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


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 Faculdade de Medicina Veterinária e Zootecnia, Departamento de Medicina Veterinária Preventiva e Saúde Animal, USP, São Paulo, SP, Brazil

c Faculdade de Medicina Veterinária e Zootecnia, Departamento de Clínica Médica, USP, São Paulo, SP, Brazil

d Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, RS, Brazil

e Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, RS, Brazil

f Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845, USA

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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 /C21 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.

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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).
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). Patients indicated that unusual 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′ 49.42′′S and 47°5′ 19.2′′W, Inhangapi—1°25′ 48.9′′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, Tururu—31°25′ 19.2′′S and 52°10′ 40.8′′W, Canguçu—31°23′ 42.0′′S and 52°40′ 03.6′′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

<table>
<thead>
<tr>
<th>Expt no. and chicken no.</th>
<th>Farmhold location</th>
<th>MAT titer</th>
<th>No. infecteda</th>
<th>No. died</th>
<th>Day of death</th>
<th>Isolate ID</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tx 233</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Santarém</td>
<td>≥1280</td>
<td>4</td>
<td>3</td>
<td>14, 18, 19</td>
<td>TgCbBr107</td>
<td>I(4), III(4), III(4), III(4), II(4) u-1, I, I, I, III, III</td>
</tr>
<tr>
<td>6</td>
<td>Santarém</td>
<td>1280</td>
<td>4</td>
<td>14, 16, 21, 23</td>
<td>TgCbBr108</td>
<td>I(4), III(4), III(4), III(4), II(4) u-1, I, I, I, III, III</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Santarém</td>
<td>320</td>
<td>4</td>
<td>14, 16, 16, 17</td>
<td>TgCbBr109</td>
<td>I(4), I(4), I(4), III(4), III(4) II, III, I, III, I, III</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Santarém</td>
<td>320</td>
<td>4</td>
<td>14</td>
<td>1</td>
<td>TgCbBr110</td>
<td>I(4), I(4), I(4), I(4), I(4) III, III, I, I, I, I</td>
</tr>
<tr>
<td>12</td>
<td>Santarém</td>
<td>640</td>
<td>4</td>
<td>3</td>
<td>21, 23, 23</td>
<td>TgCbBr111</td>
<td>I(4), III(4), I(4), I(4), I(4) II, III, III, III, I, I</td>
</tr>
<tr>
<td>15</td>
<td>Santarém</td>
<td>≥1280</td>
<td>4</td>
<td>2</td>
<td>23</td>
<td>TgCbBr112</td>
<td>I(4), I(4), III(4), III(4), I(4) III, III, III, III, I</td>
</tr>
<tr>
<td><strong>Tx 239</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Inhangapi</td>
<td>640</td>
<td>4</td>
<td>3</td>
<td>13, 15, 33</td>
<td>TgCbBr113</td>
<td>I(3), I(3), I(3), I(3), I(3) III, III, III, III, III, III</td>
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<tr>
<td>5</td>
<td>Terra Alta</td>
<td>160</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>TgCbBr114</td>
<td>I(1), I(1), III(1), I(1), I(1) II, III, III, I, I</td>
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<tr>
<td>7</td>
<td>Terra Alta</td>
<td>640</td>
<td>4</td>
<td>1</td>
<td>20</td>
<td>TgCbBr115</td>
<td>I(4), I(4), I(4), I(4), I(4) II, III, I, I, III</td>
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<td>9</td>
<td>Castanhal</td>
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<td>1</td>
<td>0</td>
<td></td>
<td>TgCbBr116</td>
<td>u-1(1), I(1), I(1), III(1), I(1) II, nd, II, II, I</td>
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<td>640</td>
<td>4</td>
<td>19, 19, 19, 19</td>
<td>TgCbBr141</td>
<td>I(3), I(3), I(3), I(3) u-1, I, I, III, I, III</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Santa Isabel</td>
<td>80</td>
<td>4</td>
<td>1</td>
<td>24</td>
<td>TgCbBr142</td>
<td>I(4), I(4), I(4), I(4), I(4) II, III, I, I, III</td>
</tr>
<tr>
<td>15</td>
<td>Santa Isabel</td>
<td>320</td>
<td>4</td>
<td>3</td>
<td>21, 23, 28</td>
<td>TgCbBr143</td>
<td>I(4), I(4), I(4), I(4), I(4) u-1, III, III, III, II, I</td>
</tr>
<tr>
<td>16</td>
<td>Santa Isabel</td>
<td>80</td>
<td>1</td>
<td>0</td>
<td>21</td>
<td>TgCbBr144</td>
<td>I(1), I(1), III(1), I(1), I(1) u-1, I, I, I, I</td>
</tr>
<tr>
<td>17</td>
<td>Marituba</td>
<td>160</td>
<td>4</td>
<td>1</td>
<td>20</td>
<td>TgCbBr145</td>
<td>I(4), I(4), I(4), I(4), I(4) II, III, I, I, III</td>
</tr>
</tbody>
</table>

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.
with cold packs by air to Beltsville, MD. Five 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 eight dilutions, from 1:10 to 1:1280 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 39 (20 from Pará, 19 from Rio Grande do Sul) chickens with titers of 1:20 or higher were bioassayed individually in outbred female Swiss Webster 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 four mice (Dubey, 1998).

Brains and hearts from 45 (14 from Pará, 31 from Rio Grande do Sul) seronegative (MAT < 1:10) chickens were pooled and fed separately to two *T. gondii*-free cats. Feces of the cats were examined for shedding of *T. gondii* oocysts 3–14 days post-ingesting chicken tissues as previously described (Dubey et al., 2002). Fecal floats were incubated in 2% sulfuric acid for 1 week at room temperature on a shaker to allow sporulation of oocysts and were bioassayed orally in mice (Dubey and Beattie, 1988). Tissue imprints of lungs and brains of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 41 post-inoculation (p.i.) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 43 days p.i. 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.

2.4. Genetic characterization for *T. gondii*

*T. gondii* DNA was extracted from the tissues of all infected mice from each group and strain typing was performed using genetic markers SAG1, SAG2, SAG3, BTUB and GRA6 as previously described (Dubey et al., 2006b). These five markers allow us to quickly characterize all samples and to identify potential mixed infection in chickens. One representative DNA extract from mice infected with the same chicken sample was

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![Map of Brazil showing the sampled states, Pará (PA) and Rio Grande do Sul (RS).](image-url)
### Table 2

Isolation of *T. gondii* from free-range chickens from Rio Grande do Sul, Brazil

<table>
<thead>
<tr>
<th>Chickens</th>
<th>Farmhold location</th>
<th>MAT titer</th>
<th>Isolation in mice</th>
<th>Genotype</th>
<th>Genotype ID</th>
<th>SAG1, SAG2, SAG3, BTUB, GRA6</th>
<th>c22-8, c29-2, L358, PK1, SAG2 (new), Apico</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. TX 242</td>
<td></td>
<td></td>
<td>No. infected</td>
<td>No. died</td>
<td>Day of death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A1</td>
<td>Pelotas farm A</td>
<td>160</td>
<td>4</td>
<td>4</td>
<td>14, 14, 14, 15</td>
<td>TgCkBr146</td>
<td>I(4), I(4), I(4), I(4), I(4)</td>
</tr>
<tr>
<td>1A2</td>
<td>Pelotas farm A</td>
<td>320</td>
<td>4</td>
<td>4</td>
<td>14, 14, 15, 16</td>
<td>TgCkBr147</td>
<td>u-1(4), I(4), III(4), III(4), III(4)</td>
</tr>
<tr>
<td>1A3</td>
<td>Pelotas farm A</td>
<td>≥1280</td>
<td>4</td>
<td>4</td>
<td>14, 14, 15</td>
<td>TgCkBr148</td>
<td>u-1(4), I(4), III(4), III(4), III(4)</td>
</tr>
<tr>
<td>1B1</td>
<td>Pelotas farm B</td>
<td>160</td>
<td>4</td>
<td>0</td>
<td></td>
<td>TgCkBr149</td>
<td>II or III(4), III(4), III(4), III(3), III(4)</td>
</tr>
<tr>
<td>1B2</td>
<td>Pelotas farm B</td>
<td>160</td>
<td>4</td>
<td>0</td>
<td></td>
<td>TgCkBr150</td>
<td>II or III(4), III(4), III(4), III(3), III(3)</td>
</tr>
<tr>
<td>1B3</td>
<td>Pelotas farm B</td>
<td>80</td>
<td>4</td>
<td>4</td>
<td>13, 15, 15, 15</td>
<td>TgCkBr151</td>
<td>u-1(4), I(4), III(4), III(4), III(4)</td>
</tr>
<tr>
<td>1B4</td>
<td>Pelotas farm B</td>
<td>20</td>
<td>4</td>
<td>1</td>
<td>20</td>
<td>TgCkBr152</td>
<td>II or III(4), III(4), III(4), III(4)</td>
</tr>
<tr>
<td>1B5</td>
<td>Pelotas farm B</td>
<td>320</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>14, 16</td>
<td>TgCkBr153</td>
<td>I(2), III(2), III(2), III(2), III(2)</td>
</tr>
<tr>
<td>2B5</td>
<td>Capão do Leão farm B</td>
<td>80</td>
<td>4</td>
<td>4</td>
<td>14, 14, 15, 15</td>
<td>TgCkBr154</td>
<td>u-1(4), I(4), III(4), III(4), III(4)</td>
</tr>
<tr>
<td>4A1</td>
<td>Canguçu farm A</td>
<td>640</td>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td></td>
<td>TgCkBr155</td>
<td>u-1(2), III(2), III(2), III(2), III(2)</td>
</tr>
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<td>Canguçu farm A</td>
<td>320</td>
<td>4</td>
<td>0</td>
<td></td>
<td>TgCkBr156</td>
<td>I(4), III(4), III(4), III(4), III(4)</td>
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<tr>
<td>4A3</td>
<td>Canguçu farm A</td>
<td>80</td>
<td>4</td>
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<td>TgCkBr157</td>
<td>II or III(4), III(4), III(4), III(4), III(4)</td>
</tr>
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<td>4</td>
<td>0</td>
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<td>TgCkBr158</td>
<td>II or III(4), III(4), III(4), III(4), III(4)</td>
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<td>4A5</td>
<td>Canguçu farm A</td>
<td>80</td>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td></td>
<td>TgCkBr159</td>
<td>u-1(3), III(3), III(3), III(3), III(3)</td>
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<tr>
<td>5A1</td>
<td>Rio Grande farm A</td>
<td>≥1280</td>
<td>4</td>
<td>1</td>
<td>23</td>
<td>TgCkBr160</td>
<td>u-1(4), I(4), III(4), III(4), III(4)</td>
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<tr>
<td>5A2</td>
<td>Rio Grande farm A</td>
<td>40</td>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
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<td>II or III(2), III(2), III(2), III(2), III(2)</td>
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<tr>
<td>5B1</td>
<td>Rio Grande farm B</td>
<td>640</td>
<td>3</td>
<td>2</td>
<td>18, 22</td>
<td>TgCkBr162</td>
<td>u-1(3), III(3), III(3), III(3), III(3)</td>
</tr>
<tr>
<td>5B3</td>
<td>Rio Grande farm B</td>
<td>640</td>
<td>4</td>
<td>2</td>
<td>19, 19</td>
<td>TgCkBr163</td>
<td>u-1(4), I(4), III(4), III(4), III(4)</td>
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<tr>
<td></td>
<td>Pool chicken tissues&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td>4</td>
<td>2</td>
<td></td>
<td>TgCkBr164</td>
<td>II or III(1), III(1), III(1), III(1), III(1)</td>
</tr>
</tbody>
</table>

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* a Chickens from Turuçú were seronegative.

* b Four mice were inoculated with tissues of each chicken.

* c Two mice inoculated with tissues of each of these three chickens died within 6 days of inoculation and were considered to have died of other causes.

* d One mouse died 6 days p.i. and was considered to have died of other causes.

* e Of the four mice inoculated one died 5 days after inoculation and was discarded; of the three mice that survived one was found to be not infected with *T. gondii*.

* f Tissues from chickens with titers of 1:10 or less were fed to cat no. 276, which shed oocysts.
genotyped with six additional genetic markers including c22-8, c29-2, L358, PK1, a new SAG2 and Apico (Su et al., 2006) to further identify isolates with high resolution. For these six markers, the targeted DNA sequences were first amplified by multiplex PCR using external primers for all markers. The external primers are: c22-8Fext, TGATGCAATCATGCGTATAT; c22-8Rext, CCTCTACCTCTCGTCTCA; c29-2Fext, ACCACCTAGCGAAAAAAGAA; c29-2Rext, AGGGTCTCTTGCAGACTACAT; L358-Fext, TCTCTCGAC-TTGGCTCTTCT; L358-Rext, GCAATTCCTCGAGACAGG; PK1-Fext, GAAAGCTGTCACACCCTGAA; PK1-Rext, AGGAAGCTCCGTGCAGTGAT; SAG2-Fext, GGAACGCGAACAATGAGTTT; SAG2-Rext, GACAGG; PK1-R, AGAAAGCTGTCCACTGTTCGCCTCTTC; L358-R2, CCCTCTGGGCAGAGCGAAAAGAAA; c29-2Rext, AGGGG8Rext, CCTCCACTTCTTCGGTCTCA; c29-2Fext, TGATGCATCCATGCGTTTAT; c22-8Fext, TGCAATTTCCTCGATCTCTTGCGCATACAT; L358-F2, AGGAGGCTGTCAGAGTGTCGC and c29-2R, TGTCTAGGAATCTCATTGC for marker PK1; SAG2-Fa, ACCC-CCAGCGGGAGACAATCAGT and PK1-R, TCATCGCTGCAGTGCT for marker L358; PK1-F, CGCAAAAGCTCTCGAATTCTCAGTT and Apico-Fext, TGGTTTTAACCTAGATTGTTG and Apico-Rext, AACACGGAATTAAGAGATTTG. The multiplex PCR reaction was carried out in 25 μl of volume containing 1X PCR buffer, 2 mM MgCl2, 200 μM each of the dNTPs, 0.10 μM each of the forward and reverse primers, 0.5 units of FastStart DNA polymerase (Roche, Indianapolis, IN) and 1.5 μl of DNA extract. The reaction mixture was treated at 95°C for 4 min, followed by 25 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1.5 min. Multiplex PCR amplified products were 1:1 diluted in water and then used for second round of amplification (nested PCR) with internal primers for each marker separately. The internal primers are: c22-8F, TCTCTTACGTGGAGCC and c22-8R, AGGT-GCTTGAGATATTCGC for marker c22-8; c29-2F, AGTTCTGAGTAGTGGC and c29-2R, TGTTTTAACCTAGATTGTTG and Apico-Rext, AACACGGAATTAAGAGATTTG. The nested PCR reaction was carried out in 25 μl of volume containing 1X PCR buffer, 2 mM MgCl2, 200 μM each of the dNTPs, 0.10 μM each of the forward and reverse primers, 0.5 units of FastStart DNA polymerase and 1.5 μl of DNA extract. The reaction mixture was treated at 95°C for 4 min, followed by 35 cycles of 94°C for 30 s, 60°C for 1 min and 72°C for 2 min. The nested PCR products were analyzed to reveal their genotypes by the method described previously (Su et al., 2006).

3. Results

3.1. Pará state chickens

Antibodies to T. gondii were found in 20 chickens with titers of 1:10 in one, 1:20 in one, 1:40 in two, 1:80 in two, 1:160 in two, 1:320 in three, 1:640 in five and 1:1280 or higher in four. T. gondii was isolated from 15 chickens with titers of 1:20 or higher. It is remarkable that 51 of 60 (84%) of mice inoculated with tissues of 15 infected chickens acquired T. gondii infection indicating high density of infection in chicken tissues. Thirty-one of 51 (60.7%) mice that became infected after inoculation with tissues of infected chickens died of toxoplasmosis, mostly due to toxoplastic pneumonia. All infected mice from four strains died of toxoplasmosis (Table 1). The T. gondii isolates were designated as TgCkBr 107–116 and 141–145 (Table 1). The cat fed seronegative chickens did not shed oocysts.

Genotyping of these 15 isolates using polymorphisms at the loci SAG1, SAG2, SAG3, BTUB and GRA6 did not find mixed infection in chickens. The results of genotyping by all 11 markers are summarized in Tables 1 and 3. Eleven genotypes were revealed for the 15 isolates. All genotypes contained different combinations of allele I, II and III. Some genotypes had unique alleles (u-1) at loci SAG1 and c22-8. No clonal types I, II and III genotypes were found.

3.2. Rio Grande do Sul

Antibodies to T. gondii were found in 19 (38.0%) of 50 chickens with titers of 1:20 in one, 1:40 in two, 1:80 in five, 1:160 in three, 1:320 in three, 1:640 in three and 1:1280 or higher in two. T. gondii was isolated from 19 chickens with titers of 1:20 or higher. These isolates were designated as TgCkBr146-163 (Table 2). Of the 72 mice inoculated with tissues of infected chickens seven died within 6 days p.i and were considered to have died of bacterial infections. Of the remaining 65 mice 64 were found to be infected with T. gondii (Table 2). All 20 infected mice from five isolates died of acute toxoplasmosis between 12 and 16 days p.i. The cat (no. 276) fed tissues from 31 seronegative chickens shed T. gondii oocysts. The two mice fed oocysts from cat 276 died of acute toxoplasmosis 4 days later and numerous tachyzoites were found in their mesenteric lymph nodes; these tachyzoites were infective to mice by the subcutaneous route; this isolate was designated as TgCkBr164.
Genotyping of these 19 isolates using polymorphisms at the loci SAG1, SAG2, SAG3, BTUB and GRA6 did not find mixed infection in chickens. The result of genotyping by all 11 markers is presented in Tables 2 and 3. Seven genotypes were identified among the 19 isolates. Five genotypes contain different combinations of allele I, II and III. Isolate TgCkBr146 had type I allele at all 11 loci, while isolates TgCkBr158, 161 and 164 had type III allele for all markers, suggesting these isolates may be identical or very closely related to clonal types I or III lineages.

4. Discussion

Two important conclusions can be drawn from our study. First, the genetic makeup of *T. gondii* in Brazil is highly diverse. From a total of 34 chicken isolates, 18 different genotypes were identified by 11 genetic markers. There were 11 different genotypes among the 15 isolates from Pará, and seven genotypes among 19 isolates from Rio Grande do Sul. Such a high genetic diversity within a relatively small number of isolates is unusual. Our result supports findings from a few recent studies that *T. gondii* in Brazil is diverse and genetically different from that of North America and Europe (Ferreira et al., 2006; Khan et al., 2006; Lehmann et al., 2004, 2006). At present, it is not clear the level of diversity in Brazil is the result of frequent genetic recombination among a limited pool of alleles or from a large number of lineages in the background. Further study by multilocus DNA sequencing for nuclear and organelle genomes will facilitate our understanding of *T. gondii* population in this region.

Second, *T. gondii* populations are distinct among different locations in Brazil. In this study, there are no overlapping genotypes between the states of Pará and Rio Grande do Sul, suggesting the two populations were separated and there was no genetic flow between them. This is in sharp contrast to the parasite population in North America and Europe, where one genotype (type II) predominate broad geographical regions (Ajzenberg et al., 2002; Dardé et al., 1992; Howe and Sibley, 1995). To fully understand *T. gondii* population structure in Brazil, it is necessary to collect a large number of samples that cover the whole region with high density. It is interesting to point out that we do see clonal types I- and III-like genotypes in this study. Isolate TgCkBr146 from Rio Grande do Sul had type I allele at all 11 loci, and isolates TgCkBr158, 161 and 164 had type III allele for all markers, suggesting these isolates may be identical or very closely related to clonal types I or III lineages.

### Table 3

Summary of genotyping

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<th>Genotype</th>
<th>Pará SAG1</th>
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<th>GRA6</th>
<th>c22-8</th>
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Rio Grande do Sul

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nd, data not available.
unique niche for studying *T. gondii* population genetics and molecular evolution.

**Acknowledgements**

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**References**


