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Characterization of *Toxoplasma gondii* isolates in free-range chickens from Chile, South America

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Abstract

The prevalence of *Toxoplasma gondii* in free-ranging chickens is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. The prevalence of *T. gondii* in 85 free-range chickens (*Gallus domesticus*) from Chile was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT), and found in 47 of 85 (55.3.9%) chickens with titers of 1:5 in six, 1:10 in four, 1:20 in four 1: 40 in three, 1: 80 in nine, 1: 160 in four 1:320 in nine, and 1: 640 or higher in eight. Hearts and brains of 47 chickens with titers of 1:5 or higher were pooled for each chicken and bioassayed in mice. Tissues from 16 seronegative (MAT < 1:5) chickens were pooled and fed to one *T. gondii*-free cat. Feces of the cat were examined for oocysts but none was found based on bioassay of fecal floats in mice. Hearts and brains from seven seronegative (<1:5) were pooled and bioassayed in mice; *T. gondii* was not isolated. *T. gondii* was isolated by bioassay in mice from 22 chickens with MAT titers of 1:20 or higher. Genotyping of these 22 isolates using polymorphisms at the loci SAG1, SAG2, SAG3, BTUB and GRA6 revealed three genotypes. Seventeen isolates had type II alleles and four isolates had type III alleles at all loci. One isolate contained the combination of type I and III alleles. This is the first report of genetic characterization of *T. gondii* isolates from Chile, South America.

Keywords: *Toxoplasma gondii*; Chickens; *Gallus domesticus*; Free-range; Chile; South America; 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,

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or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans develop clinical signs. 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) (Ajzenberg et al., 2002a, 2002b, 2004; Aspinall et al., 2003; Boothroyd and Grigg, 2002; Dubey et al., 2004a,d; da Silva et al., 2005; Ferreira et al., 2004, 2006; Fuentes et al., 2001; Grigg et al., 2001; Howe and Sibley, 1995; Howe et al., 1997; Jungersen et al., 2002; Mondragon et al., 1998; Owen and Trees, 1999). The parasite was previously considered clonal with very low genetic variability. However, most of the information was derived from isolates from Europe and North America. Using newer markers for genetic characterization and using recently isolated strains from Brazil and French Guiana, higher genetic variability was revealed than previously reported (Ajzenberg et al., 2004; Lehmann et al., 2004).

We have initiated a worldwide study of *T. gondii* population structure. For this we have chosen the free-range chicken as the indicator host for soil contamination with *T. gondii* oocysts because they feed from the ground. Thus far, we have characterized strains from South America (Brazil (Dubey et al., 2002, 2003a,d, 2006b), Peru (Dubey et al., 2004b), Venezuela (Dubey et al., 2005f), Argentina (Dubey et al., 2003e, 2005c), Colombia (Dubey et al., 2005i), Central America and the Caribbean (Guatemala (Dubey et al., 2005b), Grenada, West Indies (Dubey et al., 2005e), Costa Rica (Dubey et al., in press), North America (USA (Dubey et al., 2003c; Lehmann et al., 2003), Mexico (Dubey et al., 2004c)), Africa and Middle East (Egypt (Dubey et al., 2003b), Israel (Dubey et al., 2004e), Mali, Kenya, Burkina Faso, and Democratic Republic of Congo (Dubey et al., 2005a)), Asia (Sri Lanka (Dubey et al., 2005d), India (Sreekumar et al., 2003)), and Europe (Austria (Dubey et al., 2005g), and Portugal (Dubey et al., 2006a)). These studies are still not complete, nevertheless, a pattern is emerging that isolates from Brazil are genetically distinct (Lehmann et al., 2004).

In the present paper, we attempted to isolate and genotype *T. gondii* from chickens from Chile, South America.

2. Materials and methods

2.1. Naturally infected chickens

Chickens ($n = 85$) were obtained from free-range chickens in rural farms from 85 different properties that were at least 500 m apart. They were purchased in four (A–D) batches in June to November 2005 (Table 1). Samples of brain, whole heart, and blood were collected from each chicken, and kept at 4 °C until sent by air to Beltsville, MD. Three to eleven days elapsed between killing of chickens and receipt of samples at Beltsville. Chickens from batches A, and C were badly autolysed when received at Beltsville, MD.

Table 1
Summary of chickens from Chile used for isolation of *T. gondii*

Batch number (experiment)	Chickens				Bioassay for *T. gondii*	
	Month 2005 received	Number of chickens	MAT titer		Number of seropositive chickens bioassayed in mice ≥1:5	Number of chickens positive
			<1:5	≥1:5		
A (Tx 189)	June	26	9	17 (14)[a]	17	4
B (Tx 192)	August	28	16[b]	12 (9)	12	5
C (Tx 205)	October	16	6	10 (8)	10	5
D (Tx 209)	November	15	7[c]	8 (8)	8	8
Total		85	28	47 (39)	47	22

[a] Number of chickens with titers of 1:20 or higher.
[b] Sixteen chicken tissues were fed to a cat.
[c] Seven chicken tissues were bioassayed in mice.