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Seroprevalence of *Toxoplasma Gondii* in Sheep and Goats in Grenada, West Indies

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A B S T R A C T

Toxoplasma gondii (*T. gondii*) is a coccidian parasite which infects all warm blooded animals, including humans, worldwide. Definitive host is felid which voids infectious oocysts in feces contaminating environment. Like other intermediate hosts, sheep and goats infected with oocysts, develop tissue cysts in their tissues. Consumption of undercooked infected meat from sheep and goats and consumption of goat milk are important routes of infection to humans. An earlier study conducted in Grenada on sheep and goats showed serological evidence of exposure to *Toxoplasma gondii* in these ruminants. Modified agglutination test (MAT) was the serological test used in the previous study. The present study was conducted on the same blood samples of sheep and goats using another serological test (ELISA) Toxoplasmosis indirect multi-species, IDvet, France to compare the superiority of serological test. Both tests confirmed the exposure of sheep and goats to *T. gondii* infection in Grenada. Blood samples from 138 sheep and 138 goats tested earlier by MAT when retested by indirect ELISA revealed 71.0% (95% Confidence interval (CI): 64.77% to 77.23% sheep and 49.3% (95% CI: 42% to 56.6%) goats positive for *T. gondii* antibodies. There was statistically significant difference in prevalence for *T. gondii* in sheep vs goats (two tailed p value equals 0.0003). Seropositivity was higher in female (71.7%) and male (69.2%) sheep compared to goats (female 55.9% and male 40.0%) The gender value in male and female sheep was not statistically significant (fisher exact test: two tailed p value equals 0.8358.); where as in goats there was statistically significant difference in male and female (fisher exact test: the two tailed p value equals 0.0290). Results show a high prevalence of antibodies for *T. gondii* in sheep and goats in Grenada and the sister Island Carriacou, based on MAT and ELISA. Public health authorities of the country need a strong campaign for public awareness for the prevention of infection to human population.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite of the Phylum Apicomplexa. *T. gondii* infections

are widely prevalent in humans and animals worldwide (Dubey, 2010). *T. gondii* has been the focus of many studies worldwide

due to its economic importance in causing reproductive losses in animals and a zoonotic risk to humans. Ingestion of oocysts shed by cats is the main source of infection for herbivores, where tissue cysts develop in infected animals. Humans become infected post natively mainly by ingesting tissue cysts from undercooked meat and goat milk (Jones *et al.*, 2009; Dubey, 2010) in infected animals. Limited data, on seroprevalence of *T. gondii*, in small ruminants from Caribbean region are available. First report on the prevalence of *T. gondii*, in animals, in Caribbean is from Trinidad (Adesiyun and Cazabon, 1996) who found *T.gondii* agglutinin antibodies in 42.9% goats and 5.5% pigs.

Chikweto *et al.* (2011) in Grenada using modified agglutination test (MAT) found prevalence of antibodies in sheep (44.1%) and in goats (42.8%). In this research we also tested the sera by ELISA which were used in MAT (Chikweto *et al.*, 2011). Recently, Clare *et al.* (2014) using ELISA reported seroprevalence of *T.gondii* in sheep and goats from four Caribbean nations viz Dominica, Grenada, Montserrat and St. Kitts and Nevis.

Of many procedures used in the diagnosis of *T. gondii*, various serological tests have been used to detect antibodies to *T. gondii*: Sabin-Feldman Dye test (DT), indirect hemagglutination (IHA), Indirect fluorescent antibody test (IFAT), modified agglutination test (MAT), Latex agglutination test (LAT), Enzyme linked immunosorbent assay (ELISA) and complement fixation test (CF) (Dubey, 2010). Barring a few researchers, comparison of the serological tests with their merits and demerits has not been reported. The aim of this study is to report the seroprevalence of *T. gondii* in sheep and goats in Grenada comparing the results obtained by MAT and ELISA.

Materials and Methods

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

Grenada is the southern most of the Windward Islands of Eastern Caribbean. Its area is approximately 344Km². It is divided in 6 parishes. Carriacou is the largest island of Grenadines, approximately 34 Km² with dependency on Grenada. To cover the representative samples, blood was collected from sheep and goats from all 6 parishes of Grenada and Carriacou. Carriacou is equal to one parish of Grenada, hence results have not been shown separately, but considered for Grenada as a whole. Blood was centrifuged at 3,000g for 5 minute at 25⁰C and sera were stored at -20⁰ C until tested.

A total of 204 sheep and 180 goat sera were tested by MAT and results published (Chikweto *et al.*, 2011). For ELISA test, sera from this pool were selected randomly, without identifying positive and negative results with MAT. One hundred and thirty eight samples from goats and same number from sheep were tested by ELISA. To make up the number of samples equal, 8 sera from sheep from Grenada and 3 sera from goats from Carriacou were obtained and included in the study. Both species of animals were identified by gender.

A commercial ELISA *Toxoplasma gondii* multispecies ELISA kit from Pourquier laboratories (IDvet, France) was used for detection anti-*T.gondii* antibodies according to manufacturer's instructions.

Result and Discussion

Seroprevalence for anti-*T.gondii* was detected in 98 of 138 sheep (71.0%); 95% Confidence interval (CI): 64.77% to 77.23%

and 68 of 138 goats (49.3%, 95% CI: 42% to 56.6%). The results are presented in Table 1. In both species of animals females had higher seroprevalence (sheep 71.7%; goats

55.9%). The seroprevalence in males was lower in both species (Sheep 69.2% and goats 40.0%). The results according to gender are presented in Table 2.

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

Species of animal	Number of tested	Number of positive	Percents positive
Sheep	138	98	71.0
Goats	138	68	49.3

Sheep vs goats

Fisher's exact test

The two-tailed P value equals 0.0003

Considered to be extremely statistically significant.

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

Sheep				Goats		
Sex	No. tested	No. positive	Percent	No. tested	No. positive	Percent
Male	39	27	69.2	45	16	40.0
Female	99	71	71.7	93	52	55.9

Male vs female sheep

Fisher's exact test:

The two-tailed P value equals 0.8358

Not statistically significant.

Male vs Female goats

Fisher's exact test:

The two-tailed P value equals 0.0299

Considered to be statistically significant.

In the first study, on seroprevalence of *T. gondii* in food animals, in Grenada, Chikweto *et al.*, (2011) found antibodies in goats (42.8%) and in sheep (44.1%) using MAT. Clare *et al.*, (2014) using ELISA reported high seroprevalence for *T. gondii* in Grenada (48% in sheep and 57% in goats) compared to observations of Chikweto *et al.*, (2011). In the present study using ELISA, we found still higher seroprevalence for *T. gondii* in sheep (71.0%) than in goats (49.3%) in Grenada.

Of many serological tests used for seroprevalence of *T. gondii* antibodies, ELISA has been adopted in most domestic animals including sheep and goats (Dubey, 2008; Dubey, 2009).

Langoni *et al.*, (2011) while testing antibodies to *T. gondii* in sheep, reported superiority of MAT compared to Immunofluorescent antibody test (IFAT). Van der Puije *et al.*, (2000) testing *T. gondii* antibodies in sheep and goats by IFAT and

ELISA, found ELISA more sensitive (92%) and specific (91%). Abu- Samara *et al.*, (2007) reported a higher rate of seroprevalence in sheep for *T. gondii* using IFAT compared to ELISA. However, Mustaq and Zahida, (2010) did not find difference in seroprevalence of *T. gondii* in sheep using LAT and ELISA. More studies to compare the result of other serological tests with ELISA are needed to prove the suitability of ELISA for *T. gondii* seroprevalence in animals.

In the present study, we found higher seroprevalence of *T.gondii* antibodies in sheep (71%) compared to goats (49.3%). Van der Puije *et al.*,(2000); Hasemi, F., (1996); Masala *et al.*, (2003); Bekele and Kasali, (1989); Sharif *et al.*, (2007); Anamaria *et al.*, (2012) also reported higher seroprevalence of *T. gondii* in sheep than goats. Clare *et al.*, (2014) found higher positivity in sheep from Dominica, Montserrat and St Kits Nevis islands of Caribbean region except in Grenada where they found higher prevalence in goats. Higher seropositivity in sheep than in goats is advocated by many researchers because of the difference in feeding habits of these two species. Goats feed on shrub leaves with minimum chance of contamination with oocysts from cats, where as sheep feed on ground grasses with more chances of contamination from cat feces (Sharif *et al.*2007). Contrary to reports of higher seropositivity in sheep many researchers found higher seroprevalence of *T. gondii* antibodies in goats (Dubey, 1985; Pita Gondim *et al.*, 1999; Prelezov *et al.*, 2008, Hove *et al.*, 2005; Renate and Aspock 1996). Prelezov *et al.*, (2008) suggested the reason for higher prevalence in goats was because of an immunosuppressive state in goats while others advocate the oocyst from cat contaminated environment where goats were kept.

Sera tested by ELISA in the present study showed higher seroprevalence in females in both sheep and goats. Previous researchers (Van der Puije, *et al.*, (2000); Sathaporn, *et al.*, (2005), Sharma, *et al.*, (2008), Sharif, *et al.*, (2007) had similar findings. Contrary to this, Mustaq *et al.*, (2010) found seroprevalence more in males than in females, in sheep. Samad *et al.*, (1993), Eduardo *et al.*,(2007), Bahreini *et al.*, (2008) and Herbert *et al.*, (2009) reported no significant sex differences in both sheep and goats. No solid reason to explain the susceptibility of females over male is available. However, Alexander and Stinson (1988) reported that female animals are more susceptible than males to infection with protozoan parasites.

Conclusion

In Grenada, following chicken meat, there is great liking for goat and sheep meat (mutton). Our result shows a high rate of exposure, of sheep and goats, to *Toxoplasma gondii*, in Grenada. Thus, consumption of undercooked meat from sheep and goats and milk from infected goats poses a significant risk for humans. Government and health personnel have great responsibility to make public aware of this risk factor for the prevention of Toxoplasmosis in humans.

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