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# Genetic and biologic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Colombia, South America

J.P. Dubey [a,*], Jorge E. Gomez-Marin [b], Angela Bedoya [b], Fabiana Lora [b], M.C.B. Vianna [a], D. Hill [a], O.C.H. Kwok [a], S.K. Shen [a], P.L. Marcet [c], T. Lehmann [c]

[a] *United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA*
[b] *Grupo de Estudio en Parasitologia Molecular (GEPAMOL), Centro de Investigaciones Biomedicas, Facultad de Ciencias de la Salud, Universidad del Quindio, Av. Bolivar 12N, Armenia (Quindio),Colombia, South America*
[c] *Division of Parasitic Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, MS: F22, Chamblee, GA 30341,USA*

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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 77 free-range chickens (*Gallus domesticus*) from Colombia, South America was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT), and found in 32 (44.4%) of 72 chickens with titers of 1:5 in 4, 1:10 in 3, 1:20 in 1, 1:40 in 1, 1:80 in 8, 1:160 in 8, 1:320 in 3, and 1:640 or higher in 4. Hearts and brains of 31 seropositive chickens were pooled and bioassayed in mice. Tissues from 32 (16 + 16) seronegative chickens were pooled and fed to two, *T. gondii*-free cats, and tissues from nine chickens without matching sera were fed to one *T. gondii*-free cat. Feces of cats were examined for oocysts. *T. gondii* oocysts were excreted by a cat that was fed tissues of 16 seronegative chickens. *T. gondii* was isolated by bioassay in mice from 23 chickens with MAT titers of 1:20 or higher. All infected mice from 16 of the 23 isolates died of toxoplasmosis. Overall, 82 (81.1%) of 101 mice that became infected after inoculation with chicken tissues died of toxoplasmosis. Genotyping of these 24 isolates using polymorphisms at the SAG2 locus indicated that seven *T. gondii* isolates were Type I, 17 were Type III, and none was Type II. Phenotypically, *T. gondii* isolates from chickens from Colombia were similar to isolates from Brazil but different from the isolates from North America; most isolates from chickens from Brazil and Colombia were lethal for mice whereas isolates from North America did not kill inoculated mice. Genetically, none of the *T. gondii* isolates from Colombia and Brazil was SAG2 Type II, whereas most isolates from chickens from North America were Type II. This is the first report of genetic characterization of *T. gondii* isolates from Colombia, South America.
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*Keywords:* *Toxoplasma gondii*; Chickens; *Gallus domesticus*; Free-range; Columbia; South America; Genotype

* Corresponding author. Tel.: +1 301 504 8128; fax: +1 301 504 9222.
*E-mail address:* jdubey@anri.barc.usda.gov (J.P. Dubey).

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## 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. 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) (Howe and Sibley, 1995; Howe et al., 1997; Mondragon et al., 1998; Owen and Trees, 1999; Fuentes et al., 2001; Grigg et al., 2001; Ajzenberg et al., 2002, 2004; Boothroyd and Grigg, 2002; Jungersen et al., 2002; Aspinall et al., 2003; Dubey et al., 2004a,d; da Silva et al., 2005). The parasite was previously used to be 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, in press-e), Peru (Dubey et al., 2004b), Venezuela (Dubey et al., in press-d), Argentina (Dubey et al., 2003e, in press-b)), Central America and the Caribbean (Guatemala (Dubey et al., in press-a), Grenada, West Indies (Dubey et al., 2005b)), 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., in press-c), India (Sreekumar et al., 2003)), and Europe (Austria (Dubey et al., 2005c) and Portugal (Dubey et al., in press-g)). 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 Colombia, South America.

## 2. Materials and methods

### 2.1. Naturally-infected chickens

Chickens were obtained from free-range chickens in rural farms in Quindio region (center west of Colombia) 75°9′W 4°22′N, altitude 1483 m. Chickens ($n = 72$) were purchased during 12–16 April 2005. Chickens were collected, identified, and killed on one farm. Samples of brain, whole heart, and blood were collected from each chicken, and kept at 4 °C until sent with cold packs by air to Beltsville, MD. Three days elapsed between killing of chickens and receipt of samples at Beltsville. Samples were received in excellent condition.

### 2.2. Serological examination

Sera of chickens were tested for *T. gondii* antibodies using 4 dilutions, from 1:5 to 1:640 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

### 2.3. Bioassay of chickens for T. gondii infection

Tissues of all chickens were bioassayed for *T. gondii* infection. Brains, and hearts of 31 chickens with MAT titers of 1:5 or higher were bioassayed individually in outbred female Swiss Webster (SW) mice obtained from Taconic Farms, Germantown, New York, as described (Dubey et al., 2002). Tissues were homogenized, digested in acidic pepsin, washed, and homogenate inoculated subcutaneously into five mice (Dubey, 1998).

Brains and hearts from 32 (16 + 16) seronegative chickens were pooled and fed to two *T. gondii*-free cats (Dubey et al., 2002). Tissues from nine chickens without sera were fed to another cat. Feces of cats were examined for shedding of *T. gondii* oocysts 3–14 days post-ingesting chicken tissues as