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Multi-locus DNA sequencing of *Toxoplasma gondii* isolated from Brazilian pigs identifies genetically divergent strains

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Abstract

Five *Toxoplasma gondii* isolates (TgPgBr1–5) were isolated from hearts and brains of pigs freshly purchased at the market of Campos dos Goytacazes, Northern Rio de Janeiro State, Brazil. Four of the five isolates were highly pathogenic in mice. Four genotypes were identified. Multi-locus PCR-DNA sequencing showed that each strain possessed a unique combination of archetypal and novel alleles not previously described in South America. The data suggest that different strains circulate in pigs destined for human consumption from those previously isolated from cats and chickens in Brazil. Further, multi-locus PCR-RFLP analyses failed to accurately genotype the Brazilian isolates due to the high presence of atypical alleles. This is the first report of multi-locus DNA sequencing of *T. gondii* isolates in pigs from Brazil.

Keywords

Toxoplasma gondii; Pigs; Isolation; Genetic typing; Brazil

1. Introduction

Early studies in Europe and North America showed that *Toxoplasma gondii* strains were remarkably clonal and possessed very little genetic variability. These archetypal isolates were classified into three lineages designated Types I, II and III (Dardé et al., 1992; Howe and Sibley, 1995; Ajzenberg et al., 2002 a,b). Recent studies indicate that the isolates from Brazil are biologically and genetically different from the ones found in USA and Europe (Dubey et al., 2002; Lehmann et al., 2006; Belfort-Neto et al., 2007; Dubey et al., 2007a,b,c; Dubey et al., 2008). Archetypal strains also predominate in chickens from Africa, with higher prevalence of types II and III (Velmurugan et al., 2008). The Type II lineage is rare in Brazil, but has recently been collected from the Brazilian island of Fernando de Noronha

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(Dubey et al., 2010). Instead, there is considerable genetic variation, with the presence of many atypical alleles inherited in new combinations not found in other regions of the globe (Howe and Sibley, 1995; Khan et al., 2006; Dubey et al., 2007a,c; Dubey et al., 2008; Dubey and Su, 2009).

Pigs are considered to be important sources of human *T. gondii* infections in many countries, including Brazil (Dubey, 2009a). Although *T. gondii* has been isolated from pork or pork products in Brazil (Frazão-Teixeira et al., 2006), there are only limited molecular data on *T. gondii* isolates from pigs in Brazil (dos Santos et al., 2005; Ferreira et al., 2006; Belfort-Neto et al., 2007). In the present paper we report detailed genetic analysis of *T. gondii* isolates infecting pork from local retail meat stores, including the report of new genetic types of *T. gondii*.

2. Materials and Methods

2.1 Isolation of viable T. gondii

Fresh hearts (n=16) and brains (n=19) of pigs from five butcherhouses in the main popular market of Campos dos Goytacazes, Northern Rio de Janeiro State, Brazil were purchased for this study. Each of these 35 tissues were digested in pepsin and their homogenates were inoculated in outbred albino mice (Dubey, 1998) at the Laboratório de Sanidade Animal (LSA), Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, RJ, Brazil. Viable *T. gondii* was isolated from five tissues and the strains were designated TgPgBr 1 to 5 (Table 1). To obtain tissue cysts, tissues of infected mice were sub-inoculated into another two mice at UENF. Sick mice received sulfadiazine (1mg/ml) in their water until the mice recovered from illness. Brains of these mice containing tissue cysts were sent to the Animal Parasitic Diseases Laboratory (APDL), United States Department of Agriculture (USDA), Beltsville, MD, USA for biologic and genetic characterization. At APDL, brains were homogenized in antibiotic saline and inoculated subcutaneously in groups of two outbred swiss webster mice and two C57Bl/6 IFNγ-knockout (KO) mice. Lung smears and brain squashes of dead mice were examined for the presence of *T. gondii*. All surviving mice were bled at six weeks and sera tested for the presence of anti-*T. gondii* antibodies by modified agglutination test (MAT, titer ≥ 25; Dubey and Desmonts, 1987). Infected lung tissues were stored at −20°C for genetic characterization.

2.2 Pathogenicity of tissue stages and oocysts of porcine T. gondii isolates to mice

In order to obtain oocysts for each *T. gondii* isolate, tissues of infected mice were each fed to one of five pathogen free cats at APDL as described (Dubey, 1995). Cats were fed a pool of tissues from mice inoculated with parasites from the second or third passages; day post inoculation of mice is indicated in Table 1. Oocysts were collected from feline feces, sporulated in 2% sulfuric acid at room temperature for seven days, and kept at 4° C until bioassayed in mice.

For bioassay in mice, oocysts were counted in a hemocytometer, the solution neutralized with sodium hydroxide and diluted ten-fold until the last dilution was estimated to have no oocysts. The last infective dilution was considered to have 1 viable oocyst. Aliquots from each oocyst dilution were inoculated orally into four mice. The mice were observed for six-eight weeks. Smears of tissues (mesenteric lymph nodes of mice that died during the first week, and lungs of mice that died during the second and third week after feeding oocysts) were examined microscopically for tachyzoites. All surviving mice were bled at six weeks and their sera (1:25 dilution) tested for MAT antibodies. All mice were killed eight weeks p.i. and their brains examined microscopically for tissue cysts (Dubey, 2009b).

2.3 Genetic characterization

Toxoplasma gondii DNA was extracted from lungs of infected mice using the DNeasy® Qiagen kit.

DNA from the five isolates was amplified using the polymerase chain reaction (PCR) at 11 genotyping markers: SAG1, SAG2, SAG3, BTUB, c22-8, c29-2, L358, PK1, Apico, GRA6 and B1 (Grigg and Boothroyd, 2001; Su et al., 2006; Dubey et al., 2008). PCR products were incubated for 15 minutes with ExoSAP-IT (USB Corporation, Cleveland, OH) prior to DNA sequencing. DNA sequencing was carried out by the RML Genomics Unit, Hamilton, MT. Results were compared against published archetypal strain sequences in GenBank using the DNA STAR SeqMan application.

3. Results

3.1 Biologic characterization of T. gondii isolates

Toxoplasma gondii was isolated from five of 35 pig tissues (3 brains; 2 hearts) and these isolates were designated TgPgBr1–5 (Table 1). When infected in mice, the tissue stages and oocysts of four isolates (TgPgBr1–3, TgPgBr5) were lethal to mice (Table 1). Outbred mice inoculated with tissue stages (tachyzoites or tissue cysts) died of acute toxoplasmosis within 20 days. Oral inoculation with as few as 1 sporulated oocyst of these strains was lethal; infected mice died of acute toxoplasmosis within 8–13 days p.i. (Table 1). The fifth isolate (TgPgBr4), however, was less pathogenic. Mice inoculated with tissue stages or 1–10 oocysts remained asymptomatic; but 100 oocysts were lethal (Table 1).

3.2.Genetic characterization

PCR-RFLP analysis at 10 genetic loci revealed four genotypes; one of the four genotypes was archetypal, and two isolates (TgPgBr1 and TgPgBr2) were indistinguishable from each other (Table 2).

DNA sequencing confirmed that TgPgBr1 and TgPgBr2 were genetically identical. However, DNA sequencing was significantly more resolved and detected 15 atypical alleles across the 10 PCR-RFLP loci examined, which was substantially higher than the 2 detected by multi-locus PCR-RFLP analyses (Table 3). Further, DNA sequencing at an additional locus, B1, identified 2 more unique alleles that were circulating in pigs indigenous to Brazil (Table 3). Figure 1 shows the genetic polymorphisms encoded by each of the 17 unique alleles found in the pig isolates versus archetypal alleles expressed by clonal types I, II and III.

4. Discussion

This study was undertaken to determine whether *T. gondii* strains infecting pigs destined for human consumption in the state of Rio de Janeiro, Brazil possessed the same genotypes as those already known to infect cats, chickens and people in Brazil. Previous multi-locus PCR-RFLP typing of cats and chickens has identified four main clonal genotypes, specifically Types BrI, BrII, BrIII and BrIV (Su et al., 2006; Dubey et al, 2008; Pena et al, 2008) causing patent infections in both animal groups, in addition to a large array of other nonarchetypal lines that apparently cluster geographically (Table 2). In contrast, none of the four pig genotypes resolved here, either by low resolution PCR-RFLP or high resolution PCR-DNA sequence typing, have been described previously in Brazil, perhaps identifying a role for natural selection in the pig intermediate host. By PCR-RFLP typing, the pig isolates possessed many of the same alleles present in other Brazilian genotypes, but the alleles had segregated differently across the loci examined. These data suggest several possible

scenarios including: 1) the PCR-RFLP typing is not sufficiently resolved and that the lines are not natural recombinants expressing different combinations of the same alleles circulating in clonal BrI–IV lines, but rather exist as novel lines that possess a diversity of divergent alleles that reflect the tremendous genetic diversity indigenous to Brazil; or 2) that the pig isolates represent novel recombinants that arose by sexual recombination among extant Brazilian lineages, and were naturally selected for optimal expansion in the pig intermediate host. Ultimately, to resolve these possible scenarios will require additional sampling, and genotyping to proceed by direct DNA sequencing using at least 10 predominantly unlinked genetic markers.

Prior to genetically grouping North American and European *T. gondii* isolates into three clonal archetypes, commonly referred to as I, II and III, *T. gondii* strains were often classified based on their virulence in mice: either acutely virulent (LD_{100} = 1 parasite) or relatively avirulent (LD_{50} >1000 parasites); the latter strains being capable of establishing chronic, transmissible infections in mice. Type I isolates were later identified as a clonal lineage that is acutely virulent to mice, whereas types II and III were less so (Howe and Sibley, 1995; Howe et al., 1996). At present, no concrete evidence exists in Brazil correlating mouse pathogenicity and *T. gondii* strain genotype with clinical outcome during human infection in South America; most *T. gondii* strains isolated from asymptomatic animals are virulent in mice (Dubey et al., 2002; Pena et al., 2008). In this regard, four of the five isolates recovered from chronically infected pigs in the present study were highly pathogenic for mice, and 100 oocysts of the fifth isolate killed all inoculated mice.

With the introduction of multi-locus PCR-RFLP genotyping, population level analyses investigating the genetic structure of *T. gondii* strains circulating in North America and Europe were tremendously successful and established that the vast majority of infections in people and livestock were caused by just three genetically related, apparently recombinant lines that had expanded clonally only recently (Grigg et al, 2001; Su et al., 2003). When applied appropriately, this technique is very powerful for large-scale sampling, however, it is not as resolved as PCR-DNA sequencing methodology, and when applied in regions of the world where parasite genetic diversity is substantial, it fails to accurately resolve the true genetic “types” in, for instance, the strains isolated in this study from pigs from Campos dos Goytacazes, Rio de Janeiro State, Brazil. Indeed, Table 2 shows that many previous studies have reported an abundance of “recombinant” genotypes in South America that bear new combinations of archetypal alleles across the 10 PCR-RFLP loci examined (Dubey et al., 2008; Pena et al., 2008). However, it is likely that the majority of these I, II or III allele designations are not correct, and that the Brazilian isolates should not be classified as “recombinants” of archetypal strains, but rather as “atypical” because they possess altogether new combinations (or mixtures) of predominantly unique and some archetypal alleles. The high number of unique alleles identified by DNA sequencing among the 5 pig isolates genotyped in this study poignantly illustrates this fact (Table 3) and establishes that DNA sequencing is the preferred technique to infer the true genetic relationship and population structure of *T. gondii* strains circulating in Brazil.

Previous multi-locus PCR-RFLP analyses applied against isolates derived from chickens and cats identified four, widespread clonotypes, designated BrI, II, III and IV. None of the pig isolates in this study possessed a BrI-IV clonotype. This was not likely the result of any geographic bias, as clonotypes BrI and IV have been identified infecting chickens in Rio de Janeiro state (Table 2), and all four clonotypes have been identified infecting chickens and cats in neighboring São Paulo state (Dubey et al., 2008;Pena et al., 2008). Whether these strains infect swine, or humans, is currently unclear, chiefly because too few isolates have been examined from these two sources, and genotyping markers have not been consistently applied. Epidemiologic and environmental studies have suggested that the main routes of

human infection are via ingestion of water from untreated sources or the barbecue eating habits among the Brazilian population (Bahia-Oliveira et al., 2003;Silva et al., 2003;Dubey et al., 2003;Frazão-Teixeira et al., 2006). Because pigs in this region of Brazil are bred free-ranging and are not kept under good hygiene conditions, this animal species may represent an ideal sentinel population to examine whether consumption of undercooked pork is contributing to the diversity of strains infecting people or represent a significant risk for causing human disease.

Unfortunately, the majority of studies performed to genotype *T. gondii* strains infecting people have used too few or different markers from the ones used in the animal studies, so comparative analyses to investigate sources of human infection cannot be drawn. In research performed by Khan et al. (2006), 17 specimens from clinical cases of human ocular toxoplasmosis in Brazil were characterized via multi-locus PCR-RFLP using the markers SAG2, SAG3, BTUB, and GRA6. Comparing their results with the multi-locus PCR-RFLP genotypes detected in our present work identified one human infection with the same multi-locus type as TgPgBr1 and 2. Vallochi et al. (2005) genotyped 11 human ocular toxoplasmosis patients at SAG2, all possessed a Type I allele by PCR-RFLP, the same as TgPgBr1 and 2. However, this does not prove that these strains were genetically identical to the ones infecting the pig samples indicating that further studies requiring larger datasets, and DNA sequencing using at least 10 genetic markers are required to interrogate whether consumption of undercooked pork is a risk factor for human disease in Brazil.

In the present study, four of the five *T. gondii* isolates from pigs were virulent to mice, but cats fed tissues of acutely infected mice shed oocysts, indicating that bradyzoites had formed within 8 days and so it is conceivable that these isolates could cycle between cats and mice in nature. The isolates were obtained from pig brains and hearts that were not individually identified at the butcher shop (Table 1), therefore we are uncertain if they belonged to 3 or 5 pigs. However, our genetic data suggests that they came from different pigs.

In conclusion, this is the first research study to DNA sequence *T. gondii* isolates acquired from pig tissues in Brazil at multiple genetic loci. Multi-locus DNA sequencing is more resolved than PCR-RFLP and is capable of detecting genetic differences nucleotide by nucleotide, which is required to accurately delineate the true genetic relationship among isolates circulating in regions of the world where tremendous genetic variation exists. While the multi-locus PCRRFLP technique is easy to perform, it was originally developed to resolve only those polymorphic sites that differentiate among extant, archetypal strains circulating in North America, Europe and Africa. As a result, it typically fails to detect unique polymorphisms naturally present in the majority of alleles circulating in strains isolated from South America (Pena et al., 2008). Hence, this technique should not be applied to interrogate the *Toxoplasma* population genetic structure in Brazil, where allelic diversity is substantial. Otherwise, the multi-locus genotype reported will be inaccurate and misinterpreted as either archetypal or possibly “recombinant”, which would not be correct. In this study, the predominance and inheritance pattern of atypical alleles resolved by multi-locus DNA sequencing indicated that the genotypes isolated from pigs destined for human consumption possessed new combinations of polymorphic alleles different from archetypal strains, and these genotypes possessed different multi-locus genotypes from those found circulating in chickens and cats in Brazil.

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A

Marker	c22-8-ChrIb				
Nucleotide	73	103	175	383	ALLELE
Consensus	A	A	T	G	
RH	.	.	C	A	I
Me49	.	.	.	.	II
VEG	T	G	.	.	III
TgPgBr1	T	G	C	.	u1
TgPgBr2	T	G	C	.	u1
TgPgBr3	T	.	.	.	u2
TgPgBr4	T	G	.	.	III
TgPgBr5	T	G	C	.	u1

Marker	C29-2-ChrIII				
Nucleotide	69	263	373	392	ALLELE
Consensus	A	C	T	T	
RH	T	.	G	.	I
Me49	.	T	.	.	II
VEG	.	.	.	.	III
TgPgBr1	T	.	G	.	I
TgPgBr2	T	.	G	.	I
TgPgBr3	.	.	.	C	u1
TgPgBr4	.	.	.	.	III
TgPgBr5	.	.	.	C	u1

Marker	L358-ChrV							
Nucleotide	35	101	110	157	197	234	205	ALLELE
Consensus	T	C	C	G	G	C	C	
RH	.	.	.	.	.	.	G	I
Me49	C	.	.	A	.	.	.	II
VEG	.	.	.	.	.	.	.	III
TgPgBr1	.	.	.	.	.	.	G	I
TgPgBr2	.	.	.	.	.	.	G	I
TgPgBr3	.	G	T	.	.	.	G	u1
TgPgBr4	.	.	.	.	.	.	.	III
TgPgBr5	.	.	.	.	A	T	.	u2

Marker	PK1-ChrVI																							
Nucleotide	1442	1459	1531	1588	1636	1786	1873	1898	1974	2079	2148	2150	2167	2192	2205	2255	2316	2373	2379	2383	2394	2428	2429	ALLELE
Consensus	C	G	A	C	G	T	C	G	T	C	C	T	G	C	A	A	T	C	G	A	G	T	C	
RH	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	.	.	.	C	C	.	.	.	I
Me49	G	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	.	.	.	.	II
VEG	.	.	.	.	-	A	T	A	.	.	.	.	T	.	.	G	.	T	.	.	.	A	T	III
TgPgBr1	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	.	.	.	C	G	.	.	.	u1
TgPgBr2	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	.	.	.	C	G	.	.	.	u1
TgPgBr3	.	A	.	.	.	.	.	.	G	T	.	.	.	T	.	.	.	.	C	G	.	.	.	u1
TgPgBr4	.	A	T	.	.	.	.	.	G	.	.	.	.	T	G	.	.	.	C	G	.	.	.	u2
TgPgBr5	.	A	.	.	.	.	.	.	G	.	A	.	.	T	.	.	.	.	C	G	C	.	.	u3

B

Marker	SAG1-ChrVIII					
Nucleotide	211	246	263	356	444	ALLELE
Consensus	T	C	G	G	C	
RH	C	T	C	A	T	I
Me49	.	.	.	.	.	II
VEG	.	.	.	.	.	III
TgPgBr1	C	T	C	A	T	I
TgPgBr2	C	T	C	A	T	I
TgPgBr3	C	T	.	A	.	u1
TgPgBr4	.	.	.	.	.	II/III
TgPgBr5	C	T	.	A	.	u1

Marker	SAG2-ChrVIII																	
Nucleotide	76	319	348	458	459	470	479	487	592	593	594	718	913	1042	1111	1255	1275	ALLELE
Consensus	T	T	A	A	C	C	G	T	-	-	-	A	A	C	C	C	T	
RH	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Me49	.	G	.	.	A	G	C	.	T	G	G	G	C	.	T	G	C	
VEG	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
TgPgBr1	.	.	.	.	.	.	.	.	T	G	G	.	.	.	.	.	.	u1
TgPgBr2	.	.	.	.	.	.	.	.	T	G	G	.	.	.	.	.	.	u1
TgPgBr3	.	.	G	G	.	G	C	A	T	G	G	.	.	G	.	.	.	u2
TgPgBr4	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	III
TgPgBr5	.	.	G	G	.	G	C	A	T	G	G	.	.	G	.	.	.	u2

Marker	BTUB-ChrIX					
Nucleotide	21	86	123	126	286	ALLELE
Consensus	C	C	C	G	C	
RH	.	.	.	.	T	I
Me49	G	G	G	C	.	II
VEG	.	.	.	.	.	III
TgPgBr1	.	.	.	.	T	I
TgPgBr2	.	.	.	.	T	I
TgPgBr3	.	.	.	.	T	I
TgPgBr4	.	.	.	.	.	III
TgPgBr5	.	.	.	C	.	u1

Marker	B1-Chr-IX			
Nucleotide	366	413	504	ALLELE
Consensus	t/c	C	g/c	
RH	T	.	G	I
Me49	.	.	.	II
VEG	.	.	.	III
TgPgBr1	T	.	G	I
TgPgBr2	T	.	G	I
TgPgBr3	T	a/c	G	u1
TgPgBr4	.	.	.	II/III
TgPgBr5	T	A	G	u2

C

Marker	GRA6-ChrX																																		
Nucleotide	162	170	171	295	304	336	561	576	614	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	658	659	660	669	671	674	677	690	692	ALLELE
Consensus	G	A	A	A	T	T	A	A	G	T	A	C	G	G	A	G	G	C	A	G	A	G	G	T	G	A	G	N	A	A	C	C	A	C	
RH	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	I
Me49	.	.	G	.	.	C	.	G	.	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	G	A	II
VEG	A	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	G	.	.	III
TgPgBr1	.	C	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	G	.	.	u1
TgPgBr2	.	C	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	G	.	.	u1
TgPgBr3	.	.	.	.	.	C	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	G	.	G	A	u2
TgPgBr4	A	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	G	.	.	u3
TgPgBr5	.	.	G	.	.	C	.	G	.	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	G	A	II

Fig. 1.

Polymorphisms at 9 genetic markers by direct PCR-DNA sequencing of *Toxoplasma gondii* isolates from pigs from Brazil

DNA Sequence analysis at **A)** c22-8, c29-2, L358, PK1; **B)** SAG1, SAG2, BTUB, B1 and **C)** GRA6. Consensus sequence is defined as the nucleotide shared by at least two of the three archetypal Type I, II and III strain alleles. Numerical positions refer to sites in the published sequences (GenBank accession no. EU258475, EU258490, L42007, X14080, M33572, AY143127, AF179871 and F239283 for c22-8, L358, PK1, SAG1, SAG2, BTUB, B1 and GRA6 respectively). For c29-2, the amplicon was blasted (using the BlastN algorithm) at ToxoDB (www.toxodb.org) and the corresponding RH sequence used as the reference. "." indicates identity with consensus; "-" indicates an insertion/deletion "u" indicates a nonarchetypal allele; I, II or III refers to an archetypal allelic sequence from a Type I, II or III strain.

Table 1

Biological characteristics of *Toxoplasma gondii* isolates from fresh pork from a meat shop in Campos dos Goytacazes, RJ, Brazil

Isolate designation	Pig tissue	Oocysts shed (DPI)	Mortality in mice			
			Tissue stages inoculated[d]	Oocyst fed[f]		
				1	10	100
TgPgBr1	brain[a]	67[c] (11)	2/2	3/3	4/4	4/4
TgPgBr2	heart[a]	20 (8)	2/2	1/1	2/2	4/4
TgPgBr3	brain[b]	13 (11)	2/2	2/2	4/4	4/4
TgPgBr4	brain[b]	X (39)	0/2[e]	0/0[g]	0/4[e]	4/4
TgPgBr5	heart[b]	24 (24)	2/2	2/2	3/3	4/4

[a] Samples obtained on August 1, 2008.

[b] Samples obtained on August 25, 2008.

[c] Cat number. The data in parenthesis are DPI=day post inoculation of mice before being fed to cat.

[d] No. of mice dead out of two mice inoculated with tissue cysts.

[e] Mice were seropositive and had tissue cysts in their brains.

[f] No. of mice dead out of four mice orally inoculated with oocysts. The dose is based on the assumption that each viable oocyst is infective to mice.

[g] Mice were seronegative and determined to be not infected.

Table 2

Multi-locus genotypes of *Toxoplasma gondii* isolates from Brazil by PCR-RFLP analysis

Genotypes	Isolates	Origin	Genetic markers										References
			c22-8	C29-2	L358	PK1	SAG1	SAG2	BTUB	GRA6	SAG3	APICO	
Chromosome			**Ib**	**III**	**V**	**VI**	**VIII**	**VIII**	**IX**	**X**	**XII**	**plastid**	
Type I	RII88	USA	I	I	I	I	I	I	I	I	I	I	
Type II	PTG	USA	II	II	II	II	II/III	II	II	II	II	II	
Type III	CTG	USA	III	III	III	III	II/III	III	III	III	III	III	Dubey et al. (2008)
BrI	TgCkBr123, 124, 55 79, 86, 87, 10, 98, 101, 102, 104, 144	Brazil											Dubey et al. (2008)
	TgCatBr42, 47, 53, 54, 55, 62, 71, 75		u-1	I	I	I	I	I	I	II	III	I	Pena et al. (2008)
BrII	TgCkBr57, 64, 97	Brazil											Dubey et al. (2008)
	TgCatBr39, 51, 52, 56, 61, 68, 77, 78		I	III	I	II	I	II	III	III	III	III	Pena et al. (2008)
BrIII	TgCkBr11, 7, 17, 131, 132, 133, 134	Brazil											Dubey et al. (2008)
	TgCatBr58, 59, 60, 73, 74		II	III	III	III	I	III	III	III	III	III	Pena et al. (2008)
BrIV	TgCkBr81, 147, 148, 151, 154, 160, 162, 163	Brazil	u-1	I	I	III	u-1	II	III	III	III	I	Dubey et al. (2008)
#1	TgPgBr1, TgPgBr2	Rio de Janeiro	I	I	I	I	I	I	I	II	III	I	this research
#2	TgPgBr3		II	I	I	I	u-1	u-1	I	II	III	I	this research
Type III	TgPgBr4		III	III	III	III	II/III	III	III	III	III	III	this research
#4	TgPgBr5		I	I	III	III	u-1	u-1	III	II	III	III	this research
	TgCkBr48, 88		II	I		III	u-1	II	III	III	III	III	Dubey et al. (2008)
	TgCkBr75, 76, 92		III	III		III	u-1	II	III	III	III	I	Dubey et al. (2008)
BrIV	TgCkBr81		u-1	I		III	u-1	II	III	III	III	I	Dubey et al. (2008)
	TgCkBr41, 42, 49, 60, 62		u-1	I	I	I	u-1	II	I	III	III	I	Dubey et al. (2008)
	TgCkBr46		II	I	I	I	u-1	II	I	III	III	I	Dubey et al. (2008)
	TgCkBr38, 27, 44, 51, 65, 66, 78, 80		u-1	I	III	III	u-1	II	III	III	III	III	Dubey et al. (2008)
	TgCkBr45		II	I	III	III	u-1	II	III	III	III	I	Dubey et al. (2008)
BrI	TgCkBr55, 79, 86, 87		u-1	I	I	I	I	I	I	II	III	I	Dubey et al. (2008)
	TgCkBr40, 47		u-1	I	I	I	I	I	III	II	III	I	Dubey et al. (2008)
	TgCkBr54	Rio de Janeiro	II	I	I	I	I	I	I	III	III	III	Dubey et al. (2008)

Genotypes	Isolates	Origin	Genetic markers										References
			c22-8	C29-2	L358	PK1	SAG1	SAG2	BTUB	GRA6	SAG3	APICO	
Chromosome			**Ib**	**III**	**V**	**VI**	**VIII**	**VIII**	**IX**	**X**	**XII**	**plastid**	
	TgCkBr59, 30, 34, 67		II	I	III	I	I	I	I	III	III	III	Dubey et al. (2008)
	TgCkBr74		III	I	III	III	u-1	III	III	III	III	III	Dubey et al. (2008)
	TgCkBr82, 90		III	I	III	III	I	III	III	III	III	III	Dubey et al. (2008)
	TgCkBr31, 56		III	III	III	III	II/III	III	III	III	III	III	Dubey et al. (2008)
	TgCkBr26, 69		II	I	III	III	I	III	III	III	III	I	Dubey et al. (2008)
	TgCkBr28, 33, 50, 52, 58		I	I	I	u-1	I	III	III	III	III	I	Dubey et al. (2008)
	TgCkBr57, 64		I	III	I	II	I	II	III	III	III	III	Dubey etal. (2008)
	TgCkBr61		u-1	I	I	III	I	II	III	II	I	I	Dubey et al. (2008)
	TgCkBr32, 36, 84, 85		u-1	I	I	III	I	II	III	III	III	I	Dubey et al. (2008)
	TgCkBr89		u-1	I	I	III	I	II	III	III	III	I	Dubey et al. (2008)

“u” indicates a nonarchetpyal allele; I, II or III refers to an archetypal allele from a Type I, II or III strain

Isolates TgCkBr7–24 were collected in the state of Sao Paulo

Isolates TgCkBr26–92 were collected in the state of Rio de Janeiro

Isolates TgCkBr93–187 were collected in other states of Brazil (please see Dubey et al., 2008)

Table 3

Multi-locus genotypes of *Toxoplasma gondii* isolates from pigs from Brazil, TgPgBr1–5, by PCR-DNA sequencing

Isolates	Genetic markers										
	c22-8	c29-2	L358	PK1	SAG1	SAG2	BTUB	B1	GRA6	SAG3	Apico plastid
Chromosome	Ib	III	V	VI	VIII	VIII	IX	IX	X	XII	
TgPgBr1	u-1	I	I	u-1	I	u-1	I	I	u-1	III	I
TgPgBr2	u-1	I	I	u-1	I	u-1	I	I	u-1	III	I
TgPgBr3	u--2	u-1	u-1	u-1	u-1	u-2	I	u-1	u-2	III	I
TgPgBr4	III	III	III	u-2	II/III	III	III	II/III	u-3	III	III
TgPgBr5	u-1	u-1	u-2	u-3	u-1	u-2	u-1	u-2	II	III	III