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Short communication

Biologic and genetic comparison of *Toxoplasma gondii* isolates in free-range chickens from the northern Pará state and the southern state Rio Grande do Sul, Brazil revealed highly diverse and distinct parasite populations

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Abstract

The prevalence of *Toxoplasma gondii* in 84 free-range chickens (34 from the northern Pará state, and 50 from Rio Grande do Sul, the southern state) from Brazil, South America was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT), and found in 39 (46.4%) of 84 chickens with titers of 1:10 in one, 1:20 in two, 1:40 in four, 1:80 in seven, 1:160 in five, 1:320 in six, 1:640 in eight and ≥1:1280 in six. Hearts and brains of 45 chickens with titers of 1:20 or less were pooled and fed to two *T. gondii*-free cats. Hearts and brains of 39 chickens with titers of 1:10 or higher were bioassayed in mice. Feces of cats were examined for oocysts. One cat fed tissues from 31 chickens with titers of less than 1:10 from Rio Grande do Sul shed *T. gondii* oocysts. *T. gondii* was isolated by bioassay in mice from 33 chickens with MAT titers of 1:20 or higher. All infected mice from 10 isolates died of toxoplasmosis. All 34 isolates (15 from Pará, 19 from Rio Grande do Sul) were genotyped using 11 genetic markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, a new SAG2 and Apico. Eleven genotypes were revealed for Pará isolates and seven genotypes for Rio Grande do Sul. No genotype was shared between the two geographical locations. These data suggest that *T. gondii* isolates are highly diverse and genetically distinct between the two different regions in Brazil that are 3500 km apart.

Keywords: *Toxoplasma gondii*; Chickens; *Gallus domesticus*; Free-range; Pará state; Rio Grande do Sul state; Brazil; Genotype

1. Introduction

The prevalence of *Toxoplasma gondii* infection in humans in Brazil is unusually high, reaching to 100% in some areas (Dubey and Beattie, 1988; Bahia-Oliveira et al., 2003; de Moura et al., 2006; Sobral et al., 2005).

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In Erechim, which is located in the northern part of the southern state of Rio Grande do Sul, Brazil 17% of 1000 unselected humans examined were found to have ocular toxoplasmosis; this is the highest prevalence of ocular disease in any city of the world (Glasner et al., 1992). In a well controlled epidemiologic study of 131 persons with recently acquired ocular toxoplasmosis with demonstrable IgM antibodies in Erechim, and 110 matched uninfected controls indicated that working in the garden and eating lamb were two important risk factors for acquired toxoplasmosis (Jones et al., 2006). *T. gondii* DNA isolated from blood or ocular fluids of some of these patients indicated that unusual *T. gondii* isolates may have caused the ocular disease (Khan et al., 2006).

We have recently found that the isolates of *T. gondii* from Brazil are biologically and genetically different than those from North America and Europe (Dubey et al., 2002, 2003a,b, 2006a; Lehmann et al., 2004, 2006). In the present paper, we attempted to isolate, genotype, and compare *T. gondii* from chickens from the very north, Pará state, with chickens from Rio Grande do Sul, the very southern state of Brazil, approximately 3500 km apart one from other. For this study, we chose free-range chickens as the indicator for soil contamination with *T. gondii* oocysts because chickens feed from the ground. Direct detection of oocysts in soil is technically difficult and only 1% of cats are found shedding *T. gondii* oocysts at any time (Dubey, 2004).

2. Materials and methods

2.1. Naturally infected chickens

During February and March, 2006 a total of 84 free-range chickens were obtained for the present study. The Pará state chickens ($n = 34$) were from six municipalities (Castanhal—1°17′ 49.2″S and 47°5′ 19.2″W, Inhangapi—1°25′ 48.″S and 47°5′ 1.2″W, Marituba—1°2′ 18.0″S and 48°20′ 31.2″W, Santa Isabel do Pará—1°17′ 56.4″S and 48°09′ 39.6″W, Santarém—2°26′ 34.8″S and 54°42′ 28.8″W and Terra Alta—1°2′ 16.8″S and 47°54′ 28.8″W Table 1; Fig. 1).

The Rio Grande do Sul chickens ($n = 50$) were from 10 farms from five municipalities (Pelotas—31°46′ 19.2″S and 52°20′ 34.8″W, Capão do Leão—31°45′ 46.8″S and 52°29′ 02.4″W, Turuçu—31°25′ 19.2″S and 52°10′ 40.8″W, Canguçu—31°23′ 42.0″S and 52°40.0′ 33.6″W and Rio Grande—32°02′ 06.0″S and 52°05′ 56.4″W) with two farms from each municipality (Table 2; Fig. 1—only four municipalities are shown).

Chickens were purchased, killed by cervical dislocation, and samples of brain, whole heart, and blood were collected from each chicken, kept at 4 °C until sent

Table 1
Isolation of *T. gondii* from free-range chickens from Pará State, Brazil

Chickens			Isolation in mice			Genotype		
Expt no. and chicken no.	Farmhold location	MAT titer	No. infected[a]	No. died	Day of death	Isolate ID	SAG1, SAG2, SAG3, BTUB, GRA6[b]	c22-8, c29-2, L358, PK1, SAG2 (new), Apico
Tx 233								
5	Santarém	≥1280	4	3	14, 18, 19	TgCkBr107	I(4), III(4), III(4), III(4), II(4)	u-1, I, I, I, III, III
6	Santarém	1280	4	4	14, 16, 21, 23	TgCkBr108	I(4), III(4), III(4), III(4), II(4)	u-1, I, I, I, III, III
8	Santarém	320	4	4	14, 16, 16, 17	TgCkBr109	I(4), I(4), I(4), III(4), III(4)	II, III, I, III, II, III
9	Santaéem	320	4	1	14	TgCkBr110	I(4), III(4), I(4), III(4), III(4)	III, III, III, I, III, I
12	Santarém	640	4	3	21, 23, 23	TgCkBr111	I(4), III(4), III(4), III(4), III(4)	III, III, III, III, III, I
15	Santarém	≥1280	4	2	23, 23	TgCkBr112	I(4), III(4), III(4), III(4), III(4)	III, III, III, III, III, I
Tx 239								
4	Inhangapi	640	4	3	13, 15, 33	TgCkBr113	I(3), III(3), I(3), III(3), III(3)	III, III, III, I, III, III
5	Terra Alta	160	1	0		TgCkBr114	I(1), I(1), III(1), I(1), III(1)	II, I, III, III, II, I
7	Terra Alta	640	4	1	20	TgCkBr115	I(4), I(4), I(4), I(4), I(4)	II, I, III, I, I, III
9	Castanhal	20	1	0		TgCkBr116	u-1(1), II(1), III(1), III(1), II(1)	II, nd, II, II, II, I
12	Castanhal	640	4	4	19, 19, 19, 19	TgCkBr141	I(3), I(3), I(3), I(3), I(3)	u-1, I, I, III, I, III
14	Santa Isabel	80	4	1	24	TgCkBr142	I(4), I(4), I(4), I(4), I(4)	II, I, III, I, I, III
15	Santa Isabel	320	4	3	21, 23, 28	TgCkBr143	I(4), I(4), III(4), III(4), II(4)	u-1, III, III, III, II, I
16	Santa Isabel	80	1	1	21	TgCkBr144	I(1), I(1), III(1), I(1), II(1)	u-1, I, I, I, I, I
17	Marituba	160	4	1	20	TgCkBr145	I(4), I(4), I(4), I(4), I(4)	II, I, III, I, I, III

nd, data not available.
[a] Four mice were inoculated with tissues of each chicken.
[b] Numbers in parenthesis are the number of mice used separately for genotyping.