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Virulence and molecular characterization of *Toxoplasma gondii* isolated from goats in Ceará, Brazil

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Abstract

One hundred and sixty-nine fragments of heart muscle collected from goats in the State of Ceará, Brazil, were analyzed by mouse bioassay with the aim of isolating *Toxoplasma gondii*. Two *T. gondii* isolates, named G1 and G2, were obtained and were characterized by PCR-RFLP. In addition, their virulence was evaluated by experimental inoculation into BALB/c mice. The G1 isolate presented high virulence leading to 100% mortality of mice after inoculations with 10^1, 10^2, and 10^3 tachyzoites. The G2 isolate presented low virulence and none of the doses tested lead to mortality of mice. The PCR-RFLP analysis showed that the two isolates are recombinants of the types I/III. However, they differ in the haplotype combination for the genotypes analyzed.

Keywords: *Toxoplasma gondii*; Goats; Virulence; PCR-RFLP

1. Introduction

Detection of anti-*Toxoplasma gondii* antibodies have been reported in goats from many countries, including Brazil (Tenter et al., 2000). In this species, the main clinical and economic impact of toxoplasmosis is the abortion (Dubey, 1987).

Howe and Sibley (1995) showed that *T. gondii* present a clonal structure consisting of three lineages known as types I, II, and III. The strains classified as type I are highly virulent, while types II and III have low virulence in mice. These authors estimated that 95% of the isolates are within this classification and that 5% of them are related to recombinant strains.

A study of chicken parasites from rural areas in São Paulo, demonstrated that 64% of the *T. gondii* isolates were type I, and 34% were type III (Dubey et al., 2002). Fux et al. (2003) classified a *T. gondii* strain isolated from a dog as a recombinant of the types I/III, which was the first *T. gondii* report of a recombinant strain in Brazil. Ferreira et al. (2006), using a multilocus PCR-RFLP, demonstrated that Brazilian strains present recombinant genotypes with typical alleles of the strains types I, II, and III in the majority of the loci. The RAPD-PCR and SSR-PCR analysis of these same strains placed them into two different clusters correlated with virulence for BALB/c mice (Ferreira et al., 2004).

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The purpose of this study was to isolate *T. gondii* from heart tissue of goats in Ceará, aiming to determine their virulence and to characterize them by PCR-RFLP.

2. Materials and methods

One hundred and sixty-nine heart muscle fragments were collected from goats from different regions of the State of Ceará, Brazil. The heart muscle samples (20 g each) were obtained at time of slaughter and were bioassayed individually in two Swiss mice, according to Dubey et al. (1995). The mice were examined following the infectivity criteria established by Vitor et al. (1992).

Serum samples were collected from each goat and were tested by the indirect fluorescent antibody test (IFAT) and by the enzyme-linked immunosorbent assay (ELISA) (Vitor et al., 1992, 1999).

Tachyzoites were obtained from *T. gondii* isolates to carry out virulence assays and genotypic characterization as described by Ferreira et al. (2001).

The virulence of each isolate was assessed by the same criteria adopted by Ferreira et al. (2001). Four groups of five 6 to 8-week-old females BALB/c mice were inoculated intraperitoneally (i.p.) with either 10^0, 10^1, 10^2 or 10^3 tachyzoites. Mouse mortality was observed over a 30-day period. As a comparison, the mortality dependent-dose of *T. gondii* RH type I (highly virulent strain) and of the ME49 type II (non-virulent strain) was also assessed. Five mice were inoculated i.p. with PBS pH 7.2 and served as negative controls.

For DNA extraction, the *T. gondii* tachyzoites were resuspended in 200 μL of lysis buffer and 100 μg/mL of proteinase K, as described by Ferreira et al. (2006). After an overnight incubation period in a water bath at 37 °C, the DNA was extracted with phenol/chloroform/isoamylic alcohol (25:24:1), according to the procedure described by Sambrook et al. (1989). The PCR amplification was performed as described by Howe and Sibley (1995) and Ferreira et al. (2006).

T. gondii isolates were genotyped by PCR-RFLP from amplifications of the specific segments of the *SAG2*, *SAG3*, *GRA6*, *L363*, *cS10-A*6, and *cB21-4* genes, using restriction enzymes for each locus: *Hha*I and *Sau*3AI for *SAG2* locus, *Nci*I for *SAG3*, *Mse*I for *GRA6*, *Msp*I and *HpyCH4*IV for *L363*, *Rsa*I and *HpyCH4*IV for *cS10-A6*, and *Hae*III for *cB21-4* (Fux et al., 2003). The reference strains RH (type I), ME49 (type II) and VEG (type III) were used as controls. The digested products were purified by extraction with an equal volume of phenol/chloroform and stained with silver nitrate (Santos et al., 1993). The 100 bp ladder (Promega, Brazil) was used as molecular marker. All experiments were carried out in duplicate.

3. Results

Ten out of the 169 serum samples (5.9%) were positive for *T. gondii* by IFAT, with titers ranging from 1:16 to 1:256, while eight samples (4.7%) were positive by ELISA. Only 2 of the 169 goat samples were positive for *T. gondii* in the bioassay. The two isolates obtained were named G1 and G2. The G1 isolate killed all mice that had been inoculated with 10^1, 10^2, and 10^3 tachyzoites. At a concentration of 10^0 tachyzoites, two mice survived and they were sacrificed 30 days later. Both mice were negative for anti-*T. gondii* antibodies and for brain cysts. The G2 isolate was non-lethal to mice at all concentrations tested. All of the mice were positive for *T. gondii* antibodies by IFAT. Brain cysts were detected only in animals inoculated with 10^1, 10^2 and 10^3 tachyzoites. Mice inoculated with the RH and ME49 reference strains gave 100% and 0% mortality, respectively.

Genotyping the G1 isolate showed restriction patterns identical to those of the RH strain (type I) for the *cS10-*

Table 1
Genotyping of the *SAG2*, *SAG3*, *GRA6*, *L363*, *cS10-A6*, and *cB21-4* genes from *Toxoplasma gondii* isolated from goats in Ceará, Brazil

Locus	Restriction enzyme	Alleles					Haplotypes	
		RH (I)	ME49 (II)	VEG (III)	G1	G2	G1	G2
SAG2	*Hha*I	1	2	1	1	1	III	I
	*Sau*3AI	1	1	2	2	1		
SAG3	*Nci*I	1	2	3	3	1	III	I
GRA6	*Mse*I	1	2	3	3	3	III	III
L363	*Msp*II	1	2	3	3	3	III	III
	*HpyCH4*IV	1	1	2	2	2		
cS10-A6	*Rsa*I	1	1	2	1	2	I	III
	*HpyCH4*IV	1	2	1	1	1		
cB21-4	*Hae*III	1	2	1	1	3	I	III