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Population structure of *Toxoplasma gondii* in Argentina

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ABSTRACT:

The protozoan *Toxoplasma gondii* is worldwide distributed showing a particular population structure that may differ among continents and countries. The aim of this study was to analyze the *T. gondii* population structure in Argentina and compare it with genotyping information from other South American countries. For the analysis, 39 samples from Argentina (isolates from the provinces of Buenos Aires, Misiones, Entre Ríos and San Luis) were genotyped using 10 multilocus PCR-RFLP markers including SAG1, SAG2 (5'-3'SAG2, alt. SAG2), SAG3, BTUB, GRA6, C22-8, C29-2, L358, PK1, and Apico. The *T. gondii* DNA samples were obtained from domestic animals (chickens $n = 20$; cats $n = 3$; pigs $n = 2$; goat $n = 1$; rabbit $n = 1$), humans ($n = 6$), zoo animals ($n = 5$) and a rat ($n = 1$). Phylogenetic relationship of these Argentinean isolates together with representative reference genotypes was determined by phylogenetic network analysis. Thirty-seven Argentinean samples belonged to 21 genotypes and two samples were genotyped at 8 of the 10 loci and considered incomplete characterized. Among these 37 typed samples, five genotypes were not previously reported. The majority of the samples grouped with the Type III (ToxoDB PCR-RFLP genotype #2) lineage. The clonal Type II (ToxoDB genotypes #1 and #3) was also identified. Our results suggest a unique population structure with combination of unique genotypes and the common Type II and Type III lineages in Argentina. Nevertheless, different regions showed distinctive pattern of genotypes, revealing a higher variability in Northern provinces.

KEYWORDS: *T. gondii*; genotyping; phylogenetic network; South America; Argentina.

1. INTRODUCTION

Toxoplasma gondii is an Apicomplexan parasite with a worldwide distribution, capable of infecting all warm-blooded animals (including humans) (Dubey, 2010). Domestic and wild felines are the definitive hosts and several species of mammals and birds act as intermediate hosts. Transmission to all animals can occur by ingestion of food and water contaminated with oocysts, by ingestion of raw or undercooked meat by carnivorous animals and transplacentally in mammals (Dubey, 2010). Susceptible animals present different clinical signs; neuromuscular disorders in dogs, abortion in small ruminants, stillborn in neonatal pigs and fatal toxoplasmosis in Australian marsupials and New World monkeys (Dubey, 2010). In human health, especially in immunocompromised patients, clinical signs of infection include retinal lesions (ocular toxoplasmosis), central nervous system lesions (mainly associated with congenital toxoplasmosis) and multisystem failure in immunocompromised individuals (Weiss and Kim, 2014). However, there is a variant that can affect immunocompetent adults known as Amazonian Toxoplasmosis, which requires immediate medical intervention and can be fatal (Demar et al., 2012).

Numerous previous reports revealed genetic diversity of the parasite, with clonal population in the northern continents of the world, but highly diverse population in Central and South America (Dubey and Su, 2009; Shwab et al., 2014; Weiss and Kim, 2014). Different techniques were used to identify and classify the parasite (Su et al., 2012). Nested Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (nPCR-RFLP) of 10 markers has been widely used and showed high power of discrimination. Different genotypes identified by this method are deposited in the ToxoDB database (www.toxodb.org). Microsatellites (MS) markers consist of 15 markers with higher resolution, was also widely used to genotype *T. gondii*, together with nPCR-RFLP typing defined 16 Haplogroups within 6 clades (Su et al., 2012; Weiss and Kim, 2014). In North America (USA and Canada) the predominant genotypes are type II (ToxoDB genotypes #1 and #3), type III

(#2) and haplogroup 12 (#4 and #5) which is considered as a clonal lineage in North America. Clonal types II and III are considered low to mild virulent in mice model. In Europe type II predominates followed by type III isolates. In Asia, *Chinese I* (also known as ToxoDB genotype #9) is dominant in the central and eastern region of the continent (Chaichan et al., 2017). In Africa, type II predominates in North and East Africa along with ToxoDB genotype #20, whereas in the central and west region of the country *Africa I* (identified by MS, also known as ToxoDB genotype #6) is dominant (Galal et al., 2018). South American isolates, the majority obtained from Brazil, are widely variable with predominance of non-clonal types. In Brazil, 4 genotypes described in multiple isolates have been considered as Brazilian clonal lineages named: BrI, BrII, BrIII and BrIV (ToxoDB genotypes #6, #11, #8 and #17, respectively) with different virulence in mice model (Pena et al., 2008). In fact, some of these isolates are extremely virulent being fatal for the mouse (BrI), similar to the reference strain RH (clonal type I). Clonal type II genotypes predominate in Chile different to the patterns observed in South America (Rajendran et al., 2012). In Argentina, non-clonal allele combinations of type II and type III genotypes (using 9 markers) have been described in isolates obtained from zoo animals like meerkats, kangaroos, wallabies and monkeys (Basso et al., 2009; Basso et al., 2007; Moré et al., 2010; Pardini et al., 2015) and also from domestic animals such as pigs, rabbits and chickens from different regions of the country (Bacigalupe et al., 2011; Moré et al., 2012; Pardini et al., 2011; Pardini et al., 2016). In southern Brazil a high rate of human ocular toxoplasmosis (five times higher than reported in Europe) has been reported and is suggested to be associated with atypical genotypes infection (Silveira et al., 2015). In the state of Minas Gerais it was found that genotypes isolated from humans overlap with those found in domestic animals, suggesting a common source of infection between them (Silva et al., 2014). In Argentina, in the central and east region of Misiones province (near Brazil), the rate of patients with ocular toxoplasmosis is around 20% of clinic attendees (Rudzinski and Meyer, 2011) and non-clonal isolates of *T. gondii* were obtained from chickens belonging to the farms of the

infected patients (Pardini et al., 2016). In addition, *T. gondii* isolates with non-clonal genotypes were obtained from congenital toxoplasmosis cases in the country (Pardini et al., in preparation).

At present, there is no predominant genotype in Central and South America and correlation between all the genotypes and virulence in humans, domestic and wild animals remains uncertain (as a challenge) (Pena et al., 2008; Weiss and Kim, 2014). Different genotypes, close related in phylogenetic studies like networks, could indicate similar virulent traits (Morrison, 2005; Pena et al., 2008).

The aim of this study was to analyze the *T. gondii* population structure in Argentina and compare it with genotyping information from other South American countries.

2. MATERIALS AND METHODS

To reveal *T. gondii* population structure in Argentina, a total of 39 samples were used in phylogenetic network analysis: 26 from previous studies published by our group, 10 retrieved by ToxoDB and published by other authors and 3 from oocysts in cat feces (present study). Eight reference genotypes, 4 common genotypes from Brazil, and additional 4 diverse or non-clonal genotypes were included in this analysis. All the samples used ($n = 55$) are described in table 1.

2.1 Argentinean *T. gondii* samples from previous studies

A total of 36 samples from Argentina obtained in previous studies were used. Five samples were isolated from zoo animals; Bennett's Wallaby (*Macropus rufogriseus*) (TgWb1Arg) (Basso et al., 2007), Meerkat (*Suricata suricatta*) (TgMk1Arg) (Basso et al., 2009), Kangaroos (*Macropus rufus* and *Macropus giganteus*) (TgKg1Arg / TgKg2Arg) (Moré et al., 2010), and Squirrel Monkey (*Saimiri boliviensis*) (TgMy1Arg) (Pardini et al., 2015). The zoo is located in La Plata, Buenos Aires province. All animals died of acute toxoplasmosis and were submitted to the LAINPA for confirmation of diagnosis. The meerkats came from Bester Birds & Animal Zoo Park, South Africa, the kangaroos from a natural reserve in USA, the Bennett's Wallaby from Australia and the squirrel

monkey from natural habitat (west coast of Central and South America). Chicken (*Gallus gallus domesticus*) samples were isolated from different locations; 3 (TgCkN21Arg, TgCkC25Arg and TgCkP22Arg) from Las Flores, Buenos Aires province (Moré et al., 2012) and 7 from Misiones province (Pardini et al., 2016). The goat sample (*Capra aegagrus hircus*) (TgGtArg) belongs to San Luis province (Unzaga et al., 2014), the pigs samples (*Sus scrofa*) (TgPig10Arg and TgPig15Arg) (Pardini et al., 2011), the rabbit sample (*Oryctolagus cuniculus*) (Rabbit 2Arg) (Bacigalupe et al., 2011) and the rat sample (*Ratus rattus*) (Rat1Arg) (Dellarupe et al., in preparation) are from Buenos Aires province. Human samples (TgHm12-1Arg, TgHm14-4Arg, TgHm15-02Arg, TgHm16-01Arg, TgHm16-02Arg and TgHm17-01Arg) were obtained in the German Hospital from Buenos Aires city from congenital toxoplasmosis cases (Pardini et al., in preparation). All the DNA were processed by nested PCR-RFLP including the markers SAG1 and 5'3'SAG2 (not performed in the original reports).

Additionally, 10 genotypes isolated from chickens were retrieved from ToxoDB (www.toxodb.org) when searching for Argentinean isolates and added to the analysis (TgCkAr1, 2, 6, 7, 16, 18, 24, 25, 27 and 28) (Rajendran et al., 2012). These isolates belong to Buenos Aires and Entre Ríos provinces, and 2 of them have an unknown origin location.

2.2 *T. gondii* oocysts from cat feces

Three fecal samples were obtained from domestic cats (*Felis silvestris catus*) from Buenos Aires province. The samples were examined at DIAP laboratory and oocysts compatible with *T. gondii* (10-12 µm diameter) were detected. The samples belong to different animals and were collected in 3 different years. Oocysts were concentrated by water sedimentation-sugar flotation-water sedimentation as previously described (Ortega-Mora et al., 2007). The DNA was extracted using the ZR fecal DNA kit (Zymo research) according manufacturer instructions. The samples were identified as *T. gondii* by TOX5-TOX8 PCR as previously described (Pardini et al., 2014). The 3 samples were genotyped for 10 genetic markers as described below.

Geographic distribution and number of the Argentinean *T. gondii* samples used in this study is represented in Figure 1.

2.3 *T. gondii* reference strains

Reference strains selected were: type I (RH/GT1), type II (ME49/PTG), type III (VEG/CTG), MAS, TgCgCa1, TgCatBr5, TgCatBr64 and TgToucan (TgRsCr1), also used in the genotyping process. Strains selected were BrI, BrII, BrIII and BrIV from Brazil (Pena et al., 2008) and 4 diverse or non-clonal genotypes: CASTELLS from Uruguay (Su et al., 2006), TgCatBr3 from Brazil (Pena et al., 2008), VAND from French Guiana and BOF from Belgium (Weiss and Kim, 2014).

2.4 nPCR-RFLP and phylogenetic network

Genotyping of all *T. gondii* samples was performed by nPCR-RFLP using 10 genetic markers: SAG1, SAG2 (5'3'SAG2, altSAG2), SAG3, BTUB, GRA6, C22-8, C29-2, L358, PK1, and Apico as described previously by Su et al., 2006. Markers SAG1 and 5'3'SAG2 were processed at Molecular Parasitology and Pathogenesis Laboratory in The University of Tennessee, Knoxville, USA. The rest of the markers were conducted in both LAINPA and Knoxville laboratory and consensus genotypes achieved were used for the phylogenetic network.

Phylogenetic network was inferred using the software SplitsTree4 (Huson and Bryant, 2006). Genotyping data were coded for each marker with a combination of 0s and 1s. The concatenated coded genotype for each sample was used to infer the network as previously described (Rajendran et al., 2012). Network analysis has been suggested as a useful tool for represent the *T. gondii* population structure (Morrison, 2005; Pena et al., 2008).

3. RESULTS

A total of 39 DNA samples from Argentina were genotyped and classified according to ToxoDB PCR-RFLP genotypes. Genotypes were grouped into 21 allele combinations, allele types for two samples were not determined at two loci and considered incomplete characterized (TgHm16-01Arg and TgCk14-5Arg). The 37 genotyped samples were identified and belong to 21 genotypes, including: ToxoDB #2 or clonal type III ($n = 8$), ToxoDB #48 ($n = 3$), and ToxoDB #7, #17, #14, #19 (each with $n = 2$). Most of these genotypes have a predominance of type III alleles. Ten genotypes were represented by a single sample (#1, #3, #8, #11, #15, #116, #123, #138, #163, #182). Six samples with different allele combinations (TgCk14-6Arg and TgCk14-7Arg, TgGtArg, TgHum12-1Arg, chicken 13-3Arg and TgHum16-02Arg) were not found in ToxoDB and identified as five new genotypes (#283, #284, #285, #286 and #287, respectively). Additionally, two samples (TgPig10Arg and TgKg2Arg) lack either SAG1 and/or 5'3'SAG2, and they could represent already identified or even new genotypes (Table 1). The phylogenetic network obtained is shown in Figure 2.

Network analysis clustered most of the samples in 3 groups, having each one of them a clonal type as reference (I, II, III) and named as groups 1, 2 and 3, respectively (Figure 2). Groups 1 and 3 concentrated most of the samples (12/55 and 25/55, respectively). The group 2 clustered 6 samples showing the highest genetic divergence (distance) with the other groups. A total of 12 samples were not grouped, however 6 samples (TgCk11-9Arg, chicken11-12 Arg, TgCatBr5, TgHm15-02Arg, TgHm16-01Arg, and TgGtArg) positioned near group 3, and 5 samples positioned near group 1 (VAND, TgCatBr64, TgCkAr1, type BrII and chicken13-3Arg). TgCkAr1 and type BrII positioned in the same node with the closest distance to group 1. One sample (TgCgCa1) positioned between groups 1 and 2. Samples from Brazil clustered in group 1 (type BrI and type BrIV) and group 3 (type BrIII).

Samples from Buenos Aires province were grouped with clonal type II (5/25) and the majority with clonal type III (17/25), sample TgCkAr1 was associated with type BrII, and samples TgHm15-

02Arg and TgHm16-01Arg were not grouped but positioned near to group 3. Isolates from Misiones province were grouped with clonal type I (2/7), with clonal type III (2/7), samples chicken11-2Arg and TgCk11-9Arg (identical genotype) were associated with Brazilian isolate TgCatBr5 and sample chicken13-3Arg was positioned close to group 1. Isolates belonging to Entre Ríos grouped with clonal type III (3/4) and clonal type I (1/4). The sample from San Luis positioned near to group 3. Samples TgCkAr27 and TgCkAr28 (undefined location) were grouped with clonal type I.

T. gondii samples from chickens in Argentina ($n = 20$), were isolated from 3 different provinces: Buenos Aires (7/20), Entre Ríos (4/20), Misiones (7/20) and 2 of the samples had an unknown location. Clonal type II (TgCkP22Arg) and type III (TgCkAr2, 6 and 24) were isolated from chickens of Buenos Aires and only 1 from Entre Ríos province. TgCkP22Arg was the only chicken sample grouped with clonal type II. The rest of the samples ($n = 16$) were non-clonal and distributed in groups 1 (5/20) and 3 (7/20); the samples TgCkAr1, chicken 11-12Arg, TgCk11-9Arg and chicken13-3Arg clustered as mentioned above.

Samples from humans ($n = 6$), all from Buenos Aires province, were identified as non-clonal genotypes and positioned in group 3 (3/6), 2 samples near group 3 and 1 sample in group 2.

T. gondii samples from zoo animals ($n = 5$) such as kangaroos, wallabies, meerkats and monkeys belonging to La Plata Zoo, Buenos Aires province, were positioned in the group 3 (4/5) and 1 sample in the group 2.

T. gondii was isolated from 3 cats from Buenos Aires province and all resulted clonal type III genotype. Samples from pigs in Buenos Aires (TgPig10Arg and TgPig15Arg) were located in group 2. The sample from a goat in San Luis province (TgGtArg) presented a non-clonal genotype, identified as new and positioned close to clonal type III. *T. gondii* isolated from a rabbit

(Rabbit2Arg) and a rat (Rat1Arg) both from Buenos Aires presented genotype #48 and were grouped with clonal type III.

4. DISCUSSION

In the present study the population structure of *T. gondii* in Argentina was analyzed, 39 samples from animals and humans were genotyped using nPCR-RFLP and classified according ToxoDB. Twenty-one different genotypes were found, being most of them represented by a single isolate, suggesting a highly diverse population like in other countries of South America (Pena et al., 2008; Rajendran et al., 2012; Shwab et al., 2014). Out all samples, 8 were identified as clonal type III (TgMk1Arg, TgKg1Arg, TgCat1Arg, TgCat2Arg, Cat14-1Arg, TgCkAr2, TgCkAr6 and TgCkAr24), 1 as clonal type II (TgCkP22Arg) and 1 as clonal type II variant (TgPig15Arg). Twenty-nine samples were identified as non-clonal. In this study 10 genetic markers were used, and several isolates previously assumed as “clonal” were now identified as atypical or non-clonal (Moré et al., 2010; Pardini et al., 2011; Pardini et al., 2014). Our studies highlight the importance of complete genotyping in order to obtain trustable and comparable results (Su et al., 2006). Moreover, using microsatellite pattern analysis and sequencing, could allow us to observe genetic differences among isolates sharing the same nPCR-RFLP pattern (Su et al., 2012). In addition, the high genetic variability of *T. gondii* in Argentina was also demonstrated by 5 non-clonal allele combinations that were not registered in ToxoDB, and therefore considered new genotypes. The complete 5 genotypes obtained in this publication have been added to ToxoDB (#283, #284, #285, #286, and #287).

Phylogenetic networks have been extensively used to identify population structure and genetic distance of several species, including *T. gondii* (Huson and Bryant, 2006; Morrison, 2005). Network analyses performed in the present study revealed that the majority of the samples were grouped with clonal type III lineage, consistent with other studies conducted in South America (Rajendran et al., 2012; Shwab et al., 2014). Clonal type III and related genotypes (with predominance of type III

allele): #7, #8, #14 and #48 were found in accordance to Pena et al., 2008 and Rajendran et al., 2012. In addition, genotypes #11, #15, #17, #19, #116, #123, #138, #163 and #182 are also present in Argentina. The 3 isolates from cats from Buenos Aires province obtained in the present study showed a clonal type III genotype as well as other samples from other animals obtained in the region. A second group of samples ($n = 5$) from Misiones and Entre Ríos provinces clustered with clonal type I and reference strains from Brazil: type BrI (considered highly virulent in mice) and type BrIV (considered to have an intermediate virulence phenotype in mice) (Pena et al., 2008). Also, sample TgCkAr1 and type BrII (intermediate virulent phenotype) clustered together. The exception was chicken13-3Arg that did not cluster with other samples, but is closely related in distance with group 1. In addition, samples TgPig10Arg, TgCk14-5Arg, TgKg2Arg, chicken13-3Arg, TgHm16-02Arg and TgHm16-01Arg could be positioned more accurately once all markers get analyzed. The association of samples in the network due to similar genotypes may also be extended to other traits (Morrison, 2005). The close association on the network suggests that the Argentinean samples could be intermediate to highly virulent in mice model. Future studies in mice bioassay are needed to confirm this hypothesis. Some samples from Argentina were not grouped, however, they showed the closest genetic distance with the type III ($n = 5$; TgCk11-9Arg, chicken11-12Arg, TgHm15-02Arg, TgHm16-01Arg, and TgGtArg) and type I ($n = 2$; TgCkAr1 and chicken13-3Arg). This close distance could be interpreted as phylogenetically related strains (Morrison, 2005).

On the other hand, the presence of a group including clonal type II and closely related genotypes confirms a different population structure of *T. gondii* in Argentina in comparison with other South American countries (Pena et al., 2008; Rajendran et al., 2012). Interestingly, only samples isolated in Buenos Aires province were grouped with clonal type II. This genotype is very rare to find in Central and South America, however, it was isolated in Chile (Pena et al., 2008; Rajendran et al., 2012). Chile's geographical characteristics may have allowed clonal genotypes arriving through the harbor to spread in the region, keeping the country excluded for exchange with the rest of South

America due to the Andes Mountains (Rajendran et al., 2012). All these samples from Argentina and Chile positioned with a highest phylogenetic distance in relation to the remaining Argentinean samples grouped with type III and I genotypes. It is possible to suggest that the genotypes clonal type II and type II variant found in Argentina may have probably arrived through Buenos Aires harbor, and remained in a restricted region. These results reinforce the hypothesis of the "importation" of the type II strains in the areas closest to international ports due to animal trading (Rajendran et al., 2012), but also could be due to infected mice and cats traveling among continents as well as migratory birds (Chaichan et al., 2017; Lehmann et al., 2006). Nevertheless, remains unclear why in Brazil these "importation" has not been detected, in spite of having an extensive coast, with several harbors used for animal trading.

Interestingly, we see an association of genotypes with the geographical location in Argentina, in contrast with previous suggestions (Rajendran et al., 2012). A differential pattern of genotypes is present from the central-east region of Argentina (Buenos Aires province) to the north-east region of the country (Misiones province) in contact with Southern Brazil. Clonal type II and type II variant are only present in Buenos Aires, where was also observed the highest frequency of clonal type III and related genotypes. Moving at north towards Entre Ríos and Misiones provinces, genotypes are still closely related to type III and potentially new genotypes arise. As mentioned before, several samples from Entre Ríos and Misiones provinces are phylogenetically related with type I isolates, being an important difference in relation with samples from Buenos Aires.

The high genetic variability detected makes difficult to establish an association between the host species and *T. gondii* genotype. Most of the samples were obtained from chickens and these could be a bias on selecting genotypes (Ajzenberg et al., 2004). Chickens are resistant to clinical toxoplasmosis, rarely have clinical symptoms and are very good indicators of the *T. gondii* genotype present in the environment, that's why they have been used as sentinel animals in previous studies (Moré et al., 2012). Our findings strongly support that chicken samples from Argentina

indicate the genotype present in the environment from different regions. *T. gondii* isolated from a human in Argentina overlaps with an isolate from a wallaby from Buenos Aires (ToxoDB #14). This overlapping between animal and human genotypes has been proposed as frequent in defined regions of Brazil, and helpful to identify potential infection sources (Silva et al., 2014). Besides, related genotypes (grouped in the phylogenetic network) have been detected in domestic animals (pigs, goats, chickens and rabbits) as well as in humans from Argentina.

As seen in the network, *T. gondii* samples from Misiones clustered with reference strains from Brazil considered intermediate to highly virulent. This is in association with the high rate of ocular toxoplasmosis in the province of Misiones (Rudzinski and Meyer, 2011), probably caused by some of these non-clonal genotypes. Unfortunately, there are no human isolates of *T. gondii* from patients in Misiones to confirm such hypothesis. Nevertheless, in Southern Brazil (close to Misiones) has been demonstrated a high rate of ocular toxoplasmosis associated with non-clonal genotypes; however, genotypes comparison is not feasible since only few markers were characterized (Silveira et al., 2015). All this information should be taken into account to implement toxoplasmosis control plans, which may be adapted to regions and predominant genotypes.

Previous studies showed both clonal and non-clonal genotypes of *T. gondii* can infect zoo animals and be fatal for susceptible species, even though clonal types II and III are not virulent in mice (Dubey, 2010). It is likely that most animals from La Plata Zoo, Argentina, got infected with local strains, which were positioned with other samples from Buenos Aires province in the network (Basso et al., 2007; Basso et al., 2009; Moré et al., 2010; Pardini et al., 2015).

Hypothesis made about the South American origin of the parasite and subsequent founding of other regions with the development of clonal populations are still being tested (Lehmann et al., 2006; Shwab et al., 2014; Bertranpetit et al., 2018). Clonal type III might have arisen in South America, together with clonal type I, our data supports this hypothesis being most of Argentinean isolates grouped with III and I genotypes. As suggested before, we considered the detected clonal type II

and its variants in Argentina as “imported” from abroad. This point deserves further genetic analyses, probably using microsatellite patterns to identify genetic distance among different type II allele combinations from different countries (Verma et al., 2015).

The pattern of *T. gondii* genotypes in Argentina could be considered as highly variable as in other South American countries but with the existence of clonality, especially in Buenos Aires province where type II is present. Nevertheless, different regions showed distinctive pattern of genotypes, showing a higher variability in Northern provinces. Probably the sampling may not be representative of all regions of the country, making important to obtain new isolates and genotyping from different regions. The “regionalization” of certain genotypes could be a point to discuss on reference to potential regionalization of control programs.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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Figure captions:

Figure 1: Distribution and quantity of *T. gondii* samples within Argentinean provinces are identified in a map of Argentina. Two samples with undefined location are not represented (total represented $n=37$).

Figure 2: Phylogenetic network analysis of *T. gondii* samples from Argentina and reference strains (software SplitsTree4). The samples clustered in 3 groups and were named as groups 1, 2 and 3. Group 1 (red circle; including clonal type I) and group 3 (blue circle; including clonal type III) concentrated most of the samples. Group 2 (green circle; including clonal type II) showed the highest genetic divergence with the other groups.

Table 1: Detailed description of samples from Argentina and reference strains used in this study.

Host name	Strain ID	ToxoDB ID PCR-RFLP genotype	SAG1	5'-3'SAG2	alt.SAG2	SAG3	BTUB	GRA6	C22-8	C29-2	L358	PK1	Apico	Location	Reference
Human (<i>Homo sapiens</i>)	RH ¹	#10	I	I	I	I	I	I	I	I	I	I	I	USA	Khan et al., 2007
Sheep (<i>Ovis aries</i>)	ME49 ²	#1	II or III	II	II	II	II	II	II	II	II	II	I	USA-CA	Khan et al., 2007
Human (<i>Homo sapiens</i>)	VEG ³	#2	II or III	II I	II I	II I	II I	II I	II I	II I	II I	II I	I I	USA-CA	Khan et al., 2007
Human (<i>Homo sapiens</i>)	MAS	#17	u-1	I	II	II I	II I	II I	u-1	I	I	II I	I	France	Khan et al., 2007
Cougar (<i>Puma concolor cougar</i>)	TgCg Cal	#66	I	II	II	II I	II	II	II	u-1	I	u-2	I	Canada	Khan et al., 2007
Cat (<i>Felis silvestris catus</i>)	TgCat Br5	#19	I	II I	II I	II I	II I	II I	I	I	I	u-1	I	Brazil	Khan et al., 2007
Cat (<i>Felis silvestris catus</i>)	TgCat Br64	#111	I	I	u-1	II I	II I	II I	u-1	I	II I	II I	I	Brazil	Pena et al., 2008
Toucan (<i>Ramphastida e</i>)	TgToucan (TgRs Cr1)	#52	u-1	I	II	II I	I	II I	u-2	I	I	II I	I	Costa Rica	Rajendran et al., 2012
Sheep (<i>Ovis aries</i>)	CAST ELLS	#15	u-1	I	II	II I	II I	II I	II I	I	I	II I	I	Uruguay	Su et al., 2006
Human (<i>Homo sapiens</i>)	VAND	#60	I	I	II	I	II I	II I	II I	I	II I	II I	I	French Guiana	Weiss and Kim, 2014
Cat (<i>Felis silvestris catus</i>)	TgCat Br3	#8	I	II I	II I	II I	II I	II I	II I	II I	II I	II I	I I	Brazil	Pena et al., 2008
Human (<i>Homo sapiens</i>)	BOF	#6	I	I	I	II I	I	II	u-1	I	I	I	I	Belgium	Weiss and Kim, 2014

Several species	type BrI	#6	I	I	I	II	I	II	u-I	I	I	I	I	Brazil	Pena et al., 2008
Several species	type BrII	#11	I	I	II	II	II	II	I	II	I	II	I	Brazil	Pena et al., 2008
Several species	type BrIII	#8	I	II	II	II	II	II	II	II	II	II	I	Brazil	Pena et al., 2008
Several species	type BrIV	#17	u-I	I	II	II	II	II	u-I	I	I	II	I	Brazil	Pena et al., 2008
Chicken (<i>Gallus gallus domesticus</i>)	TgCkP 22Arg	#1	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires Province (Las Flores)	Moré et al., 2012
Pig (<i>Sus scrofa</i>)	TgPig 10Arg	#1 likely	II or III	n d	II	II	II	II	II	II	II	II	I	Buenos Aires Province	Pardini et al., 2011
Pig (<i>Sus scrofa</i>)	TgPig 15Arg	#3	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires Province	Pardini et al., 2011
Meerkat (<i>Suricata suricatta</i>)	TgMk 1Arg	#2	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires Province (LaPlata Zoo)	Basso et al., 2009
Kangaroo (<i>Macropus rufus</i>)	TgKgl Arg	#2	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires Province (LaPlata Zoo)	Moré et al., 2010
Cat (<i>Felis silvestris catus</i>)	TgCat 1Arg	#2	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires Province	Present study
Cat (<i>Felis silvestris catus</i>)	TgCat 2Arg	#2	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires Province	Present study
Cat (<i>Felis silvestris catus</i>)	Cat 14-1Arg	#2	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires Province	Present study
Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar2	#2	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires province (surroundings city of La Plata)	Dubey et al., 2003
Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar24	#2	II or III	II	II	II	II	II	II	II	II	II	I	Entre Ríos Province (Ceibas)	Dubey et al., 2005
Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar6	#2	II or III	II	II	II	II	II	II	II	II	II	I	Buenos Aires province (surroundings city of La Plata)	Dubey et al., 2003

Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar16	#7	I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	Entre Ríos Province (Gualedguaychu)	Dubey et al., 2005
Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar18	#7	I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	Entre Ríos Province (Gualedguaychu)	Dubey et al., 2005
Chicken (<i>Gallus gallus domesticus</i>)	TgCk N21Arg	#8	I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	Buenos Aires Province (Las Flores)	Moré et al., 2012
Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar1	#11	I	I	II I	II I	II I	II I	I	II I	I	II I	I	I	Buenos Aires province (surroundings city of La Plata)	Dubey et al., 2003
Bennett's Wallaby (<i>Macropus rufogriseus</i>)	TgWb 1Arg	#14	I	II I	II I	II I	II I	II I	II I	I	II I	II I	II I	II I	Buenos Aires Province (LaPlata Zoo)	Basso et al., 2007
Human (<i>Homo sapiens</i>)	TgHm 17-01Arg	#14	I	II I	II I	II I	II I	II I	II I	I	II I	II I	II I	II I	Buenos Aires Province	Pardini et al., in preparation
Chicken (<i>Gallus gallus domesticus</i>)	TgCk1 4-5Arg	incomplete	nd	n d	II I	II I	II I	II I	II I	II I	I	II I	II I	II I	Misiones Province	Pardini, et al. 2016
Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar25	#15	u- I	I	II I	II I	II I	II I	II I	I	I	II I	I	I	Entre Ríos Province (Ceibas)	Dubey et al., 2005
Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar27	#17	u- I	I	II I	II I	II I	II I	II I	u- I	I	I	II I	I	Argentina (unknown province)	Rajendra n et al., 2012
Chicken (<i>Gallus gallus domesticus</i>)	TgCk Ar28	#17	u- I	I	II I	II I	II I	II I	II I	u- I	I	I	II I	I	Argentina (unknown province)	Rajendra n et al., 2012
Chicken (<i>Gallus gallus domesticus</i>)	TgCk1 1-9Arg	#19	I	II I	II I	II I	II I	II I	II I	I	I	I	u- I	I	Misiones Province	Pardini et al., 2016
Chicken (<i>Gallus gallus domesticus</i>)	chicken 11-12Arg	#19	I	II I	II I	II I	II I	II I	II I	I	I	I	u- I	I	Misiones Province	Pardini et al., 2016
Rabbit (<i>Oryctolagus cuniculus</i>)	Rabbit 2Arg	#48	I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	Buenos Aires Province	Bacigalup e et al., 2011
Rat (<i>Rattus rattus</i>)	Rat1A rg (Reti4)	#48	I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	II I	Buenos Aires Province	Dellarupe et al., in preparation

Chicken (<i>Gallus gallus domesticus</i>)	TgCkAr7	#48	I	II	II	II	II	II	II	II	II	II	I	Buenos Aires province (surroundings city of La Plata)	Dubey et al., 2003
Chicken (<i>Gallus gallus domesticus</i>)	TgCk13-5Arg	#116	I	II	II	II	I	II	II	II	II	II	I	Misiones Province	Pardini et al., 2016
Chicken (<i>Gallus gallus domesticus</i>)	TgCkC25Arg	#123	I	II	II	II	II	II	I	II	II	II	I	Buenos Aires Province (Las Flores)	More et al., 2012
Human (<i>Homo sapiens</i>)	TgHm14-4Arg	#138	u-1	II	II	II	II	II	II	I	II	II	I	Buenos Aires Province	Pardini et al., in preparation
Squirrel Monkey (<i>Saimiri boliviensis</i>)	TgMy1Arg	#163	I	II	II	II	II	II	II	I	II	II	I	Buenos Aires Province (LaPlata Zoo)	Pardini et al., 2015
Human (<i>Homo sapiens</i>)	TgHm15-02Arg	#182	I	II	II	II	II	II	II	I	I	I	I	Buenos Aires Province	Pardini et al., in preparation
Chicken (<i>Gallus gallus domesticus</i>)	TgCk14-6Arg	#283, new	u-1	I	II	II	II	II	I	I	II	I	I	Misiones Province	Pardini et al., 2016
Chicken (<i>Gallus gallus domesticus</i>)	TgCk14-7Arg	#283, new	u-1	I	II	II	II	II	I	I	II	I	I	Misiones Province	Pardini et al., 2016
Goat (<i>Capra aegagrus hircus</i>)	TgGtArg	#284, new	I	II	II	II	II	II	II	II	I	II	I	San Luis Province	Unzaga et al., 2014
Human (<i>Homo sapiens</i>)	TgHm12-1Arg	#285, new	u-1	I	II	II	II	II	II	II	II	II	I	Buenos Aires Province	Pardini et al., 2014
Kangaroo (<i>Macropus giganteus</i>)	TgKg2Arg	#285 likely	nd	I	II	II	II	II	II	II	II	II	I	Buenos Aires Province (LaPlata Zoo)	More et al., 2010
Chicken (<i>Gallus gallus domesticus</i>)	chicken 13-3Arg	#286, new	u-1	nd	II	II	II	II	I	I	II	II	I	Misiones Province	Pardini et al. 2016
Human (<i>Homo sapiens</i>)	TgHm16-02Arg	#287, new	u-1	nd	II	II	II	II	II	II	II	II	I	Buenos Aires Province	Pardini et al., in preparation
Human (<i>Homo</i>)	TgHm16-	incomplete	nd	nd	II	II	II	II	II	II	I	II	I	Buenos Aires Province	Pardini et al., in preparation

<i>sapiens</i>)	01Arg													I			n

Reference table 1:

Identical allele combination to reference strain ¹GT1, ²PTG and ³CTG. Strain ID for the Argentinean samples is referred as “Tg” or a host name to identify isolates or only DNA sample, respectively.

ACCEPTED MANUSCRIPT

HIGHLIGHTS

- Thirty-nine *T. gondii* samples from Argentina were genotyped using nPCR-RFLP.
- Network analysis revealed 3 groups with clonal types I, II and III as reference.
- Twenty-one different genotypes were identified including 5 new genotypes.
- A combination of unique genotypes and type II and III lineages was observed.
- A unique population structure of *T. gondii* was identified in Argentina.

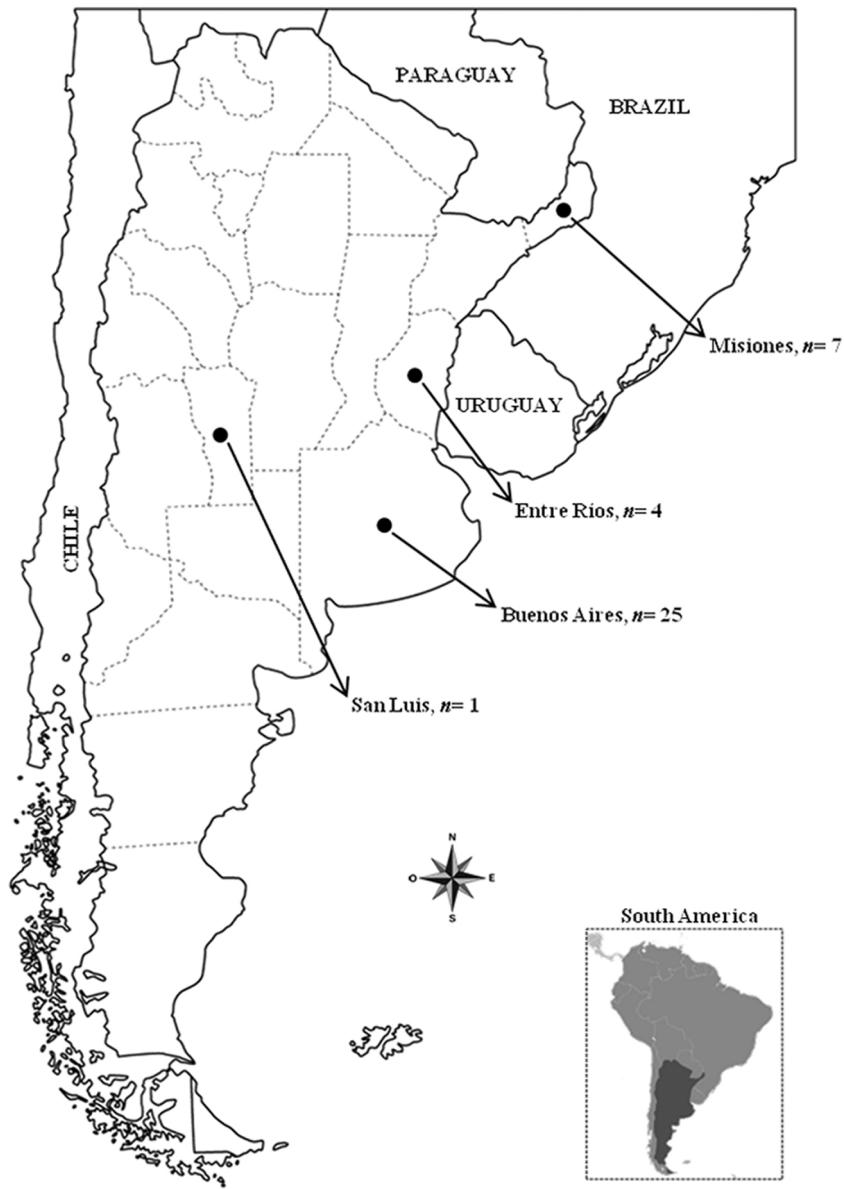


Figure 1

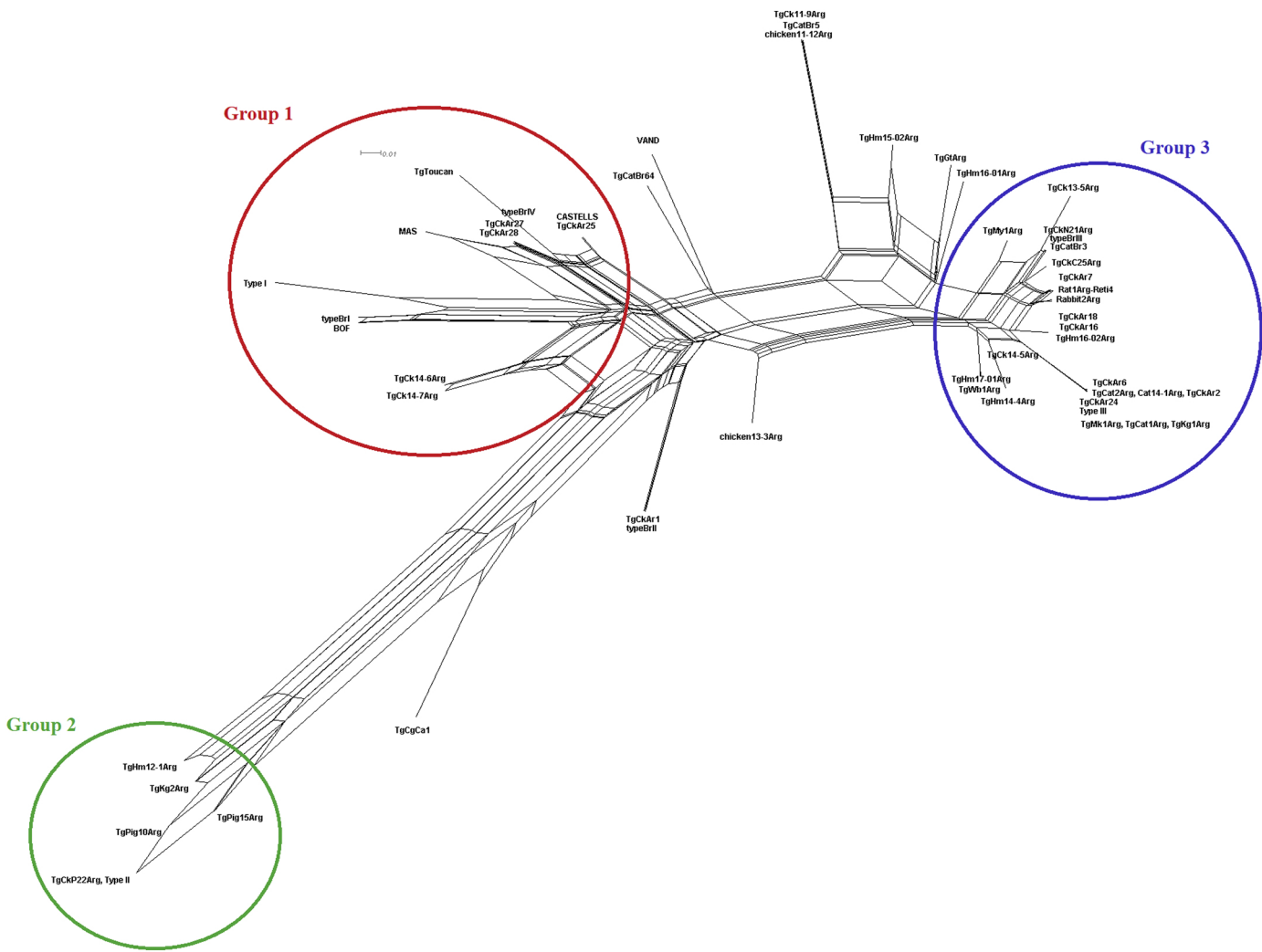


Figure 2