

A systematic review of *Toxoplasma gondii* genotypes in *Gallus gallus domesticus* worldwide: The focus is Brazil

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Abstract

Toxoplasma gondii was initially classified in three main lineages related to its virulence: Types I, II and III. The recombination of genes during sexual cycle in felids gut led to more than 200 genotypes, found in ToxoDB database, using 11 RFLP markers. Free-range chickens are good bioindicators of soil contamination with *T. gondii* oocysts. In this sense, there are systematic reviews regarding data of genetic characterization of this parasite in felines and ruminants, but not in chickens heretofore, what makes this work necessary. A systematic review in the literature was performed with papers published prior to September 21st, 2020. The main inclusion criteria was the presence of *T. gondii* genotypes, isolated strictly from free-range chickens, in experimental works. Initially, a total of 1,343 studies related to the terms were identified on databases and 30 studies were selected to be systematically reviewed. A total of 561 isolates of *T. gondii* from 6,356 free-range chickens were analyzed for genotyping, revealing 190 genotypes. ToxoDB #59 and #2 were the most frequent in America, #1 was the most frequent in Africa and 3 atypical isolates from genotype ToxoDB #9 were found in Asia. There is not data from Europe and Oceania. The majority of studies were Brazilian (16/30). A total of 68 RFLP genotypes were recognized among the 561 isolates' DNAs analyzed from the 30 studies. Some studies show new genotypes never described before, which reinforces the idea that some years from now, even more new genotypes will be isolated, due to progressive genetic recombination. The large amount of undefined genotypes makes it necessary to perform Nested PCR technique when genotyping. Moreover, the lack of data in Continents such as Europe, Asia and Oceania makes it necessary to perform new isolating and genotyping studies in these places.

A systematic review of *Toxoplasma gondii* genotypes in *Gallus gallus domesticus* worldwide: The focus is Brazil

T. gondii genotypes in *Gallus gallus domesticus*

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SUMMARY

Toxoplasma gondii was initially classified in three main lineages related to its virulence: Types I, II and III. The recombination of genes during sexual cycle in felids gut led to more than 200 genotypes, found in

ToxoDB database, using 11 RFLP markers. Free-range chickens are good bioindicators of soil contamination with *T. gondii* oocysts. In this sense, there are systematic reviews regarding data of genetic characterization of this parasite in felines and ruminants, but not in chickens heretofore, what makes this work necessary. A systematic review in the literature was performed with papers published prior to September 21st, 2020. The main inclusion criteria was the presence of *T. gondii* genotypes, isolated strictly from free-range chickens, in experimental works. Initially, a total of 1,343 studies related to the terms were identified on databases and 30 studies were selected to be systematically reviewed. A total of 561 isolates of *T. gondii* from 6,356 free-range chickens were analyzed for genotyping, revealing 190 genotypes. ToxoDB #59 and #2 were the most frequent in America, #1 was the most frequent in Africa and 3 atypical isolates from genotype ToxoDB #9 were found in Asia. There is not data from Europe and Oceania. The majority of studies were Brazilian (16/30). A total of 68 RFLP genotypes were recognized among the 561 isolates' DNAs analyzed from the 30 studies. Some studies show new genotypes never described before, which reinforces the idea that some years from now, even more new genotypes will be isolated, due to progressive genetic recombination. The large number of undefined genotypes makes it necessary to perform Nested PCR technique when genotyping. Moreover, the lack of data in Continents such as Europe, Asia and Oceania makes it necessary to perform new isolating and genotyping studies in these places.

Keywords: continent, free-range chicken, genetic diversity, genotype, *Toxoplasma gondii* .

1 INTRODUCTION

Toxoplasma gondii is a protozoan able to infect all warm-blooded animals, causing toxoplasmosis, and is widely distributed around the world. In general, the infection is asymptomatic in immunocompetent individuals but can be more severe in congenital transmission, specially when the infection happens in the first third of pregnancy, and in immunodeficient people (Dubey et al., 2010; Cortés et al., 2019).

Humans often get infected by ingesting undercooked or raw meat contaminated with tissue cysts with bradizoytes, or by accidentally ingesting food or water contaminated with oocysts from felids' feces. In some cases, tachyzoites are viable in milk and are infectant for those who drink it as well (Hill & Dubey, 2002; Cortés et al., 2019).

Researchers characterize *T. gondii* in three major clonal lineages (Types I, II and III) related to high, medium and low mouse virulence. The strains RH88, ME49 and VEG are important representants of Type I, Type II and Type III, respectively. Nonetheless, recent literature revealed huge genetic diversity among strains specially in South America, Asia and Africa, where atypical are predominant. Atypical strains are also related to high virulence, in some cases, can cause congenital ocular toxoplasmosis and acute systemic toxoplasmosis even in immunocompetent individuals (Howe & Sibley, 1995; Saeij et al., 2005; Cortés et al., 2019). Mixed infections are rare, but may occur when more than one strain of *T. gondii* are found simultaneously with molecular assays in a host (Moreno et al., 2019).

Therefore, the combination of genes during sexual cycle in felids gut led to more than 200 genotypes, which can be characterized by ToxoDB database. Once, genotyping was performed by analysing only SAG2 marker, which made it possible to classify the isolate as being part of one of the three major lineages. Usually, more than 11 markers are used, and it is not trustworthy to classify by using less than 5 (Ajzenberg et al., 2004; Su et al., 2006; Rajendran et al., 2012; Shwab et al., 2014).

Free-range chickens are good bioindicators of soil contamination with *T. gondii* oocysts, and one of the most important source of infections for humans, due to large consumption (Dubey, 2010; Dubey et al., 2015). By studing *T. gondii* genotypes isolated from chickens, it is possible to determine the frequency of specific strains belonging to a determined genotype correlated with the locations where they were identified from other hosts. In this sense, there are systematic reviews regarding data of genetic characterization of this parasite in felines and ruminants (Sharif et al., 2017; Amouei et al., 2020), but not in chickens heretofore, what makes this work necessary.

2 METHODS

2.1 Protocols

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were followed in order to systematically review 30 cross-sectional studies. The whole methodological protocol of this study was registered on OSF with DOI 10.17605/OSF.IO/FJU94 and might be accessed by the link <https://osf.io/fju94/>.

2.2 Systematic search strategy

Four English Databases were used including *Scopus*, *PubMed*, *ScienceDirect* and *Web of Science*, for literature searching of articles published prior to September 21st, 2020. All scientific publications related to genetic characterization of *T. gondii* isolated from free-range chickens (*Gallus gallus domesticus*) were sought.

The terms used in the searching process were “toxoplasmosis”, “*Toxoplasma gondii*”, “*T. gondii*”, “type”, “typing”, “genotype”, “genotyping”, “molecular characterization”, “chicken”, “fowl”, “poultry” and “RFLP”. Only studies published in English were considered. All citations were imported to Mendeley (by Elsevier) and the studies were tabulated on Sheets (by GSuite – Google). Further studies were checked by scanning the reference list of the selected papers to prevent missing valuable data.

2.3 Study selection criteria

The papers were screened independently by three reviewers. The studies were selected as they corresponded to inclusion criteria, and those which did not contemplate the requirements were excluded. Duplicated articles and review works were excluded from the process. The main inclusion criteria was the presence of genotypes of *T. gondii* isolated strictly from free-range chickens (*Gallus gallus domesticus*) in experimental works, which was observed in the titles and abstracts or full texts, if necessary.

Thus, articles about seroprevalence, diagnostic methods, or those which did not perform the genotyping of their isolates using RFLP-PCR and did not present a table showing their markers along the result of ToxoDB # genotype or clonal type, were excluded. Studies that used less than 5 markers were not included (Amoui et al., 2020).

2.4 Data extraction

Data from all of the selected articles were extracted independently according to PRISMA checklist standardized form developed for molecular studies by trained data extractors. Data contemplated general characteristics: first author, year of publication and country; and methodological characteristics: sample size, tissue location, host (only free-range chickens), examination techniques used to identify infected hosts (MAT, IFAT, ELISA, PCR, Nested-PCR, bioassays), number of DNA isolates, number of genotypes/types/clonal types, and ToxoDB # evidenced.

3 RESULTS

3.1 Study characteristics and search results

Initially, a total of 1,343 studies related to the terms were identified on databases. All papers used for analysis were published in English language from 2006 onwards. After applying inclusion criteria, 30 studies were selected to be systematically reviewed. **Figure 1** illustrates the searching process, selection and exclusion.

The general and methodological characteristics of the included studies are presented in **Table 1**. A total of 561 isolates of *T. gondii* from 6,356 (Total sample) free-range chickens were analysed for genotyping, revealing 190 genotypes. The results from the literature review were classified based on country and continent (**Table**

2). Out of the retrieved articles, 27, 2, 1, 0 and 0 studies were from America, Africa, Asia, Europe and Oceania, respectively.

3.2 Genetic patterns of continents

The genotyping results among the three continents (America, Africa and Asia) are listed in **Table 3** and illustrated in **Figure 2**.

3.2.1 America

27/30 of the articles were from America, revealing 517 isolates classified in 166 different genotypes. 3 (0,5%), 22 (4,3%), 35 (6,8%), 210 (40,6%), 1 (0,2%) and 246 (47,6%) were from Clonal Type I, II, III, Atypical, Mixed and Not defined, respectively. Overall, genotypes ToxoDB #1, #2, #3, #6, #7, #8, #10, #11, #13, #14, #16, #19, #21, #24, #26, #30, #32, #36, #44, #48, #53, #55, #59, #60, #61, #62, #64, #65, #75, #88, #94, #95, #97, #98, #108, #109, #111, #116, #120, #122, #140, #146, #152, #162, #163, #170, #175, #206, #213, #214, #215, #235, #248, #251, #252, #253, #259, #264, #265, #273, #274, #277, #278, #279, #280, #281 and #282 were identified from this Continent. ToxoDB #59 and #2 were the most frequent.

3.2.2 Africa

2/30 of the articles were from Africa, revealing 41 isolates classified in 23 different genotypes. 1 (2,5%), 8 (19,5%), 6 (14,6%), 0 (0,0%), 5 (12,2%) and 21 (51,2%) were from Clonal Type I, II, III, Atypical, Mixed and Not defined, respectively. Overall, genotypes ToxoDB #1, #2 and #10 were identified from this Continent. ToxoDB #1 was the most frequent.

3.2.4 Asia

Only one study was from Asia, which revealed 3 atypical isolates from genotype ToxoDB #9.

3.2.5 Europe and Oceania

There were not data from these Continents in the literature.

3.3 Genetic patterns in Brazil

According to databases sought, Brazil owns the major quantity of studies, a total of 16/30 articles. For this reason, it is suitable to describe the genetic patterns found specifically in this Country, among its five geographical regions (North, Northeast, Middle-West, Southeast and South). Genotypes are listed in **Table 4** by region, and illustrated in **Figure 3**. One of the articles studied chickens from Pará (North) and Rio Grande do Sul (South).

A total of 259 isolates were identified in Brazil, classified in 107 different genotypes. 1 (0,4%), 5 (2,0%), 1 (0,4%), 142 (56,1%), 1 (0,4%) and 103 (40,7%) were from Clonal Type I, II, III, Atypical, Mixed and Not defined, respectively. Overall, genotypes ToxoDB #1, #2, #6, #7, #8, #10, #11, #13, #14, #16, #19, #21, #24, #26, #30, #32, #36, #44, #48, #53, #55, #59, #60, #61, #62, #64, #65, #75, #88, #94, #95, #97, #98, #108, #109, #111, #116, #120, #122, #140, #146, #152, #162, #163, #175, #206, #213, #214, #215, #216, #235, #248, #251, #252, #253, #273, #274, #277, #278, #279 and #280 were identified. ToxoDB #59 was the most frequent genotype.

3.3.1 North

Data from North was extracted by an article which shared genotypes from States of Pará (North) and Rio Grande do Sul (South). All of the 15 isolates from North (like the ones from South in this paper) were not possible to determine a genotype, being considered Not defined (100%).

3.3.2 Northeast

6/16 studies were from States in Northeast, reaching 33 different genotypes from 97 isolates. 5 (5,2%), 1 (1,0%), 64 (66,0%) and 27 (27,8%) isolates were classified as Clonal Type II, III, Atypical and Not defined,

respectively. Genotypes ToxoDB #1, #2, #6, #8, #7, #11, #13, #36, #48, #59, #60, #61, #62, #88, #109, #116, #122, #146, #163, #235, #273, #274, #277 and #279 were found. The most frequent were #59 and #163.

3.3.3 Middle-West

Data from Middle-West was extracted by 1/16 study, in which 12 different genotypes were identified from 22 isolates in chickens of Pantanal Area, which consists on Mato Grosso and Mato Grosso do Sul States. 21 (95,5%) and 1 (4,5%) isolates were genotyped as Atypical and Mixed, respectively. Genotypes ToxoDB #16, #30, #32, #44, #59, #60, #94, #95, #97 and #98 were found, and #59 was the most recurrent.

3.3.4 Southeast

4/16 studies were from Southeast Region, whose 58 isolates result in 17 different genotypes. 25 (43,1%) were Atypical and 33 (56,9%) were Not defined. Genotypes ToxoDB #6, #11, #14, #24, #65, #75, #108, #109, #162, #206, #213, #214, #215, #216 and #280 were found. Out of them, #6 and #24 were the most repeated.

3.3.5 South

5/16 studies were from South Region, and 1/5 shared results among North and South, as it was described on item 3.3.1. They showed 32 different genotypes in 61 isolates. 1 (1,6%) was Clonal Type I, 32 (52,5%) Atypical and 28 (45,9%) were Not defined. Genotypes ToxoDB #6, #10, #11, #19, #21, #26, #26, #53, #55, #64, #111, #120, #140, #152, #163, #175, #248, #251, #252, #253 and #278 were found, and #120 was the predominant.

3.4 Multiloci analysis of *Toxoplasma gondii* genotypes defined by network phylogeny

A total of 68 RFLP genotypes (ToxoDB) were recognized among the 561 isolates' DNAs analyzed from the 30 studies. Phylogenetic network was inferred by the software SplitsTree4 (Huson e Bryant, 2006) using the coded genotyping data for 11 multiloci RFLP-PCR markers and 1 apicoplast marker (**Figure 4**). Most of the samples were clustered in three genetic groups, according to kinship with control samples (GT1-Type I, PTG-Type II and CTG-Type III). Most of the strains are related to Types I and III, and some from Type II common ancestry.

4 DISCUSSION

Many genotypes were not defined because there were not enough markers in the results. This is due to insufficient DNA in the samples, leading to inconclusive RFLP genotyping. This explains why some studies realized Nested-PCR before RFLP-PCR, to increase DNA of the samples.

This systematic study used papers From America 27/30 (Brazil 16/27) majorly, and this was one of the reasons that the genetic characteristics of *T. gondii* isolated from chickens around the world is not conclusive yet. More genotyping studies must be performed in order to increase data, especially in Europe (no data), Asia (1 article), Oceania (no data), Africa (2 articles with many inconclusive genotypes) and North America (only USA has studies - 2).

Shwab et al. (2014) described that #1 (Type II clonal), #2 (Type III), #3 (Type II variant) and #10 (Type I) are identified all over the world. Genotypes #2 and #3 are most found in Africa, whereas genotypes #9 (Chinese 1) and #10 are prevalent in Asia. The present study did not find papers from Europe strains, however they described genotypes #1, #2 and #3 as prevalent. ToxoDB #1, #2, #3, #4 and #5 dominate in North America, and the present study showed predominance of #1 (31,4%) and #2 (34,3%) in USA.

Amouei et al. (2020) also had difficult to find studies regarding genotyping of *T. gondii* isolates in Oceania, when studying felids, they described only one study in which 8 isolates were genotyped into 2 genotypes, and the majority of the strains (97,5%, n=7) were genotype ToxoDB #3.

There are some strains of *T. gondii* which circulates in many species of animals concomitantly. Melo et al. (2020) performed a study in Fernando de Noronha Island, in Brazil, in which they isolated *T. gondii* genotypes ToxoDB #146 from sheep and pigs. This strain was also reported in chickens by Silva et al. (2010), in Brazil.

Besides, Pena et al. (2020) isolated ToxoDB #8 (Br III type) from giant anteater (*Myrmecophaga tridactyla*), which was also isolated in chickens by Rocha et al. (2018), Feitosa et al. (2017) in Brazil and Lindstrom et al. (2008) in Uganda. This find supports the theory postulated by Pena et al. (2020) and Dubey et al. (2010) whom say that giant anteater highly contributes to Brazil's common lineages circulation and that chickens are good bioindicators of *T. gondii* contamination, respectively.

The systematic review demonstrated the presence of genotypes ToxoDB #11, #162 and #206 in chickens (Table 2) and a Brazilian study by Carneiro et al. (2013) identified such genotypes in cases of congenital toxoplasmosis in humans, in the state of Minas Gerais, corroborating the hypothesis that free-range chickens are sources of contamination in humans and this affection may cause diseases. Such study also showed that isolates #11 and #206 are highly virulent in female mice of the BALB/c strain whereas isolate #162 shows intermediate virulence.

North America and Europe have a predominance of archetypal strains of type I, II and III, while South America has atypical strains without genotype pattern prevail. This distribution of the diverse population structure from *T. gondii* strains, is closely associated with virulence patterns, and the severity of infections in humans (Xiao e Yolken, 2015; Saraf et al., 2017). According to Shwab et al. (2016), strains commonly found in Europe, North America, North Africa and Asia are non-lethal to mice when used at low doses for infection, while those prevalent in South America are highly virulent and lethal.

5 CONCLUSION

The present study presented the first data investigating *T. gondii* genotyping from *Gallus gallus domesticus*, and revealed the existence of a large amount of atypical genotypes, in special in South America. Some studies show new genotypes never described before, which reinforces the idea that some years from now, even more new genotypes will be isolated, due to progressive genetic recombination.

The large amount of not defined genotypes due to inconclusive markers suggests the need of Nested-PCR technique in genotyping studies. The knowledge of the genotyping of the strains prevailing in free-range chickens denotes the virulence patterns found in each continent. The lack of data in Continents such as Europe, Asia and Oceania makes it necessary to perform new isolating and genotyping studies in these places.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

No ethical approval was required as this is a review article with no original research data.

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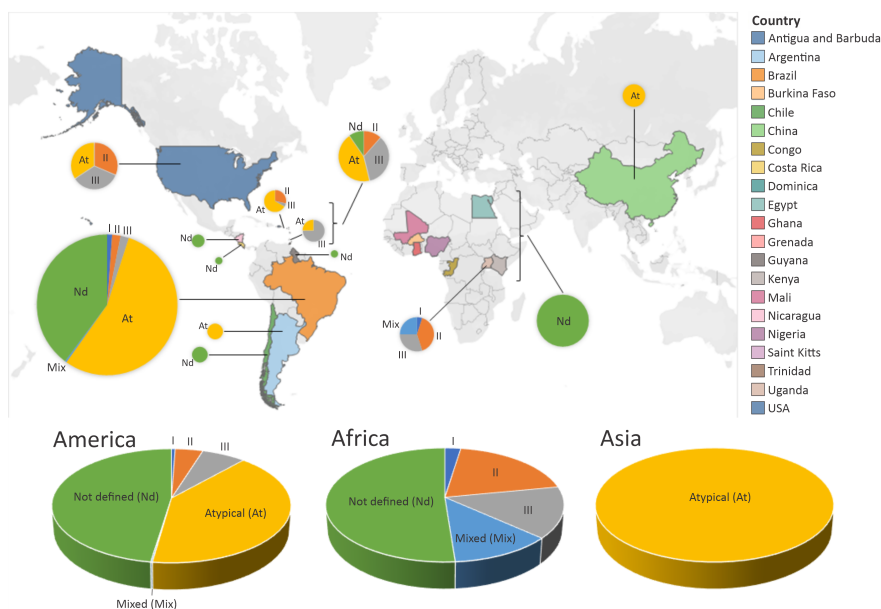
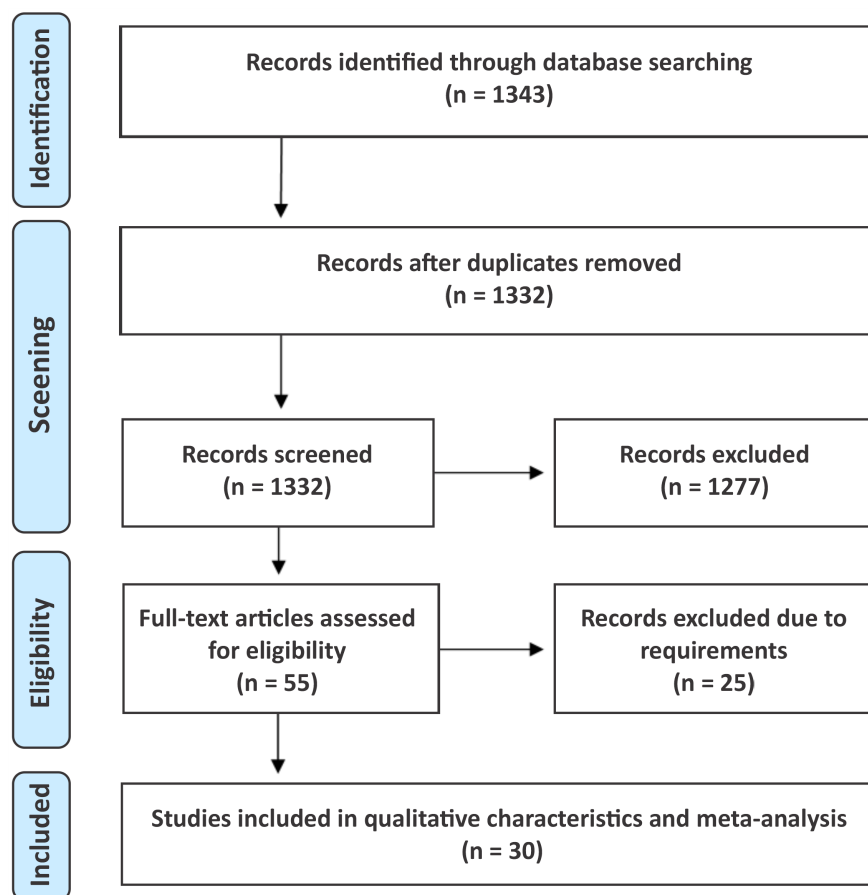
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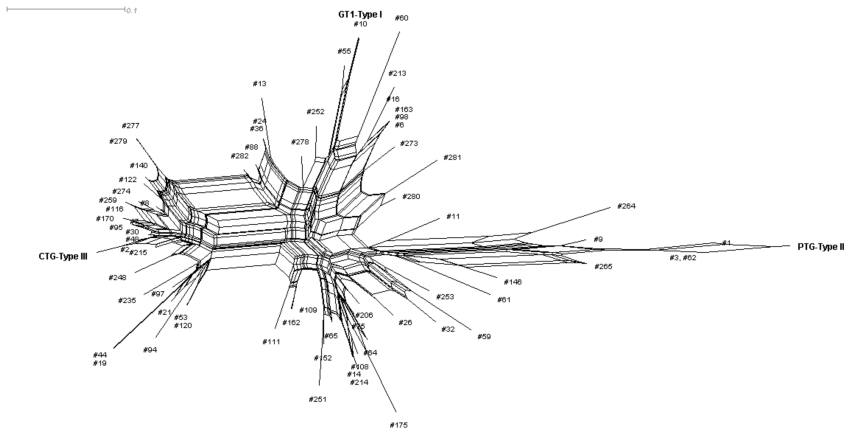
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