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Genotyping of *Toxoplasma gondii*: DNA extraction from formalin-fixed paraffin-embedded autopsy tissues from AIDS patients who died by severe disseminated toxoplasmosis.

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This study investigated the genetic features of *T. gondii* DNA in autopsies of HIV-infected patients who died with severe disseminated toxoplasmosis

Patient - Code [1]	Gender /Age (years)	Diagnosis [2] (clinical, images and laboratory data)	PCR *T. gondii*	HIV serology	Autopsy histopathology for *T. gondii*	Genotype
Patient 1 A04/05	M/39	disseminated toxoplasmosis and cytomegalovirus encephalitis	pos	pos	pos	**TgHuDis1**
Patient 2 A13/05	F/31	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis2**
Patient 3 A21/05	F/24	disseminated toxoplasmosis and neurotuberculosis	pos	pos	pos	**TgHuDis3**
Patient 4 A35/05	M/39	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis1**
Patient 5 A6/06	M/40	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis1**
Patient 6 A17/06	F/31	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis4**
Patient 7 A21/07	M/50	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis5**
Patient 8 A11/08	F/38	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis5**
Patient 9 A21/08	F/48	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis3**
Patient 10 A31/08	F/42	disseminated toxoplasmosis	pos	pos	pos	**ToxoDB #11**
Patient 11 A12/09	M/30	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis1**
Patient 12 A39/09	M/40	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis1**
Patient 13 A02/10	M/43	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis1**
Patient 14 A6/11	F/30	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis1**
Patient 15 A21/11	M/59	disseminated toxoplasmosis	pos	pos	pos	**TgHuDis1**

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Abstract

This study investigated the genetic features of *T. gondii* isolated directly in autopsies of HIV-infected patients who died with severe disseminated toxoplasmosis. This retrospective analysis was conducted in a cohort of 15 HIV-infected patients with clinical and laboratory data. They had previous cerebral toxoplasmosis at least 6 months before the disseminated toxoplasmosis episode. The hypothesis was that they were infected with highly virulent parasites due to the condition in which they died. *T. gondii* genotyping was done directly in DNA extracted from 30 autopsy brain and lung samples (2 per patient) and mutilocus PCR-RFLP genotyping was done using 12 molecular markers. The 30 clinical samples were genotyped successfully in 8 or more loci and six suggestive genotypes were identified. One of them was Toxo DB #11, previously identified in different domestic animals and virulent in experimental animals. The other five suggestive genotypes identified in 14 patients were not described. **TgHuDis1** was the most frequent and was determined in 8 patients. **TgHuDis3** and **TgHuDis5** were identified in two patients each. **TgHuDis2** and **TgHuDis4** have been identified in one patient each. These suggestive genotypes could be considered as virulent, since they caused severe tissue damage and had similar characteristics as **Toxo # DB 11**.

Keywords**:** Disseminated toxoplasmosis, Brazilian AIDS patients, *Toxoplasma gondii* genotyping.

1. Introduction

Toxoplasma gondii has been reported to infect all warm-blooded mammals worldwide, including one third of the global human population (Montoya and Liesenfeld, 2004; Dubey, 2008). Intermediate hosts infected with *T. gondii* develop tissue cysts in a number of organs, such as in the central nervous system and skeletal muscle (Montoya and Liesenfeld, 2004). In asymptomatic humans, mild symptoms occasionally occur during the first few weeks. However, around 10-20% of cases of *T. gondii* infection become symptomatic (Dubey, 2008; Dubey et al., 2012; Hill et al. 2012).

In immunocompromised patients, especially those with human immunodeficiency virus (HIV) infection, the reactivation of latent infection can cause life-threatening encephalitis (Montoya and Liesenfeld, 2004; Pereira-Chioccola et al., 2009). Although toxoplasmosis, most often, presents as a localized central nervous system infection, patients are also exposed to disseminated toxoplasmosis. This form affects more than two organ systems, being a devastating opportunistic infection with very high mortality rates. Although its occurrence is rare, its clinical evolution is similar to sepsis and is correlated with a bad prognosis (Pomeroy and Filice, 1992; Medeiros et al., 2001; Bossi and Bricaire, 2004, Saadatnia and Golkar, 2012; Schmidt et al., 2013).

Genetic diversity of *T. gondii* strains has been an interesting and important subject in medical research, since it is possible to study the potential correlation between parasite genotype and disease patterns in infected patients (Dubey, 2008; Pereira-Chioccola et al., 2009). Studies using multi-locus markers showed a higher genetic variability with distinct genotypes (Su et al., 2006). In Brazil, analysis of the isolates from domestic animals revealed that the majority of the strains were grouped

into four genotypes considered common clonal lineages (BrI BrII, BrIII, and BrIV) (Pena et al., 2008).

Several genotyping studies have been performed on Brazilian *T. gondii* strains isolated in experimental mice and the majority of them were obtained from animal infection (Dubey et al., 2007; Dubey et al., 2008; Pena et al., 2008; Ragozo et al., 2010). Studies on *T. gondii* strains obtained from human infection are uncommon due to the difficulty of strain isolation in experimental animals (Carneiro et al., 2013). In addition, ethical animal committees around the world recommend moderate or minimal use of experiential animals. Despite these difficulties, many genotyping studies analyzed *T. gondii* DNA extracted directly from clinical sample, that provided important information on *T. gondii* genotyping in human congenital, ocular, and cerebral infections (Fuentes et al., 2001; Grigg et al., 2001; Ajzenberg et al., 2002; Vallochi et al., 2005; Gallego et al., 2006; Nowakowska et al., 2006; Ferreira et al., 2008; Alzenberg et al., 2009; Delhaes et al., 2010; Ferreira et al., 2011).

According to Ferreira et al. (2011), patients with good clinical evolution including ocular, congenital, and cerebral infections were infected with strains belonging to genotype Toxo DB 65. However, HIV- infected patients who developed diffuse toxoplasmosis and died were infected with *T. gondii* strains belonging to different genotypes (Toxo DB 6 and Toxo DB 7). These findings led us to suppose that some genotypes could be more virulent in humans. Based on the idea of correlation, the severity of the infection and parasite isolate, this study aims to investigate the genetic features of *T. gondii* isolated directly in autopsy samples from HIV-infected patients died of severe disseminated toxoplasmosis.

2. Materials and Methods

2.1. Ethics statement

The Ethics Committee of all involved Institutions approved the entire study, which was performed according to recommendations of the same Committee (CONEP-IAL number: 186 971).

2.2. Patients and autopsy samples

This retrospective study was conducted using 30 autopsy samples extracted from brain and lung tissues from 15 HIV-infected patients who died at the Instituto de Infectologia Emilio Ribas located in Sao Paulo, Brazil from severe disseminated toxoplasmosis. These formalin-fixed paraffin-embedded (FFPE) tissues were sectioned in 4-µm-thick fragments for histopathological, immunohistochemical and molecular diagnosis, as well as, *T. gondii* genotyping. Pre-mortem diagnosis of cerebral toxoplasmosis was defined according to previously reported "clinical and radiological criteria" (Vidal et al., 2005). Disseminated toxoplasmosis was defined since all patients had *T. gondii* infection in, at least, two non-contiguous organs (brain and lung). Severe disease was defined because disseminated toxoplasmosis was the direct cause of the deaths, which occurred between 2005 and 2011. The autopsy samples were sent to Instituto Adolfo Lutz for histopathological analyses.

2.3. Histopathological and immunohistochemical diagnosis

For histopathological analyses, FFPE sections were stained with hematoxylin-eosin. For immunohistochemical analyses, positive and negative controls consisted of known positive human tissue samples for *T. gondii* and the primary-antibody omission,

respectively. Immunostained procedures were performed after removing paraffin in xylene and rehydrating in baths with decreasing concentrations of ethanol and in distilled water, and then submitted to an antigen retrieval procedure in a 10-mM citrate buffer, pH 6.0 in a pressure cooker for 3 minutes, under pressure. Endogenous peroxidase activity blockage was obtained with 6% peroxide hydrogen solution treatment. The sections were immediately incubated with a mouse polyclonal anti-*T. gondii* diluted in 1% bovine soroalbumine in phosphate buffered saline (PBS) and tissue sections were incubated overnight at 4° C. An amplification signal was obtained by peroxidase conjugated polymer (Spring Biosciences, USA), in a 30-minute incubation step at 37°C. Color development was obtained with diaminobenzidine (Sigma, USA, 100 mg% in PBS, 0.1% peroxide hydrogen) chromogenic substrate, followed by light counterstaining with Harry's hematoxylin and permanent mounting with Entellan (Merck, Germany).

2.4. *T. gondii* reference strains

The reference strains GTI, RH, PTG, ME49, CTG, VEG, TgCgCa1 (COUGAR) and MAS, TgCatBr5 were used as positive control and as genotype indication in each genetic marker. RH, ME49 and VEG parasites were grown and maintained in VERO tissue cells. Tachyzoites were harvested from culture supernatants, centrifuged and washed twice at 2,000*g* for 10 min in PBS, pH 7.2. The parasite pellets were used for DNA extraction. Samples of DNA from the other strains were kindly provided by Dr Chunlei Su from Department of Microbiology, The University of Tennessee, Knoxville, USA.

2.5. DNA extraction

DNA molecules were extracted from 5 sections of 4-µm-thick FFPE/each from brain and lungs using DNA extraction kit QIAamp® DNA FFPE Tissue (Qiagen), according to the manufacturer's instructions. Before, autopsy fragments were dissolved in xylene (1 mL) for 30 seconds and, centrifuged at 13, 000 g for 2 minutes. Supernatants were removed and pellets incubated for 10 minutes, at room temperature for complete xylene evaporation. DNA molecules from tachyzoites were extracted using QIAamp® DNA Mini Kit (Qiagen). DNA pellets were dissolved in ultra-pure water. All DNA sample purities were determined by the ratio of O. D. at 260 and 280 nm in a NanoDrop ND1000 (Thermo Scientific).

2.5. PCR for diagnosis

The amplifications and primer selection were carried out as previously described (Colombo et al., 2005; Ferreira et al., 2011). The DNA samples (or controls) and 25 pmol of each primer were added to a kit purchased from Promega (Go Taq Green Master Mix). The PCR mix (12.5 µL) was composed of 1 unit of Taq DNA polymerase, 10 mM Tris-HCl, pH 8.5; 50 mM KCl; 1.5 mM $MgCl_2$; and 200 mM of each of each dNTP. Each amplification run contained two negative controls (ultrapure water and a negative DNA for toxoplasmosis) and one positive control (DNA extracted from RH strain). The primer pair used was B22/B23, which amplified a 115-bp sequence from a specific repetitive region of the B1 gene as target (Colombo et al., 2005; Burg et al., 1989). To control the course of extraction and check for PCR inhibitors, all samples were assayed using the primer pair β1/β2, which amplified a 140-bp fragment of the human β-globulin gene. After thermal cycles, PCR products were electrophoresed in 2% agarose gel and stained with ethidium bromide and visualized under UV illumination.

2.7. PCR-RFLP for genotyping

The genotypes of *T. gondii* were performed using multilocus PCR-RFLP, as previously described (Su et al., 2006; Su et al., 2010; Ferreira et al., 2011). The genetic markers were SAG1, SAG2 (5'-and 3'-SAG2, alt.SAG2) SAG3, BTUB, GRA6, C22-8, c29-2, L358, PK1 and Apico. The set of reaction included: (i) multiplex PCR; (ii) nested PCR; and (iii) amplified product treatment with restriction enzymes. Each reaction set included two negative controls (ultrapure water and a negative DNA for toxoplasmosis); and at least 5 positive controls (reference strains).

2.8. Data analysis

The DNA profiles after restriction enzyme digestion were compared with the reference strains. The genotyping results were named as "*suggestive genotypes*" since analyses were made in *T. gondii* DNA extracted from clinical samples and isolation of live parasites was impossible. Next, they were compared, identified and matched to those listed in ToxoDB at http://toxodb.org/toxo/ .

3. Results

The 15 deceased patients had diagnosis of severe disseminated toxoplasmosis, according to the clinical diagnosis, as well as, the histopathological and immunohistochemical analysis in the autopsy fragments. The fragments had numerous *T. gondii* cysts, especially in the 30 brain and lung samples used in this study. In the same way, the 30 DNA samples extracted from autopsy samples were positive in PCR using the primer pair B22/B23. The quality and purity of the extractions were checked by positive reaction using the primer pair β1/β2.

In order to investigate whether these patients could have been infected with more than one *T. gondii* strain, the genotyping experiments were carried out analyzing DNA extracted from two different sites: brain and lung. As shown in Table 1, no different genotyping was shown in the same patient.

The 30 clinical specimens (two of each patient) were successfully genotyped at 8 or more genetic loci. Among them, six suggestive genotypes were identified. According to analysis carried out in ToxoDB, the suggestive genotype of Patient 11 was identified as Toxo DB genotype #11. The other 5 suggestive genotypes identified in 14 patients were considered new types not reported previously. **TgHuDis1** was the most common, being found in 8 patients, including patient 1 (10 loci), patient 2 (11 loci), patients 5, 6, 12, 13, 14, 15 (8 loci). **TgHuDis3** was shown in patients 4 (9 loci) and 10 (8 loci); **TgHuDis5**, in patients 8 and 9 (8 loci). **TgHuDis2** and **TgHuDis4** were seen in patients 3 (9 loci) and 7 (8 loci) respectively. Table 1 shows the genotypes in details.

The clinical and laboratory data are shown in Table 2. The clinical records reported that all of them had brain histopathological results consistent with toxoplasmosis, including the identification of *T. gondii* antigens. They previously had an episode of cerebral toxoplasmosis and were successfully treated. Next they developed the disseminated infection, at least 6 months after the cerebral toxoplasmosis episode. Even with the second round of treatment (sulphadiazine, pyrimetamine and folinic acid), they died. Two patients had co-infections (patient 1, cytomegalovirus encephalitis; and patient 3, neurotuberculosis).

4. Discussion

Toxoplasmosis is an asymptomatic disease in the majority of infected individuals. The association of toxoplasmosis-AIDS usually occurs in the neurological

disease due to the opportunistic nature of the parasite. In Brazil, despite advances in the treatment of the co-infection, in the post-HAART era, the prevalence of symptomatic toxoplasmosis in AIDS patients is still high and with considerable morbidity and mortality, but the worst form of the infection, disseminated toxoplasmosis, is rare (Vidal et al., 2005; Pereira-Chioccola et al., 2009; Saadatnia and Golkar, 2012).

The cohort of 15 patients consisted in 8 males and 7 females, aged 24-59 years and the deaths occurred over six years, between 2005 and 2011. These cohort characteristics are rare and important for clinical epidemiological studies.

This study reports the genetic characteristics of *T. gondii* DNA extracted directly from human autopsies preserved in paraffin. The Brazilian studies showed high genetic diversity in Brazilian *T. gondii* populations (Dubey et al., 2012). The majority of these studies were done in animals. Parasites that survived in studied clinical samples were previously isolated in several experimental mice before mutilocus PCR-RFLP genotyping (Dubey et al., 2007; Pena et al., 2008; Dubey, et al., 2008a).

The study of *T. gondii* diversity selecting parasite DNA directly from clinical specimens is challenging, since chronic infection is characterized by tissue cysts and the absence of circulating parasites. Nevertheless, in symptomatic infection caused by any immunosuppression, *T. gondii* DNA can be detected in biological fluids (Vallochi et al., 2005; Khan et al., 2006; Ferreira et al., 2006; Ferreira et al., 2008).

The first studies using multilocus PCR-RFLP genotyping directly in human clinical samples analyzed two to four loci. The results were limited because recombinant strains were not diagnosed (Vallochi et al., 2005; Khan et al., 2006; Ferreira et al., 2006; Ferreira et al., 2008). Recent studies analyzed a larger number of loci (around 11 molecular markers) (Su et al., 2006). The results revealed a diverse population structure with large number of genotypes with typical Brazilian strains

(Dubey et al., 2007; Dubey et al., 2008a; Pena et al., 2008; Ferreira et al., 2011). Multilocus PCR-RFLP genotyping allowed determination of multiple loci using a small amount of DNA extracted from tissues, such as cerebrospinal fluid, amniotic fluid or blood, but only a small portion of clinical samples are genotyped (Ferreira et al., 2011). The advantage of multilocus PCR-RFLP genotyping in this study was that it allowed for extracting DNA from tissues preserved for at least 10 years. However, only a small portion of clinical samples was totally genotyped by multilocus PCR-RFLP genotyping using the 11 molecular markers.

Studies using DNA extracted directly from clinical specimens have disadvantages (Su et al., 2010; Ferreira et al., 2011). Some clinical samples have disadvantages of being *T. gondii* DNA; thus, molecular markers that amplify the locus with a single copy, such as APICO, PK1 and L358 are rarely amplified. These molecular markers have low sensitivity in PCR-RFLP compared with the B1 gene that is highly repetitive (35 copies in the genome) and used in PCR for molecular diagnosis (Burg et al., 1989; Colombo et al., 2005; Su et al., 2010). The suggestive genotypes in this study were determined when, at least, 8 loci were identified. The molecular markers SAG1, SAG2 (5'-SAG2 and 3'SAG2), SAG2 (New) and SAG3 showed good sensitivity (30/30). On the other hand, the molecular marker APICO, which amplifies a sequence in apicoplast showed poor sensitivity (12/30). The other disadvantage in this study was the impossibility of checking the virulence of *T. gondii* strains in experimental animals, since the autopsies were preserved in paraffin for at least 4 years. In addition, strain isolation in experimental mice from human clinical samples is very difficult and many animals are used. DNA controls were included in each reaction to control contamination in all experiment stages. The controls included *T. gondii* DNA and negative DNA for toxoplasmosis. Questionable results were retested to prevent contamination. The 30

clinical samples were genotyped successfully in 8 or more loci, but the same suggestive genotype was identified in autopsies of brain and lung of each patient.

Despite that multilocus PCR-RFLP genotyping for *T. gondii* DNA was collected from autopsy samples fixed in formal and embedded in paraffin, 6 genotypes were identified. Thus, these autopsy samples provided important information.

According to the analysis carried out in Toxo DB, one of them was identified as Toxo DB # 11 (Patient 11, died in 2009). This genotype was previously identified in chickens, capybaras, dogs, sheep, rabbits and cats and from different geographic regions of Brazil, Argentina and the USA (Dubey et al., 2007; Pena et al., 2008; Yai et al., 2009; Ragozo et al., 2010; Silva et al., 2014; Dubey et al., 2012). In another study, one strain belonging to Toxo DB # 11 was isolated from a baby infected through the congenital route (Carneiro et al., 2013). The strain was isolated in experimental mice and histopathological analysis verified that lung tissues were seriously injured causing mortality in the animals (Pinheiro et al., 2015). These occurrences confirm the importance of these animals as reservoirs for human infection also described by others (Ferreira et al., 2011; Silva et al, 2014; Pinheiro et al., 2015).

The other five identified suggestive genotypes had not been described in ToxoDB. We named them **TgHuDis** (*T, gondii*HumanDisseminated) **1, 2, 3, 4**, and **5**. The condition in which the patients died suggests that these suggestive genotypes can also be virulent. **TgHuDis1** was the most frequent as it was determined in 8 patients (1, 2, 5, 6, 12, 13, 14, 15). The deaths occurred in 2005 (2 patients), 2006 (2 patients), 2009 (1 patient), 2010 (1 patient) and 2011 (2 patients). **TgHuDis3** was identified in one patient (4) died in 2005 and the other (10) in 2008. Similarly, **TgHuDis5** was identified in 2 patients (8, 9), who died in 2008. **TgHuDis2** and **TgHuDis4** were identified in 1 patient (3), who died in 2005 and another (7), who died in 2007, respectively. Despite

the impossibility in characterize *in vivo* the virulence of isolated strains, these suggestive genotypes could be considered virulent since they caused severe tissue damage and had similar characteristics as Toxo# DB11. However, the immunological and genetic deficiencies of patients should also be regarded.

The 15 patients were diagnosed and treated in São Paulo at different times, with the site of infection being uncertain. These data can contribute to better understanding of the epidemiology of toxoplasmosis in humans. This study verified the possibility of performing multilocus PCR-RFLP genotyping in autopsy paraffin embedded and stored for a long time. The use of experimental animals was not necessary since the isolation of *T. gondii* strains in experimental mice was not possible. Thus, good practices in research and animal ethics were followed, as they prescribe that laboratory animals should only be used when they are really needed.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Legends to tables

Table 1. *T. gondii* genotypes determined in 30 human autopsy samples preserved in paraffin from 15 deceased patients (with disseminated toxoplasmosis)

Table 2. Clinical and laboratory diagnostic results of the 15 dead patients

Table 1

Clinical samples	Code/year of sample collection	Markers											Genotype
		SAG1	5'+3' SAG2	SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico	
Patient 1	A04/05/Lung	I	I	I	III	III	III	III	I	I	I	nd	**TgHuDis1**
	A04/05/Brain	I	I	I	III	III	III	III	I	I	I	nd	
Patient 2	A13/05/Lung	I	I	I	III	III	III	III	I	I	I	I	**TgHuDis1**
	A13/05/Brain	I	I	I	III	III	III	III	I	I	I	I	
Patient 3	A21/05/Lung	II	I	I	III	III	III	III	I	nd	nd	I	**TgHuDis2**
	A21/05/Brain	II	I	I	III	III	III	III	I	nd	nd	I	
Patient 4	A35/05/Lung	I	II	I	III	III	III	III	I	I	nd	nd	**TgHuDis3**
	A35/05/Brain	I	II	I	III	III	III	III	I	I	nd	nd	
Patient 5	A6/06/Lung	I	I	I	III	III	III	III	I	nd	nd	nd	**TgHuDis1**
	A6/06/Brain	I	I	I	III	III	III	III	I	nd	nd	nd	
Patient 6	A17/06/Lung	I	I	I	III	III	III	IIII	I	I	nd	nd	**TgHuDis1**
	A17/06/Brain	I	I	I	III	III	III	nd	I	I	nd	nd	
Patient 7	A21/07/Lung	I	II	I	III	I	nd	nd	I	III	I	nd	**TgHuDis4**
	A21/07/Brain	I	II	I	III	I	nd	nd	I	III	I	nd	
Patient 8	A11/08/Lung	I	II	II	III	nd	III	nd	nd	I	I	I	**TgHuDis5**
	A11/08/Brain	I	II	II	III	nd	III	nd	nd	I	I	I	
Patient 9	A21/08/Lung	I	II	II	III	nd	III	nd	nd	I	I	I	**TgHuDis5**
	A21/08/Brain	I	II	II	III	nd	III	nd	nd	nd	I	I	
Patient 10	A31/08/Lung	I	II	I	III	III	III	nd	nd	I	nd	I	**TgHuDis3**
	A31/08/Brain	I	II	I	III	III	III	nd	nd	I	nd	I	
Patient 11	A12/09/Lung	I	I	II	III	III	III	I	III	I	II	nd	**ToxoDB #11**
	A12/09/Brain	I	I	II	III	III	III	I	III	I	nd	nd	
Patient 12	A39/09/Lung	I	I	I	III	III	III	I	I	nd	nd	I	**TgHuDis1**
	A39/09/Brain	I	I	I	III	III	III	nd	I	nd	nd	I	
Patient 13	A02/10/Lung	I	I	I	III	III	III	I	nd	I	I	nd	**TgHuDis1**
	A02/10/Brain	I	I	I	III	III	III	I	nd	I	I	nd	
Patient 14	A6/11/Lung	I	I	I	III	III	III	nd	I	I	nd	nd	**TgHuDis1**
	A6/11/Brain	I	I	I	III	III	III	nd	I	I	nd	nd	
Patient 15	A21/11/Lung	I	I	I	III	nd	III	III	I	I	I	nd	**TgHuDis1**
	A21/11/Brain	I	I	I	III	nd	III	III	I	I	I	nd	

ToxoDB #11 was previously identified in chickens (TgCkBr89) and cats (TgCatBr82) in Brazil. This genotype is also known as type BrI. (published in ToxoDB http://toxodb.org/toxo/). nd, not determined.

Table 2.

Patient - Code [1]	Gender /Age (years)	Diagnosis [2] (clinical, images and laboratory data)	PCR *T. gondii*	Toxoplasmosis serology	CD4+ Lym/ counts[3]	HIV serology	Autopsy histopathology for *T. gondii*	Genotype
Patient 1 A04/05	M/39	disseminated toxoplasmosis and cytomegalovirus encephalitis	pos	nd	nd	pos	pos	**TgHuDis1**
Patient 2 A13/05	F/31	disseminated toxoplasmosis	pos	nd	159	pos	pos	**TgHuDis2**
Patient 3 A21/05	F/24	disseminated toxoplasmosis and neurotuberculosis	pos	nd	115	pos	pos	**TgHuDis3**
Patient 4 A35/05	M/39	disseminated toxoplasmosis	pos	nd	nd	pos	pos	**TgHuDis1**
Patient 5 A6/06	M/40	disseminated toxoplasmosis	pos	nd	21	pos	pos	**TgHuDis1**
Patient 6 A17/06	F/31	disseminated toxoplasmosis	pos	pos	83	pos	pos	**TgHuDis4**
Patient 7 A21/07	M/50	disseminated toxoplasmosis	pos	nd	nd	pos	pos	**TgHuDis5**
Patient 8 A11/08	F/38	disseminated toxoplasmosis	pos	nd	70	pos	pos	**TgHuDis5**
Patient 9 A21/08	F/48	disseminated toxoplasmosis	pos	nd	nd	pos	pos	**TgHuDis3**
Patient 10 A31/08	F/42	disseminated toxoplasmosis	pos	nd	236	pos	pos	**ToxoDB #11**
Patient 11 A12/09	M/30	disseminated toxoplasmosis	pos	nd	93	pos	pos	**TgHuDis1**
Patient 12 A39/09	M/40	disseminated toxoplasmosis	pos	nd	nd	pos	pos	**TgHuDis1**
Patient 13 A02/10	M/43	disseminated toxoplasmosis	pos	nd	117	pos	pos	**TgHuDis1**
Patient 14 A6/11	F/30	disseminated toxoplasmosis	pos	pos	nd	pos	pos	**TgHuDis1**
Patient 15 A21/11	M/59	disseminated toxoplasmosis	pos	pos	nd	pos	pos	**TgHuDis1**

[1] Paraffin block code/year of death and autopsy collection; [2] Diagnosis was defined by clinical, images and laboratory data as described in Materials and Methods section;[3] Number of CD4+ T lymphocytes/μl of blood; nd, not determined; neg, negative; pos, positive.

Highlights

- This study investigated the genetic features of *T. gondii*
- DNA was isolated directly in 30 autopsies of brain and lung
- Cohort of 15 HIV and disseminated toxoplasmosis patients were investigated
- The analysis was conducted by Mutilocus PCR-RFLP genotyping
- Six suggestive genotypes of *T. gondii* were determined