Toxoplasma gondii Genotypes Isolated from Humans: A Systematic Review

Genótipos de Toxoplasma gondii isolados de seres humanos: Uma Revisão Sistemática

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ABSTRACT
Toxoplasma gondii has the capacity to infect several animals, including humans. This parasite has great genetic variability due to crossing over in felines’ gut. These new atypical and exotic genotypes are complex, requiring genotyping research to help elucidate virulence mechanisms. For the purpose of the study, five English language databases reporting data on T. gondii genotyping in humans were searched. The searching process resulted in the inclusion of 28 articles published from 2000 to September 2020. The data revealed that 1,167 samples were genotyped into 470 T. gondii isolates. In the world, type II was predominant (53.65%, n = 257). Atypical genotypes were the second most common (22.33%, n = 107). The results suggest greater genetic diversity in Asia and America than in the European and African continents. The genotype #9 was prevalent in Asia. Furthermore, atypical strains were predominant in 50 isolates from patients with ocular toxoplasmosis (52%, n = 26). In individuals with congenital toxoplasmosis, type II was predominant presenting a prevalence of 46.49% (n = 73). Type II strains were predominant in immunocompromised with a prevalence of 75.6% (n = 31). In cancer patients, there was a predominance of atypical strains with 67.14% (n = 47) and genotype #17 was specific for this group. In general, this systematic review indicated a great degree of genetic diversity and circulation of virulent T. gondii strains in humans. However, further studies are needed to better understand the population structure of T. gondii and its clinical characteristics.

Keywords: genetic diversity, genotype, human, toxoplasmosis, Toxoplasma gondii.
predominantes em imunocomprometidos, com uma prevalência de 75,6% (n = 31). Em pacientes
com câncer, houve uma predominância de cepas atípicas com 67,14% (n = 47) e o genótipo nº
17 foi específico para esse grupo. Em geral, essa revisão sistemática indicou um grande grau de
diversidade genética e circulação de cepas virulentas de T. gondii em humanos. Entretanto, são
necessários mais estudos para entender melhor a estrutura populacional do T. gondii e suas
características clínicas.

**Palavras-chave:** diversidade genética, genótipo, humano, toxoplasmose, Toxoplasma gondii.

1 INTRODUCTION

Toxoplasmosis is an infection caused by the protozoan Toxoplasma gondii, which has
three evolutionary forms: tachyzoites, bradyzoites and sporozoites. The parasite has an
heterogeneous biological cycle in which the asexual phase occurs in intermediate hosts, such as
humans, and the coccidian phase occurs in definitive hosts, the felids (Weiss & Dubey, 2009).
Cats have great relevance in maintaining the urban cycle, due to their ability to eliminate oocysts
in their feces, especially stray cats, responsible for environmental contamination, promoting the
spread of the parasite (Teixeira et al., 2019).

The infection in immunocompetent individuals is mostly asymptomatic, however it can
present mild and nonspecific symptoms, such as headaches, malaise, muscle pain and diarrhea
(Filha & Oliveira, 2009). Symptomatic toxoplasmosis has serious consequences for the
individual, such as the development of ophthalmic, neurological and lymphadenopathic
manifestations. Fetuses, newborns, pregnant women and immunocompromised patients are at
risk of infection. In addition, nutrition, immune status, parasitic burden, virulence and genotype
found in humans have been identified as factors that influence disease progression (Dubey et al.,

For a long time, the theory of a clonal population of T. gondii was believed. However,
through the emergence of molecular techniques, use of microsatellites and analysis of
polymorphism of DNA fragments - generated by restriction enzymes by the polymerase chain
reaction (RFLP-PCR) - it has been proven that the parasite in question has great genetic diversity.
Therefore, it should not be considered just a clone, as it was described by initial genetic studies
in 2001. The appearance of atypical strains, which are responsible for serious and lethal
infections in immunocompetent individuals, corroborate this fact (Robert-Gangneux & Dardé,
The first studies of the genotypic population of T. gondii occurred in North America and Europe and presented three main clonal strains (Types I, II and III) related to virulence in mice. However, research has shown that strains from South America, Africa and Asia have a higher genetic polymorphism when compared to other continents. According to experimental studies, this diversity occurs when felids become infected and the parasites realize crossing over in the enterocytes of these hosts. These new atypical genotypes are even more complex, requiring genotyping research to help elucidate virulence mechanisms (Su, Howe & Dubey, 2002; Amouei et al., 2020). Therefore, the objective of the present study is to demonstrate the various genotyping studies of T. gondii in humans from different geographic regions and the techniques used in the genetic epidemiology of the parasite.

2 METHODS

2.1 SYSTEMATIC REVIEW PROTOCOLS

This systematic review was based on the methods proposed by the Cochrane collaboration (Moher, Liberati, Tetzlaff, Altman & Prisma, 2009; Higgins et al., 2019) and standards guided by the PRISMA protocol (Preferred Reporting Items for Systematic reviews and Meta-analysis).

2.2 SEARCH STRATEGY

Five databases were used to search for the articles: Scopus (Elsevier), Pubmed, ScienceDirect, Google Scholar and Web of Science from 2000 to September 30th, 2020.

2.3 STUDY SELECTION CRITERIA

For the selection of articles, the descriptors genotyping OR genotype OR isolation OR RFLP OR PCR OR molecular AND biology OR nested-PCR OR T. and gondii OR Toxoplasma and gondii OR toxoplasmosis OR acquired and toxoplasmosis OR congenital AND toxoplasmosis OR neurotoxoplasmosis OR human AND toxoplasmosis OR host.

Experimental studies, only in English, that genotyped T. gondii isolates strictly from humans were included. Studies that did not perform genotyping of the parasite in humans, duplicated articles, review articles, research before 2000 and studies that used less than five molecular markers were excluded.
2.4 DATA EXTRACTION

Data were extracted from all eligible studies and then summarized according to the PRISMA manual. The information included: title, first author, year of publication, country where the study was carried out, types of samples, number of samples, conventional diagnostic methods, number of individuals with positive tests, molecular markers, numbers of isolates, identification test molecular and isolated type or genotype (TOXODB).

3 RESULTS

3.1 GENERAL CHARACTERISTICS OF THE STUDY AND TOTAL RESULTS

Initially, a total of 5,918 articles were identified in the literature search, related to genotyping. After reading the abstracts, 28 studies were selected for systematic review. All articles used in the analysis were published in English since 2002. Figure 1 clarifies the order of the search and selection process and Table 1 presents the general characteristics of the selected articles. In data collection 4, 6, 7, 10, 1 studies were from Africa, America, Asia, Europe and Oceania, respectively.

In total, 1,167 samples were analyzed for inclusion in the genotyping, which consisted of amniotic fluid, cerebrospinal fluid, blood, fetal placental tissue, nervous tissue and trophoblast. The use of several molecular techniques was observed, such as: conventional-PCR, nested-PCR, multiplex-PCR, RFLP-PCR and Mn-RFLP-PCR. The country where the study was carried out, type of sample, diagnostic methods, molecular identification techniques and molecular markers used to identify 479 isolates in humans are described in Table 1. The results of the literature review were classified according to the country and continent of origin of the study.
Figure 1: Research strategy and study selection flowchart.

Table 1: Overview of studies included in the systematic review and molecular markers associated with their respective molecular identification methods.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Sample</th>
<th>Number of samples</th>
<th>Method</th>
<th>Molecular Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajzenberg et al., 2002</td>
<td>France</td>
<td>Blood</td>
<td>86</td>
<td>ELISA, multiplex-PCR</td>
<td>TUB2, TgM-A, AA, N61, N83, N82, N60 and W35</td>
</tr>
<tr>
<td>Khan, 2005</td>
<td>USA</td>
<td>Cerebrospinal fluid</td>
<td>10</td>
<td>multiplex-PCR and RFLP-PCR</td>
<td>5’-SAG2, 3’-SAG2, BTUB, GRA6 and SAG3</td>
</tr>
<tr>
<td>Khan et al., 2006</td>
<td>Brazil</td>
<td>Blood</td>
<td>11</td>
<td>RFLP-PCR</td>
<td>SAG1, SAG2, SAG3, B1, cB21-4, cS10-A6, GRA6 and L363</td>
</tr>
<tr>
<td>Nowakowska et al., 2006</td>
<td>Poland</td>
<td>Cerebrospinal and blood</td>
<td>19</td>
<td>TAD, ELISA and RFLP-PCR</td>
<td>5-SAG2, 3-SAG2, SAG3, GRA6 and BTUB</td>
</tr>
<tr>
<td>Genot et al., 2007</td>
<td>France</td>
<td>Blood</td>
<td>1</td>
<td>Multiplex-PCR</td>
<td>TUB2, TgM-A, W35, B17 and B18</td>
</tr>
<tr>
<td>Zhou et al., 2009</td>
<td>China</td>
<td>Blood</td>
<td>3</td>
<td>RFLP-PCR</td>
<td>SAG1, SAG2, SAG3, BTUB, GRA6, L358, PK1, c22-8, c29-2 and Apico</td>
</tr>
<tr>
<td>Boughattas et al., 2011</td>
<td>Tunisia</td>
<td>Blood</td>
<td>1</td>
<td>RFLP-PCR</td>
<td>3’ SAG2, 5’ SAG2, SAG3, BTUB, GRA6 and Apico</td>
</tr>
<tr>
<td>Authors</td>
<td>Location</td>
<td>Test Type</td>
<td>Technique</td>
<td>Details</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------</td>
<td>-----------</td>
<td>--------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Fekkar et al., 2011</td>
<td>France</td>
<td>Blood</td>
<td>Multiplex-PCR</td>
<td>TUB2, W35, TG-MA, B18 and B17</td>
<td></td>
</tr>
<tr>
<td>Ferreira et al., 2011</td>
<td>Brazil</td>
<td>Blood</td>
<td>RFLP-PCR</td>
<td>SAG1, SAG2, 5'- and 3'- SAG2, alt. SAG2, SAG3, BTUB, GRA6, C22-8, c29-2, L358, PK1 and Apico</td>
<td></td>
</tr>
<tr>
<td>Carneiro et al., 2013</td>
<td>Brazil</td>
<td>Blood</td>
<td>RFLP-PCR</td>
<td>SAG1, 5' 3' SAG2, [alt.] SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 e Apico</td>
<td></td>
</tr>
<tr>
<td>Costache et al., 2013</td>
<td>Romania</td>
<td>Amniotic fluid</td>
<td>ELISA and multiplex-PCR</td>
<td>TUB2, W35 TgM-A, B18, B17, M33, IV.1, XI.1, M48, M102, N60, N82, AA, N61 and N83</td>
<td></td>
</tr>
<tr>
<td>Döşkaya et al., 2013</td>
<td>Turkey</td>
<td>Blood</td>
<td>Multiplex-PCR</td>
<td>TUB 2, W35 TgM-A, B18, B17, M33, IV.1, XI.1, M48, M102, N60, N82, AA, N61 and N83</td>
<td></td>
</tr>
<tr>
<td>Štajner et al., 2013</td>
<td>Serbia</td>
<td>Blood</td>
<td>Multiplex-PCR and RFLP-PCR</td>
<td>SAG1, SAG2, GRA6, GRA7, TUB2, W3,5 TgM-A, B18, B17, M33, IV.1, XI.1, M48, M102, N60, N82, AA, N61 and N83</td>
<td></td>
</tr>
<tr>
<td>Wang et al., 2013</td>
<td>China</td>
<td>Blood</td>
<td>Mn-RLP-PCR</td>
<td>SAG1, SAG2, SAG3, BTUB, GRA6, C22-2, c29-2, L358, PK1 and Apico</td>
<td></td>
</tr>
<tr>
<td>Herrmann et al., 2014</td>
<td>Germany</td>
<td>Blood</td>
<td>PCR convencional, nested-PCR and RFLP-PCR</td>
<td>SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico</td>
<td></td>
</tr>
<tr>
<td>Higa, Su &amp; Rossini, 2014</td>
<td>Brazil</td>
<td>Blood</td>
<td>PCR convencional and RFLP-PCR</td>
<td>SAG1, 5'3'SAG2, alt. SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, Apico and CS3</td>
<td></td>
</tr>
<tr>
<td>Yera et al., 2014</td>
<td>French Polynesian</td>
<td>Amniotic fluid</td>
<td>Multiplex-PCR</td>
<td>TUB2, W3,5 TgM-A, B18, B17, M33, IV.1, XI.1, M48, M102, N60, N82, AA, N61 and N83</td>
<td></td>
</tr>
<tr>
<td>Cong et al., 2015</td>
<td>China</td>
<td>Blood</td>
<td>Mn-PCR-RFLP</td>
<td>SAG1, SAG2, SAG3, BTUB, GRA6, C22-2, C29-2, L358, PK1 and Apico</td>
<td></td>
</tr>
<tr>
<td>Wang et al., 2015</td>
<td>China</td>
<td>Blood</td>
<td>ELISA and RFLP-PCR</td>
<td>SAG1, SAG2, 5'- e 3'-SAG2, alt. SAG2, SAG3, BTUB,</td>
<td></td>
</tr>
</tbody>
</table>
3.2 GENETIC PATTERNS OF ISOLATES ON CONTINENTS

The results of genotyping between the five main continents: Africa, America, Asia, Europe and Oceania, are contained in Tables 2 and 3 and in Figure 2.

In Africa, 110 isolates obtained from 650 samples were from Ghana and Egypt, the predominant strain was type II (70.9%, n = 78), followed by type I (22.72%, n = 25), type III (0.9% n = 1), atypical (0.9% n = 1) and mix / recombinant (4.54%, n = 5). In addition, the TOXODB # 17 genotype was found.

In America, 80 isolates were genotyped, of which atypical were predominant (66.25%, n = 53). Types I, II, III and mix / recombinant strains were responsible for 13.75% (n = 11), 5% (n = 4), 3.75% (n = 3) and 3.75% (n = 3) of the genotypic population, respectively. The reports
occurred in Argentina, Brazil and the United States. Overall, TOXO DB genotypes # 6, # 8, # 10 (type I) # 11, # 17, # 36, # 41, # 65, # 67, # 71, # 108, # 162, # 166, # 206, # 207, # 208, # 209, # 211, # 212 and # 210 have been identified for this continent. Of these, the most frequent were TOXO DB # 11 and # 65.

In Asia, 95 isolates resulted in atypical as predominant strain with 49.47% (n = 47). In addition, types I, II and mix / recombinant had a prevalence of 21.05% (n = 20), 27.36% (n = 26), and 2.1% (n = 2), respectively. The review did not show type III isolates. In addition, the TOXO DB # 9 genotype was the only one reported in this region.

On the European continent, the genotyping of 192 human isolates revealed that Type II (77.6%, n = 149) had a higher frequency. Type I, III, atypical and mix / recombinant genotypes had frequency rates of 10.93% (n = 21), 5.72% (n = 11), 2.08% (n = 4) and 3.64% (n = 7), respectively.

In Oceania, there was only one survey conducted in French Polynesia. In the mentioned study, a total of 2 isolates were atypical (100%, n = 2).

Figure 2: Archetypal strains of Toxoplasma gondii found worldwide.

Source: from the authors.
Table 2: Summary of the main lineages of viable isolates of T. gondii in humans in the world.

<table>
<thead>
<tr>
<th>Continent</th>
<th>Country</th>
<th>Clinical forms</th>
<th>Number of Samples</th>
<th>Lineage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>Tunisia (Boughattes et al., 2011)</td>
<td>Congenital toxoplasmosis</td>
<td>1</td>
<td>Atypical: 1 (100)</td>
</tr>
<tr>
<td></td>
<td>Ghana (Ayi et al., 2016)</td>
<td>Undefined</td>
<td>81</td>
<td>I: 1 (1.3), II: 75 (93.8), I e III: 5 (4.9)</td>
</tr>
<tr>
<td></td>
<td>Ghana (Pappoe et al., 2017)</td>
<td>Immunocompromised</td>
<td>6</td>
<td>I: 2 (33), II: 3 (50) e III: 1 (17)</td>
</tr>
<tr>
<td></td>
<td>Egito</td>
<td>Congenital toxoplasmosis</td>
<td>22</td>
<td>I: 22 (100)</td>
</tr>
<tr>
<td>America</td>
<td>Estados Unidos (Khan et al., 2005)</td>
<td>Immunocompromised</td>
<td>8</td>
<td>I: 6 (75), II: 2 (25)</td>
</tr>
<tr>
<td></td>
<td>Brasil (Khan et al., 2006)</td>
<td>Ocular toxoplasmosis</td>
<td>11</td>
<td>I: 3 (27), II: 2 (19), III: 3 (27) e III: 3 (27)</td>
</tr>
<tr>
<td></td>
<td>Brasil (Ferreira et al., 2011)</td>
<td>Ocular toxoplasmosis</td>
<td>18</td>
<td>Atypical: 18 (100)</td>
</tr>
<tr>
<td></td>
<td>Brasil (Carneiro et al., 2013)</td>
<td>Congenital toxoplasmosis</td>
<td>25</td>
<td>Atypical: 25 (100)</td>
</tr>
<tr>
<td></td>
<td>Brasil (Higa et al., 2014)</td>
<td>Congenital toxoplasmosis</td>
<td>4</td>
<td>Atypical: 4 (100)</td>
</tr>
<tr>
<td></td>
<td>Brasil (Cunha et al., 2016)</td>
<td>Asymptomatic</td>
<td>8</td>
<td>I: 8 (100)</td>
</tr>
<tr>
<td></td>
<td>Argentina (Pardini et al., 2019)</td>
<td>Congenital toxoplasmosis</td>
<td>6</td>
<td>Atypical: 6 (100)</td>
</tr>
<tr>
<td>Asia</td>
<td>China (Zhou et al., 2009)</td>
<td>Asymptomatic</td>
<td>3</td>
<td>I: 3 (100)</td>
</tr>
<tr>
<td></td>
<td>Turquia (Döşkay et al., 2013)</td>
<td>Congenital toxoplasmosis</td>
<td>2</td>
<td>Mix: 2 (100)</td>
</tr>
<tr>
<td></td>
<td>China (Wang et al., 2013)</td>
<td>Cancer patient</td>
<td>4</td>
<td>I: 1 (25), II: 1 (25) Atypical: 2 (50)</td>
</tr>
<tr>
<td></td>
<td>China (Cong et al., 2015)</td>
<td>Cancer patient</td>
<td>17</td>
<td>I: 8 Atypical: 9 (100)</td>
</tr>
<tr>
<td>Country</td>
<td>Source</td>
<td>Clinical Form</td>
<td>No. of Isolates</td>
<td>Atypical:</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------------</td>
<td>----------------------------------------</td>
<td>----------------</td>
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</tr>
<tr>
<td>China (Wang et al., 2015)</td>
<td>Cancer patient</td>
<td>36</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Índia (Vijaykumar et al., 2018)</td>
<td>Immunocompromised</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Irã (Ajzenberg et al., 2019)</td>
<td>Ocular toxoplasmosis</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polônia (Nowakoska et al., 2006)</td>
<td>Congenital toxoplasmosis</td>
<td>8</td>
<td>III: 8 (100)</td>
<td></td>
</tr>
<tr>
<td>França (Genot et al., 2007)</td>
<td>Immunocompromised</td>
<td>1</td>
<td>I e III: 1 (100)</td>
<td></td>
</tr>
<tr>
<td>França (Fekkar et al., 2011)</td>
<td>Ocular toxoplasmosis</td>
<td>13</td>
<td>II: 10 (77.5), III: 1 (7.5) II e III: 2 (15)</td>
<td></td>
</tr>
<tr>
<td>Romênia (Costache et al., 2013)</td>
<td>Congenital toxoplasmosis</td>
<td>1</td>
<td>I: 1 (100)</td>
<td></td>
</tr>
<tr>
<td>Sérvia (Štajner et al., 2013)</td>
<td>Immunocompromised</td>
<td>1</td>
<td>II: 1 (100)</td>
<td></td>
</tr>
<tr>
<td>Alemanha (Herrmann et al., 2014)</td>
<td>Undefined</td>
<td>17</td>
<td>II: 15 (88), Mix/recombinant: 2 (12)</td>
<td></td>
</tr>
<tr>
<td>Portugal (Vilares et al., 2017)</td>
<td>Asymptomatic</td>
<td>48</td>
<td>I: 13, II: 35</td>
<td></td>
</tr>
<tr>
<td>Dinamarca (Jokelainen et al., 2018)</td>
<td>Asymptomatic</td>
<td>15</td>
<td>II: 15 (100)</td>
<td></td>
</tr>
<tr>
<td>Oceania</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polinésia Francesa (Yera et al., 2014)</td>
<td>Congenital toxoplasmosis</td>
<td>2</td>
<td>Atypical: 2 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Source: from the authors.

3.3 GENETIC DIVERSITY IN DIFFERENT CLINICAL FORMS OF TOXOPLASMOSIS

In the present study, types and genotypes were classified by clinical groups. The atypical strains were predominant in the 50 isolates from patients with ocular toxoplasmosis (52%, n = 26), types I, II, III and mix showed prevalence of 4%, 24%, 8% and 10%, respectively.
In 157 isolates from cases of congenital toxoplasmosis, type II had a prevalence of 46.49% (n = 73) while types I, III, atypical and mix had a prevalence of 19.10% (n = 30), 6.36% (n = 10), 26.75% (n = 42) and 1.27% (n = 2), respectively.

In cancer patients who presented reactivation of toxoplasmosis, there was a predominance of atypical strains with 67.14% (n = 47) followed by strains of type II (n = 11), type I (n = 9), type III (n = 1) and mix / recombinant (n = 2). Type II strains were predominant in immunocompromised patients with a prevalence of 75.6% (n = 31) whereas strains of types I, III and mix / recombinant showed a prevalence of 19.51% (n = 8), 2.43% (n = 1) and 2.43% (n = 1). No atypical strains were found in this group.

There was also the distribution of genotypes by RFLP-PCR found in the different groups, Table 3 shows the genotypic characterizations of each of them, performed by RFLP-PCR. Thus, the genotypes TOXO DB # 9 (n = 36) in cancer patients and # 65 (n = 10) in patients with congenital toxoplasmosis had the highest frequency rates. Furthermore, the comparison between the genotypes showed that TOXO DB # 8, # 11, # 17, # 36, # 41, # 65, # 67, # 108, # 162, # 166, # 206, # 207, # 208, # 209, # 210, # 211 and # 212 were identified only in patients with congenital toxoplasmosis. The genotypes TOXO DB # 45 and # 141 were specific in cases of toxoplasmosis in immunocompromised individuals. And the TOXO DB # 9 genotype was observed only in cancer patients. The genotype TOXO DB # 65 was common among all groups and TOXO DB # 1 in immunocompromised and cancer patients. There were not specific genotypes for ocular toxoplasmosis.

Table 3: Genotypic diversity in several clinical forms of toxoplasmosis.

<table>
<thead>
<tr>
<th>Category</th>
<th>Patient group</th>
<th>GenOTYPE TOXO DB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All genotypes</td>
<td>Ocular toxoplasmosis</td>
<td>#65 (0.56)</td>
</tr>
<tr>
<td></td>
<td>Congenital toxoplasmosis</td>
<td>#8 (0.56), #11 (0.56), #36 (0.56), #41 (0.56), #65 (0.56), #67 (0.56), #108 (0.56), #162 (0.56), #166 (0.56), #206 (1.68), #207 (0.56), #208 (0.56), #209 (0.56), #210 (0.56), #211 (0.56) e #212 (0.56)</td>
</tr>
<tr>
<td></td>
<td>Reactivation of toxoplasmosis in a cancer patient</td>
<td>#1 (5.61), #9 (24.71), #10 (4.49), #65 (1.12) e #204 (0.56)</td>
</tr>
<tr>
<td></td>
<td>Immunocompromised</td>
<td>#17 (0.56), #65 (2.24) and (0.56)</td>
</tr>
<tr>
<td>Genotypes found simultaneously</td>
<td>All clinical forms</td>
<td>#65</td>
</tr>
<tr>
<td></td>
<td>Ocular toxoplasmosis and Immunocompromised</td>
<td>#65</td>
</tr>
<tr>
<td>Ocular toxoplasmosis and Congenital toxoplasmosis</td>
<td>#65</td>
<td></td>
</tr>
<tr>
<td>Immunocompromised and congenital toxoplasmosis</td>
<td>#65</td>
<td></td>
</tr>
<tr>
<td>Immunocompromised and reactivation of toxoplasmosis in a cancer patient</td>
<td>#65</td>
<td></td>
</tr>
</tbody>
</table>

**Specific genotypes**

| Ocular toxoplasmosis | #8, #11, #36, #41, #67, #108, #162, #166, #206, #207, #208, #209, #210, #211 and #212 |
| Congenital toxoplasmosis | - |

| Reactivation of toxoplasmosis in a cancer patient | #9 |
| Immunocompromised toxoplasmosis | #17 |

Source: from the authors.

## 4 DISCUSSION

The systematic review demonstrated that the biological samples used in genotyping studies are varied, which helps to obtain *T. gondii* isolates. In addition, a wide use of molecular methods was identified in genotyping studies. Such techniques allow greater reliability in the result, mainly in immunocompromised patients since the serological diagnosis is difficult (Pardini et al., 2014).

This study demonstrated that the type II strain was predominant in 479 isolates with a prevalence of 53.65% (257 in 479). Type I strains were the second most found genotype in the world, with 77 isolates and a prevalence rate of around 16.07%. In Africa and Europe, the archetypal lineage II obtained a higher prevalence as in the study by Shwab et al. (2014). In addition, previous studies have shown that archetypal clonal lineages, I, II and III, have a higher prevalence in Europe (Silva, Andrade, Carneiro & Vitor, 2014). Atypical strains were more frequent in America (66.25%, 53 in 80), which indicates great genetic variability when compared to European countries (29.68%, 57/192), corroborating the findings of Sharif et al. (2017) in ruminants.

In Africa, only one atypical strain has been identified, thus showing a highly clonal population in this Continent. In Oceania, only atypical strains have been identified. However, the
amount of sample (n = 2) is not sufficient to determine the presence of genetic polymorphism in this region, therefore, further genotyping studies are needed. Worldwide, clonal types III and mix / recombinant with prevalences of 3.13% (15/479) and 3.54% (17/479), respectively, were the least frequent. Type III strains, such as VEG, are associated with toxoplasmosis in animals and cause mild and asymptomatic infections in humans (Sharif et al., 2017).

Only Brazil and France presented all archetypal strains, atypical isolates and mix/recombinant simultaneously. Brazilian genotyping revealed a higher prevalence of atypical genotypes when compared to France. This fact is consistent with the study by Silva et al., 2014 in which the Brazilian genotypic profile of T. gondii varies considerably, demonstrating a great genetic recombination. The isolates identified in South America are highly virulent and lethal to rats, for example, the BrI strain (Grigg, Dubey & Nussenblatt, 2015). In addition, severe symptoms associated with ocular toxoplasmosis are seen more frequently in Brazil than in European (Chaichan et al., 2017).

The genotypes found in this study showed a great genetic diversity worldwide due to the contrast clearly observed in the five continents. These data suggest that genotype # 9, known as Chinese I, had a higher frequency in humans, in Asia (n = 44). It is suggested that Chinese I and types I, II and III are the main clonal lineages found in Asia (Khan et al.,2007). Types I, II and III are believed to have emerged with the advent of agriculture and domestication of animals, in the region of the fertile crescent, a factor that favored the appearance of strains adaptable to domestic animals (Dardé, 2008). Furthermore, no TOXODB # genotype was found simultaneously on the five continents.

As for virulence, type I strains have an absolute lethal dose (Ld100) in 1 mouse, whereas types II and III have lower rates with LD100 ≥ 103 (Pomares et al., 2018). The data from this study indicated the high prevalence of type II strains in individuals with chronic infection who have reactivation of toxoplasmosis47. In order to reduce contamination by T. gondii, socio-educational actions are needed to inform the population about basic prophylactic measures.

Atypical strains were predominant in the 50 isolates from patients with ocular toxoplasmosis (52%, n = 26). A survey conducted by Pomares et al. (2018) also revealed that atypical strains were prevalent in this population and are strongly associated with severe cases of ocular toxoplasmosis. In individuals with congenital toxoplasmosis, type II was predominant, with a prevalence of 46.49% (n = 73). Cases of congenital toxoplasmosis can result in
hydrocephalus, mental retardation and retinochoroiditis (Rico-Torres, Vargas-Villavicencio & Correa, 2016). Generally, neonates infected with type II strains in the first half of pregnancy have severe symptoms of the disease (Delhaes et al., 2010).

The genetic diversity of T. gondii may be related to the severity of clinical signs in infected children during pregnancy. Most cases of congenital toxoplasmosis with atypical strains have an unsatisfactory prognosis, regardless of treatment. In addition, the consequences caused to the fetus by the infections of these strains in the third trimester of pregnancy appear to be more severe when compared to type II strains (Benamrouz et al., 2012).

In cancer patients who presented reactivation of toxoplasmosis, there was a predominance of atypical strains with 67.14% (n = 47). A study has shown that protozoa from the phylum Apicomplexa probably aggravate neoplasms in the host's tissue (Sibley, Khan, Ajioka & Rosenthal, 2009). Infection with T. gondii in cancer patients is a serious problem, therefore, integrative measures such as association of molecular identification with virulence studies are needed for better control of toxoplasmosis in these individuals. Type II strains were predominant in immunocompromised patients with a prevalence of 75.6% (n = 31). This result confirms the findings by Sibley et al. 55 who found that type II strains have a higher prevalence in immunocompromised individuals and neurotoxoplasmosis.

Genotype # 65 was common in all clinical groups in the study, in addition to being more frequent in patients with congenital toxoplasmosis. In 2015, this genotype was first isolated from pigs. It is believed that it comes close to the BrIV and MAS types, isolated in other regions of the country, suggesting a wide territorial distribution56. Genotypes # 8, # 11, # 17, # 36, # 41, # 67, # 108, # 162, # 166, # 206, # 207, # 208, # 209, # 210, # 211 and # 212 were specific for congenital toxoplasmosis. It is worth mentioning that identifying the genotype helps in determining the type of treatment, management and prognosis of the disease in newborns (Pardini et al., 2014).

Genotype # 17 occurred only in immunocompromised individuals. The study of genetic variations in clonal populations in different clinical forms of toxoplasmosis can have important health implications due to the possibility of future outbreaks of drug-resistant strains (Pardini et al., 2019). There were no specific TOXODB # genotypes for patients with ocular toxoplasmosis. This fact can be explained by the reduced number of studies that address isolates in individuals with ocular toxoplasmosis. Thus, there is a need for further studies to learn about genotyping as
it helps control transmission and reduces the spread of toxoplasmosis between humans and animals (Su, Shwabe, Zhu & Dubey, 2010).

5 CONCLUSION

Given the survey of studies about the genotyping of T. gondii, it was possible to conclude that currently a wide variety of molecular techniques are used. The research demonstrated great genetic diversity among the isolates from different clinical samples. In general, the results obtained showed that strains of T. gondii, highly virulent in mice, are circulating in humans, mainly in South America. Furthermore, it is necessary that genotyping studies continue to be carried out in order to determine the circulating genotypes. The knowledge of predominant strains in the various clinical forms of toxoplasmosis is important in the field of public health, as it helps both in the creation of measures to control toxoplasmosis and in therapeutic decisions.
REFERENCES


