HHS Public Access

Author manuscript

Vet Parasitol. Author manuscript; available in PMC 2015 May 29.

Published in final edited form as:

Vet Parasitol. 2008 November 7; 157(0): 299–305. doi:10.1016/j.vetpar.2008.07.036.

Genetic diversity of *Toxoplasma gondii* isolates from chickens from Brazil

J.P. Dubey[a,*], **G.V. Velmurugan**[a], **A. Chockalingam**[b], **H.F.J. Pena**[c], **L. Nunes de Oliveira**[c], **C.A. Leifer**[b], **S.M. Gennari**[c], **L.M.G. Bahia Oliveira**[d], and **C. Su**[e]

[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]Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, VMC C5-153, Ithaca, NY 14853, USA

[c]Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brazil

[d]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Biociências e Biotecnologias, Laboratório de Biologia do Reconhecer, Av. Alberto Lamego 2000, Horto, Campos dos Goytacazes, RJ Brazil 28013-600, Brazil

[e]Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845, USA

Abstract

Until recently, *Toxoplasma gondii* was considered clonal with very little genetic variability. Recent studies indicate that *T. gondii* isolates from Brazil are genetically and biologically different from *T. gondii* isolates from USA and Europe. In the present study, we retyped 151 free range chicken isolates from Brazil including 117 newly isolated samples from 11 geographically areas (Alagoas, Bahia, Ceará, Maranhão, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Norte, São Paulo, Sergipe, and Rondonia) and 34 previously reported isolates from the very north (Pará) and the very south (Rio Grande do Sul). Ten PCR-RFLP markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico were used to genotype all isolates. Overall analysis of 151 *T. gondii* isolates revealed 58 genotypes. Half (29/58) of these genotypes had single isolate and the other half of the genotypes were characterized with two or more isolates. Only 1 of 151 isolates was clonal Type I strain and 5 were clonal Type III strains. Two isolates had mixed infections. Clonal Type II strain was absent. One strain was Type II at all loci, except BTUB. The results confirm high genetic diversity of *T. gondii* isolates from Brazil.

Keywords

Toxoplasma gondii; Chickens; Genotype; PCR-RFLP; Brazil

[*]Corresponding author. Tel.: +1 301 504 8128; fax: +1 301 504 9222. jitender.dubey@ars.usda.gov (J.P. Dubey).

1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and other animals worldwide (Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans or other animals develop clinical signs of disease. Whether the severity of toxoplasmosis in immunocompetent hosts is due to the parasite strain, host variability, or to other factors is largely unknown. Recently, attention has been focused on the genetic variability among *T. gondii* isolates from apparently healthy and sick hosts. In humans in French Guiana and Suriname, severe cases of toxoplasmosis in immuno-competent patients have been related to *T. gondii* strains with unusual genetic characteristics (Ajzenberg et al., 2004; Demar et al., 2007). An unusually high prevalence of clinical ocular toxoplasmosis in Erechim, Brazil is thought to be related to characteristics of prevailing *T. gondii* isolates (Glasner et al., 1992; Khan et al., 2006, 2007).

Most *T. gondii* isolates from human and animal sources have been grouped into one of three clonal lineages (Type I, II and III) by multilocus enzyme electrophoresis, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and microsatellite typing (Dardé et al., 1992; Howe and Sibley, 1995; Ajzenberg et al., 2002a,b). We have started a worldwide evaluation of genetic diversity of *T. gondii* isolates based on DNA derived from live parasites. Our attention has been focused on South America, especially Brazil, because of the collaborative success in obtaining tissues from animals for the isolation of *T. gondii*. Initial results indicate that the isolates of *T. gondii* from Brazil are biologically and genetically different from those in North America and Europe (Dubey et al., 2002; Lehmann et al., 2006; Dubey et al., 2007a,b,c). *T. gondii* isolates from asymptomatic chickens from Brazil were more pathogenic to mice than isolates from Europe or North America, irrespective of the genotype (Dubey et al., 2006). Additionally, most isolates from chickens from Brazil were not clonal, and Type II lineage was absent (Dubey et al., 2007a,c).

Our initial studies were based on one marker (SAG2) (Dubey et al., 2002) and six microsatellites (Lehmann et al., 2006). In the present paper we genotyped 94 *T. gondii* isolates (previously analyzed with only SAG2) using 10 PCR-RFLP markers to achieve a high resolution in identification and evaluated the entire PCR-RFLP data set on chickens from Brazil (Table 1). Results indicate a very high genetic diversity among isolates of *T. gondii*, irrespective of the geographic location.

2. Materials and methods

For the present study, in total 151 *T. gondii* isolates from 11 different regions of Brazil (Fig. 1) were genotyped (Table 1). Of these SAG2 data on 94 isolates were previously reported (Dubey et al., 2002, 2003a,b, 2006). For the present study, these cryopreserved 94 *T. gondii* isolates were revived in mice at the Animal Parasitic Diseases Laboratory, Beltsville, MD (Dubey et al., 2002). Viable *T. gondii* parasites were collected from lung tissue of mice that died, and from the brains of mice that survived for more than 30 days after inoculation with cryopreserved material were processed for the present study, and were cryopreserved in

liquid nitrogen with DMSO (Dubey and Beattie, 1988) for future studies. Additionally, 23 isolates were recently obtained at the Universidade de São Paulo, São Paulo, SP, Brazil (de Oliveira et al., in press); these isolates were genetically characterized for the first time. Genetic data on the remaining 34 isolates had been published previously (Dubey et al., 2007a) and incorporated in the present study. Details of *T. gondii* isolation in mice have been published in detail in the papers listed in Table 1 and not repeated here.

T. gondii DNA was extracted from tissues of positive mice using DNeasy kit (Qiagen) and genotyped using the genetic markers SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico (Dubey et al., 2006; Su et al., 2006). Data on 34 *T. gondii* isolates recently published (Dubey et al., 2007a) from chickens from Pará and Rio Grande do Sul (Table 1) were combined with data on newly genotyped 117 isolates and analyzed by SplitsTree4 (Huson, 1998; Huson and Bryant, 2006) in the present study.

3. Results

Multilocus PCR-RFLP genotyping of a total of 151 *T. gondii* isolates from free range chickens in 13 geographically areas including the states of Alagoas, Bahia, Ceará, Maranhão, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Norte, São Paulo, Sergipe, Rondönia, Pará and Rio Grande do Sul identified 58 genotype groups (Genotype #1–58) and two isolates with mixed infection (Table 2). Twenty-nine of the 58 multilocus genotypes identified are characterized with two or more isolates, while the other 29 genotypes have single isolate each. Only 1 (TgCkBr 146, now lost) of 151 isolates was clonal Type I strain, and 5 were clonal Type III strains. No clonal Type II lineage was found. One strain (TgCkBr 168) was Type II at all loci, except BTUB. The four major *T. gondii* lineages (Type BrI, BrII, BrIII and BrIV) previously identified from different animal hosts in Brazil (Pena et al., 2008) were also identified from chickens from much wider geographical regions in this study. In addition, the genotype group #21 was also identified in several states in Brazil (Table 2). Phylogenetic relationships of these 149 chicken isolates (exclude the two with mixed infection) were analyzed by SplitsTree4 (Huson, 1998; Huson and Bryant, 2006). The result is presented as reticulated network in Fig. 2. This results show that there is no clear clustering of genotypes with different geographical regions, and there is lack of type II alleles in the parasite population in Brazil. In addition, most genotypes are found only in one of the 13 states analyzed in this study, with exception of the lineages Type III, BrI, BrII, BrIII, BrIVand Genotype groups #21 (with 10 isolates from six different states in Brazil (Table 1, Table 2 and Fig. 2).

4. Discussion

Multilocus genotyping data on these 151 chicken isolates (including two mixed infections) showed that half (29/58) of the identified genotype groups have a single isolate, indicating high diversity of *T. gondii* isolates in Brazil. This is in supporting of recent findings from cat and dog isolates in Brazil (Pena et al., 2008). The current study of chicken isolates confirms the previous finding in that the Type I strain is rare and there is lack of the clonal Type II strain in Brazil, which is in sharp contrast in that these two lineages are highly prevalent in North America and Europe (Dardé et al., 1992; Howe and Sibley, 1995; Ajzenberg et al.,

2002a,b). Identification of the Type III, BrI, BrII, BrIII, BrIV and the genotype group #21 strains from different states of Brazil do suggest that these lineages are wide spread in different regions. The high proportion of genotypes with single isolate indicates high diversity of parasite strains in Brazil. This would suggest there could be many more unique genotypes circulating in the environment. To better understand molecular epidemiology and population structure of *T. gondii* in Brazil, a much deeper sampling of a variety of animal hosts is necessary.

References

Ajzenberg D, Bañuls AL, Tibayrenc M, Dardé ML. Microsatellite analysis of *Toxoplasma gondii* shows considerable polymorphism structured into two main clonal groups. Int J Parasitol. 2002a; 32:27–38. [PubMed: 11796120]

Ajzenberg D, Cogné N, Paris L, Bessières MH, Thulliez P, Filisetti D, Pelloux H, Marty P, Dardé ML. Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. J Infect Dis. 2002b; 186:684–689. [PubMed: 12195356]

Ajzenberg D, Bañuls AL, Su C, Dumètre A, Demar M, Carme B, Dardé ML. Genetic diversity, clonality and sexuality in *Toxoplasma gondii*. Int J Parasitol. 2004; 34:1185–1196. [PubMed: 15380690]

Dardé ML, Bouteille B, Perstreal M. Isoenzyme analysis of 35 *Toxoplasma gondii* isolates and the biological and epidemiologic implications. J Parasitol. 1992; 78:909–912. [PubMed: 1403437]

Demar M, Ajzenberg D, Maubon D, Djossou F, Panchoe D, Punwasi W, Valery N, Peneau C, Daigre JL, Aznar C, Cottrelle B, Terzan L, Dardé ML, Carme B. Fatal outbreak of human toxoplasmosis along the Maroni River: epidemiological, clinical, and parasitological aspects. Clin Infect Dis. 2007; 45:e88–e95. [PubMed: 17806043]

de Oliveira LN, Costa LM, de Melo CB, Silva JCR, Bevilaqua CML, Azevedo SS, Muradian V, Araújo DAFV, Dubey JP, Gennari SM. *Toxoplasma gondii* isolates from free-range chickens from the northeast region of Brazil. J Parasitol. in press.

Dubey, JP.; Beattie, CP. Toxoplasmosis of animals and man. CRC Press; Boca Raton, FL: 1988. p. 1-220.

Dubey JP, Graham DH, Blackston CR, Lehmann T, Gennari SM, Ragozo AMA, Nishi SM, Shen SK, Kwok OCH, Hill DE, Thulliez P. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo Brazil: unexpected findings. Int J Parasitol. 2002; 32:99–105. [PubMed: 11796127]

Dubey JP, Graham DH, Silva DS, Lehmann T, Bahia-Oliveira LMG. *Toxoplasma gondii* isolates of free-ranging chickens from Rio de Janeiro, Brazil: mouse mortality, genotype, and oocyst shedding by cats. J Parasitol. 2003a; 89:851–853. [PubMed: 14533703]

Dubey JP, Navarro IT, Graham DH, Dahl E, Freire RL, Prudencio LB, Sreekumar C, Vianna MC, Lehmann T. Characterization of *Toxoplasma gondii* isolates from free range chickens from Paraná. Brazil Vet Parasitol. 2003b; 117:229–234.

Dubey JP, Gennari SM, Labruna MB, Camargo LMA, Vianna MCB, Marcet PL, Lehmann T. Characterization of *Toxoplasma gondii* isolates in free-range chickens from Amazon. Brazil J Parasitol. 2006; 92:36–40.

Dubey JP, Sundar N, Gennari SM, Minervino AHH, Farias NAR, Ruas JL, dos Santos TRB, Cavalcante GT, Kwok OCH, Su C. 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. Vet Parasitol. 2007a; 143:182–188. [PubMed: 16982151]

Dubey JP, Applewhaite L, Sundar N, Velmurugan GV, Bandini LA, Kwok OCH, Hill R, Su C. Molecular and biological characterization of *Toxoplasma gondii* isolates from free-range chickens from Guyana. South America identified several unique and common parasite genotypes. Parasitology. 2007b; 134:1559–1566. [PubMed: 17572931]

Dubey JP, Gennari SM, Sundar N, Vianna MCB, Bandini LM, Yai LEO, Kwok OCH, Su C. Diverse and atypical genotypes identified in *Toxoplasma gondii* from dogs in São Paulo. Brazil J Parasitol. 2007c; 93:60–64.

Glasner PD, Silveira C, Kruszon-Moran D, Martins MC, Burnier M, Silveira S, Camargo ME, Nussenblatt RB, Kaslow RA, Belfort R. An unusually high prevalence of ocular toxoplasmosis in southern Brazil. Am J Ophthalmol. 1992; 114:136–144. [PubMed: 1642287]

Howe DK, Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. J Infect Dis. 1995; 172:1561–1566. [PubMed: 7594717]

Howe DK, Honoré S, Derouin F, Sibley LD. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. J Clin Microbiol. 1997; 35:1411–1414. [PubMed: 9163454]

Huson DH. SplitsTree: a program for analyzing and visualizing evolutionary data. Bioinformatics. 1998; 14:68–73. [PubMed: 9520503]

Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. Mol Biol Evol. 2006; 23:254–267. [PubMed: 16221896]

Khan A, Jordan C, Muccioli C, Vallochi AL, Rizzo LV, Belfort R Jr, Vitor RWA, Silveira C, Sibley LD. Genetic divergence of *Toxoplasma gondii* strains associated with ocular toxoplasmosis. Brazil Emerg Infect Dis. 2006; 12:942–949.

Khan A, Fux B, Su C, Dubey JP, Darde ML, Ajioka JW, Rosenthal BM, Sibley LD. Recent transcontinental sweep of *Toxoplasma gondii* driven by a single monomorphic chromosome. Proc Natl Acad Sci (Washington, DC). 2007; 104:14872–14877.

Lehmann T, Marcet PL, Graham DH, Dahl ER, Dubey JP. Globalization and the population structure of *Toxoplasma gondii*. Proc Natl Acad Sci. 2006; 103:11423–11428. [PubMed: 16849431]

Pena HFG, Gennari SM, Dubey JP, Su C. Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. Int J Parasitol. 2008; 38:561–569. [PubMed: 17963770]

Su C, Zhang X, Dubey JP. Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. Int J Parasitol. 2006; 36:841–848. [PubMed: 16643922]

Fig. 1.
Map of Brazil with sources of chickens sampled.

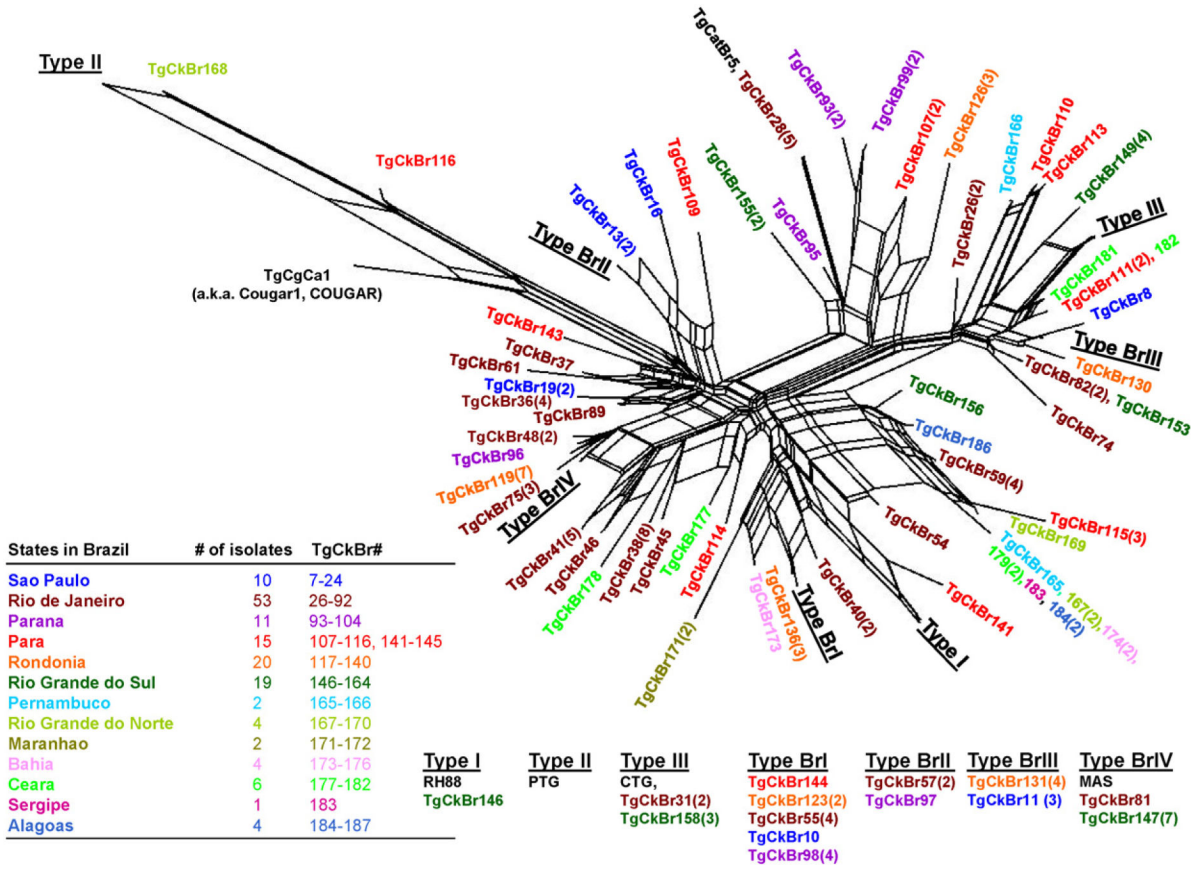

Fig. 2.

NeighborNet phylogenetic network of *T. gondii* isolates in chickens from Brazil. Isolates from different states of Brazil are color-coded for clarity. RH88, PTG, CTG, TgCgCa1 (a.k.a. Cougar1, COUGAR), MAS and TgCatBr5 are used as reference strains. Numeric number in parenthesis following each isolate's identification number is the number of strains with the identical genotype from the same states.

Table 1

Genetic diversity of *T. gondii* isolates from different regions of Brazil

***T. gondii* isolate**	**No. of isolates**	**States in Brazil**	**Reference for isolates**	**Genotype**
TgCkBr 117, 119, 120, 122, 123, 124, 126, 127, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140	20	Rond?nia	Dubey et al. (2006)	7 genotypes – this study
TgCkBr 7, 8, 10, 11, 13, 16, 17, 19, 23, 24	10	São Paulo	Dubey et al. (2002, 2006)	6 genotypes – this study
TgCkBr 26, 27, 28, 30, 31, 32, 33, 34, 36, 37, 38, 40, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 69, 74, 75, 76, 78, 79, 80, 81, 82, 84, 85, 86, 87, 88, 89, 90, 92.	53	Rio de Janeiro	Dubey et al. (2003a,b, 2006)	29 genotypes – this study
TgCkBr 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 104	11	Paraná	Dubey et al. (2003a,b, 2006)	7 genotypes – this study
TgCkBr 107–116, 141–145	15	Pará	Dubey et al. (2007a)	11 genotypes – Dubey et al. (2007a)
TgCkBr 146–164	19	Rio Grande do Sul	Dubey et al. (2007a)	9 genotypes – Dubey et al. (2007a)
TgCkBr 165, 166	2	Pernambuco	de Oliveira et al. (in press)	2 genotypes – this study
TgCkBr 167–170	4	Rio Grande do Norte	de Oliveira et al. (in press)	3 genotypes – this study
TgCkBr 171, 172	2	Maranhão	de Oliveira et al. (in press)	1 genotype – this study
TgCkBr 173–176	4	Bahia	de Oliveira et al. (in press)	3 genotypes – this study
TgCkBr 177–182	6	Ceará	de Oliveira et al. (in press)	5 genotypes – this study
TgCkBr 183	1	Sergipe	de Oliveira et al. (in press)	1 genotype – this study
TgCkBr 184–187	4	Alagoas	de Oliveira et al. (in press)	3 genotypes – this study

Table 2

Summary of *T. gondii* PCR-RFLP genotypes from chickens from Brazil

Genotypes (clonal types)	*T. gondii* isolate	Genetic markers										
		SAG1*	(5′ + 3′) SAG2[a]	SAG2[b]	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
Reference (I)	RH88	I	I	I	I	I	I	I	I	I	I	I
Reference (II)	PTG	II or III	II	II	II	II	II	II	II	II	II	II
Reference (III)	CTG	II or III	III	III	III	III	III	III	III	III	III	III
Reference	COUGAR	I	II	II	III	II	II	II	u-1	I	u-2	I
Reference	MAS	u-1	I	II	III	III	III	u-1	I	I	III	I
Reference	TgCatBr5	I	III	III	III	III	III	I	I	I	u-1	I
#1	7 (TgCkBr 119, 120, 122, 129, 135, 137, 140)	u-1	I	II	III	III	III	III	I	I	III	I
#2	2 (TgCkBr 48, 88)	u-1	I	II	III	III	III	II	I	I	III	III
#3	1 (TgCkBr 96)	u-1	I	II	III	III	III	II	I	I	III	I
#4	3 (TgCkBr 75, 76, 92)	u-1	I	II	III	III	III	III	III	I	III	I
#5 (BrIV)	8 (TgCkBr 81, 147, 148, 151, 154, 160, 162, 163)	u-1	I	II	III	III	III	u-1	I	I	III	I
#6	5 (TgCkBr 41, 42, 49, 60, 62)	u-1	I	II	III	I	III	u-1	I	I	I	I
#7	1 (TgCkBr 46)	u-1	I	II	III	I	III	II	I	I	I	I
#8	1 (TgCkBr 178)	u-1	I	II	III	I	III	II	III	III	I	III
#9	8 (TgCkBr 38, 27, 44, 51, 65, 66, 78, 80)	u-1	I	II	III	III	III	u-1	I	III	III	III
#10	1 (TgCkBr 45)	u-1	I	II	III	III	III	II	I	III	III	I
#11	1 (TgCkBr 177)	I	I	II	III	III	III	III	I	III	III	III
#12	1 (TgCkBr 114)	I	I	II	III	I	III	II	I	III	III	I
#13	2 (TgCkBr 171, 172)	I	I	I	III	I	II	u-1	I	III	II	III
#14	1 (TgCkBr 173)	I	I	I	III	I	II	u-1	I	I	III	III
#15	3 (TgCkBr 136, 138, 139)	I	I	I	III	I	II	I	I	I	I	I
#16 (BrI)	12 (TgCkBr 123, 124, 55, 79, 86, 87, 10, 98, 101, 102, 104, 144)	I	I	I	III	I	II	u-1	I	I	I	I
#17	2 (TgCkBr 40, 47)	I	I	I	III	III	II	u-1	I	I	I	I
#18	1 (TgCkBr 146)	I	I	I	I	I	I	I	I	I	I	I
#19	1 (TgCkBr 141)	I	I	I	I	I	I	u-1	I	I	III	III
#20	1 (TgCkBr 54)	I	I	I	III	I	III	II	I	I	I	III

Genotypes (clonal types)	*T. gondii* isolate	Genetic markers										
		SAG1*	(5′ + 3′) SAG2[a]	SAG2[b]	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
#21	10 (TgCkBr 165, 167, 170, 174, 176, 179, 180, 183, 184, 185	I	I	I	I	I	III	II	III	III	I	III
#22	1 (TgCkBr 169)	I	I	I	I	I	III	II	I	III	I	III
#23	3 (TgCkBr 115, 142, 145)	I	I	I	I	I	I	II	I	III	I	III
#24	4 (TgCkBr 59, 30, 34, 67)	I	I	I	III	I	III	II	I	III	I	III
#25	1 (TgCkBr 186)	I	I	I	III	III	III	II	I	III	I	III
#26	1 (TgCkBr 156)	I	I	I	III	III	III	I	I	III	I	III
#27	1 (TgCkBr 74)	u-1	III	III	III	III	III	III	I	III	III	III
#28	3 (TgCkBr 82, 90, 153)	I	III	III	III	III	III	III	I	III	III	III
#29	1 (TgCkBr 130)	I	III	III	III	I	III	II	III	III	III	III
#30 (BrIII)	7 (TgCkBr 11, 7, 17, 131, 132, 133, 134)	I	III	III	III	III	III	II	III	III	III	III
#31	1 (TgCkBr 8)	I	III	III	III	III	III	II	III	III	u-2	III
#32	3 (TgCkBr 111, 112, 182)	I	III	III	III	III	III	III	III	III	III	I
#33	1 (TgCkBr 181)	I	III	III	III	III	III	III	III	III	III	III
#34	5 (TgCkBr 31, 56, 158, 161, 164)	II or III	III	III	III	III	III	III	III	III	III	III
#35	4 (TgCkBr 149, 150, 152, 157)	II or III	III	III	III	III	III	I	III	III	I	III
#36	1 (TgCkBr 113)	I	III	III	I	III	III	III	III	III	I	III
#37	1 (TgCkBr 110)	I	III	III	I	III	III	III	III	III	I	I
#38	1 (TgCkBr 166)	I	III	III	I	III	III	III	I	III	I	I
#39	2 (TgCkBr 26, 69)	I	III	III	III	III	III	II	I	III	III	I
#40	3 (TgCkBr 126, 127, 117)	I	III	III	III	I	II	II	III	I	I	III
#41	2 (TgCkBr 107, 108)	I	III	III	III	III	II	u-1	I	I	I	III
#42	2 (TgCkBr 99, 100)	I	III	III	III	III	II	u-1	I	I	II	I
#43	2 (TgCkBr 93, 94)	I	III	III	III	III	II	I	III	I	II	I
#44	5 (TgCkBr 28, 33, 50, 52, 58)	I	III	III	III	III	III	I	I	I	u-1	I
#45	1 (TgCkBr 95)	I	III	III	III	III	III	I	I	I	III	III
#46	2 (TgCkBr 155, 159)	u-1	III	III	III	III	III	u-1	I	I	III	I
#47	1 (TgCkBr 109)	I	I	II	I	III	III	II	III	I	III	III
#48	1 (TgCkBr 16)	I	I	II	I	III	III	I	I	I	II	I
#49	2 (TgCkBr 13, 23)	I	I	II	III	III	III	I	III	I	II	I
#50 (BrII)	3 (TgCkBr 57, 64, 97)	I	I	II	III	III	III	I	III	I	II	III

Genotypes (clonal types)	*T. gondii* isolate	Genetic markers									
		SAG1*	(5′ + 3′) SAG2[a]	SAG2[b]	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
#51	1 (TgCkBr 116)	u-1	II	II	III	III	II	II	nd	II	II	I
#52	1 (TgCkBr 168)	II or III	II	II	II	III	II	II	II	II	II	II
#53	1 (TgCkBr 143)	I	I	II	III	III	II	u-1	III	III	III	I
#54	1 (TgCkBr 37)	I	I	II	III	III	II	u-1	I	I	III	I
#55	1 (TgCkBr 61)	I	I	II	I	III	II	u-1	I	I	III	I
#56	2 (TgCkBr 19, 24)	I	I	II	III	III	III	u-1	I	I	u-2	I
#57	4 (TgCkBr 36, 32, 84, 85)	I	II	II	III	III	III	u-1	I	I	III	I
#58	1 (TgCkBr 89)	I	I	II	III	III	III	u-1	I	I	III	I
Mixed infections	1 (TgCkBr 175)	I	I + III	I + III	I + III	I	II + III	II	I + III	I + III	I	I + III
	1 (TgCkBr 187)	I	I	I	I + III	I + III	III	II	I + III	III	I	III

u-1 and u-2 are new alleles that are different from the clonal Type I, II and III alleles.

[a] *SAG2* marker based on 5′- and 3′-ends of the gene sequence (Howe et al., 1997).

[b] A new *SAG2* marker based on the 5′-end of the gene sequence (Su et al., 2006).