Isolation, Tissue Distribution, and Molecular Characterization of *Toxoplasma gondii* From Free-Range Chickens From Guatemala

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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* was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT). Antibodies were found in 37 (74%) chickens with titers of 1:5 (11), 1:10 (7), 1:20 (11), 1:40 (1), 1:80 (1), 1:160 (3), 1:2,280 (2), and 1:2,560 (1). Heads, pectoral muscles, and hearts of 19 chickens with MAT titers of 1:20 or more were bioassayed individually in mice. Tissues from the remaining 31 chickens with titers of 1:10 or lower were pooled and fed to 4 *T. gondii*-free cats (13 chickens with titers of less than 1:5 to 1 cat, 11 chickens with titers of 1:5 to 2 cats, and 7 chickens with titers of 1:10 to 1 cat). Feeces of cats were examined for oocysts; they did not shed oocysts. *Toxoplasma gondii* was isolated from 8 chickens with MAT titers of 1:20 or more (from 1 of 11 chickens with a titer of 1:20 and all 7 chickens with a titer of 1:80 or more) from the heart, brain, and pectoral muscle (3); heart and pectoral muscle (1); and heart alone (4). Genotyping of these 8 isolates with the SAG2 locus indicated that 5 were type III and 3 were type I. This is the first report of isolation of *T. gondii* from chickens from Guatemala.

*Toxoplasma gondii* infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected postnatally by ingesting tissue cysts from undercooked meat, by 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 other factors. *Toxoplasma gondii* isolates have been classified into 3 genetic types (I, II, III) on the basis of restriction fragment length polymorphism (RFLP; Howe and Sibley, 1995; Howe et al., 1997). It has been suggested that type I strains, or recombinants of types I and III, are more likely to result in clinical ocular toxoplasmosis (Howe et al., 1997; Feuntes et al., 2001; Grigg et al., 2001; Boothroyd and Grigg, 2002; Aspinall et al., 2003), but genetic characterization has been limited essentially to isolates from patients ill with toxoplasmosis (Ajzenberg et al., 2004). Unlike these reports, Ajzenberg et al. (2002) found that most (73 of 86) isolates from cases of congenital toxoplasmosis in humans from France were type II. Nothing is known of the genetic diversity of *T. gondii* isolates circulating in the general human population. In animals, most isolates of *T. gondii* were type II or type III irrespective of clinical status (Howe and Sibley, 1995; Mondragon et al., 1998; Owen and Trees, 1999; Jungersen et al., 2002; Dubey, Navarro et al., 2004; Dubey, Parnell et al., 2004). *Toxoplasma gondii* isolates differ markedly in their virulence to out-bred mice. Type I isolates are more virulent to mice than types II and III. Because chickens become infected mostly by feeding from ground contaminated with oocysts, prevalence of *T. gondii* in chickens is a good indicator of the strains prevalent in their environment (Ruiz and Frenkel, 1980).

Recently, we found that 70% of 73 *T. gondii* isolates obtained from asymptomatic free-range chickens from Brazil were type I (Dubey et al., 2002; Dubey, Graham, Silva et al., 2003; Dubey, Navarro et al., 2003), whereas samples from the United States and Egypt were dominated by either type II or type III but had no type I (Dubey, Graham, Dahi, Hilali et al., 2003; Dubey, Graham, Dahl, Streekumar et al., 2003). Type II isolates of *T. gondii* have not been found in chickens from Brazil. All 3 types were found in chickens from Argentina (Dubey, Venturini et al., 2003). Nothing is known of the characteristics of isolates of *T. gondii* from animals or humans from Guatemala. In this paper, we attempted to isolate and genotype *T. gondii* from chickens from Guatemala. Additionally, the distribution of *T. gondii* in the heart, brain, and pectoral muscles of chickens was compared.

The chickens (n = 50) were from different households ([14'(46–50'1–60'N, 90'34–40'3–57'W, height 974–1,589 m) in Guatemala. The properties were at least 200 m apart, and at least 1 cat was present at each property. The chickens were 6–18 mo old. They were purchased, bled, and then killed by cervical dislocation on 2 April 2004. Serum, heart, pectoral muscle, and brain from each chicken were sent cold by air to Beltsville, Maryland. Five days elapsed between killing of chickens and receipt of samples at Beltsville. Samples were received in excellent condition.

Sera of chickens were tested for *T. gondii* antibodies in 4 serum dilutions; 1:5, 1:10, 1:20, and 1:200 by the modified agglutination test (MAT) as described by Dubey and Desmons (1987). After the completion of the bioassays, all positive chicken sera were rerun in 2-fold dilutions from 1:5 to 1:5,128.

Tissues of all chickens were bioassayed for *T. gondii* infection. Brains, pectoral muscles, and hearts of 19 chickens with MAT titers of 1:20 or more were each bioassayed individually in out-bred female Swiss Webster mice obtained from Taconic Farms (Germantown, New York) as described (Dubey et al., 2002). Each tissue was homogenized individually, digested in acidic pepsin, and washed, and homogenate was inoculated subcutaneously into 5 mice; in total, 15 mice were inoculated with tissues of each chicken.

Tissues from 31 chickens with titers of 1:10 or less were pooled into 4 batches of 13, 4, 7, and 7 chickens with titers of less than 1:5, doubtfully at 1:5, 1:10, respectively, and fed separately to 4 *T. gondii*-free cats (Dubey et al., 2002). Feeces of cats were examined for shedding of *T. gondii* oocysts 3–14 days postinfection of chicken tissues as previously described (Dubey, 1995). Fecal floats were incubated for 1 wk at room temperature to allow sporulation of oocysts and were bioassayed in mice (Dubey and Beattie, 1988). Tissue imprints of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were fed on day 41 postinoculation (PI), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 49 days PI, and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

*Toxoplasma gondii* DNA was extracted from mouse tissue as described previously (Lehmann et al., 2000). The RFLP strain type of *T. gondii* isolates was determined by nested PCR on the SAG2 locus according to Howe et al. (1997).

Antibodies were found in 37 (74%) chickens with titers of 1:5 (11), 1:10 (7), 1:20 (11), 1:40 (1), 1:80 (1), 1:160 (3), 1:1,280 (2), and 1:2,560 (1). *Toxoplasma gondii* was isolated from 8 chickens with MAT titers of 1:20 or more (from 1 of 11 chickens with a titer of 1:20 and all 7 chickens with a titer of 1:80 or more). It was isolated from the heart, brain, and pectoral muscle (3), heart and muscle (1), and heart alone (4; Table I).

Genotyping of these 8 isolates with *T. gondii* indicated that 5 were type III, and 3 were type I (Table I); the results are based on DNA analysis from 1 mouse in each group. The cats fed tissues of chickens with MAT titers of 1:10 or less did not shed oocysts.

The threshold MAT titer indicative of *T. gondii* infection in chickens has not been determined. Data comparing serology and recovery of viable *T. gondii* from chickens are now accumulating (Dubey et al.,...
Table I. Isolation of Toxoplasma gondii from tissues of seropositive chickens from Guatemala.

<table>
<thead>
<tr>
<th>Chicken no.</th>
<th>Age (mo)</th>
<th>MAT titer</th>
<th>Brain</th>
<th>Heart</th>
<th>Muscle</th>
<th>Genotype (isolate designation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>12</td>
<td>2,560 (1)</td>
<td>5 (3)</td>
<td>5</td>
<td>III (TgCrGa1)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>1,280 1</td>
<td>5</td>
<td>5</td>
<td>III (TgCrGa2)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>1,280 1</td>
<td>5</td>
<td>4</td>
<td>III (TgCrGa3)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>160 5</td>
<td>5</td>
<td>0</td>
<td>I (TgCrGa4)</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>36</td>
<td>160 5</td>
<td>5 (1)</td>
<td>0</td>
<td>I (TgCrGa5)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>24</td>
<td>160 5</td>
<td>0</td>
<td>0</td>
<td>III (TgCrGa6)</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>36</td>
<td>80 5</td>
<td>5</td>
<td>0</td>
<td>III (TgCrGa7)</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>180</td>
<td>20 5</td>
<td>5 (1)</td>
<td>0</td>
<td>I (TgCrGa8)</td>
<td></td>
</tr>
</tbody>
</table>

* Five mice inoculated with each tissue.
† No. of mice that died are in parentheses.

2002; Dubey, Graham, Dahl, Hilali et al., 2003; Dubey, Graham, Dahl, Sreekumar et al., 2003; Dubey, Graham, Silva et al., 2003; Silva et al., 2003; Dubey et al., 2005). In this study, T. gondii was isolated from all 7 chickens with a titer of 1:80 or more and only 1 of 12 chickens with a titer of 1:20 or 1:40. Lack of shedding of oocysts by 4 cats that consumed entire hearts, brains, and 20–25 g of pectoral muscles of 31 chickens with titers of 1:10 or less supports the validity of the MAT. The mouse virulence data indicate that the isolates of T. gondii from Guatemala are similar to isolates from Egypt, India, Mexico, United States, and Grenada. None of the isolates from Egypt (Dubey, Graham, Dahl, Hilali et al., 2003), India (Sreekumar et al., 2003), Mexico (Dubey, Morales et al., 2004), United States (Dubey, Graham, Dahl, Sreekumar et al., 2003; Lehmann et al., 2003), or Grenada (Dubey et al., 2005) was virulent for mice. Only 10% (6 of 66) mice infected with T. gondii isolates from Guatemala died, irrespective of the genotype. The isolates from Guatemala were different from those in Brazil (Dubey et al., 2002; Dubey, Graham, Silva et al., 2003; Dubey, Navarro et al., 2003), Argentina (Dubey, Venturini et al., 2003), and Peru (Dubey, Levy et al., 2004); isolates from these countries were virulent for mice. Toxoplasma gondii isolates from chickens from Brazil were predominantly type I, whereas they were predominantly type III from Guatemala, although data are limited to 8 isolates.


LITERATURE CITED


———. 2000. Strain typing of Toxoplasma gondii: Comparison...


