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ORIGINAL PAPER

Toxoplasmosis in the Caribbean islands: literature review, seroprevalence in pregnant women in ten countries, isolation of viable *Toxoplasma gondii* from dogs from St. Kitts, West Indies with report of new *T. gondii* genetic types

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Abstract Little is currently known of clinical toxoplasmosis in humans and animals in the Caribbean. We investigated the prevalence of IgG and IgM antibodies in 437 pregnant women from 10 English speaking Caribbean countries. Overall, antibodies (IgG) to *Toxoplasma gondii* (modified agglutination test, MAT, cut-off 1:6) were found in 174 (39.8 %) of 437 human sera; specifically 12 of 38 from Antigua-Barbuda, 26 of 52 from Belize, 9 of 50 from Bermuda, 29 of 49 from Dominica, 18 of 49 from Grenada, 16 of 47 from Jamaica, 5 of 15 from Montserrat, 8 of 44 from St. Kitts/Nevis, 24 of 45 from St. Lucia, and 27 of 50 from St. Vincent/Grenadines were seropositive. All IgG-positive sera were tested for IgM antibodies using the immunocapture method; all sera were negative for IgM antibodies. Additionally, tissues and sera of 45 dogs from St. Kitts were examined for *T. gondii* infection. Antibodies (IgG, MAT, 1:≥25) were found in 19 (42.2 %) of 45 dogs. Muscle samples (tongue, leg) of 19 seropositive dogs were digested in pepsin, and homogenates were bioassayed in mice. Viable *T. gondii* were isolated from 6 dogs. *T. gondii* isolates were further propagated in cell culture. PCR-RFLP genotyping of cell culture derived tachyzoites using 10 genetic markers, SAG1, SAG2 (5′ and 3′ SAG2, and alt.SAG2) SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico revealed that 4 isolates were ToxoDB PCR-RFLP genotype #2, and 2 were new genotypes #264 and #265. Review of 22 viable *T. gondii* isolates from chickens, dogs, and cats from Grenada and St. Kitts revealed that 1 isolate was type II, 13 were type III, and 8 were atypical. Thus, type III strains were predominant. Overall, the study revealed high prevalence of *T. gondii* in the Caribbean islands.

Keywords *Toxoplasma gondii* · Human · Dog · Caribbean · Bioassay · Genotype

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Introduction

Toxoplasma gondii infections are prevalent in animals and humans worldwide (Dubey 2010). Although each Caribbean island is separated from the others by the Caribbean and Atlantic oceans, tourism and the presence of multiple education institutions ensure that these islands are not isolated from each other. Further, food and water-borne illness in travelers returning from trips to these countries remains a public health concern (Sepulveda-Arias et al. 2014). Relatively, little is

known of toxoplasmosis in humans in Caribbean countries. The available reports of *T. gondii* infection in the Caribbean countries are listed in Table 1. Some of these surveys were conducted more than two decades ago, and different serological tests and sampling methods were used; thus, results are not directly comparable among countries.

One of the objectives of the present study was to test sera from pregnant or child-bearing age women from 10 English speaking

Table 1 Reports of *T. gondii* antibody prevalence and toxoplasmosis in humans from the Caribbean countries

Country	No. tested	Category	Year samples collected	Serological test, cut-off	Positive no. (%)	Remarks	References
Trinidad	504	Newborn cord blood	2002–2003	EIA Kit[a]	IgG 220 (43.7), IgM 2 (0.4)	Seroprevalence increased with age. Prevalence was higher in East Indian (54.1 %) versus 36.4 % of African descent.	Adesiyun et al. (2007)
Trinidad and Tobago	450	Women	2002–2005	EIA Kit[a]	159 (35.3)	IgG in 83 (35.8 %) and IgM in 8 (3.4 %) of 232 pregnant women in antenatal clinics, of 218 women in health centers, 76 (34.9 %) had IgG, and 26 (11.9 %) had IgM	Ramsewak et al. (2008)
Trinidad and Tobago	407	Adults, healthy sugar cane workers	Not stated	EIA Kit[a], IgM	64 (15.7)	High percentage with IgM, suggesting recent infection	Adesiyun et al. (2010)
Grenada	534	Pregnant women	1995	IMX ELISA[b]	305 (57)	Prevalence increased with age.	Asthana et al. (2006)
Saint Lucia	54	All ages	1964	Dye test, 1:16	23 (42.5)	Blood on filter papers tested.	De Roever-Bonnet et al. (1969)
Trinidad	300	15–40 years	1991–1992	ELISA[d]	130 (43.3)	No evidence of seroconversion during pregnancy.	Orrett (1993)
Jamaica	3267	15–45-year-old pregnant women	1986	ELISA[c]	911 (56.8)	129 (7.5 %) were considered to have recent infection, based on arbitrarily selected level of IgG antibody concentration.	Prabhakar et al. (1991)
Jamaica	511	All ages	1987	IgG ELISA[c]	230 (45)	26 % prevalence in 0–12 months old, 24 % in 13–24 month old, 42–48 % in lactating and pregnant. Higher prevalence in rural (58 %) than urban (46 %).	Rawlins and Prabhakar (1989)
Jamaica	1	Newborn	1956	Autopsy		Child born weak died 2 h after birth. Disseminated toxoplasmosis.	Sterlin and Dixon (1959)
Jamaica	603	Adults	2007	CT scan	2 toxoplasmic encephalitis	Patients enrolled in HIV/AIDS clinic. Authors mentioned 6 other patients with toxoplasmic encephalitis. However, based on CT scan, the diagnosis is only presumptive.	Barrow et al. (2010)
La Guadeloupe, French West Indies	3238	All ages	1979	IFAT, 1:50	2043 (60)	1 % of random population sampled. Prevalence rates 40 % in 0–5-year-old, 50 % in 6–10-year-olds. No difference in seroprevalence with respect to meat consumption. Prevalence higher in houses with cats (61 %) versus homes without cats.	Barbier et al. (1983)
La Guadeloupe, Martinique, French West Indies	9950	All ages		CFT; 1:1, IHAT; 1:1	56.9, 65.6	Increasing prevalence during teens.	Tribouley et al. (1978)

[a] Diamedix, Miami, USA

[b] Toxo IgG 2.0 kit, Abbott Laboratories, Abbott Park, Illinois, USA. Samples were collected in 1995

[c] Labsystems, Finland, Helsinki

[d] Pharmacia, Milton Keyes, UK

Caribbean countries (Table 2). The target was 50 samples from each of the participating country. This research was a 5-year program funded by the Canadian Global Health Research Initiative in the Caribbean with a focus on research, capacity building, and knowledge translation. The second objective was to isolate and genotype viable *T. gondii* from dogs from St. Kitts to supplement the reports on toxoplasmosis in cats and dogs from St. Kitts and Grenada (Dubey et al. 2008, 2009b; Sharma et al. 2014).

Humans become infected postnatally mostly by the ingestion of infected meat or food and water contaminated with oocysts. Although only cats can excrete the environmentally resistant oocysts, petting of dogs has been linked epidemiologically as risk factor for *T. gondii* infection in humans (Frenkel and Parker 1996; Lindsay et al. 1997). Dogs are known to rollover and eat cat feces, and oocysts can pass unchanged through the dog gut; viable *T. gondii* oocysts have been isolated from dog feces (Schares et al. 2005).

As stated earlier, little is known of clinical toxoplasmosis in humans in the Caribbean, and there is no report of it from St. Kitts. In general, why some species and some individuals become ill whereas most remain asymptomatic is unknown. Genetic characteristics of *T. gondii* are thought to be associated with clinical illness in humans. Therefore, we genotyped viable isolates of *T. gondii* from dogs from St. Kitts to supplement the information on *T. gondii* isolates from cats.

Materials and methods

Human sera

Blood samples were collected from up to 50 healthy, pregnant women from 10 countries (Table 2) over a 3-year period from 2009 to 2011. The recruitment of pregnant women for this study used the same strategy that was used in the Arctic Monitoring and Assessment Programme (AMAP, www.amap.no), carried out in circumpolar countries, which focused on assessing exposures to persistent organic pollutants (Van Oostdam et al. 2004). Following this protocol, pregnant and delivering women ≥18 years coming to the main hospital or health clinics during their last prenatal visits or to deliver were invited to participate in this study by the local nurses. In most cases, blood samples were taken before delivery; however, in some cases where this was not possible, the sampling was done within 2 weeks of delivery. In accordance with the AMAP protocol, a sample size of 50 mothers ≥18 years for each country was set. Informed consent was obtained from all participants, and all serum samples were processed at Ross University School of Veterinary Medicine in St. Kitts (Wood et al. 2014).

Aliquots of sera were transported cold to the National Reference Centre on Toxoplasmosis, Reims, France for *T. gondii* testing. All sera were diluted 1:6 to 1:800 (Table 2) and tested for *T. gondii* IgG antibodies by the modified agglutination test (MAT) as described (Desmonts and Remington 1980; Dubey and Desmonts 1987). Whole formalin preserved tachyzoites and mercaptoethanol were used in this test. The MAT detects only IgG because the mercaptoethanol destroys IgM like factors in the sera. The MAT is considered highly specific for *T. gondii* antibodies in all hosts. Viable *T. gondii* has been isolated from animals with titer as low as 1:5 (Dubey et al. 2016; Richomme et al. 2009; Villena et al. 2012). Sera that were positive in MAT were further tested for IgM antibodies using the procedure described (Pinon et al. 1996).

Dog samples

The samples were from 45 feral dogs euthanized at the Clinical Pathology Laboratory of Ross University School

Table 2 Seroprevalence of *T. gondii* in pregnant women in 10 Caribbean countries

Country	No. tested	No. positive[a]	% positive	No. of sera with MAT titers of:					
				6	10	25	50	100	≥200
Antigua-Barbuda	38	12	32	1	0	4	3	0	4
Belize	50	26	52	2	4	6	6	2	6
Bermuda	50	9	18	2	3	0	2	0	2
Dominica	49	29	59	5	10	3	4	5	2
Grenada	49	18	37	4	4	3	1	3	3
Jamaica	47	16	34	1	6	2	2	2	3
Montserrat	15	5	33	1	0	2	1	1	0
St. Kitts/Nevis	44	8	18	1	2	2	0	3	0
St. Lucia	45	24	53	1	10	4	5	3	1
St. Vincent/Grenadines	50	27	54	4	9	4	6	2	2
Total	437	174	40	22	48	30	30	21	23

[a] MAT = 1:6 or higher

Table 3 Isolation of *T. gondii* from muscle of dogs from St. Kitts, West Indies by bioassay in mice

Dog			*Toxoplasma gondii*			
No.	Date euthanized	Sex	MAT titer	Bioassay in SW mice	Isolate designation	ToxoDB genotype #
2	10/17/2007	F	100	4/4[a]	TgDogStK1	#2
15	10/17/2007	F	200	4/4	TgDogStK2	#2
10	11/02/2007	F	100	4/4	TgDogStK3	#2
7	02/13/2008	F	200	4/4	TgDogStK4	#2
6	03/04/2008	F	50	4/4	TgDogStK5	#264
10	03/04/2008	F	100	4/4	TgDogStK6	#265

[a] No. of mice *T. gondii* positive/No. of mice inoculated with dog tissues

of Veterinary Medicine collected from St. Kitts, West Indies during October 2007-March 2008. All procedures involving handling, sample collection, surgery and euthanasia were performed according to the Institutional Animal Care and Use Committee from Ross University, School of Veterinary Medicine. No animal was euthanized solely for the present study. Blood for serology, and muscle from tongue and leg were submitted to the Animal Parasitic Diseases Laboratory, United States Department of Agriculture in Beltsville, Maryland, USA for *T. gondii* examination.

Sera of dogs were tested for *T. gondii* antibodies by the same MAT used to test human sera. A titer of 1:≥25 was considered as evidence of *T. gondii* exposure.

Bioassay in mice

Muscle (50 g pool of tongue and leg muscle) of individual dogs were homogenized, digested in acidic pepsin, and washed, and aliquots of each homogenate were inoculated subcutaneously into 4 outbreed Swiss Webster (SW) mice (Table 3), and one homogenate (dog #15) was also inoculated in to 3 interferon gene knockout (KO) mice (Dubey 2010). Mice were bled on 45 days post-inoculation (p.i), and a 1:25 dilution of serum was tested for *T. gondii* antibodies by MAT as described previously. Mice were killed on 46 days p.i., and an unstained brain squash of each brain was examined for *T. gondii* tissue cysts (Dubey 2010). Smears of lungs of any mouse that died were examined for tachyzoites. The inoculated mice were considered infected with *T. gondii* when tachyzoites and/or tissue cysts were found in their tissues.

In vitro cultivation

The number of tissue cysts in brains of chronically infected mice is often low. Therefore, homogenates of brains with tissue cysts were sub-inoculated in KO mice. When the KO mice died or were euthanized when ill, their lung homogenates were seeded on to CV1 cell culture flasks. Infected flasks were incubated at 37 °C and 2 % CO_2 and observed on alternate days under an inverted microscope. Tachyzoites from successfully grown cultures were harvested from the medium for DNA isolation and cryopreserved in liquid nitrogen for future studies as described (Dubey 2010).

Extraction of DNA and multilocus PCR-RFLP genotyping of *T. gondii*

Toxoplasma gondii DNA was extracted from cell culture derived tachyzoites. Briefly, tachyzoites were pelleted, washed with phosphate buffer saline, and subjected to genomic DNA extraction. Genomic DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA quantification and quality were determined by Thermo Scientific NanoDrop Lite Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

The multiplex PCR of *T. gondii* isolates were performed using the 10 PCR-RFLP genetic markers; SAG1, SAG2 (5′-3′ SAG2, alt.SAG2), SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico as previously described (Su et al. 2010). Appropriate positive and negative controls were included for different genetic types in all batches respectively (Table 4). The profiles of DNA fragments found after restriction endonucleases digestion were compared with the reference strains profiles (Su et al. 2010).

Ethics

All research was performed using protocols approved by all participating institutions.

Results

Antibodies (IgG, MAT) to *T. gondii* were found in 174 (39.8 %) of 437 human sera with titers of 1:6 or higher (Table 2). All sera were negative for IgM antibodies.

Toxoplasma gondii antibodies were found in the sera of 19 (42.2 %) of 45 dogs with titers of 25 in 6, 50 in 6, 100 in 5, and 200 or higher in 2 dogs. Viable *T. gondii* was isolated from 6 dogs (Table 3). All SW mice of each group remained asymptomatic, and tissue cysts were found in brains of 24 of 24 mice (Table 3). The 3 KO mice inoculated with muscle homogenate of dog #15 died of acute toxoplasmosis on 21 days p.i., and tachyzoites were found in smears of their lungs.

All 6 isolates of *T. gondii* were further propagated in KO mice and then in cell culture. PCR-RFLP genotyping of cell culture derived tachyzoites revealed three genotypes. Four isolates had characteristics of Type III (ToxoDB PCR-RFLP genotype #2). Two isolates had different new genotypes, designated ToxoDB PCR-RFLP genotypes #264, and #265, respectively (Table 4).

Discussion

As mentioned earlier, humans become infected postnatally mostly by the ingestion of infected meat or food and water contaminated with oocysts. Cats (domestic and wild felids) are the only definitive hosts for *T. gondii*. There is no information on felids, other than the domestic cat (*Felis catus*) in Caribbean countries. We are aware of only 4 reports of *T. gondii* infection in domestic cats in the Caribbean. Antibodies to *T. gondii* were found in 35 % of 40 and 28.9 % of 176 cats from Grenada (Asthana et al. 2006; Dubey et al. 2009a) and 84.9 % of 106 (Moura et al. 2007), and 73.9 % of 96 cats from St. Kitts (Dubey et al. 2009b). The high prevalence of *T. gondii* seropositivity in St. Kitts cats indicates that the environment is highly contaminated with oocysts. Finding *T. gondii* antibodies in 42.2 % of 45 dogs in the present study further attests to the high environmental contamination in St. Kitts. A recent study of *T. gondii* prevalence in livestock on St. Kitts and Nevis reported seropositivity using an in-house ELISA in 48 % of 124 pigs, 34 % of 66 goats, and 26 % of 116 sheep from the abattoir (Hamilton et al. 2015). *Toxoplasma gondii* DNA was detected in hearts of 21 % pigs, 16 % of sheep, and 23 % of goats. Using MAT, *T. gondii* antibodies were found in 23.1 % of 247 pigs, 44.1 % of 204 sheep, 42.8 % of 180 goats, and 8.4 % of 119 cattle from Grenada and Carriacou (Chikweto et al. 2011). Because herbivores become infected postnatally mostly with ingestion of oocysts, these data indicate high *T. gondii* oocyst contamination of farms in Grenada and Carriacou. Currently, nothing is known of *T. gondii* infection in farm cats in the Caribbean.

Little is known of genotypes of *T. gondii* circulating in the Caribbean. Initial studies using microsatellites indicated that the *T. gondii* isolates from chickens in Grenada were different than in the USA and Europe (Lehmann et al. 2006). Two decades ago, *T. gondii* was considered clonal with three types: I, II, and III (Howe and Sibley 1995), with type II strains being the most prevalent strain in Europe and the USA. Recent studies indicated that the

Table 4 PCR-RFLP genotyping of *T. gondii* isolates from dogs samples of St. Kitts, West Indies

Strain ID	ToxoDB PCR-RFLP Genotype #	Genetic markers										
		SAG1	(5′-3′) SAG2	alt. SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
GT-1	#10 (type I)	I	I	I	I	I	I	I	I	I	I	I
PTG	#1 (type II)	II or III	II	II	II	II	II	II	II	II	II	II
CTG	#2 (type III)	II or III	III	III	III	III	III	III	III	III	III	III
MAS	#17	u-1	I	II	III	III	III	u-1	I	I	III	I
TgCgCa1	#66	I	II	II	III	II	II	II	u-1	I	u-2	I
TgCtBr5	#19	I	III	III	III	III	III	I	I	I	u-1	I
TgCtBr64	#111	I	I	u-1	III	III	III	u-1	I	III	III	I
TgRsCr1	#52	u-1	I	II	III	I	III	u-2	I	I	III	I
Present study												
TgDogStK1	#2	II or III	III	III	III	III	III	III	III	III	III	III
TgDogStK2	#2	II or III	III	III	III	III	III	III	III	III	ND	III
TgDogStK3	#2	II or III	III	III	III	III	III	III	III	III	ND	III
TgDogStK4	#2	II or III	III	III	III	III	III	III	III	III	ND	ND
TgDogStK5	#264 (new)	II or III	II	II	II	III	I	II	I	I	II	I
TgDogStK6	#265 (new)	II or III	II	II	III	II	II	II	III	III	II	III

ND no data available

isolates from Brazil are mostly atypical, and the type II strain is rare (Shwab et al. 2014). Genotyping of strains from the Caribbean using 10 markers, including the present study, indicate that 8 of the 22 isolates were atypical, one is type II (ToxoDB PCR-RFLP genotype #1), and 13 are type III (ToxoDB PCR-RFLP genotype #2) (Table 5). Thus, there is a higher genetic variability among isolates from Caribbean than in the USA or Europe. It is of interest to note that the type III strains seem to be a common type in this region. The clinical significance of *T. gondii* genotypes in the Caribbean is unknown because little is known of clinical toxoplasmosis in the Caribbean countries. Sepulveda-Arias et al. (2014) mentioned a case of glandular toxoplasmosis in a patient from Bogota, in the North of Colombia, 1 month after returning from travel to the Caribbean region.

A review of data in Table 1 indicates that a sizable human population in the Caribbean becomes infected with *T. gondii* during childhood (Barbier et al. 1983; Rawlins and Prabhakar 1989). By the time of pregnancy, nearly half of women in the Caribbean are exposed to *T. gondii* and thus are immune to giving births to infected children (Table 1). Detection of toxoplasmic infection during pregnancy is difficult and requires extensive serological testing using tests that can discriminate between recent and past exposure. Detection of IgM antibodies is helpful to detect beginning of infection, but results are not definitive because IgM antibodies can persist for several months. Based on testing of one sample, we did not detect IgM antibodies in any of the women tested, suggesting that infections were old. Orrett (1993) also found no evidence for seroconversion during pregnancy in Jamaican women. However, IgM antibodies were reported to be frequent in women in Trinidad and Tobago. A planned screening program will be needed to assess the risk of toxoplasmosis during pregnancy.

One of the shortcomings of the present study was that a nonrandomized population-based sampling strategy was used. It is possible that selection bias may have occurred in the recruitment of pregnant women in at least two of the Caribbean countries, Jamaica and Belize, included in this study. Jamaica's population is much higher (2.8 million inhabitants) compared to the other Caribbean countries that participated in this study which have populations approximately around 100,000. The Belizean population (327,000 inhabitants) has multiple different subgroups differentiated by culture, language, and ethnicity for which a sample size of 50 pregnant women may not provide a representative snapshot of the entire population. For the other eight Caribbean countries, however, given that almost all delivering women utilize one or two major healthcare centers in these islands, and given that the populations on these islands are much smaller (<100,000), as well as more homogenous, it is very likely that the samples collected in this study are representative of the population from which they were drawn. Further, given that the dates of conception and delivery are more or less inherently random events, and

Table 5 Genetic diversity of *T. gondii* isolates from two Caribbean countries, using PCR-RFLP 10 markers

Country	Host	No. of isolates	*T. gondii* types		References
			ToxoDB # (*N*)	Conventional (*N*)	
Grenada	Chicken	9	#2 (5) #13 (2) #187 (2)	Type III (5) Atypical (4)	Rajendran et al. (2012)
St. Kitts	Cat	7	#1 (1) #2 (4) #13 (1) #141 (1) #1, and #2 Mix (1)	Type II (1) Type III (4) Atypical (2)	Dubey et al. (2009b); Shwab et al. (2014)
St. Kitts	Dog	6	#2 (4) #264 (1) #265 (1)	Type III (4) Atypical (2)	Present study
Total		22	#1 (1) #2 (13) #13 (3) #187 (2) #141 (1) #264 (1) #265 (1) #1, and #2 Mix (1)	Type II (1) Type III (13) Atypical (8)	

N No. of isolates

no evidence was found to suggest that the pregnant women who participated in this study differed in any material way from those who were not sampled, the samples collected in this study could be viewed as very close proxies of randomly population-based samples.

Although there are no published reports of clinical toxoplasmosis from St. Kitts, the data from this study expands the previous epidemiological work done on *T. gondii* and sheds light on its genetic characterization throughout several Caribbean islands. In summary, the environment of St. Kitts is highly contaminated with *T. gondii* oocysts, and greater estimates of emerging recombinant strains are circulating in domestic animals.

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