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Short communication

Prevalence of *Toxoplasma gondii* in dogs from Sri Lanka and genetic characterization of the parasite isolates

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Abstract

The prevalence of *Toxoplasma gondii* in 86 street dogs from Sri Lanka was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT) and found in 58 (67.4%) of 86 dogs with titers of 1:20 in eight, 1:40 in four, 1:80 in 10, 1:160 in 22, 1:320 in six, 1:640 in five, and 1:1280 or higher in three. Hearts, tongues, and brains (either separately or pooled) of 50 dogs with MAT titers of 1:40 were selected for isolation of *T. gondii* by bioassays in mice. For bioassays, canine tissues were digested in pepsin and homogenates were inoculated subcutaneously into mice; the mice receiving canine tissues were examined for *T. gondii* infection. In all, *T. gondii* was isolated from 23 dogs. Interestingly, dog organs varied in their capacity to induce *T. gondii* infection in mice, muscles producing more positive results than the brain. The *T. gondii* isolates obtained from 23 seropositive dogs were PCR-RFLP genotyped using polymorphisms at 10 nuclear markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, a new SAG2, and an apicoplast marker Apico. Mixed infection with two genotypes was observed in one dog. Four genotypes were revealed, including three unique genotypes in addition to one belonging to the predominant Type III lineage. The 24 isolates were designated as TgDgSl 1–24.
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Keywords: *Toxoplasma gondii*; Dogs; Sri Lanka; Bioassays; Antibodies; Genotype

1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment (Dubey and Beattie, 1988). However, only a small percentage of exposed adult humans develop clinical symptoms (Dubey and Beattie, 1988). It is unknown whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or to other factors.

T. gondii isolates have been classified into three genetic Types (I, II, III) based on restriction fragment length polymorphism (RFLP) (Howe and Sibley, 1995; Howe et al., 1997) and until recently, *T. gondii* was considered to be clonal with very little genetic variability. Based on recent studies, a higher genetic variability has been revealed than previously reported

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(Ajzenberg et al., 2004; Ferreira et al., 2006; Khan et al., 2006; Lehmann et al., 2006).

We have initiated a study on the genetic diversity of *T. gondii* worldwide with ultimate objective to understanding the protein variability among strains for immunoprophylaxis. In the present study, we attempted to isolate and characterize *T. gondii* from dogs from Sri Lanka. We also examined distribution of *T. gondii* in tissues of asymptomatic dogs to improve biological diagnosis.

2. Materials and methods

2.1. Naturally infected dogs

Eighty-six street dogs that were caught by the municipality were euthanized by intravenous injection of sodium thiopentone. The study group comprised of 48 males and 38 females and were of mixed breed and different age groups. At necropsy, brain, heart, tongue and blood samples were collected and kept at 4 °C until sent refrigerated by air to Beltsville, MD. The samples were received in March (batch 1) and June (batch 2) 2006. Six days elapsed between the killing of dogs and the receipt of samples in Beltsville.

2.2. Serological examination

Sera of dogs were tested for *T. gondii* antibodies using two-fold serum dilutions from 1:20 to 1:1280 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

2.3. Bioassay of canine tissues for T. gondii infection

Tissues of 50 dogs (24 in batch 1 and 26 in batch 2) with titers of 1:40 or higher were bioassayed for *T. gondii* infection in mice, 1–3 days after the results of serologic examination were available (Table 1). For the dogs in batch 1, brains were bioassayed separately and the hearts and tongues from each were pooled together. For batch 2 dogs, brains, hearts, and tongues were bioassayed separately for each dog into out-bred female Swiss Webster mice obtained from Taconic Farms, Germantown, New York, as described by Dubey et al. (2002). Each tissue (20–25 g) was homogenized individually, digested in acidic pepsin, neutralized, and washed (Dubey, 1998); the sedimented homogenate was suspended in antibiotic saline and an aliquot was inoculated subcutaneously into four mice (Table 1).

The mice receiving canine tissues were examined for *T. gondii* infection. Mice were bled on days 40–42 post-inoculation (p.i.) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 6-week p.i. and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). If tissue cysts were not found in seropositive mice, their brain homogenates were inoculated into interferon gamma gene knock out (KO) as described (Dubey and Lindsay, 1998); these KO mice are highly susceptible to intracellular protozoan infections because they lack the cytokine important for developing protective immunity. The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

2.4. Genetic characterization

T. gondii DNA was extracted from tissues of infected mice from each group and strain typing was initially performed using PCR-RFLP genetic markers SAG1, SAG2, SAG3, BTUB and GRA6 (Dubey et al., 2006). In brief, the target DNA sequences were amplified by multiplex PCR using external primers for all five markers. Multiplex PCR amplified products were then used for nested PCR with internal primers for each marker separately. Nested PCR products were treated with restriction enzymes and resolved in agarose gel by electrophoresis to reveal the RFLP patterns of the isolates. These five markers allow us to quickly characterize all samples and to identify potential mixed infection in dogs. The samples with low DNA concentration and cannot be reliably genotyped by

Table 1
Prevalence of *T. gondii* infection in dogs from Sri Lanka

Batch number	Number of dogs	Number of seropositive (MAT > 40)	Total bioassay-positive dogs	*T. gondii* isolation from canine		Genotypes			
				Brain	Muscle	#1	#2	#3	#4
1	36	24	13[a]	5	12	3	6	5	0
2	50	26	10	3	10	0	5	4	1

[a] Two genotypes were obtained from one dog.