming the streets, fresh open markets, and public places. These stray dogs can act as source of many zoonotic diseases.

The present study assessed the prevalence of *T. gondii* antibodies in two populations of dogs (stray and owned) from all the six parishes of Grenada, West Indies.

Materials and Methods

Ethical approval

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

Test procedure

Blood samples were collected by venipuncture in owned and stray dogs. The owned dogs were part of the "one health one medicine" fair and vaccine clinics organized by veterinary students and the faculty from the School of Veterinary Medicine, St. George's University Grenada. Stray dogs captured by the Ministry of Health in conjunction with the Ministry of Agriculture, Land, Fisheries and Environment,

Government of Grenada under the stray dog control program were used in the study. A volume of 2 ml of blood was obtained from each animal. The blood samples were transported, within 2 h of collection, to the research laboratory at seminal vesicle mesenchyme and centrifuged for 10 min at 1500 g. The separated sera were stored frozen at (−20°C) until analysis. The presence of *T. gondii* antibodies was analyzed in duplicate by indirect enzyme linked immunosorbent assay (ELISA) (ID screen toxoplasmosis-indirect IDVet, France). Frozen serum samples were thawed and diluted 1:10 before ELISA test. The protocol was followed as directed in the IDVet indirect ELISA kit.

Statistical analysis

Confidence intervals (CI) were calculated using the following website: (http://www.mccallumlayton.co.UK/states/confidence intervalCalcProportion.aspx).

Results

In the present study, a total of 625 dogs (368 stray dogs and 257 owned dogs) were examined. Based on this sample population seropositivity for *T. gondii* was 123 (33.4%; 95% CI: 28.58-38.22%) for the stray dogs and 64 (25%; 95% CI: 19.71-30.29%) for the owned dogs (Table-1). This investigation also showed that the prevalence in female stray dogs was 37.7% (95% CI: 31.3-44.1%) and 27% (95% CI: 19.85-34.15%) in male stray dogs, whereas that for owned dogs 22.5% (95% CI: 15.03-29.97%) in female and 27% (95% CI: 19.57-34.43%) in male dogs (Table-2).

Discussion

Researchers have used different laboratory methods to detect serprevalence of *T. gondii* in dogs in different counties. MAT is most widely used [7-10]. MAT is now indicated to give false positives due to cross reactivity. Latex agglutination test (LAT) has also been used [11-14]. However, Ahmad *et al.* [14] indicated that LAT might give false positive due to interfering factors (rheumatoid factor and IgG antibodies). Immunoflourescent antibody test has been used by others [15-18]. Indirect hemagglutination antibody (IHA) test has also been used [3,19]. ELISA has recently been used by many researchers [17,19-21]. Limited studies comparing merits and demerits of various methods in serology of *T. gondii* are available. Miereles *et al.* [21] comparing ELISA with IHA recommended superiority of ELISA. We also used ELISA in this study, with ID screen toxoplasmosis indirect multispecies kit. The manufacturer (IDvet France) reports 100% specificity and 100% sensitivity of their ELISA kit.

In the present study, the overall seropositivity for *T. gondii* in stray dogs was significantly higher (33.4%; 95% CI: 28.58-38.22%) than in owned dogs (25%; 95% CI: 19.71-30.29%) (p=0.026). In Grenada large numbers of stray dogs are found roaming the streets, fresh open markets and public places allowing for potential exposure to *T. gondii* oocyst contaminated environment, compared to owned dogs which are mostly indoor and are less likely to be exposed to *T. gondii*.

Similar results have been found in other studies. In Pakistan, Ahmad *et al.* [14] reported 78.5% in stray dogs and 34.6% in owned dogs; whereas Jadoon *et al.* [12] found 83.33% positivity in stray dogs and 37.95% in owned dogs. In Iran a seroprevalence of 31.2% in stray dogs and 9.03% in owned dogs was reported by Hosseinininejad *et al*. [22], while Shadfar *et al*. [20] in Iran found 77.7% in stray dogs and 40% in owned dogs. Thuy *et al.* [13] in Korea, while comparing the prevalence of antibodies in stray dogs with owned dogs, found a higher proportion in stray (18.5%) than in owned dogs (5.1%). Other researchers, although not comparing two population of dogs (stray vs. owned) reported high positivity in stray dogs (51.3%) in Turkey [17]; 40.3% in an urban rural gradient in China [20]; 67.4% in Sri Lanka [7]; 38.0% in Portugal [8]; 50.5% in Brazil [23]; 16.8% in Colombia [22]. A low seropositivity (10.81%) has been found in owned dogs in Northwest China [9]; 3.50% in Southwestern China [3]; 10.0% in Northwestern China [20]; 30.9% in Northeastern China [10] and 25.9% in Czech Republic [16].

The difference in seroprevalence amongst different parts of the world suggests that climatic changes influence the prevalence of *T. gondii*, in which warmer and moist conditions have a higher seroprevalence.

Table-1: Seroprevalence of *T. gondii* in stray and owned dogs from Grenada.

Type of dog	Number of tested	Number of positive	Percent positive
Stray dog	368	123	33.5
Owned dog	257	64	25.0

Fisher's exact test (stray dogs vs. owned dogs): p=0.0263. There is a significant difference in seropositivity to *T. gondii* between stray and owned dogs. *T. gondii* = *Toxoplasma gondii*

Table-2: Seroprevalence of *T. gondii* in stray and owned dogs according to gender

Sex	Stray dogs	Number of positive	Percent	Owned dogs	Number of positive	Percent
Male	148	40	27.0	137	37	27.0
Female	220	83	37.7	120	27	22.5

Fisher's exact test (stray dogs) male versus female: p=0.04, There is a significant difference in seropositivity to *T. gondii* between stray female dogs and stray male dogs. Fisher's exact test (pet dogs-male versus female): p=1.0000, There is no significant difference in seropositivity to *T .gondii* between owned female dogs and owned male dogs. *T. gondii*=*Toxoplasma gondii*

The moist and warm conditions that we have in Grenada make the oocysts more viable than in dry, hot, or cold climates [17].

Since cat is the primary host, seropositivity of *T. gondii* in cats bears a relationship with seropositivity in dogs [15,16]. In Grenada, antibodies to *T. gondii* were found in 57% of pregnant women and 35% of cats [24]. Yet again in 2009, screening for *T. gondii* antibodies in domestic and feral cats was undertaken using a MAT, with seropositivity in 30.6% of domestic cats and 27.7% of feral cats [25]. In Grenada a decreasing rate of seropositivity in cats from these studies correlates well with a concurrent decrease in seropositivity in dogs observed in the current study and also an earlier report [5].

Statistical analysis showed that difference in *T. gondii* infection between female and male stray dogs was significant (p=0.04), whereas in owned dogs there was no significant difference in gender (p=1.0). Previous researchers [8,9,13,14,16,22,26,27] did not find a significant difference in *T. gondii* antibodies between male and female stray and owned dogs. Shadfar *et al* [20] reported higher antibody titers in male owned dogs; and Alain *et al*. [28] in owned dogs found bitches 2.5% more infected with *T. gondii*.

Conclusion

The overall seropositivity for *T. gondii* in dogs in Grenada in this investigation is lower than that reported in 2008, and the percentage remains higher in stray dogs than in owned dogs and is therefore of a concern to both animal and human health.

Recommendation

Since stray dogs and cats may act as reservoirs of many zoonotic diseases in addition to *T. gondii*, a stray dog and cat control program should be instituted in Grenada.

Authors' Contribution

TP and CA collection of blood from owned and stray dogs respectively, AC, KT and GO conducted ELISA, AC also did statistical analysis, RNS preparation of project, overlooked all aspects of the project and manuscript writing with GO and MIB draft and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The research was supported by funds from Small Research Grant Initiative (SRGI 12010) at St. Georges University, Grenada.

Competing Interests

The authors declare that they have no competing interests.

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