REVIEW ARTICLE

Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology†

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SUMMARY

Infections by the protozoan parasite *Toxoplasma gondii* are widely prevalent in humans and animals in Brazil. The burden of clinical toxoplasmosis in humans is considered to be very high. The high prevalence and encouragement of the Brazilian Government provides a unique opportunity for international groups to study the epidemiology and control of toxoplasmosis in Brazil. Many early papers on toxoplasmosis in Brazil were published in Portuguese and often not available to scientists in English-speaking countries. In the present paper we review prevalence, clinical spectrum, molecular epidemiology, and control of *T. gondii* in humans and animals in Brazil. This knowledge should be useful to biologists, public health workers, veterinarians, and physicians. Brazil has a very high rate of *T. gondii* infection in humans. Up to 50% of elementary school children and 50–80% of women of child-bearing age have antibodies to *T. gondii*. The risks for uninfected women to acquire toxoplasmosis during pregnancy and fetal transmission are high because the environment is highly contaminated with oocysts. The burden of toxoplasmosis in congenitally infected children is also very high. From limited data on screening of infants for *T. gondii* IgM at birth, 5–23 children are born infected per 10 000 live births in Brazil. Based on an estimate of 1 infected child per 1000 births, 2649 children with congenital toxoplasmosis are likely to be born annually in Brazil. Most of these infected children are likely to develop symptoms or signs of clinical toxoplasmosis. Among the congenitally infected children whose clinical data are described in this review, several died soon after birth, 35% had neurological disease including hydrocephalus, microcephaly and mental retardation, 80% had ocular lesions, and in one report 40% of children had hearing loss. The severity of clinical toxoplasmosis in Brazilian children may be associated with the genetic characteristics of *T. gondii* isolates prevailing in animals and humans in Brazil.

Key words: *Toxoplasma gondii*, Brazil, toxoplasmosis, humans, animals, clinical, congenital.

INTRODUCTION

Brazil is a large country with a human population of more than 190 million, and a booming economy. It is divided in to 26 states and a Federal district (Fig. 1). Most of the population is concentrated in the south with 41% of the population in the state of São Paulo. We have used abbreviated names of states in the following review; full names with human population are given in Fig. 1. We review the current status of *Toxoplasma gondii* infection in humans and animals. We have attempted to incorporate all published reports available to us on natural *T. gondii* infections, especially papers in Portuguese. We consulted original papers because in many instances information online was not correct. Detailed historical, serological, parasitological, clinical and genetic information on *T. gondii* infections in humans and other animals are summarized in Tables throughout the review.

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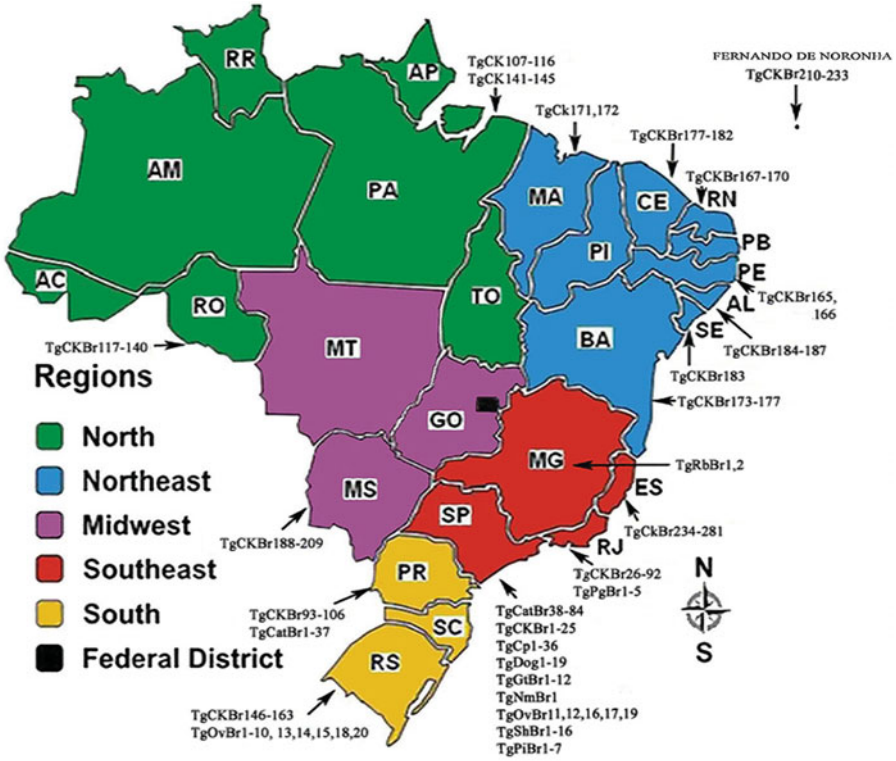

Fig. 1. Map of Brazil with 5 regions and distribution of human population, and sources of *Toxoplasma gondii* isolates genotyped. Figures in parenthesis are millions of people and % of the total population. State abbreviation—(population million, %): **AC** – Acre (0·7, 0·38%), **AL** – Alagoas (3·1, 1·64%), **AM** – Amazonas (3·4, 1·83%), **AP** – Amapá (0·6, 0·35%), **BA** – Bahia (14·0, 7·35%), **CE** – Ceará (8·4, 4·43%), **DF** – Distrito Federal (2·5,1·35%), **ES** – Espírito Santo (3·5, 1·84%), **GO** – Goiás (6·0, 3·15%), **MA** – Maranhão (6·5, 3·45%) **MS** – Mato Grosso do Sul (2·4, 1·28%) **MG** – Minas Gerais (19·5, 10·27%), **MT** – Mato Grosso (3·0, 1·59%) **PA** – Pará (7·5, 3·97%), **PB** – Paraíba (3·7, 1·97%), **PE** – Pernambuco (8·7, 4·61%), **PI** – Piauí (3·1, 1·63%), **PR** – Paraná (10·4, 5·48%), **RN** – Rio Grande do Norte (3·2, 1·66%), **RJ** – Rio de Janeiro (15·9, 8·38%), **RO** – Rondônia (1·5, 0·82%), **RR** – Roraima (0·4, 0·24%), **RS** – Rio Grande do Sul (10·6, 5·60%), **SC** – Santa Catarina (6·2, 3·28%) **SE** – Sergipe (2·0, 1·08%) **SP** – São Paulo (41·2, 21·63%) **TO** – Tocantins (1·3, 0·73%).

HISTORY

The parasite now called *Toxoplasma gondii* and the disease it causes called toxoplasmosis were first noted in 1908 in the rodent *Ctenodactylus gundi* in Tunisia by Nicolle and Mancaeux (1908, 1909), and in the domestic rabbit (*Oryctolagus cuniculus*) in Brazil by Splendore (1908). Dr Alfonso Splendore was a physician immigrant from Italy (Splendore, 1908, translated in 2009 into English). It is a remarkable coincidence that this disease was first recognized in laboratory animals and was first thought to be *Leishmania* by both groups of investigators. The parasite was named *Toxoplasma gondii* by Nicolle and Manceaux (1909).

Congenital toxoplasmosis was probably first recognized in Brazil in 1927 by Carlos Bastos Magarinos Torres (1927) who performed an autopsy on a 2-day-old girl in Rio de Janeiro. The child was born at term and had generalized muscular twitching and convulsions soon after birth. Predominant lesions were meningo-encephalomyelitis, myocarditis and myositis. Numerous protozoal bodies were found in histological sections of the central nervous system, heart, skeletal muscles, and subcutaneous tissue. Torres (1927) named the parasite *Encephalitozoon chagasi*. In retrospect the lesions and the morphology of the parasite are indicative of toxoplasmosis.

The first proven case of congenital toxoplasmosis was described by Drs Wolf, Cowen, and Page (1939) in a Caesarian-derived infant on 23 May 1938 at the Babies Hospital, New York, USA. Guimarães (1943) extensively reviewed worldwide reports of toxoplasmosis in humans and first described confirmed congenital toxoplasmosis in a Brazilian 14-month-old girl. She was born with hydrocephalus, suffered convulsions and ocular tremor, and subsequently radiographical examination revealed intracerebral calcification. The diagnosis was confirmed by bioassay in mice and guinea pigs inoculated with cerebro-spinal fluid (CSF) from the child. The strain of *T. gondii* from the child was virulent for dogs, pigeons, mice, rabbits, and guinea pigs. Guimarães (1943) also reported acute fatal toxoplasmosis in an 18-year-old Brazilian male from rural Rio de Janeiro. This man had fever, mononucleosis, headache, paresis of lower limbs, dysphasia, dyspnea, and died after an illness of 37 days. A post-mortem examination revealed pericarditis, hepatitis, splenitis, nephritis, and

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Table 1. Historical landmarks of *Toxoplasma gondii* and toxoplasmosis in Brazil

Finding	Reference
Aetiological agent	
Toxoplasma-like parasite found in rabbits	Splendore (1908)
Toxoplasma gondii propagated in cell culture	Guimarães and Meyer (1942)
Cytoskeleton of *T. gondii* tachyzoite detailed by electronmicroscopy	de Souza (1974)
Brazilian isolates of *T. gondii* found phenotypically and genetically different	Dubey *et al.* (2002); Lehmann *et al.* (2006)
***T. gondii* infection in humans**	
Congenital toxoplasmosis-like illness recognized in an human infant	Torres (1927)
First confirmed fatal case of congenital toxoplasmosis	Guimarães (1943)
First nationwide serological survey of Brazilian military recruits	Lamb and Feldman (1968)
Fatal toxoplasmosis in an adult described	Guimarães (1943)
Toxoplasma gondii isolated from an affected eye for the first time in Brazil	Melamed (1983, cited in Osorio,1986); Melamed (1992)
High prevalence of post-natally acquired ocular toxoplasmosis recognized	Silveira *et al.* (1988); Glasner *et al.* (1992*b*); Melamed (2009)
Neonatal screening of congenital toxoplasmosis initiated	Neto *et al.* (2000)
Major waterborne outbreak reported with isolation of viable *T. gondii* from municipal water	de Moura *et al.* (2006)
***Toxoplasma gondii* infection in other animals**	
Clinical toxoplasmosis diagnosed in a dog and a pigeon	Carini (1911)
Viable *T. gondii* isolated from food animal	Jamra *et al.* (1969); do Amaral and Macruz (1968, 1969)
Serological surveys in food animals initiated	do Amaral *et al.* (1978*a*, *b*)
Free range chickens used to estimate soil contamination due to oocysts nationwide	Dubey *et al.* (2002)
Toxoplasma gondii reported in aborted goat fetuses	Pescador *et al.* (2007)

bronchopneumonia. *Toxoplasma gondii* stages were seen in sections of several organs including encephalitic lesions in the brain. The histological picture was typical of recently acquired acute toxoplasmosis. Alencar and Schäffer (1971) histologically confirmed fatal congenital toxoplasmosis in 2 children in Rio de Janeiro. A few landmarks of the history of toxoplasmosis in Brazil are given in Table 1 and also reported by de Souza *et al.* (2009), Melamed (2009) and Fialho *et al.* (2009).

TOXOPLASMOSIS IN HUMANS

Prevalence of T. gondii *infection*

The discovery of a novel and specific serological test, the dye test, by Sabin and Feldman (1948) made it possible to conduct population-based surveys for this parasite. Different serological techniques used in Brazilian studies and their abbreviation are listed in Table S1 (online version only). In many instances commercial test kits were used and the manufacturers have changed over time. Cut-off values for serological tests are listed wherever the authors provided the information. Details of in-house tests are not listed in Table S1 or any subsequent tables.

Reports of serological surveys in Brazilians are summarized in Table 2. Among these reports, the study based on military recruits is most noteworthy because results could be compared with prevalence data in the USA (Feldman, 1965; Lamb and Feldman, 1968; Walls and Kagan, 1967; Walls *et al.* 1967). In this survey, sera were collected from young adult males (18–21 years old) in Brazil and the USA and sera from both countries were deposited at a World Health Organization Center in the USA where they were tested in an identical manner in 2 US laboratories (Feldman's lab [co-inventor of the dye test], and the Centers for Disease Control [CDC], Atlanta, Georgia). Results indicated that the seroprevalence of *T. gondii* was (and still is, Dubey, 2010*a*) 4 times (56% versus 13%) higher in Brazil than in the USA, and the magnitude of antibody titres were also higher (27% versus 1% at a titre of 1:256) in Brazil than in the USA. Results based on the dye test are shown in Table S2 (online version only). Similar results were obtained with the IHA test conducted at CDC on sera from both countries (Walls and Kagan, 1967; Walls *et al.* 1967); seroprevalence at IHA titre of 1:64 was 56·4% (Brazil) versus 24·4% (USA). Such a direct comparison of seroprevalence of *T. gondii* among countries has never been made elsewhere.

Serological prevalence data in children are summarized in Table 3. Up to 32% of 0–5 year olds, 19·5–59% of 6–10 year olds, and 28·4–84·5% of 11–15 year old children in Brazil were seropositive (Table 3). Limited data indicate that in certain areas approximately 50% of pre-teenage children have been exposed to the parasite (Table 3). Among these reports, Jamra and Guimarães (1981) provided

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Table 2. Serological prevalence of *Toxoplasma gondii* in the general population in Brazil

Year of sampling	Age group	Source of sera	Place	No. tested	% positive	Test	Cut-off value	Reference
1962	10 years or older	General sampling	Amapá, Macapá, Amazon	354	68·1	DT	1:16	Deane (1963)
1964	Males, 18–21 years	Military recruits	Nationwide	2022	56·0	DT	1:16	Lamb and Feldman (1968)
				2889	59·1	IHA	1:64	Walls and Kagan (1967)
No data	Adults	Blood donors	MG	729	50·3	DT, and or IFA	1:16	Araujo (1970)
1966	All ages	Indian tribe (total population 600, no cats)	Upper Xingu river, Indian reserve, central Brazil	254	51·6	IFA	1:16	Baruzzi (1970)
No data	Adults	Blood donors	RJ	284	83·8	IFA or DT	1:16	Coutinho *et al*. (1970)
1971–1975	20–50 years, urban	Out patient clinic	Ribeirão Preto, SP	115	83·5	IFA 1:16		Gomes *et al*. (1975)
	20–50 years, rural			115	77·4			
	20–50 years, rural, urban			115	73·0			
No data	All ages	General public	20 states	1410	61·0	IFA	1:16	Ricciardi *et al*. (1975, 1978)
No data	All ages	General public	Manasus, Amazon	51	59·0	IHA	1:28	Ferraroni *et al*. (1980)
			Roraima	51	54·0			
1971–1977	Adults	Ambulatory clinic	RJ	6079	78·7	IFA	1:16	Coutinho *et al*. (1981)
No data	3–21 years	Patients with tonsilitis	RJ	203	51·7	IFA	1:16	Amendoeira and Coutinho (1981)
1996–1997	5–78 years	Residents in rural area	Jaguatipã/PR	345	66·0	IFA	1:16	Garcia *et al*. (1999*c*)
1996–1998	All ages	Hospitalized patients (for any reason) in a teaching general hospital	Florianópolis/ SC	2994	41·9	IFA	1:16	Cantos *et al*. (2000)
1998	Students	Public schools	Rolândia/ PR	343	42·4	IFA	1:16	Giraldi *et al*. (2002)
1997–1999	Women, 12–49 years	Community	Goiânia/GO	3564	65·8	IFA	1:20	Avelino *et al*. (2003)
1997–1999	All ages	Low socioeconomic population from poor communities	Campos dos Goytacazes/RJ	316	84·0	UMELISA	15 IU/mL	Bahia-Oliveira *et al*. (2003)
		Middle-class population: children and adult staff from public schools, soldiers and adult members of their families	Campos dos Goytacazes/RJ	819	62·0			
		Upper socioeconomic population: children and adult staff from private schools	Campos dos Goytacazes/RJ	301	23·0			

1999	NS	Veterinary students	Campo Grande/MS	145	30·3	IHA[1]	1:16	de Araújo *et al*. (2000)
1999	All ages	Rural area	Governador Valadares/ MG	599	49·5	IFA	1:16	Portela *et al*. (2004)
2001	18–56 years	Blood donors (men)	Recife/PE	94	79·0	EIA[2]	1·0 (index)	Coêlho *et al*. (2003)
		Blood donors (women)		26	63·4			
2001	5–21 years	Students (of all socioeconomic levels)	Natal/ RN	959	46·0	MEIA	3 IU/mL	de Amorim Garcia *et al*. (2004)
		Students whose mothers were illiterate/elementary incomplete		509	53·2			
		Students whose mothers were university graduates		65	21·5			
2001	6–76 years	Amerindians Enawenê-Nawê	Mato Grosso State	148	80·4	IFA or ELISA	Not stated	Amendoeira *et al*. (2003)
2001	All ages	Amerindians from Iauareté region	São Gabriel da Cachoeira/AM	260	73·5 (95·6% of 46 >50 years)	IFA or ELISA	1:16	Bóia *et al*. (2008)
2001	11–77 years	Renal transplant patients	Belém/PA	82	82·9	EIA[1]	6 UI/mL	do Carmo *et al*. (2004)
2002	8 months-76years	Farmers	Monte Negro/ RO	266	73·0	IFA	1:16	Cavalcante *et al*. (2006*a*)
2003	15–18 years	High school students	São Jerônimo da Serra/ PR	133	50·4	IFA	1:16	Lopes *et al*. (2005)
2003	Adults	Slaughterhouse workers	Northern Paraná State	150	70·0	IFA	1:16	Gonçalves *et al*. (2006)
2006–2007	0–90 years	Potential solid organ donors	Santa Catarina State	96	68·0	Not stated	Not stated	do Amaral *et al*. (2008)
2008	18–35 years, females	Undergraduate students	Presidente Prudente/SP	80	33·8	ELISA[1]	15 UI/mL	Souza *et al*. (2010)
No data	Adults	Farm workers	Jauru micro-region/MT	116	97·4	IFA	1:40	Santos *et al*. (2009)
2008	All ages	Hospital laboratory	São Luis/MA	3037	66·3	ELFA	6 UI/mL	Costa *et al*. (2010)
No data	⩾ 17 years	Undergraduate students	Campo Grande/MS	100	39·0	EIA[4]	Not stated	de Figueiredo *et al*. (2010)

Table 3. Serological prevalence of *Toxoplasma gondii* antibodies in children in Brazil

Year	Place/state	Source	Test and cut-off	**Age in years**, % positive (number tested) **0–5**	**6–10**	**11–15**	Reference
No data	Rio de Janeiro/ RJ	Random	IFA 1:16	32·0 (63)	59·0 (103)	69·0 (58)	Coutinho *et al*. (1972)
No data	Presidente Prudente/ SP	Urban	IFA 1:256	No data	50·7 (71)	75·0 (52)	Corrêa *et al*. (1972)
		Rural			33·9 (103)	36·6 (112)	
No data	São Paulo city/ SP		DT 1:16	12·2 (90)	19·4 (163)	28·4 (144)	Jamra and Guimarães (1981)
1986	Bonsucesso, Rio de Janeiro/ RJ	Elementary school	IFA[1] 1:16	No data	36·5 (81)	70·4 (81)	Souza *et al*. (1987)
	Jacarepaguá, Rio de Janeiro/ RJ				64·0 (222)	84·5 (220)	
1997–1999	Campos dos Goytacazes/ RJ	Population based survey	UMELISA >15 IU/mL	No data	39·7 (189)[a]	45·5 (585)	Bahia-Oliveira *et al*. (2003)
1998	Rolândia/ PR	Elementary school	IFA 1:16	No data	42·0 (276)[b]	No data	Giraldi *et al*. (2002)
2007	Jataizinho/ PR	Elementary school	IFA 1:16	No data	46·4 (276)[c]	No data	Lopes *et al*. (2008)
2002	São Paulo county/ SP	Low income	IFA 1:16	12·5 (88)	33·8 (142)	51,5 (99)	Francisco *et al*. (2006)
1997	Fortaleza/ CE	Pre-school and school	EIA[1] >6 UI/mL	22·8 (227)	58·4 (584)		Rey and Ramalho (1999)
1997–2003	Salvador/ BA	6–11 year old, urban	ELISA[2] >1 IU/mL	13·7 (63)	19·5 (147)	75·0 (3)	Dattoli *et al*. (2011)[d]
1999	Melquíades/ MG (rural area)	Population based survey	IFA 1:16	47·0 (49)[e]			Portela *et al*. (2004)
2001	Natal/ RN	Students	MEIA >3 UI/mL	No data	36·5 (203)	48·6 (44·5)	de Amorim Garcia *et al*. (2004)
2004	Granada/ AC (rural Amazonia)	Population based survey	ELISA[f]	35·5 (107)			Ferreira *et al*. (2009)
2008	São Luis/ MA	Hospital laboratory	ELFA >8 IU/ml	43·1 (114)		66·2 (546)[g]	Costa *et al*. (2010)
2012	Fortaleza/ CE	Pregnant teenagers	ELISA[4][h] ⩾ 10 IU/mL			44·4 (27)[i]	Costa *et al*. (2012*a*)

[a] 0 to 9-year-old.
[b] 27·2% in 756 7-year-olds, 41·7% in 115 8 to 9-year olds, 31·1% in 86 10-year-olds.
[c] 4 to 11-year-old.
[d] Data in Table supplied by authors. Published data were: 13·7% of 459 in 6-year-olds, 15·5% in 419 6 to 7-year-olds, and 25·1% in 339 8 to11-year-olds.
[e] Children were 1–9 years old.
[f] Cut-off: average absorbance reading obtained with 15 negative control sera plus 3 standard deviations.
[g] 11 to 20-year-old.
[h] Personal communication to E. Lago.
[i] 12 to14-year-old.

seroprevalence data in 450 children, 0–15 years old, from a health centre in São Paulo. The percentages of seropositives were: 53·3% in children <1 year old, 0% in 2–3 year olds, 13·3% in 3–4 year olds, and 10% in 4–5 year olds, reaching to 43·3% in 15 year olds (Table 3). Seropositivity in infants <1 year old was attributed to antibodies transferred from their infected mother. *Toxoplasma gondii* antibodies were found in 4 of 30 (13·3%) 3–4 year old children but not in 2–3 year old children. In isolated Amerindians of Mato Grosso state, 6 of 12 children, 6–9 years old, were seropositive (Amendoeira *et al*. 2003).

Very high (36–92%) seroprevalences were found in pregnant women (Table 4). These data indicate that seroprevalence of *T. gondii* in children and in pregnant women in Brazil is one of the highest worldwide (Dubey and Beattie, 1988; Tenter *et al*. 2000; Dubey, 2010*a*).

Viable *T. gondii* was isolated from the tonsils of asymptomatic humans (Jamra *et al*. 1971; Amendoeira and Coutinho, 1982).

Congenital toxoplasmosis in children. Based on one report, ocular toxoplasmosis in congenitally infected children in Brazil was more severe than in children in Europe (Gilbert *et al*. 2008). This conclusion was based on comparison of ocular lesions in 30 children in Brazil with 281 children in Europe using similar methodology. In these 30 Brazilian children, the lesions in the eyes were more extensive than in the European children and more likely to involve the area of the retina affecting the central vision, in spite of the fact that most of the Brazilian children had been treated for toxoplasmosis for 12 months (Gilbert *et al*. 2008). This study concluded that the Brazilian children had a 5 times higher risk of severe toxoplasmosis than children in Europe. In another report, the risk of intracranial lesions detected by computed tomography (CT) scan was much higher in Brazilian children than in children in Europe (The SYROCOT, 2007). Some of these differences are thought to be related to the genetic makeup of the *T. gondii* strains in humans in Brazil but direct evidence for this hypothesis is lacking and difficult to obtain. However, this subject is intriguing. Perhaps further studies using a larger sample size as well as basic studies concerning pathogenesis of infections caused by different isolates may lead to further insight concerning the observations above. In this respect, we summarize published information on prevalence, and severity of congenital toxoplasmosis in Brazil.

An estimate of incidence or prevalence, and clinically manifest neonatal toxoplasmosis may be obtained from reports of observed cases, calculations based on the infection rate during pregnancy, and screening of children at birth. In the present review, we have listed all surveys in pregnant women and serological methods used. There is only limited information on the validity of commercial kits used, especially for the detection of IgM worldwide (Wilson *et al*. 1997; Calderaro *et al*. 2008; Remington *et al*. 2011); some of these kits were also used for testing sera of Brazilian women and children. However, for the past 2 decades, reagents and manufacturers of the commercial products have changed. Additionally, the performance of kits used for diagnosis of infections with Brazilian strains of the parasite has not been studied, and could be very different from results in other countries in terms of sensitivity, specificity, and positive predictive value. The accuracy of some of the commercial diagnostic kits, especially for the detection of IgM antibodies, is unsatisfactory (Remington *et al*. 2011). There is no national reference laboratory for *T. gondii* testing in Brazil.

Congenital toxoplasmosis detected by prenatal screening. There are very few reports of prenatal screening in Brazil. In a study of 2513 consecutive periparturient women at a hospital in Porto Alegre, RS, congenital toxoplasmosis was diagnosed in 4 infants (Lago *et al*. 2009*b*). Of these women, 1667 (67·3%), were already seropositive before pregnancy and thus unlikely to deliver congenitally infected children (Remington *et al*. 2011). In the 810 susceptible women, 3 infected infants were identified through testing of the mother at delivery, and 1 fetus was found infected during the second trimester of gestation; this child had hydrocephalus on fetal ultrasound and hepato-splenomegaly, microphthalmia, and retinochoroiditis at birth. This child had severe mental retardation at 5 years of age. The second child was asymptomatic at birth but had hepatomegaly when 3 weeks old. The other 2 also had retinochoroiditis. Fetal toxoplasmosis was diagnosed by polymerase chain reaction (PCR) on amionic fluid in 12 of 72 women with acute toxoplasmosis who were followed during pregnancy at a hospital in Belo Horizonte (de Faria Couto and Leite, 2004). Ultrasound examination was performed fortnightly and children were followed clinically for up to 1 year. Eight fetuses had signs of toxoplasmosis with bilateral ventricular enlargements, some accompanied by lesions in other organs; of these, 4 fetuses were stillborn, and 3 had retinochoroiditis with neurological abnormalities. The 4 surviving fetuses without ventricular lesions remained asymptomatic as infants for the first year of life (de Faria Couto and Leite, 2004) indicating the prognostic value of fetal ultrasound examination. In another study, severe clinical toxoplasmosis with hydrocephalus was found in an infant born to 1 of 75 women who acquired toxoplasmosis during pregnancy (Higa *et al*. 2010).

Varella *et al*. (2009) recorded acute toxoplasmosis in 41 per 10 000 pregnancies among 44 112 pregnant women submitted to prenatal screening for toxoplasmosis in Porto Alegre, RS. Acute toxoplasmosis was identified by the criteria recommended by the European Research Network on Congenital

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Table 4. Serological prevalence of *Toxoplasma gondii* in pregnant/delivering, or child-bearing aged women in Brazil

Year of sampling	Group	Source of patients	Place	No. tested	% positive	Test	Cut-off value	Reference
1974–1976	Pregnant	Clinics[a]	São Paulo/SP	120	50·0	DT	1:16	Jamra *et al.* (1979)
1990	Pregnant	Clinics	São Paulo/SP	1246	68·8	IFA	1:16	Guimarães *et al.* (1993)
1993	Pregnant	Clinics	Alagoas State	200	85·0	IHA[1]	1:16	dos Santos *et al.* (1994)
1996–1998	Pregnant/delivering	Maternity ward	Londrina/PR	1559	67·0	IFA	1:16	Reiche *et al.* (2000)
1997	Pregnant/delivering	Clinics, maternity wards	Fortaleza/CE	186	71·5	EIA[1]	>6 UI/mL	Rey and Ramalho (1999)
1997–1998	Pregnant	Clinics	Municipalities from Northwest RS State	2126	74·5	IFA	1:16	Spalding *et al.* (2003, 2005)
1997–1999	12–49 year old	Urban community	Goiânia/GO	2242	51·2	IFA	1:20	Avelino *et al.* (2004)
1998–2003	Pregnant	Clinic and maternity ward	Porto Alegre/RS	10468	61·1	MEIA	>3 UI/mL	Reis *et al.* (2006)
2000	Pregnant/delivering	Maternity ward	Porto Alegre/RS	1261	59·8	MEIA	>3 UI/mL	Varella *et al.* (2003)
2000	Pregnant/delivering	Maternity ward	Cuiabá/MT	205	70·7	EIA[3]	NS[b]	Leão *et al.* (2004)
2000	Pregnant	Clinics	Belém/PA	531	73·0	IHA[1]	NS	de Oliveira (2002)
2001	Pregnant	Clinics	Campinas/SP	2199	60·4	MEIA	2 UI/mL	Stella (2004)
2001–2002	Pregnant	Clinics	Bernardino de Campos/SP	308	71·4	ELFA	>8 UI/mL	Ferreira *et al.* (2007)
2001–2002	Delivering	Maternity ward	Passo Fundo/RS	1250	48·5	IFA/MEIA/ ELFA	NI/>3UI/ mL/>8UI/mL	Mozzatto and Procianoy (2003)
2002–2003	HIV negative pregnant/ puerperal women (median 24 years, range 12–45)	Maternity ward	Porto Alegre/RS	2421	67·0	ELFA	>8 UI/mL	Lago *et al.* (2009*b*)
	HIV infected pregnant/ puerperal women (median 27 years, range 16–42)			168	72·0			
2002–2003	Pregnant	Clinics	Mato Grosso do Sul State	32512	92·0	ELISA[5] (dried blood in filter paper)	NS	Figueiró-Filho *et al.* (2005)
2003	Pregnant	Clinic	Ipatinga/MG	49	75·5	Several commercial kits	NS	Coelho *et al.* (2003)
2003	Pregnant	Community based	Cascavel/CE	231	69·7	ELFA	>8 UI/mL	Heukelbach *et al.* (2007)
2003–2004	Pregnant	Clinics	Londrina/PR	5839	56·6	MEIA	>3 UI/mL	Mandai *et al.* (2007)
2003–2004	Pregnant	Clinics	Curitiba/PR	20389	53·0	MEIA/CLIA	>3 UI/ mL/>8 UI/mL	Vaz *et al.* (2010)
2004	Pregnant, 15–49 years	Patients attended by one laboratory	Caxias do Sul/RS	1065	36·8	ELFA	>8 UI/mL	Detanico and Basso (2006)
2004–2005	Pregnant	Clinic	Recife/PE	503	77·5	IFA[1]	1:16	Porto *et al.* (2008)

2004–2005	Delivering	Maternity ward	Belo Horizonte/MG	420	57·8	Several commercial kits	NS	Carellos *et al*. (2008)
2005	Pregnant/delivering	Maternity ward	Fortaleza/CE	963	68·6	MEIA	> 3 UI/mL	Sroka *et al*. (2010)
2005–2006	Delivering	Maternity ward	Taubaté/SP	392	52·8	Several commercial kits	NS	Kawasaki *et al*. (2006)
2005–2006	Pregnant	Clinics	São José do Rio Preto/SP	232	57·3	IHA[2]	1:32	Galisteu *et al*. (2007)
2005–2006	Pregnant	Clinics	Cascavel/PR	334	54·8	IFA and ELISA	NS	Mioranza *et al*. (2008)
2005–2006	High-risk	High-risk clinic	Santa Maria/RS	408	66·4	MEIA	> 3 UI/mL	Beck *et al*. (2010)
2005–2007	High-risk	High-risk clinic	São José do Rio Preto/SP	87	64·4	IFA	1:16	de Mattos *et al*. (2011)
2006	Pregnant	Clinics	Vitória/ES	1153	73·5	CLIA	> 8·8 UI/mL	Areal and Miranda (2008)
2006	Pregnant	Clinics	Londrina/PR	492	49·2	CLIA	> 8·8 UI/mL	Lopes *et al*. (2009)
2006	Pregnant	Clinics	Pelotas/RS	425	54·8	DPC Immulite	⩾ 8 II/mL	Cademartori *et al*. (2008)
2007	Pregnant	Clinics	Sergipe State	9139	69·3	ELISA [4] and [5]	NS	Alves *et al*. (2009); Inagaki *et al*. (2009)
2007	Pregnant	Clinics	Natal/RN	190	66·3	MEIA	> 3 UI/mL	Barbosa *et al*. (2009)
2007–2009	Pregnant	Clinics	Rolândia/PR	607	55·1	IFA or ELISA[1]	1:16 or 15	Dias *et al*. (2011)
2008	Pregnant	Clinics	Bahia State	2229	58·2	ECL	> 3·0 UI/ml	Rebouças *et al*. (2011)
2008	Pregnant	Clinics	Rolândia/PR	287	55·1	IFA/MEIA/ ELISA[1]	1:16/> 3 UI/mL/ 15 UI/mL	Dias *et al*. (2011)
2008	Pregnant	Clinics	Goiânia/GO	10 316	67·7	ELISA[5] (dried blood in filter paper)	> 8 UI/ml	Sartori *et al*. (2011)
2009–2010	Pregnant, 12–19 years	Clinics	Fortaleza/CE	214	45·3	ELISA[4][c]	⩾ 10 IU/mL	Costa *et al*. (2012*b*)
2009–2010	Pregnant	Clinics	Palotina and Jesuítas/PR	422	59·9	ELISA[6] and MEIA	> 50 IU/mL and >3 UI/mL	Bittencourt *et al*. (2012)

[a] Antenatal clinics.
[b] NS: Not stated, as recommended by the manufacturer.
[c] Personal communication to E.Lago.

Toxoplasmosis (Lebech *et al*. 1996) plus IgG avidity test and PCR testing in amniotic fluid. The rates of acute toxoplasmosis decreased from 66 per 10 000 pregnancies in 2001 to 21 per 10 000 pregnancies in 2005. Twenty-five cases of congenital toxoplasmosis were diagnosed at birth, and 12 additional cases were diagnosed at follow-up during the first year of life, resulting in a prevalence of congenital toxoplasmosis of 9 per 10 000 live births (Varella *et al*. 2009).

Congenital toxoplasmosis detected by post-natal filter paper screening. Table 5 summarizes congenital toxoplasmosis identified through screening of children at birth or their mothers. Most of these reports were based on determination of IgM antibodies on blood collected on filter papers. Based on data in Table 5 the prevalence of congenital toxoplasmosis was 5–23 cases per 10 000 live births. In the largest sampling involving 800 164 infants from 27 states in Brazil (Neto *et al*. 2010), 496 infected (average 1 per 1613, range 0–20 per 10 000 infants) were identified. The variation in rate of congenital toxoplasmosis in various samples maybe partly related to the seroprevalence of *T. gondii* in pregnant women; in some regions >90% of women of child-bearing age are seropositive before pregnancy and thus not likely to deliver a *T. gondii*-infected baby. However, in most regions of Brazil the seroprevalence of *T. gondii* in pregnant women is between 50 and 80% and, although the proportion of susceptible pregnant women is still small, these women are a high risk for infection because they live in a highly contaminated environment.

Human immunodeficiency virus (HIV) infection and congenital toxoplasmosis. Concurrent HIV infection of the mother may alter the course of toxoplasmosis during pregnancy. De Azevedo *et al*. (2010*a*) and Fernandes *et al*. (2009) reported congenital transmission in 4 infants born to HIV- infected women in Brazil but indicated that this event is rare. Seroprevalences in women with or without HIV are generally similar (Neto and Meira, 2004). Seroprevalence of *T. gondii* in HIV-positive women (72% of 168) was only slightly higher than in women without HIV (67% of 1624) and no previously *T. gondii*-infected HIV-positive women delivered a *T. gondii*-infected child as documented by Lago *et al*. (2009*a*). However, *T. gondii* has been transmitted from HIV-infected women with chronic *T. gondii* infection to their children (de Azevedo *et al*. 2010*a*). Recently, Delicio *et al*. (2011) reported congenital transmission of HIV and other concurrent infections in 15 (13 women were not on highly active antiretroviral therapy [HAART]) of 452 HIV-infected women; *T. gondii* infection was found in 6 children. On the face of it this appears to be a high rate of transmission of *T. gondii*, but methods used for diagnosis were not stated.

Congenital toxoplasmosis from chronically infected women. In immunocompetent women transmission of *T. gondii* usually occurs when the mother acquires infection during pregnancy. Rarely, congenital transmission has been documented from mothers infected in the months before the pregnancy. Silveira *et al*. (2003) reported congenital toxoplasmosis in a baby whose mother had evidence of past infection before the current pregnancy; she had been diagnosed serologically with ocular toxoplasmosis 20 years previously. She had no known immunocompromise but details of methods used to evaluate immunosuppression were not provided (Remington *et al*. 2011). Elbez-Rubinstein *et al*. (2009) reported a case of severe toxoplasmosis as a result of re-infection during pregnancy, and reviewed 5 previous cases of congenital transmission from chronically infected women. One of the hypotheses for this rare event is re-infection with a highly virulent parasite with atypical *T. gondii* genotype (Lindsay and Dubey, 2011). In this respect travel to Brazil is a focus of attention (Kodjikian *et al*. 2004; Anand *et al*. 2012) because Brazilian strains of *T. gondii* are genetically different from those prevalent in Europe and North America (Lehmann *et al*. 2006). In the case described by Kodjikian *et al*. (2004) the mother was a resident of Switzerland for 6 years but born in Brazil and had travelled to Brazil during the fifth month of gestation. Recently, Andrade *et al*. (2010) described a most unusual ocular toxoplasmosis in a mother and her baby. The baby was born asymptomatic but was found to have bilateral retinochoroiditis and had IgM and IgG antibodies to *T. gondii*. The mother had clinical retinochorioditis and *T. gondii* antibodies 10 years before the current pregnancy giving birth to the infected child. During the current pregnancy the mother had clinical retinochorioditis, stable IgG antibodies and no IgM antibodies to *T. gondii*.

Clinical toxoplasmosis in congenitally infected children. Most congenitally infected children are asymptomatic at birth and some do not manifest symptoms until later in childhood, or even in adult life (McLeod *et al*. 2009; Delair *et al*. 2011; Remington *et al*. 2011). The most common manifestation of congenital toxoplasmosis is ocular disease, sometimes presenting as retinochoroiditis, cataracts, strabismus or nystagmus, and total blindness. This pattern was observed in studies in Brazil, although most children were not followed past 12 months of age. Depending on how the sample was obtained, some studies are more useful for demonstrating the prevalence of congenital toxoplasmosis. Other studies are case series reported by symptoms, which although not able to determine prevalence, demonstrate the wide range and the possible severity of clinical manifestations (Table 6). The most accurate information with respect to clinical disease during the neonatal period was provided by studies by Vasconcelos-Santos *et al*.

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Table 5. Prevalence of congenital toxoplasmosis in Brazil

Year of sampling	Source of samples	Place	Cases/number tested	Prevalence in 10 000 live born infants (Mid-P 95% confidence interval)	Confirmatory serologic test for IgM	Cut-off (index)	Reference
1995–2002	FPUS[a]	Several states in Brazil	195/364 130	5 (5–6)	MEIA	⩾ 0·600	Neto *et al*. (2004)
1998	FPUS	Campos dos Goytacazes/RJ	5/2550	20 (7–43)	ELFA	> 0·65	Bahia-Oliveira *et al*. (2001)
1998	VBSSMAC[b]	Municipalities from Northwestern RS State	3/2126	14 (4–38)	ELISA[3]	⩾ 1·10	Spalding *et al*. (2003)
2001	FPUS	Ribeirão Preto/SP	7/15 162	5 (2–9)	EIA[1]	[d]	Carvalheiro *et al*. (2005)
2001–2002	CBSSDR[c]	Passo Fundo/RS	1/1250	8 (0·4–39)	ELFA	> 0·65	Mozzatto and Procianoy (2003)
2002	FPUS	Porto Alegre/RS	6/10 000	6 (2–12)	ELFA	> 0·65	Lago *et al*. (2007)
2002–2003	VBSSMAC	Porto Alegre/RS	3/2476	12 (3–33)	ELFA	> 0·65	Lago *et al*. (2009*b*)
2003–2004	FPUS	Belo Horizonte/MG	20/31 808	6 (4–10)	ELFA	> 0·65	de Andrade *et al*. (2008)
2004–2005	CBSSDR	Belo Horizonte/MG	1/420	23 (1–114)	Not stated	Not stated	Carellos *et al*. (2008)
1998–2005	VBSSMAC	Porto Alegre/RS	37/40 727	9 (6–13)	MEIA/ELFA	⩾ 0·600/> 0·65	Varella *et al*. (2009)
1995–2009	FPUS	Several states in Brazil	496/800 164	6 (6–7)	MEIA	⩾ 0·600	Neto *et al*. (2010)
2006–2007	FPUS	Minas Gerais State	190/146 307	13 (11–15)	ELFA	> 0·65	Vasconcelos-Santos *et al*. (2009)
2009	FPUS	Belém/PA	1/1000	10 (0·5–49)	ELFA	> 0·65	Bichara *et al*. (2012)

[a] Filter paper, universal neonatal screening.
[b] Venous blood, screening survey of mothers in antenatal clinics.
[c] Cord blood, screening survey in a delivery room.
[d] Optical densities ⩾80% of the control cut-off.

Table 6. Clinical toxoplasmosis in congenitally-infected children in Brazil

Reference	Bahia *et al.* (1992)	Bahia-Oliveira *et al.* (2001)	Melamed *et al.* (2001)	Sáfadi *et al.* (2003)	Neto *et al.* (2004)	Carvalheiro *et al.* (2005)	Lago *et al.* (2007)	Gilbert *et al.* (2008)	Melamed *et al.* (2009)	Vasconcelos-Santos *et al.* (2009); de Resende *et al.* (2010)	Rodrigues *et al.* (2009)	Lago *et al.* (2009*b*)
Place	Belo Horizonte/MG	Campos dos Goytacazes/RJ	Porto Alegre/RS	São Paulo/SP	countrywide	Ribeirão Preto/SP	Porto Alegre/RS	Campos/RJ and Porto Alegre/RS	Rio Grande do Sul State	Minas Gerais State	Goiânia/GO	Porto Alegre/RS
Sample	Referred to an infectious diseases clinic, toxoplasmosis confirmed	Universal neonatal screening	Referred to an ophthalmology clinic after diagnosis	Referred to an infectious diseases clinic because of symptoms	Universal neonatal screening	Universal neonatal screening	Universal neonatal screening	Neonatal screening	Referred to an ophthalmology clinic after diagnosis	Universal neonatal screening	Mothers with acute toxo in pregnancy	Universal delivery screening
Infected (prevalence)	96	5/2550 (20/10000)	22	43	195/364130 (5/10000)	7/15162 (5/10000)	6/10000 (6/10000)	30	44	190/146307 (13/10000)	28/50	4/2476 (16/10000)
Examined	**96**	**9** (5 plus 2 from a previous pilot study and 2 identified with symptoms)	**22**	**43**	Not stated	**5**	**6**	**30**	**44**	**178** ophthalmic examination (Vasconcelos-Santos 2009), **106** (examined for hearing and language at 18 months-2 years, Resende *et al.* 2010)	**28**	**4**
Sex	52% females		54·5% males	54% males		66·7% males	50%		54·5% females	54·2% males		75% males
Birthweight				84% ⩾ 2500 g		2060–3790 g	2980–3200 g					1290–3150 g
Age at last examination	2 days-11 years	1–6 months	2 months-8 years	<1 month-5 years	1 month-7 years	2 days- 19 months	1 month-2 years	1 day-11 years	2 days-1 year	1 day-2 months	3 days-1 year	1–5 years
Deaths	No deaths	2 died with systemic generalized disease	No deaths	No deaths	1 died with immunosuppression	No deaths	No deaths	No deaths	No deaths	4 deaths	1 died soon after birth with systemic generalized disease	No deaths
Abnormal physical examination (except strabismus)		3/9 (30%)				2/5 (40%)	4/6 (66%)			101/190 (53%)	10 (36%)	2 (50%)
Symptomatic (physical examination and/or complimentary investigation)		5/9 (56%)	22 (100%)	43 (100%)	57	5/5 (100%)	5 (83%)	At least 20 (67%) – ocular involvement	At least 31 (70%) – ocular involvement	At least 142 (80%) – ocular involvement	16 (57%)	4 (100%)
General physical and laboratory findings		HS 1			HS 6, LAD 1	HS with jaundice 2 Petechiae 1 Ascites 1 Elevated ALT and AST 2	HS 3			HS		HS 2

ICC		1/9 (11%)	16 (73%)	28 (65%)	14	2 (40%)	4 (67%)		39 (21%)		4 (100%)
Other than ICC neurological findings		Psychomotor abnormality 1	Hydrocephalus 2	Cortical atrophy with ventricular dilatation 13	Hydrocephalus 1	Hydrocephalus 1	Hydrocephalus 1		Hydrocephalus 12 (6·3%)	Hydrocephalus 6 (21%)	Hydrocephalus 1
		Cerebral ventricular dilatation 1	Hydranencephaly 1	Neurological sequelae 22	Microcephaly 1	Cerebral ventricular dilatation 1	Mental retardation 1		Microcephaly 10 (5·3%)	Neuro-psychomotor disfunction 2	Mental retardation 1
		Microcephaly 1	Cerebral ventricular dilatation 4		Mental retardation 3	Microcephaly 1			Language deficits 28 (26%)		Cerebral ventricular dilatation 1
						Elevated CSF protein 2			Neurological impairment 19 (18%)		Microcephaly 1
Hearing impairment						None impaired	None impaired		46/106 (43%) Resende *et al.* 2010	2 /28 (7%)	None impaired
Eye lesions											
Examined	**96**	**7**	**22**	**43**		**5**	**6**	**30**	**44**	**178**	**28**	**4**
Sedated	No	No	Yes	No		No	No	No	Yes	No	No	No
RC	74 (77%)	2 (29%)	22 (100%)	41 (95%)	32	5 (100%)	4 (67%)	20 (67%) 31/60 eyes (52%)	29 (66%) 51/81 eyes (63%)*	142 (80%) 255/356 eyes (72%)	8 (29%)	4 (100%)
Active RC	1 (1%)					2 (40%)	2 (33%)		8/51 eyes (16%)	85/142 patients (60%) 142/255 eyes (56%)		4/4 patients (100%)
Bilateral	50/74 (68%)	1 (12%)		36 (84%)			3 (75%)	12 (60%)	22 (76%)	113 (80%)		3 (75%)
Macular	61/96 patients (82%)	1/8 patients (12%)	35/44 eyes (80%)			2/5 patients (40%)		25/31 eyes (81%)	39/51 eyes (76%)	165/255 eyes (65%)		5/8 eyes (63%)
Microphthalmia	18 (19%)	1 (12%)		4 (9%)	1		1 (17%)		6 (14%)	14 (8%)		1 (25%)
Blindness					2		1 (17%)					1 (25%)
Strabismus	44 (46%)	1 (12%)		21 (49%)		3 (60%)	2 (33%)		12 (27%)			
Nystagmus	3 (3%)	1 (12%)		20 (47%)					7 (16%)			
Cataract	12 (13%)			1 (2%)					6 (14%)	2 (1%)		
Turbid vitreous humor	3/96 patients (3%)					1/5 patients (20%)	2/4 patients (50%)		5/44 patients (11%)	26/356 eyes (7%)		1/4 patients (25%)
Visual impairment							3 patients (50%)	27/31 eyes (87%)				4 patients (100%)

CT, congenital toxoplasmosis; ICC, intracranial calcification; RC, retinochoroiditis; RS, retinal scar; HS, hepato-splenomegaly; LAD, lymphadenopathy; CSF, cerebrospinal fluid; ALT, alanine aminotransferase ; AST, aspartate aminotransferase.

* 7 eyes presented medial opacities, which prevented fundus examination.

(2009). Unlike other studies in Brazil, 178 (93·7%) of 190 children were examined ophthalmologically at a median of 56 days of age and all children were born in the state of Minas Gerais. Most (142, 79·8% of 178) infants had some evidence of ocular disease (Table 6). The authors state that some peripheral ocular lesions might have been missed because the children were not sedated during eye examinations. Viable *T. gondii* was isolated from blood of 27 children and the scientific community is waiting for results of genotyping of these isolates. Hearing was evaluated in 106 of these children (de Resende *et al*. 2010). Forty-six children had hearing dysfunction; 13 had conductive hearing loss, 4 had sensorineural hearing loss, and 29 had central hearing dysfunction. Additionally, there was an association between hearing problems and language deficits. The percentage of children with hearing loss in this study is much higher than reported for treated or untreated congenitally infected children in North America or Europe (Olariu *et al*. 2011; Remington *et al*. 2011).

Melamed *et al*. (2001, 2009) examined under sedation the eyes of 44 <1 year-old congenitally infected children. Thirty-one of 44 (70·4%) children had ocular disease and retinochoroiditis was the most common (65·9%) lesion. The retinochoroiditis was bilateral in 22 cases, lesions were active in 8 eyes and had healed in 43 children. This study indicated that, like the study from Minas Gerais, a high proportion of children with ocular disease were observed earlier than studies from other countries (McLeod *et al*. 2009; Remington *et al*. 2011). These findings are contrary to what has been seen in treated children in Europe and North America and might be related to treatment (McLeod *et al*. 2006, 2009).

Nationwide estimates of congenital toxoplasmosis in Brazil. Data summarized in Table 5 indicate a wide range of 5–23 congenital infections per 10 000 births. Most of the studies were based on selected sampling because there is no national screening of women or children for toxoplasmosis in Brazil. Often the sampling was based on who could afford the testing and under these circumstances there will be under representation of samples from low economic groups. Based on observations in Europe and USA, there is also the possibility of false negativity because many infants with congenital toxoplasmosis are negative for IgM antibodies at birth (Guerina *et al*. 1994; Lebech *et al*. 1999; Remington *et al*. 2011). It is also important to note the issue of false positivity if the newborn IgM tests are not confirmed with follow-up confirmatory tests in the mother and infant. The specificity of various IgM tests used is also of concern (Remington *et al*. 2011). As stated earlier, there is no national laboratory for confirmation of *T. gondii* serological testing in Brazil.

The most accurate figures on congenital toxoplasmosis prevalence are provided by the study by Vasconcelos-Santos *et al*. (2009). In this study, blood samples were collected from 146 307 newborns at 1560 public health care centres in 853 cities in the state of Minas Gerais. All serological testing was performed in one laboratory initially using an IgM-ELISA capture test kit (Toxo IgMQ-Preven, Symbiosis, Leme, Brazil) and results were confirmed on further testing for IgA antibodies (enzyme-linked immunosorbent assay) and IgG and IgM anti-*T. gondii* (enzyme-linked fluorometric assay, VIDAS, BioMérrieux SA, Lyon, France), using blood samples from infants and their mothers. Additionally, infected children were followed clinically months after delivery (Vasconcelos-Santos *et al*. 2009; de Resende *et al*. 2010). Congenital toxoplasmosis was suspected in 235 infants (1 in 622), and confirmed in 190 children (1 in 770 live births). This figure of 1 per 770 live births does not include *in-utero* mortality due to toxoplasmosis nor infants negative for IgM antibodies at birth.

According to the Brazilian Institute for Geography and Statistics (IBGE) (http://www.ibge.gov.br/home/estatistica/populacao/projecao_da_populacao/2008/projecao.pdf) 2, 649 396 live births are projected in Brazil in 2015. If one assumes a rate of 1 infected child per 1000 births, 2649 children infected with congenital toxoplasmosis are likely to be born yearly in Brazil. Most of these infected children are likely to develop some symptoms or signs of clinical toxoplasmosis (Table 6).

Currently, there are no estimates of cost of caring for these infected children in Brazil, but financial burden on families and the government will be high. Based on the 1992 cost of living, the human illness losses due to congenital toxoplasmosis were estimated to be up to 8·8 billion dollars in the USA based on 1 infected child per 1000 live births (Roberts *et al*. 1994). During the past 2 decades medical costs have sky rocketed (Stillwaggon *et al*. 2011). As stated earlier the morbidity due to congenital toxoplasmosis in Brazil is much higher than in the other parts of the world (Gilbert *et al*. 2008; Melamed *et al*. 2009). Clinical data on congenitally infected children in Brazil in the last 2 decades are summarized in Table 6. Based on those studies whose methodology allows us to estimate morbidity rates, approximately 35% of children had neurological disease including hydrocephalus, microcephaly and mental retardation, approximately 80% had ocular lesions, and in one report 40% of children had hearing loss. Several deaths occurring soon after birth, as a result of congenital toxoplasmosis, have been described. It is remarkable that in 2 studies from Minas Gerais, 2 decades apart, the percentages of children with retinal disease are similar (77% of 74, Bahia *et al*. 1992, and 80% of 178 Vasconcelos-Santos *et al*. 2009).

As stated earlier, currently, there are no economic estimates with respect to impact of clinical toxoplasmosis in Brazil. Recently, Stillwaggon *et al*. (2011)

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provided an extensive guideline for estimating costs of preventive maternal screening for and the social costs resulting from toxoplasmosis based on studies in Europe and the USA. While estimating these costs, the value of all resources used or lost should be considered, including the cost of medical and non-medical services, wages lost, cost of in-home care, indirect costs of psychological impacts borne by the family for life-time care of a substantially cognitively impaired child; cost of fetal death was estimated to be $5 million dollars (Stillwaggon *et al*. 2011).

Although it is unethical to value human life in terms of dollars, each nation has to balance public funding for all the needs of its people, including prevention of crippling ailments. Brazil has one of highest rates of *T. gondii* infection (50–80%) in women before their first pregnancy. Therefore, these seropositive women are considered immune, and can be excluded from future screening for toxoplasmosis if the infection in Brazil is the same as documented so carefully in France by Desmonts and Couvreur (1974) in earlier decades. At issue are pregnant women who are seronegative; their chances of acquiring *T. gondii* infection during pregnancy are high because the environment is highly contaminated with oocysts. Preventive measures (hygiene education, possible immunization if a vaccine for toxoplasmosis becomes available) should start in elementary schools since as many as 50% of 10-year-old children in many localities in Brazil had been exposed to *T. gondii* (Table 3).

Post-natal toxoplasmosis

Pregnant women. It has been suggested by Avelino *et al*. (2003) that women in Brazil are more susceptible to *T. gondii* infection during pregnancy but there is no definitive evidence for this assumption. However, it is a fact that pregnancy induces immunosuppression and thus toxoplasmosis maybe more severe clinically in pregnant than in non-pregnant women. Judging from the published information it seems that only a small proportion of women who become infected in pregnancy have recognized symptomatic clinical toxoplasmosis. In Lyon, France, where all pregnant women are tested for *T. gondii* infection, only 36 (5% of 603) recalled any clinical symptoms simulating toxoplasmosis (Dunn *et al*. 1999). In Europe, the severity of toxoplasmosis in the fetus or the infant is not related to the degree of symptoms of *T. gondii* infection in the mother (Dunn *et al*. 1999; Gilbert *et al*. 2001), but such data are not available for Brazil. Limited information suggests that clinical toxoplasmosis in the pregnant woman may be more severe in Brazil than in Europe. In a prenatal follow up of 204 women who became infected with *T. gondii* during pregnancy, 58 (28·4%) were symptomatic (Castilho-Pelloso *et al*. 2007). These symptoms included headaches in 58 (100%), concomitant symptoms in 45 (77·5%) with visual disturbances, myalgia in 35 (58%), and lymphadenopathy with fever in 24 (41·3%).

Immunocompetent persons. Clinical toxoplasmosis is rarely recognized in immunocompetent adults in Brazil, except in special circumstances. Silva *et al*. (2008) reported acute toxoplasmosis in a group of 313 patients who were seen because of toxoplasmosis like symptoms at a specialized hospital in the city of Rio de Janeiro from 1992 to 2004. Records of these patients were studied retrospectively, and the inclusion criteria were serology for acute infection including IgM and IgA testing, and symptoms. Most of these (65·5%) were child-bearing age women (27·2% pregnant). Clinical signs or symptoms were noted in 261; lymphadenopathy in 59·8%, fever in 27·2%, headache in 10·7%, weakness in 10·0%, weight loss in 8·4%, myalgia in 8%, and hepatosplenomegaly in 1·5%. Nine patients developed retinochoroiditis, 7 had ocular lesions at the time of admission and 2 developed lesions 2 years after initial visit. Of particular interest is that 26 symptomatic patients were children 10 years or younger; to our knowledge this is one of the first reports of clinically acquired toxoplasmosis in children in Brazil. Similar clinical signs were documented by Neves *et al*. (2009) who enrolled 37 symptomatic patients (22 males, 15 females) in a 30-month prospective study. To be included in the sampling, patients had to present with at least 1 of the following signs or symptoms of acquired toxoplasmosis: fever, lymph node enlargement, weight loss or retinochoroiditis. These patients in 2006–2008 attended a clinic in Rio de Janeiro and had ascending IgM and IgG antibody titres to *T. gondii*. Frequency (%) of symptoms and laboratory findings in these 37 patients were: lymph node enlargement 94·6, asthenia 86·5, headache 70·3, fever 67·6, weight loss 67·2, and retinochoroiditis 10·8.

Unusually severe acute toxoplasmosis was noted in two 41-year-old men who had fever, myalgia, nausea, and severe headache; these were unrelated reports (Leal *et al*. 2007; de Souza Neves *et al*. 2011). The first patient from São Paulo was admitted to a hospital with an 8-day history of fever, myalgia, and headache followed by 4 days of nausea and vomiting (Leal *et al*. 2007). On the second day of hospitalization he developed pulmonary insufficiency with bilateral pneumonitis. He was successfully treated with sulfadiazine, pyrimethamine, corticosteroids and folinic acid. The diagnosis was supported by positive findings of IgM and IgG antibodies to *T. gondii*.

The other patient was admitted to an emergency unit of a hospital in Rio de Janeiro also with a history of fever, myalgia, nausea, and severe headache (de Souza Neves *et al*. 2011). He later developed

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meningeal signs, pneumonia, and cervical lymphadenopathy. Diagnosis was supported by increasing levels of IgG and IgM antibodies. The patient was treated successfully with anti-*T. gondii* therapy comprised of intravenous clindamycin and oral pyrimethamine.

Acute toxoplasmosis outbreaks. An epidemic of febrile adenopathy simulating toxoplasmosis was observed in 1966 in a University in São José dos Campos, 100 km from São Paulo city (Magaldi *et al*. 1967, 1969). Between March and May 1966, 99 out of 500 students became ill. Symptoms reported were: fever in 79, lymph node enlargement in 61, asthenia in 52, headache in 32, sore throat in 17, and myalgia in 10. High titred *T. gondii* antibodies were found in most patients, and titres were still rising 6 months later. Another group of 22 people (not students) also had a similar syndrome. No risk assessment was performed because the life cycle of *T. gondii* was unknown at that time.

An outbreak of toxoplasmosis was reported in people from a farm in rural Além Paraíba, Minas Gerais (Coutinho *et al*. 1982*b*). Nine of 36 persons living on a dairy farm developed illness characterized by fever, headache, and lymphadenopathy; all of them had serological evidence of acute toxoplasmosis. The illness was noted in May 1976, one month after the farmer had a party and served barbecued pork from a pig killed on the farm. The source of *T. gondii* infection was not determined. Viable *T. gondii* was isolated from soil samples collected from the farm but the year of soil sampling was not stated (Coutinho *et al*. 1982*a*). Two outbreaks were circumstantially linked to eating mutton (Bonametti *et al*. 1997*b*) or pork (de Almeida *et al*. 2006), and in both of these instances a child developed acute toxoplasmosis after drinking mother's milk. Sixteen of 17 people who feasted on raw mutton while attending a party in Paraná, Brazil in September, 1993 became ill, all 16 developed fever, headaches, myalgia, arthralgia, and cervical lymphadenopathy, and 1 also had retinochoroiditis (Bonametti *et al*. 1997*a*, *b*). Among these patients was a mother with a nursing child. The child developed fever, malaise, and irritability, and had both IgG and IgM antibodies; and the child was fed exclusively on mother's milk. The mother's illness began 3 weeks before the child became ill.

The second case of acquired toxoplasmosis was in a 2-month-old baby diagnosed by one of the authors (Eleonor Lago). The infant was fed exclusively on mother´s milk. The mother had symptoms of acute toxoplasmosis beginning 1 month after delivery, including fever. Ten members of the same family (including this mother and her baby), and another 1-year-old child from a different mother had acute acquired toxoplasmosis. Eight of 10 were symptomatic, with cervical lymphadenopathy and myalgia in 8, fever and night sweats in 7, and headache in 6 patients. Another adult woman had acute active toxoplasmic retinochoroiditis. The family had consumed raw pork sausage at a party in Santa Vitória do Palmar, RS (de Almeida *et al*. 2006).

In May 1999, 113 people at a university campus had evidence of lymphoglandular toxoplasmosis, thought to be associated with contamination of food and water with *T. gondii* oocysts at the university cafeteria (Gattás *et al*. 2000-published only as an abstract of a meeting). There were more than 200 cats on the campus. No new cases were observed when filtered (2 μm filter to remove larger particles including *T. gondii* oocysts) water was served, and efforts were made to control the cat population.

One of the largest outbreaks of clinical toxoplasmosis occurred in Santa Isabel do Ivaí Paraná (Daufenbach *et al*. 2002; de Moura *et al*. 2006). The outbreak peaked between November 2001 and January 2002. A total of 426 persons had IgM and IgG antibodies to *T. gondii* out of 2884 serologically tested (area population 6771). Of these 156 persons participated in the clinical study. The main symptoms were headache (87%), fever (82%), myalgia (80%), lymphadenopathy (75%), anorexia (69%), arthralgia (61%), night sweats (53%), vomiting (38%), and rash (7%) (de Moura *et al*. 2006). Subsequently, 408 patients from this outbreak were examined for ocular lesions and IgG and IgM *T. gondii* antibodies; 18 had typical lesions of retinochoroiditis (15 unilateral, 3 bilateral), 24 had atypical superficial retinal lesions. Ten women seroconverted during pregnancy, 6 babies were born with congenital toxoplasmosis, 4 with ocular lesions, and 1 with neurological signs. One woman had lesions in both of her eyes and both eyes of her infant also were affected (Silveira, 2002; Dubey, 2010*a*). This outbreak was epidemiologically linked to a cistern that supplied municipal water. Viable *T. gondii* was isolated from water tanks on roof tops that temporarily stored water (de Moura *et al*. 2006). Viable *T. gondii* isolates were also obtained from a cat that was associated with a water cistern, domestic cats from homes in the city (Dubey *et al*. 2004) and feral chickens from the city centre and adjoining area in Santa Isabel do Ivaí (Dubey *et al*. 2003). Although no attempts were made to isolate *T. gondii* from sick people a seroepidemiological study based on peptide typing of sera from patients from the outbreak linked the infection to the isolate from the water tank (Vaudaux *et al*. 2010).

Ocular toxoplasmosis. Ocular disease is probably the most common potentially severe symptomatic manifestation in acute, post-natally acquired toxoplasmosis (Holland, 2009). Until the 1980s, most of *T. gondii* retinochoroiditis was thought to be congenital (Holland, 2003). Ophthalmologists from Brazil first reported retinochoroiditis in multiple siblings, and in

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patients who acquired infection later in life (Silveira *et al*. 1988, 2001). These findings have now been amply confirmed in many countries. Currently, most of the eye disease is thought to be post-natally acquired because <1% of the population becomes infected congenitally (Glasner *et al*. 1992*a*, *b*). The prevalence of ocular toxoplasmosis in Brazil is considered to be high (Table S3, online version only). Glasner *et al*. (1992*b*) reported 17·7% prevalence of ocular toxoplasmosis in patients examined at Clínica Silveira in Erechim, southern Brazil. Erechim is mainly rural with a temperate climate and predominantly Italian, German, and Polish immigrant population. A door-to-door survey identified 1042 subjects (63% of the population) who participated in the study; all were examined for ocular lesions and had blood drawn for *T. gondii* serology. Prevalence increased with age; 4·3% of those 9–12 years old, 14·3% of those 13–16 years old and 24·6% of those 17–20 years old had ocular lesions. All but 1 patient (183 of 184) had antibodies to *T. gondii* and the prevalence was similar in males and females. This prevalence of ocular toxoplasmosis in Erechim is more than 10-fold higher than the prevalence in the USA (Jones and Holland, 2010).

A follow-up study performed a decade later evaluated risk factors associated with ocular toxoplasmosis in the same locality (Jones *et al*. 2006). For this study, 131 infected and 110 uninfected controls were selected from the patients with eye disease who were evaluated at the Clínica Silveira for 12 months starting June 2003. All infected patients had IgG and IgM antibodies to *T. gondii*, indicating recently acquired infection. The controls were patients without *T. gondii* antibodies and seen at the same time as infected patients. Age, gender, race and ethinicity data were recorded, and all participants completed a detailed questionnaire. Salient risk factors associated with toxoplasmosis were: eating rare meat, eating home-made cured, dried or smoked meat, having a garden, having soil-related activity, being male, and past and present pregnancy (Jones *et al*. 2006). The association of pregnancy and the number of children as a risk factor for toxoplasmosis is intriguing and has been observed previously in Brazil (Avelino *et al*. 2003).

Another impressive population-based study on ocular toxoplasmosis prevalence was reported by Portela *et al*. (2004). A door-to-door survey was conducted in rural Melquíades, northeast Governador Valadares, MG within a 100 km area of a village. A total of 414 persons were enrolled in the study. Half (49%) of them had *T. gondii* antibodies with a very high (47% of 49) seroprevalence in children less than 9 years old. A total of 29 of 414 (7%) persons had ocular lesions; 28 of these were seropositive, and 1 was seronegative. Overall, 28 (12·9%) of 216 seropositives had ocular lesions, and only 1 (0·5%) of 198 seronegatives had ocular lesions suggestive of toxoplasmosis. None of the 49 children had ocular toxoplasmosis, although 47% were seropositive. These data affirm that most ocular toxoplasmosis is post-natally acquired. A retinal scar was the most common lesion and predominated in persons older than 50 years. Shared residence was a risk factor for ocular toxoplasmosis, suggesting a common source of seropositivity among household members. It will be seen from data summarized in Table S3 that the prevalence of ocular toxoplasmosis differs with respect to region and the age groups studied. Using similar methods prevalence was 10-fold higher in Erechim (25·5% in patients up to 21 year olds, Glasner *et al*. 1992*b*) than in Natal (1·15% in 5–21 year- olds, de Amorim Garcia *et al*. 2004). Ocular lesions were found in 11 of 959, 5 to 21-year-old students attending public schools in Natal; lesions were bilateral in 1 student but with 20/20 vision (de Amorim Garcia *et al*. 2004). Overall, lesions were less severe in these students than in patients in Erechim.

As stated earlier, although most reports of ocular toxoplasmosis were from the Clínica Silveira in Erechim, the disease is probably common in the rest of the Brazilian population, and toxoplasmosis has been recognized as an important cause of uveitis in Brazil since late 1970's (Belfort *et al*. 1978; de Abreu *et al*. 1980, 1982; Petrilli *et al*. 1987; Silveira *et al*. 1987; Pinheiro *et al*. 1990; Glasner *et al*. 1992*b*; Schellini *et al*. 1993; Reis *et al*. 1998*a*, *b*; Sebben *et al*. 1995; Abreu *et al*. 1998; de Carvalho *et al*. 1998; Jorge *et al*. 2003; Gouveia *et al*. 2004; Oliveira and Reis, 2004; do Carmo *et al*. 2005; Alvarenga *et al*. 2007; Haddad *et al*. 2007; Nóbrega and Rosa, 2007; Lynch *et al*. 2008; Aleixo *et al*. 2009; de Souza and Casella, 2009; Lynch *et al*. 2009; Melamed, 2009; Diniz *et al*. 2011; Mattos *et al*. 2011). Even though toxoplasmosis can affect any part of the eye, retinochoroiditis is its hallmark (Silveira *et al*. 1989; Hayashi *et al*. 1997; Holland *et al*. 1999; Silveira *et al*. 2001; Silveira, 2002; Yamamoto *et al*. 2003; Eckert *et al*. 2007; Oréfice *et al*. 2007; Lynch *et al*. 2008; Commodaro *et al*. 2009; Melamed *et al*. 2009; Bottós *et al*. 2009; de Souza *et al*. 2009; Belfort *et al*. 2010; Arevalo *et al*. 2010; Delair *et al*. 2011). Ocular toxoplasmosis is the main cause of uveitis worldwide, and in Brazil it is responsible for 70% of the cases (de Amorim Garcia *et al*. 2004). In retrospective studies conducted more than 20 years ago, bilateral toxoplasmic macular scars, optic atrophy, and congenital cataracts were the main cause of reduced vision in children in Brazil (Kara-José *et al*. 1988; Buchignani and Silva, 1991; de Cavalho *et al*. 1998).

In human ocular toxoplasmosis, the parasite multiplies in the retina and inflammation occurs primarily in the choroid; the choroid alone is not affected. Early lesions of acquired toxoplasmosis are unknown because eyes are often not examined until the infection is symptomatic. Holland *et al*. (1999)

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reported retinal vasculitis without necrosis in 10 Brazilian patients who had recently acquired toxoplasmosis. Eckert *et al*. (2007) reported optic nerve involvement in 5·3% of ocular toxoplasmosis in Brazil and in 23 of 51 eyes, optic nerve lesions preceded retinal lesions. Clinical diagnosis of ocular toxoplasmosis is difficult in the absence of retinal lesions, and it is often difficult to clinically distinguish congenital versus acquired toxoplasmosis, in both instances ocular lesions may develop several years after infection. However, in congenital infection ocular lesions are more often bilateral, serum IgG antibodies are often low in titre, and IgM is rarely detectable. Antibody levels in ocular patients may differ with respect to patients from different countries; levels of IgG were higher in Brazilian versus Swiss patients (Garweg *et al*. 2005). The severity of ocular toxoplasmosis may be influenced by the high virulence of the *T. gondii* genotype (Vallochi *et al*. 2005; Bottós *et al*. 2009).

Definitive cure of ocular toxoplasmosis without recurrence is not possible because available antitoxoplasmic medicines are not effective in killing tissue cysts present in the retina. Recurrences of retinochoroiditis in Brazilian patients are common in spite of treatment (Silveira *et al*. 2002). A recent study showed that viable *T. gondii* can circulate in patients with eye disease in both acutely and chronically infected patients (Silveira *et al*. 2011).

Toxoplasmosis in HIV-infected patients. The HIV epidemic in the 1980s brought recognition of cerebral toxoplasmosis in adults, resulting from reactivation of latent infection. The percentage of *T. gondii* seropositive persons with AIDS that develop clinical toxoplasmosis varies. In the USA approximately 10% of seropositives developed clinical toxoplasmosis whereas this percentage was 25–30% of the seropositives in Europe; reasons for this variability are unknown (Luft and Remington, 1992). Cerebral toxoplasmosis was definitively diagnosed in 8–34% of AIDS patients in Brazil who were examined at autopsy (Rosemberg *et al*. 1986; Chimelli *et al*. 1992; Camara *et al*. 2003; Weinstein *et al*. 1992; Cury *et al*. 2003; de Souza *et al*. 2008; Silva *et al*. 2012; Table S4, online version only). However, *T. gondii* seroprevalence was not determined in these persons that were examined post-mortem. Passos *et al*. (2000) retrospectively analysed records of 73 AIDS patients considered to have toxoplasmic encephalitis, 38 patients in 1988 (group A), and 33 patients in 1993 (group B) at the main hospital in São Paulo. *Toxoplasma gondii* antibodies were found in 81·2% (25 of 31) in group A and 61·5% (16 of 26) in group B patients; 21·1% (8 of 38) in group A and 30·3% (17 of 33) in group B died of toxoplasmosis. However, criteria for patient selection and diagnosis were ill defined. Neurological signs or symptoms, CT scan and anti-*T. gondii* therapy were considered in case selection; however, CT scan and *T. gondii* serological examination were not performed on all patients. It is worth noting that the definitive diagnosis of cerebral toxoplasmosis should not be made based solely on the CT scan because lymphomas and other conditions may be mistaken for toxoplasmosis (Mentzer *et al*. 2012).

There are other reports of toxoplasmosis in AIDS patients from different regions of Brazil (Chahade *et al*. 1994; Nascimento *et al*. 2001; Nobre *et al*. 2003; Alves *et al*. 2010*a*, *b*; Correia *et al*. 2010). The incidence of cerebral toxoplasmosis in AIDS patients is now drastically reduced after the institution of highly active antiviral therapy (HAART). In one report based on 1138 HIV-infected patients admitted to a hospital in São Paulo, 115 (10%) were diagnosed with neural toxoplasmosis (Vidal *et al*. 2005). In 35% of these patients, neural toxoplasmosis led to the diagnosis of HIV infection and in 75% cerebral toxoplasmosis was the AIDS-defining disease. Of these 115 patients, 55 were followed clinically, 40 had headache and hemiparesis, 28 had confusion, 25 had fever, 11 had alterations of cranial nerves, 8 had visual alterations and 5 were ataxic. Of these 55 patients, cerebral toxoplasmosis was diagnosed at autopsy in 2 patients who had died within 2 weeks of initiation of HAART and anti-*T. gondii* therapy. De Oliveira *et al*. (2006) reported a high (42·3%) prevalence of neural toxoplasmosis among 417 HIV patients admitted to hospital in Belo Horizonte, MG.

In most AIDS patients, toxoplasmosis is due to reactivation of latent infection and lesions are restricted to the central nervous system (CNS). Of 92 AIDS patients from a reference hospital in Brazil, examined at post-mortem in 1993–2000, 8 were diagnosed with toxoplasmosis, all with CNS involvement (Cury *et al*. 2003). In the brain, the predominant lesion is necrosis, often resulting in multiple abscesses, some of which are as large as a tennis ball. These abscesses often blend with normal tissue in which numerous tachyzoites and tissue cysts are present. As many as 1 million tachyzoites per ml or gramme of affected tissue can be present (Dubey, 2010*a*). Tissue cysts are often seen at the periphery and often differ in size. Such lesions are now rarely seen in patients treated for toxoplasmosis and HIV. Although any part of the brain may be involved, lesions are more common in the basal ganglia and appear as ring-enhancing lesions (Cota *et al*. 2008). Vidal *et al*. (2005) noted diffuse cerebral necrosis in 2 patients that were examined at autopsy. These atypical diffuse cerebral infections might be caused by atypical genotypes of *T. gondii* (Ferreira *et al*. 2011). According to Pereira-Chioccola *et al*. (2009) 20% of AIDS patients in Brazil have these atypical cerebral lesions.

In a few AIDS patients toxoplasmosis is generalized, affecting many organs. Barbosa *et al*. (2007) reported disseminated toxoplasmosis in 2 AIDS

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patients confirmed at autopsy. Severe myocarditis was found in 1 patient (Nobre *et al*. 2003). Ocular toxoplasmosis has been reported in 4–8% of AIDS patients (Rehder *et al*. 1988; Muccioli *et al*. 1994; Matos *et al*. 1999; Arruda *et al*. 2004; Zajdenweber *et al*. 2005; Alves *et al*. 2010*a*,*b*), including a 13-month-old child (Moraes, 1999).

In the early days of the AIDS epidemic, diagnosis of cerebral toxoplasmosis was confirmed at autopsy (Table S5, online version only) or by needle biopsy of lesions suspected by computer tomography (CT) scan. Currently, diagnosis is aided by attempts to demonstrate live *T. gondii* parasites, antibodies to *T. gondii* or *T. gondii* antigens or *T. gondii* DNA in blood or CSF or even in saliva (Borges and Figueiredo, 2004; Vidal *et al*. 2004; Colombo *et al*. 2005; Meira *et al*. 2008; Nogui *et al*. 2009; Correia *et al*. 2010; Mesquita *et al*. 2010*a*,*b*; Meira *et al*. 2011). Obviously, use of peripheral blood for diagnosis is less invasive and good results were obtained using quantitative serology and DNA detection in cerebral toxoplasmosis (Vidal *et al*. 2011).

EPIDEMIOLOGY

The ingestion of oocysts from the environment and the consumption of meat infected with tissue cysts are the two most important modes of transmission of *T. gondii*. Determination of sources of infection is technically difficult because by the time *T. gondii* infection is diagnosed the original source of infection may not be demonstrable. Table S5 (online version only) summarizes some of the risk assessment studies for *T. gondii* infection in Brazil. Much of this epidemiological information was dependent on the type of questions asked and the answers obtained. The environment in many areas in Brazil is highly contaminated by oocysts, and thus it is difficult to pinpoint sources of infection. We will attempt to summarize available information regarding oocyst shedding and infection in meat animals. Among pregnant women, lower socio-economic level, lower level of education, higher age, soil handling, and contact with cats were considered the most important risk factors for *T. gondii* infection (Table S5, online version only).

Transmission by oocysts

Cats (both domestic and wild) are the only animals that can excrete *T. gondii* oocysts. A cat can excrete millions of oocysts, which can survive in the environment for months, depending on moisture and temperature (Dubey, 2010*a*). Free-roaming domestic cats are abundant in public places in Brazil. Relatively little is known of the prevalence of *T. gondii* in cats in Brazil, and 7 of 15 surveys were from the São Paulo State (Table 7). Seroprevalence was low in cats sampled in clinics, but these were probably pets and were most likely fed processed food (Table 7).

Of these surveys, the most comprehensive study was that reported by Pena *et al*. (2006). In that study an equal number of male and female (118 males, 119 females) stray cats were captured from 15 counties in São Paulo State in 2003. Antibodies to *T. gondii* were found in 84 (35·4%) of 237 cats. Tissues of 71 seropositive cats were bioassayed in mice and viable *T. gondii* was isolated from 66·2% (47 cats).

During the epidemiological study of a waterborne outbreak in Santa Isabel do Ivaí, Paraná, 58 adult cats were obtained from 51 houses around this town (Dubey *et al*. 2004). All cats were serologically tested as well as by bioassay, irrespective of their antibody status. Antibodies to *T. gondii* were found in 49 (84·4%) of 58 cats, and viable *T. gondii* was isolated from 37 of 54 (68·5%) of these cats. This study indicated that more than 80% of homes in this area had a *T. gondii*-infected cat.

Cats start shedding *T. gondii* within 10 days of consuming infected tissues and they shed oocysts only for 1–2 weeks (Dubey and Frenkel, 1972). During the period of oocyst shedding cats are rarely ill and they do not have antibodies to *T. gondii*. Thus, it is a reasonable assumption that most seropositive cats have already shed oocysts. Therefore for epidemiological studies, seroprevalence data are more meaningful than determining the prevalence of oocysts in feces. Moreover, at any given time-period only 1% of cats are found shedding oocysts (Jones and Dubey, 2010). This low rate of fecal positivity of oocysts was also exemplified in the report by Pena *et al*. (2006); *T. gondii* oocysts were found in only 3 of 237 (1·2%) cats. Early reports from Brazil also indicate a low prevalence of *T. gondii*-like oocysts in cat feces (Barbosa *et al*. 1973; Nery-Guimarães and Lage 1973; do Amaral *et al*. 1976*b*; Ogassawara *et al*. 1980; Chaplin *et al*. 1991).

Based on 12 million cats, a seropositivity of 25–50%, and shedding of 1 million oocysts per cat there could be large numbers of oocysts in the environment in Brazil. In addition to domestic cats, wild felids can shed oocysts. *Toxoplasma gondii* oocysts have been demonstrated in feces of several species of naturally and experimentally infected wild felids (Jones and Dubey, 2010). Brazil is home to several species of wild Felidae, especially in zoos (Table S6, online version only).

Epidemiological surveys, especially in pre-teen children (Table 3) imply that the environment is highly contaminated with oocysts, especially in lower socio-economical communities. Dos Santos *et al*. (2010) found *T. gondii* oocysts in 7 of 31 soil samples from 31 elementary public-school playgrounds in the northwest area of São Paulo State. This is an alarming rate of soil contamination due to *T. gondii* oocysts. Coutinho *et al*. (1982*a*) also found *T. gondii* in soil samples from a farm where an outbreak of *T. gondii*

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Table 7. Prevalence of *Toxoplasma gondii* antibodies in domestic cats in Brazil

State, city or area	Type of cat		Test	No. tested	% positive	Cut- off titre	Reference
Paraná							
Jaguapitã	Stray	Rural	IFA	163	73·0	16	Garcia *et al*. (1999*b*)
51 homes around Santa Isabel do Ivaí	Pet	Urban	MAT	58	84·4	20	Dubey *et al*. (2004)
VC[a], Curitiba	Pet	Urban	IFA	282	16·3	16	Cruz *et al*. (2011)
Rio de Janeiro							
ZC[b], Niterói	Stray	Urban	IHA[1]	41	24·4	Not stated	Netto *et al*. (2003)
Zoo, Rio de Janeiro city	Stray	Urban	IHA[1]	118	72·0	16	Mendes-de-Almeida *et al*. (2007)
Rio Grande do Sul							
Porto Alegre	Pet	Urban	IHA	27	40·7	64	Chaplin *et al*. (1984)
VC, Porto Alegre	Pet	Urban	IHA	49	10·2	64	Braccini *et al*. (1992)
VC, Porto Alegre	Pet	Urban	IHA	100	37·0	64	de Araújo *et al*. (2003)
VC, Porto Alegre	Pet	Urban	IFA	245	37·9	16	Pinto *et al*. (2009)
			IHA[2]	245	26·9	64	
Pernambuco							
Fernando de	Stray	Urban	MAT	48	66·6	25	Costa *et al*. (2012*a*)
Noronha island	Pet		IFA	25	72·0	16	
	Pet		MAT	45	44·4	25	
Rondônia							
Monte Negro (western Amazon)	Stray	Rural, farms	MAT	63	87·3	25	Cavalcante *et al*. (2006*b*)
Manaus	Not stated	Rural /urban	IHA	32	81·0	128	Ferraroni *et al*. (1980)
Santa Catarina							
Lages	Pet	Urban	IFA	300	14·3	64	Rosa *et al*. (2010)
São Paulo							
São Paulo city	Stray	Urban	DT	130	5·1	2	Sogorb *et al*. (1972)
VC, University of São Paulo	Pet	Urban	IFA	248	17·7	16	Lucas *et al*. (1999)
São Paulo city	Not stated	Urban	IHA	100	59·0	64	Santos *et al*. (1983)
São Paulo city[b]	Stray, pet	urban	IFA	251	20·3	16	Sobrinho *et al*. (2012)
VC, University of Botucatu	Not stated	No data	MAT	100	19·0	16	da Silva *et al*. (2002)
ZC, São Paulo city	Stray	Urban	ELISA	100	40·0		Meireles *et al*. (2004)
15 counties	Stray	Urban/rural	MAT	237	35·4	25	Pena *et al*. (2006)
Guarulhos and São Paulo city	Stray, pet	Rural	MAT	502	26·3	20	Silva *et al*. (2002)
ZC, Araçatuba city	Pet	Urban	IFA	400	25·0	64	Bresciani *et al*. (2007)
Guarani Indian settlements, Krucutu and Morro da Saudade	Stray	Rural	IFA	28	53·5	64	Ortolani *et al*. (2005)
Andradina city	Not stated	Rural	IFA	70	15·7	64	Coelho *et al*. (2011)

[a] Veterinary clinic.
[b] Zoonosis Center.

had occurred. In earlier studies in Porto Alegre, RS, Chaplin *et al*. (1991) found *Toxoplasma*-like oocysts in feces of 13 of 15 young cats, and Braccini *et al*. (1992) reported oocysts in feces of 5 of 25 cats. However, microscopic diagnosis was not confirmed by bioassays in mice. Drinking water could be easily contaminated with oocysts (Bahia-Oliveira *et al*. 2003). Technically, it is difficult to find *T. gondii* oocysts in water because the number of oocysts in water is low due to the dilution factor (de Moura *et al*. 2006).

Another epidemiological means to assess soil contamination due to oocysts is to determine *T. gondii* prevalence in animals that feed from the ground. The authors have used free-range (FR) chickens (*Gallus domesticus*) for this purpose. This collaborative project was initiated by 2 of us (J.P. Dubey and S. Gennari) in 2000. Our initial objectives were to determine the prevalence of *T. gondii* infection in FR chickens, and isolate viable *T. gondii* to study genetic diversity. Subsequently, these studies were extended to other livestock and wild

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animals. Chickens were obtained from individual properties that were approximately 1 km apart. The number of chickens from one property was no more than 6 to minimize the clustering effect. Chickens were purchased, killed, bled, and serologically and parasitologically tested. Attempts were made to bioassay 50 or more chickens from each area irrespective of the serological status of chickens. Infected chickens were found on most properties or individual houses sampled (Table 8).

In addition to indicators of soil contamination these infected chickens could be a source of infection for cats and possibly humans. These FR chickens are frequently slaughtered at home and viscera are often not properly disposed off. Although chickens are usually cooked well before human consumption, improper hygiene while handling and cooking chickens could be a source of infection for people.

Oocyst shedding by wild felids. A large number of wild felids in most of the zoological parks and breeding centres in Brazil had antibodies to *T. gondii* (Table S6, online version only). Pena *et al*. (2011) isolated viable *T. gondii* from muscles of a captive jaguarandi that died of trauma. There is no information concerning prevalence of *T. gondii* in free-ranging wild felids in Brazil. The high seropositivity in captive wild felids suggests that they have already shed oocysts and contaminated the zoo environment. In isolated Amerindians, Mato Grosso, 80·4% of 148 people surveyed had *T. gondii* antibodies (Amendoeira *et al*. 2003). These people live on a large area with little contact with non-Indians, do not have pet cats, and do not eat meat. They eat insects and vegetables, including mushrooms. The authors speculate that *T. gondii* oocysts excreted by wild felids in the area could contaminate soil and vegetation. In one report 86·3% of 95 free-ranging Amazon River dolphins from Amazonas were seropositive to *T. gondii* (Table S7, online version only). These herbivorous dolphins most likely became infected by ingesting river waters contaminated with oocysts from wild felids (most likely jaguars) because domestic cats are unlikely in this environment.

Role of dogs in transmission of T. gondii. There have been no epidemiological studies to assess transmission of *T. gondii* from dogs to people in Brazil but antibodies to *T. gondii* have been reported widely in dogs in Brazil (Table S7, online version only). How dogs become infected with *T. gondii* is unknown. They do serve as indicators of environmental contamination with *T. gondii* because of close association with humans. Higher *T. gondii* prevalence in stray and farm dogs than in pets suggests that eating infected prey is an important source of infection (de Souza *et al*. 2003).

Transmission by infected meat

Millions of food animals are slaughtered for human consumption yearly in Brazil. Serological surveys indicate that up to 90% of domestic and wild animals had antibodies to *T. gondii*, and viable *T. gondii* was isolated from a variety of animals in Brazil. Details of isolation by bioassays are as follows:

Pigs. Up to 90% of pigs surveyed in Brazil had *T. gondii* antibodies (Table 9), and viable parasites were isolated from tissues of pigs (Table 10). Jamra *et al*. (1969) tested 83 samples of pork from butcher shops, and grocery stores in São Paulo city but there is no information on the number of pigs that were the sources for these pork samples; 5 samples contained viable *T. gondii*. At about the same time Amaral and Macruz (1968, 1969) found viable *T. gondii* in 8 of 25 diaphragms, also from São Paulo. Frazão-Teixeira *et al*. (2006, 2011) isolated viable *T. gondii* from samples of brains and hearts from the butcher shops in Campos dos Goytacazes, RJ but it is uncertain if the hearts and brains were from the same or different pigs. Bezerra *et al*. (2012) isolated viable *T. gondii* by bioassay of pooled brains and tongues of 5 of the 20 pig heads from small farms and pork butchers in Ilhéus, Bahia. In these studies, the sources of pigs, their ages, and serological status were unknown. Dos Santos *et al*. (2005) tested 286 market-age 6–8 month old pigs, from 17 small poorly managed farms in Jaboticabal, SP. Of these, 49 (17%) were seropositive by MAT. Tissues were collected for bioassay when these pigs were slaughtered. Viable *T. gondii* was isolated from tissues of 7 (MAT titres 100–1 pig, 200–4 pigs, 1600–2 pigs) pigs. Such information is needed for market-age pigs raised under different management conditions in Brazil.

Sheep. Up to 59% of sheep surveyed in Brazil had *T. gondii* antibodies (Table 11), and viable parasites were isolated from some of their tissues (Table 10). Spósito Filha *et al*. (1992) reported isolation of *T. gondii* from diaphragms of 20 of 136 sheep from the state of Rio Grande do Sul. The identification of 5 of these isolates was based on finding tissue cysts in smears of brains of mice inoculated with ovine tissues; 3 isolates were recognized in the first passage in mice, the fourth isolate on the second passage, and the fifth isolate was detected on the third passage in mice (Spósito Filha *et al*. 1992). In the remaining 15 cases, tissue cysts were identified only in haematoxylin and eosin-stained sections of the brains of mice and not by observation of live parasites; whether these parasites were *T. gondii* or not could not be confirmed. Da Silva and Langoni (2001) isolated *T. gondii* from tissues of 34 of 40 seropositive sheep. However, most data were based on finding *T. gondii* antibodies (1:16 titre by the IFA test) in sera of mice inoculated with ovine tissues. *Toxoplasma gondii*-like

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Table 8. Serological prevalence of *Toxoplasma gondii* antibodies in free-range chickens from different states, counties or areas of Brazil

State, county, area or region	No. of chickens				
	Total	Seropositive (%)	Bioassay positive (%)	*T. gondii* isolate	Reference
Alagoas	**8**	**8 (100)**	**4 (50·0)**	**TgCKBr184–187**	
Penedo	5	5 (100)	2 (40·0)	TgCKBr186–187	
Porto Real	3	3 (100)	2 (75·0)	TgCKBr184–185	de Oliveira *et al*. (2009)
Bahia	**20**	**10 (50·0)**	**3 (33·3)**	**TgCKBr173–177**	de Oliveira *et al*. (2009)
Caém	5	5 (100)	3 (60·0)	TgCKBr174–177	
Jacobina	13	4 (30·7)	1 (25·0)	TgCKBr173	
Ceará	**25**	**17 (68·0)**	**6 (35·2)**	**TgCKBr177–182**	de Oliveira *et al*. (2009)
Cascavel	15	11(73·3)	3 (27·2)	TgCKBr177–179	
Quixadá	10	6 (60·0)	3 (50·0)	TgCKBr180–182	
Espírito Santo	**490**	**196 (40·0)**	**48 (75·0)**	**TgCkBr234–281**	Beltrame *et al*. (2012)
Colatina	99	73 (73·7)	23 (85·1)	TgCkBr234–256	
Guarapari	53	13 (24·5)	4 (100)	TgCkBr257–260	
Linhares	60	24 (40·0)	6 (85·7)	TgCkBr261–266	
Marechal Floriano	41	13 (31·7)	9 (90·0)	TgCkBr267–275	
Serra	107	17 (15·9)	2(18·1)	TgCkBr276–277	
Vila Velha	130	56 (43·1)	4 (80·0)	TgCkBr278–281	
Maranhão Chapadinha	20	14 (31·7)	2 (14·2)	TgCKBr171,172	de Oliveira *et al*. (2009)
Mato Grosso do Sul	**90**	No data	**22 (24·4)**	**TgCKBr188–209**	Soares *et al*. (2011)
Aquidauna	10	No data	10 (100)	TgCKBr199–209	
Eldorado	50	No data	11 (22·0)	TgCKBr188–197	
Rio Verde	30	No data	1 (3·3)	TgCKBr188–197	
Minas Gerais Belo Horizonte	28	15 (53·6)[a]	11(39·2)	CH1–11	Brandão *et al*. (2006)
Pará	**40**	**26 (65·0)**	**15 (57·6)**	**TgCKBr107–116, 141–145**	Dubey *et al*. (2007*b*)
Castanhal	4	4 (100)	2 (50·0)	TgCKBr116,141	
Inhangapi	4	2 (50·0)	1(50·0)	TgCKBr113	
Marituba	4	2 (50·0)	1(50·0)	TgCKBr145	
Santarém	20	12 (50·0)	6 (50·0)	TgCKBr107–112	
Tera Alta	4	3 (75·0)	2 (66·6)	TgCKBr114,115	
Santa Isabel	4	3 (75·0)	3 66·6)	TgCKBr142–144	
Paraná					
Jaguapitã	155	16 (10·3)[a]	Not done	Not done	Garcia *et al*. (2000)
Santa Isabel do Ivaí	40	16 (40·0)	13 (81·2)	TgCKBr93–106	Dubey *et al*. (2003*b*) ; Vaudaux *et al*. (2010)
Toledo	65	28 (43·0)	22 (84·6)	*T. gondii* DNA	Aigner *et al*. (2010)
Pernambuco	**20**	**10**	**2 (20·0)**	**TgCKBr165,166**	de Oliveira *et al*. (2009)
Caruaru	3	2 (66·6)	1 (50·0)	TgCKBr165	
Gravatá	2	2 (100)	1 (50·0)	TgCKBr166	
Fernando de Noronha	50	42 (84·0)	24 (57·1)	TgCKBr210–233	Dubey *et al*. (2010);
island	50	38 (76·0)	Not done	Not done	Costa *et al*. (2012*a*)
Rio de Janeiro					
Campos dos Goytacazes	198	129 (65·1)	67 (69·7)	TgCKBr26–92	da Silva *et al*. (2003*b*); Dubey *et al*. (2003*a*)
Seropédica, Itaguí	20	Not stated	6 (30·0)	No	Peixoto and Lopes (1990)
Barra Mansa	316	151 (47·8)[a]	Not done		Bonna *et al*. (2006)
Rio Grande do Norte	**47**	**17 (36·1)**	**4 (23·5)**	**TgCKBr167–170**	de Oliveira *et al*. (2009)
Baraúna	4	2 (50·0)	1(50·0)	TgCKBr170	
Felipe Guerra	1	1 (100)	1(100)	TgCKBr168	
Ouro Branco	27	4 (14·8)	1 (25·0)	TgCKBr167	
Serra do Mel	4	3 (75·0)	1 (33·3)	TgCKBr169	
Rio Grande do Sul	**50**	**19 (38·0)**	**18 (94·7)**	**TgCKBr146–163**	Dubey *et al*. (2007*b*)
Canguçu	10	5 (50·0)	5 (100)	TgCKBr155–159	
Capão do Leão	10	1(10·0)	1 (100)	TgCKBr154	
Pelotas	10	8 (80·0)	8 (100)		
Rio Grande	10	5(50·0)	4 (80·0)	TgCKBr160–163	
Rondônia					
Monte Negro (western Amazon)	50	33 (66·0)	24 (72·7)	TgCKBr117–140	Dubey *et al*. (2006)

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Table 8. (*Cont.*)

State, county, area or region	No. of chickens: Total	Seropositive (%)	Bioassay positive (%)	*T. gondii* isolate	Reference
SãoPaulo	**82**	**33 (40·2)**	**22 (75·8) + 3**[b]	**TgCKBr1–25**	Dubey *et al*. (2002)
Botucatu	8	4 (50)	4 (100)	TgCKBr6,10,13,15	
Pirassununga	38	6 15·7)	5 (83·3)	TgCKBr1–3,5,8	
Pratânea	33	21 (63·6)	12 (57·1)	TgCKBr7,9,11,12, 14,16–22	
São Manuel	3	1(33·3)	1 (100)	TgCKBr4,	
Sergipe	12	5 (41·6)	1 (20·0)	TgCKBr183	de Oliveira *et al*. (2009)
Itabaiana	7	4 (57·1)	1 (25·0)	TgCKBr183	

[a] IFA, others were done by MAT.
[b] Additional isolates from tissues pooled from several chickens.

tissue cysts were detected in smears of the brains of mice inoculated with tissues of only 12 sheep and identification of tissue cysts was confirmed in only 4 cases by Giemsa-stained smears of brains of mice inoculated with ovine tissues. It needs to be stressed that identification of *T. gondii* should always be confirmed by passage of parasites to new mice or by other verifiable methods.

Ragozo *et al*. (2008) serologically tested 495 sheep from 36 counties in São Paulo State; *T. gondii* antibodies were found in 24·2% of sheep and seropositivity was present in sheep from all counties. Viable *T. gondii* was isolated from 16 of these 82 seropositive sheep bioassayed.

Recently, da Silva *et al*. (2011) found *T. gondii* antibodies in 66 (11%) of 602 sheep from 2 slaughter houses in São Paulo State. These sheep originated in RS and SP States; 51 (11·8%) of 430 sheep from RS and 15 (8·7%) of 172 sheep from SP were seropositive (da Silva *et al*. personal communication to J.P.D.). Viable *T. gondii* was isolated from 20 of 66 seropositive sheep (15 of 51 from RS and 5 of 15 from SP) bioassayed in mice (da Silva *et al*. 2011). Fifteen of these 20 isolates were from sheep from the state of Rio Grande do Sul (TgOvBr 1–4, 6–8, 10, 15, 18, 20 from Santana do Livramento, TgOvBr 5, 9, 13, 14 from Uruguaiana) and 5 were from sheep from the state of São Paulo (TgOvBr11, 16 from Ourinhos, 12, 19 from Pirajuí, TgOvBr 17 from Manduri, personal communication from authors to J.P.D., data added here not reported by da Silva *et al*. 2011).

Goat. Up to 92% of goats surveyed in Brazil had *T. gondii* antibodies (Table 12), and viable parasites were isolated from their tissues (Table 10). Spósito Filha *et al*. (1983) isolated *T. gondii* from diaphragms of 3 of 95 goats from São Paulo and Cavalcante *et al*. (2007) isolated *T. gondii* from the hearts of 2 of 169 goats from Ceará; the low recovery rate was probably related to small fragments of tissues used for bioassay. Silva *et al*. (2009) detected *T. gondii* DNA in 8 of 102 tissues of goats from Bahia: brains of 4, hearts of 4, and tongues of 3.

Ragozo *et al*. (2009) had better success when isolating viable *T. gondii*. They tested 143 goats and detected *T. gondii* antibodies in 41 (35·9%) of 114 goats from 6 counties in São Paulo State, 5 (26·3%) of 19 from the state of Rio Grande do Norte but no antibodies in 10 goats from the state of Bahia (data added here, not given by Ragozo *et al*. 2009). Tissues of 26 of these 46 seropositive goats were bioassayed in mice and viable *T. gondii* was isolated from 12.

Cattle. The high seroprevalence of *T. gondii* in some surveys of cattle in Brazil (Table 13) is puzzling because viable *T. gondii* have rarely been isolated from beef worldwide, including Brazil. Viable *T. gondii* were not isolated from 98 samples of beef from São Paulo (Jamra *et al*. 1969), and 98 diaphragms from Belo Horizonte, MG (Passos *et al*. 1984). Recently, Costa *et al*. (2011*b*) isolated *T. gondii* from 3 of 50 fetuses (brains of 2 and retina of 1) from 50 cows killed at a slaughter house in Jaboticabal, SP; whether fetuses were diseased is unknown.

Horses. Viable *T. gondii* were not isolated from diaphragms of 23 horses in RS and SP; 4 of these animals were seropositive (Spósito Filha *et al*. 1986). In general, horses are not a good host for *T. gondii* and seropositivity is low worldwide, except 31·6% seropositivity among 561 horses by Vidotto *et al*. (1997) (Table 13).

Rodents. The prevalence of viable *T. gondii* in these animals is important because they serve as sources of infection for humans and other animals. Capybara (*Hydrochoeris hydrochaeris*) is a large herbivorous rodent widely prevalent in Brazil; its meat is consumed by people. Capybaras are the largest rodents and can weigh up to 90 kg. They have been

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Table 9. Serological prevalence of *Toxoplasma gondii* antibodies in pigs in Brazil

State, city or area	Source of sera	Test	No. tested	% positive	Cut- off titre	Reference
Bahia	Farms	ELISA	465	18·2		Bezerra *et al*. (2009)
Simões Filho						
Ceará	Abattoir	IHA	37	59·4	64	do Amaral *et al*. (1978*a*)
Fortaleza						
Goiás	Farms	IHA	829	27·7	64	Matos *et al*. (1999)
Goiânia						
Mato Grosso	Farms	IFA	708	12·8	64	Muraro *et al*. (2010)
Nova Mutum and Diamantino						
Minas Gerais	Farms	IFA	198	90·4	16	Guimarães *et al*. (1992*a*)
Igarapé						
Belo Horizonte	Not stated	IFA	900	29·9	16	Schenk *et al*. (1976)
Belo Horizonte	Abattoir	IFA	652	33·4	16	Passos *et al*. (1984*a*)
Ponte Nova and Ubá	Abattoir	MAT	187	0	100	Pezerico *et al*. (2007)
Pará	Abattoir	IHA	110	50·0	16	Freitas *et al*. (2009)
Belém						
Paraíba	Abattoir	IFA	130	36·2	64	de Azevedo *et al*. (2010*b*)
Patos						
Paraná						
Cambará, Carlópolis, Cerqueira Campos, Lacerdópolis, São Jorge do Oeste, Vitorino	Abattoir	IHA	290	32·0	64	do Amaral *et al*. (1978*a*)
North region of the state	Farms	IFA	521	15·3	64	Tsutsui *et al*. (2003)
Guarapuava	Abattoir	IFA	117	8·5	64	de Moura *et al*. (2007)
Jaguapitã	Farms	IFA	267	24·0	64	Garcia *et al*. (1999*a*)
Londrina	Farms	IFA	1131	37·8	64	Vidotto *et al*. (1990)
13 counties	Abattoirs	IFA	424	4·0	64	Carletti *et al*. (2005)
Toledo microregion	Abattoir	MAT	606	13·4	25	Piassa *et al*. (2010)
Umuarama and Francisco Beltrão	Abattoir	MAT	226	1·8	64	da Silva *et al*. (2008)
	Farms		78	23·1		
25 cities	Abattoirs	IFA	408	25·5	64	Millar *et al*. (2008)
Pernambuco 11 counties	Abattoir	IFA	305	12·5	64	Fernandes *et al*. (2012)
Not stated	Farms	IFA	259	4·7	64	Caporali *et al*. (2005)
Piauí	Abattoir	IHA	60	38·3	64	do Amaral *et al*. (1978*a*)
Terezina						
Rio de Janeiro	Farms	IFA	38	65·8	16	Bonna *et al*. (2006)
Barra Mansa						
Campos dos Goytacazes	Farms	ELISA	61	11·5		Frazão-Teixeira and de Oliveira (2011*b*)
Rio Grande do Sul						
Northwest region of the state	Abattoir	IHA[1]	200	18·0	64	Grünspan *et al*. (1995)
Not stated	Abattoir	IHA	111	53·1	64	do Amaral *et al*. (1975)
Erechim	Abattoir	IFA	274	9·7	16	Araujo and Souza (1997); Araujo *et al*. (1998*a*, *b*)
		ELISA		9·4		
	Farms	IFA	278	7·9	16	
		ELISA		9·3		
	Mixed	IFA	240	32·9	16	
		ELISA		33·3		
Not stated	Abattoir	LA	2142	11·3	16	Nishikawa *et al*. (1984)
Pelotas	Farms	IHA	195	9·2	16	Pereira (2005)
		IFA		13·9		
Porto Alegre	Abattoir	IHA	54	7·4	64	Chaplin *et al*. (1984)
Porto Alegre	Abattoir	IHA	240	20·0	64	Fialho and Araújo (2003)
		IFA		33·7	16	
Roca Sales	Abattoir	IHA	497	7·2	64	Silva *et al*. (1981*b*)
Rondônia	Farms	MAT	80	37·5	25	Cavalcante *et al*. (2006*b*)
Monte Negro (western Amazon)						
Santa Catarina						
Different counties	Farms	IHA	1033	1·1	64	Wentz *et al*. (1986)
Quilombo	Abattoir	IHA	42	9·5	64	do Amaral *et al*. (1978*a*)

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Table 9. (*Cont.*)

State, city or area	Source of sera	Test	No. tested	% positive	Cut- off titre	Reference
Santa Catarina and Rio Grande Sul	Farms	MAT	115	86·0	50	Silva *et al*. (2003)
São Paulo	Abattoir	ELISA	300	9·6	NA	Suaréz-Aranda *et al*.
São Paulo city		IHA		21·0	16	(2000)
Not stated	Farms	MAT	286	17·0	25	dos Santos *et al*. (2005)
São Manuel	Abattoir	MAT	75	0	100	Pezerico *et al*. (2007)
São Paulo city	Abattoir	DT	10	60·0	2	Sogorb *et al*. (1972)
	Abattoir	IHA	95	47·3	64	do Amaral *et al*. (1975)
	Abattoir	IHA	955	30·3	64	Amaral *et al*. (1976*a*)
	Abattoir	IFA	328	32·8	16	Ishizuka (1978)
	Abattoir	IFA	273	57·8	16	Ishizuka *et al*. (1986)
		IHA		42·1	64	
Botucatu	Farm	IFA	487	19·1	20	Corrêa *et al*. (1978)
São Paulo city	Abattoir		513	25·7		
32 counties	Farms	IHA	960	24·6	64	Santos *et al*. (1978)
Jaboticabal	Farms	IFA	409	47·0	16	Vasconcelos *et al*. (1979)
	Abattoir	IFA	348	51·1	16	D'Angelino Ishizuka
		IHA		44·5	64	(1986)
São Paulo	Farms	IFA	500	0·8	64	Caporali *et al*. (2005)
	Abattoir	IFA	213	8·5	64[a]	Lima *et al*. (2007)
Registro	Farms	MAT	550	20·1	64	de Oliveira *et al*. (2007)
16 properties	Indoor farms	IFA	300	0	64	Villalobos *et al*. (2011)
	Outdoor farms		200	48·0		

[a] Personal communication.

domesticated but are also common in the wild. Antibodies to *T. gondii* were found in 42–75% of capybaras (Table S8, online version only) and viable *T. gondii* were isolated from a high percentage of seropositive animals (Table 10).

The low prevalence of *T. gondii* in feral house mice and rats in Brazil is puzzling, if one assumes that the environment is highly contaminated with oocysts. In the largest survey of rodents, *T. gondii* was isolated from only 1 of 20 *Rattus norvegicus*, but not from any of the 193 *Rattus rattus*, and 4 *Mus musculus* from São Paulo (Muradian *et al*. 2012). Tissues from all of these rodents were bioassayed in mice and also tested for *T. gondii* DNA; by PCR DNA was found in tissues of 1 *M. musculus*, 7 *R. rattus*, and 2 *R. norvegicus*. Araújo *et al*. (2010) also reported similar results in rodents from Paraná state; *T. gondii* was isolated from 1 of 19 *M. musculus* and 1 of 24 *R. rattus*; all of these animals were seronegative for *T. gondii*. Nothing is known of clinical toxoplasmosis in rats and mice under natural conditions in Brazil or anywhere else in the world.

Relative risk of T. gondii *transmission from different infected meats*

Infected pigs and pork products. Among the food animals, infected pigs are the most likely meat source of *T. gondii* infection for people in many countries, including Brazil (Dubey 2009*b*; da Silva *et al*. 2010). The ingestion of homemade sausages has long been considered a source of *T. gondii* infection in southern Brazil, particularly Erechim (Glasner *et al*. 1992*b*). In addition to reports of recovery of viable *T. gondii* from pork, *T. gondii* DNA has been frequently demonstrated in pork in Brazil. Belfort-Neto *et al*. (2007) detected *T. gondii* DNA from 34% of 50 diaphragms and 66% of 50 tongues from pigs from abattoirs in Erechim. DA Silva *et al*. (2005*a*) reported *T. gondii* DNA by PCR in 19 of 70 sausages from 55 establishments from São Paulo and Bezerra *et al*. (2012) detected *T. gondii* DNA in brains of 11 and tongues of 9 of 20 pig heads from a butcher shop in Ilhéus, Bahia. Somica Fernandes *et al*. (2012) found *T. gondii* DNA in 21 of 38 seropositive pigs from Pernambuco. However, DNA testing does not distinguish between live and dead parasites. Additionally, salting, curing, and pickling procedures used to make sausages and other preparations do often kill tissue cysts, but these procedures have not been standardized universally (Dubey, 2010*a*).

Annually in Brazil, approximately 32 million pigs are produced and 2 220 000 tons of pork are consumed. Most edible portions of pork could be infected with live *T. gondii* and one infected pig could be source of infection for many people (Dubey *et al*. 1986; Tsutsui *et al*. 2007). As stated earlier, a whole family had clinical toxoplasmosis epidemiologically linked to consumption of raw pork sausage at a party in Santa Vitória do Palmar, RS (de Almeida *et al*. 2006). High seroprevalence of *T. gondii* in pigs

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Table 10. Isolation of viable *Toxoplasma gondii* from animals in Brazil

Animal	Source (**State**, city or area)	No. of samples, tissues[a]	No. positive (isolate designation)	Reference
PIG *(Sus scrofa)*	**Bahia** Ilhéus	20 B,T	5	Bezerra *et al*. (2012)
	Minas Gerais Belo Horizonte	159 D	1	Schenk *et al*. (1977)
		98 B	4	
	Paraná Londrina	149 sausages	1	Dias *et al*. (2005)
	Rio de Janeiro Campos dos Goytacazes	12 B	6	Frazão-Teixeira *et al*. (2006)
		19 B, 16 H	5 (TgPgBr1–5)	Frazão-Teixeira *et al*. (2011*a*)
	São Paulo São Paulo city	83	5	Jamra *et al*. (1969)
		25	8	do Amaral and Mcruz (1968, 1969)
	São Paulo small farms	**28**[b] (256)[c] B,H,T	7 (TgPiBr1–7)	dos Santos *et al*. (2005)
SHEEP *(Ovis aries)*	**Rio Grande do Sul**	136 B,D,H	20	Spósito Filha *et al*. (1992)
	Rio Grande do Sul and São Paulo	**66** (602) B,D,H,L	20 (TgOvBr1–20)[f]	da Silva *et al*. (2011)
	São Paulo São Manuel	**40** (552) B,D	34	da Silva and Langoni (2001)
	São Paulo	**82** (495) B,D,H	16 (TgShBr1–16)[g]	Ragozo *et al*. (2008, 2010)
GOAT *(Capra hircus)*	**Bahia**	95 D	4	Spósito Filha *et al*. (1983)
	Ceará	169 H	2 (G1,G2)	Cavalcante *et al*. (2007)
	São Paulo	**26** (143) B,H,M	12 (TgGtBr1–12)[h]	Ragozo *et al*. (2009, 2010)
CAPYBARA *(Hydrochoeris hydrochaeris)*	**São Paulo**	**40** (64) B,H,T	36 (TgGtCp1–36)[i]	Yai *et al*. (2008, 2009)
RABBIT *(Oryctolagus cuniculus)*	**Minas Gerais** Belo Horizonte	**2** (21) B	2 (TgRbBr1,2)	Dubey *et al*. (2011)
	São Paulo	37 D (37 pools from 370 rabbits)	3	do Amaral *et al*. (1972)
GUINEA FOWL *(Numida meleagris)*	**São Paulo**	10 B	1 (TgNmBr1)	Dubey *et al*. (2011)
CAT (*Felis catus*)	**Paraná** Isabel do Ivaí	54 B,H,M	37 (TgCatBr1–37)	Dubey *et al*. (2004)
	São Paulo	54 D	3	do Amaral *et al*. (1978*b*)
	São Paulo	**71** (237) B,H,S,T	47 (TgCatBr38–84)[j]	Pena *et al*. (2006, 2008)
DOG (*Canis familiaris*)	**Minas Gerais** Belo Horizonte	25 D	8 (D1–8)	Ferreira *et al*. (2004); Brandão *et al*. (2006)
	Paraná Umuarama	34 B	9	da Silva *et al*. (2005*b*)
	São Paulo São Paulo city	36	19 (TgDgBr1–19)[k]	Dubey *et al*. (2007*a*)
HOUSE MOUSE *(Mus musculus)*	**São Paulo**	6	1	Sogorb *et al*. (1972)
	Paraná Umuarama	19[d] B,H	1	Araújo *et al*. (2010)
RAT *(Rattus rattus)*		24[d] B,H	1	
	São Paulo	193 B,H,M	0	Muradian *et al*. (2012)
(*Rattus norvegicus*)		20 B,H,M	1	

GIANT ARMADILLO (*Priodontes maximus*) OTHERS[e]	**São Paulo**	3 B	1	Sogorb *et al.* (1977)

[a] B = brain, H = heart, L = lung, M = skeletal muscle, S = spleen, T = tongue.
[b] Figures in bold are the number of seropositive animals bioassayed.
[c] Figures in parenthesis are the number of animals serologically tested.
[d] Seronegative (MAT < 1:10).
[e] Pena *et al.* (2011) isolated viable *T. gondii* from 1 BLACK-EARED OPOSSUM (*Didelphis aurita*, designatedTgOpBr1), 1 JAGUARUNDI (*Puma yagouaroundi*, designatedTgJaBr1), and 1 HOWLER MONKEY (*Alouatta belzebul*, designated TgRhHum1) that died in captivity of unrelated causes.
[f] TgOvBr 1–4, 6–8, 10, 15, 18, 20 from counties Santana do Livramento, TgOvBr 5, 9, 13, 14 Uruguaiana in state of RS, and TgOvBr11,16 from county Ourinhos, 12, 19 Pirajuí, TgOvBr 17 Mandur were from the state of Sao Paulo.
[g] Isolates from counties: Presidente Prudente (TgShBr1,2), Araçariguama (TgShBr3,4), Araçatuba (TgShBr5), Marilia (TgShBr6,7), Botucatu (TgShBr 8,9), Coronel Macedo (TgShBr10), Dracena (TgShBr11), Engenheiro Coelho (TgShBr12,13,14), Tietê (TgShBr 15,16).
[h] Isolates from counties: Botucatu, São Paulo (TgGtBr1–7,9, 11, 12), Jardim do Seridó, Rio Grande Norte (TgGtBr8) and Ouro Branco, Rio Grande Norte (TgGtBr10).
[i] Isolates from counties: Andradina (TgBrCp1–7), Cordeirópolis TgBrCp8–16), Cosmorama (TgBrCp17–22), Ribeirão Preto (TgBrCp23,24), São Paulo (TgBrCp25–31) and Valparaíso (TgBrCp32–36).
[j] Isolates from counties: Araçariguama (TgCatBr38–41), Colina (TgCatBr42), Conchas (TgCatBr 43–49), Espírito Santodo Pinhal (TgCatBr50–57), Guaíra (TgCatBr58–62),Marília (TgCatBr63,64) Osasco (TgCatBr65,66), Pirassununga (TgCatBr67–73), Ribeirão Preto (TgCatBr74–76), S.J. do Rio Peto (TgCatBr77–80), and São Paulo (TgCatBr81–84).
[k] São Paulo county.

raised in small back-yard operations is of public health concern (Table 9). As an example, da Silva *et al.* (2008) reported an alarming high rate (86% of 115) of *T. gondii* antibodies in small farms (Table 9); the prevalence was probably underestimated because the survey was based on 1:50 titre in the MAT. Little information is available concerning prevalence of *T. gondii* in pigs raised under different management conditions in Brazil. It is possible to raise *T. gondii*–free pigs indoors by proper hygiene and rodent and cat control. In a recent report, antibodies to *T. gondii* were found in 48% of 200 outdoor pigs versus zero prevalence in 300 indoor pigs using identical detection methods (Villalobos *et al.* 2011).

Infected mutton and goat's milk. There are no data on the frequency of consumption of undercooked mutton in Brazil. As stated earlier in this review an outbreak of toxoplasmosis was linked to eating mutton (Bonametti *et al.* 1997*a*,*b*). The sheep and goat population is increasing in Brazil. Although there are no documented cases of toxoplasmosis acquired through drinking unpasteurized goat's milk, *T. gondii* infection is widely prevalent in dairy goats in Brazil (Table 11). In the largest survey of 72 dairy goats, 25% of goats were seropositive (Cavalcante *et al.* 2008).

Infected beef. The role of cattle and buffaloes in transmission of *T. gondii* is uncertain because viable parasites have rarely been demonstrated in beef (Santos *et al.* 2010*b*). Cattle and buffaloes are naturally resistant to *T. gondii* infection and there is evidence that some cows become seronegative after apparently successful infection (Dubey, 2010*a*). Little is known of the specificity and sensitivity of serological diagnosis of *T. gondii* infection in cattle because several tests that are used to diagnose toxoplasmosis in other animals give erratic results with bovine sera, and it is difficult to verify specificity using naturally infected cattle. Most of the serological surveys from cattle in Brazil listed in Table 13 were based on IFA and nothing is known of its specificity for detecting *T. gondii* antibodies in latently infected cattle. Therefore, we cannot access the zoonotic significance of 71% seropositivity (IFA titre 1:40) in 1420 cattle reported by Santos *et al.* (2009). Among all serological tests evaluated, a titre of 1:100 in the MAT appears to be indicative of *T. gondii* infection in cattle (Dubey, 2010*a*).

Poultry and eggs. Raw hen's eggs are unlikely to be a source of infection for humans (Dubey, 2010*b*). Raw eggs should not be consumed by humans, not for fear of getting *T. gondii*, but more importantly salmonellosis. Data summarized in Table 8 provide ample evidence that chickens raised in back-yard operations have viable *T. gondii*. In many instances, these chickens are killed at home or in unsupervised

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Table 11. Serological prevalence of *Toxoplasma gondii* antibodies in sheep in Brazil

State, city or area	Source	Test	No. tested	% positive	Cut-off titre	Reference
Alagoas						
Statewide, 23 counties	27 farms	IFA	432	32·9	64	Pinheiro *et al*. (2009)
Bahia						
Recôncavo, Caatinga	10 farms	LA[1]	240	18·7	64	Gondim *et al*. (1999)
Bahia and Rio Grande do Sul	Abattoirs	IHA	100	23·0	64	do Amaral *et al*. (1978*c*)
Federal District						
9 rural zones	32 farms	IFA	1028	38·2	64	Ueno *et al*. (2009)
Minas Gerais						
Uberlândia	2 farms	IFA	155	46·5	64	Rossi *et al*. (2011)
Paraná						
Curitiba	3 farms	ELISA	167	25·7		Soccol *et al*. (2009)
Guarapuava	Abattoir	IFA	157	7·0	64	de Moura *et al*. (2007)
Guarapuava	9 farms	IFA	305	51·5	64	Romanelli *et al*. (2007)
Jaguapitã	Farms	IFA	228	51·8	64	Garcia *et al*. (1999*a*)
Londrina	Farms	IFA	370	47·8	64	Freire *et al*. (1995)
Londrina, Cambé, Rolândia, Ibiporã	Farms	IFA	339	54·6	64	Ogawa *et al*. (2003)
Pernambuco						
Zona da Mata	10 farms	IFA	173	35·3	16	da Silva *et al*. (2003*a*)
Different mesoregions	18 farms	IFA	124	48·4	16	Bispo *et al*. (2011)
Fernando de Noronha island	Farms	IFA	97	59·0	16	Costa *et al*. (2012*a*)
Rio Grande do Norte						
Lajes	3 Farms	ELISA	102	29·4		Clementino *et al*. (2007)
Mossoró	35 farms	IFA	409	20·7	64	Soares *et al*. (2009)
Rio Grande do Sul and Santa Catarina	One abattoir in São Paulo	IFA	522	7·7	16	da Silva and Langoni (2001)
Rio Grande do Sul						
Guaíba	Not stated	IFA	218	12·8	16	da Silva *et al*. (1981)
Livramento	Farms	LA[1]	144	30·5	64	Martins *et al*. (1998)
Rosário do Sul	Farms	IFA	123	39·0	20	Silva and de la Rue (2006)
		IHA		21·1	16	
São Lourenço	Farm	IFA	92	9·8	16	Silva *et al*. (1980)
Uruguaiana	Abattoir	DT	100	39·0	16	Larsson *et al*. (1980)
Uruguaiana, Marau	Farms	IHA	662	18·2	64	Zonta *et al*. (1987–1988)
Not stated	Abattoir	LA	655	8·0	64	Nishikawa *et al*. (1984)
Rondônia						
Monte Negro (western Amazon)	Farms	IFA	141	46·8	64	Cavalcante *et al*. (2004)
São Paulo						
Botucatu county	Not stated	IFA	100	23·0	16	da Silva *et al*. (2002)
		MAT		27·0	16	
	Abattoirs	IFA	602	10·9	16	da Silva *et al*. (2011)
Botucatu and Pardinho	8 Farms	IFA	382	18·6	25	Langoni *et al*. (2011)
Bauru, Botucatu, Pratânea, São Manuel	Farms	IFA	597	34·7	64	Figliuolo *et al*. (2004*a*)
Jaboticabal microregion	6 farms	IFA	488	52·0	64	Lopes *et al*. (2010)
São Manuel	Abattoir	ELISA	200	31·0	NS	Meireles *et al*. (2003)
	Farms	IFA	522	7·7	16	da Silva and Langoni (2001)
São Paulo county	Farms	IFA	177	22·5	16	de Oliveira-Sequeira *et al*. (1993)
36 counties	Abattoirs	MAT	495	24·2	25	Ragozo *et al*. (2008)

slaughter facilities and the viscera are left for scavengers or are improperly disposed of. *Toxoplasma gondii* infection can be transmitted if care is not taken to wash hands thoroughly after cutting meat and during cooking of meat; however, risk assessment studies have not been undertaken.

In Brazil, 2 220 000 metric tons of poultry are consumed annually, but there is virtually no information on the prevalence of *T. gondii* in chickens raised in large-scale operations. In small samples of commercially raised chickens, *T. gondii* antibodies were not found in 185 chickens in states of São Paulo

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Table 12. Serological prevalence of *Toxoplasma gondii* antibodies in goats in Brazil

State, city or area	Source/breed	Test	No. tested	% positive	Cut- off titre	Reference
Alagoas						
Agreste, Sertão, East regions	24 farms	IFA	454	39·0	64	Anderlini *et al*. (2011)
Bahia						
Recôncavo and Caatinga regions	Farms	LA[1]	439	28·9	64	Gondim *et al*. (1999)
3 regions, 7 counties	Farms, dairy	IFA	373	16·4	16	Uzêda *et al*. (2004)
Bahia and Rio Grande do Sul	Not stated	IHA	100	10	64	do Amaral *et al*. (1978*c*)
Ceará						
Different regions	72 farms, dairy, mixed	ELISA	2362	25·1	NS[a]	Cavalcante *et al*. (2008)
Different regions	Abattoir	IFA	169	5·9	16	Cavalcante *et al*. (2007)
Minas Gerais						
Belo Horizonte	Farms, dairy	IFA	343	92·4	16	Chiari *et al*. (1987)
Pedro Azul			208	70·0		
14 counties	Farms	IFA	372	36·8	16	Machado and Lima (1987)
Not stated	Sera bank	IFA	169	68·0	16	Bahia *et al*. (1993)
		dot-ELISA		70·0	16	
Uberlândia	4 farms	IHA[4]	174	18·9	64	Figueiredo *et al*. (2001)
		IFA		19·5		
		ELISA		19·5		
5 regions	115 farms	IFA	767	45·7	64	Carneiro *et al*. (2009)
Paraíba						
Patos	Abattoir	IFA	306	24·5	64	Faria *et al*. (2007)
Paraná						
Pitanga	Farms, dairy	IFA	282	44·6	64	dos Reis *et al*. (2007)
		MAT		23·0	64	
Londrina	Farms	IFA	153	30·7	64	Sella *et al*. (1994)
Curitiba	Farms	IFA	405	35·9	64	Garcia *et al*. (2012)
		ELISA		39·4		
Pernambuco						
Zona da Mata region	10 farms	IFA	213	40·4	16	da Silva *et al*. (2003*a*)
Different regions	18 farms	IFA	164	47·6	16	Bispo *et al*. (2011)
Fernando de Noronha island		IFA	11	81·8	16	Costa *et al*. (*2012a*)
Rio de Janeiro						
10 counties	Farms	IFA	202	15·8	16	da Serra-Freire *et al*. (1994)
Rio Grande do Norte						
Seridó Oriental region	12 farms	IFA	366	30·6	64	Araújo Neto *et al*. (2008)
Jardim do Seridó county	Farms, dairy, mixed	MAT	19	26·3	25	Ragozo *et al*. (2009)
Mossoró	14 farms	IFA	381	17·1	64	de Lima *et al*. (2008)
Rio Grande do Sul						
Gravataí and Viamão	Farms	IFA	360	30·0	NS	Maciel and de Araujo (2004)
		IHA[2]		19·4		
Porto Alegre	Farms	IHA	118	16·1	64	Araújo *et al*. (1984)
São Paulo						
Botucatu	Farms	MAT	100	11	16	da Silva *et al*. (2002)
		IFA		8·0	16	
Botucatu	Farms, dairy, mixed	MAT	114	35·9	25	Ragozo *et al*. (2009)
São Manuel	Abattoirs	ELISA	200	17·0		Meireles *et al*. (2003)
7 regions	Farms, dairy	IFA	442	14·5	16	Mainardi *et al*. (2003)
15 counties	19 farms	IFA	394	28·7	64	Figliuolo *et al*. (2004*b*)
17 counties	17 farms	IFA	923	23·4	16	Stachissini (2005)

[a] NS=not stated.

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Table 13. Serological prevalence of *Toxoplasma gondii* antibodies in miscellaneous domestic animals in Brazil

SPECIES, state, city or area	Test	No. tested	% positive	Cut- off titre	Reference
CATTLE *(Bos taurus)*					
Amazonas					
Manaus	IHA	25	12·0	16	Ferraroni *et al*. (1980)
Bahia					
Recôncavo, Caatinga regions	LA[1]	194	1·0	64	Gondim *et al*. (1999)
Itapetinga, Itaju do Colônia, Ipirá, Marcionílio Souza, Fátima and Macajuba	IFA	100	26·0	50	Santos *et al*. (2010*b*)
Ilhéus, Itabuna	IFA	600	11·8	64	Spagnol *et al*. (2009)
Mato Grosso do Sul					
Alto Taquari, Aquidauna, Baixo Pantanal, Bodoquena, Campo Grande, Dourados, Três Lagoas	IHA	466	4·3	64	de Araújo *et al*. (1998)
Jauru region	IFA	1420	71·0	40	Santos *et al*. (2009)
South region	IFA	78	30·7	64	Marana *et al*. (1994)
Minas Gerais					
Belo Horizonte	IFA	991	9·0	64	Passos *et al*. (1984*b*)
Poços de Caldas, Botelhos	IFA	350	12·0	64	Costa and Costa (1978)
Pernambuco					
Fernando de Noronha island	IFA	100	3·0	16	Costa *et al*. (2012*a*)
Paraná					
Jaguapitã	IFA	400	25·8	64	Garcia *et al*. (1999*a*)
Londrina	IFA	503	48·5	64	Marana *et al*. (1995)
Pato Branco	IFA	348	41·3	64	Daguer *et al*. (2004)
12 counties in north of the state	IFA	385	26·0	64	Ogawa *et al*. (2005)
North, West, Central regions	IFA	256	31·2	64	Marana *et al*. (1994)
Rio de Janeiro					
Resende, Rio Claro	IFA	589	14·8	64	Albuquerque *et al*. (2005)
Rio Grande do Sul					
Guaporé	IHA	112	5·4	64	Chaplin *et al*. (1984)
Porto Alegre	IHA	134	6·7	64	Braccini *et al*. (1992)
Porto Alegre	IHA	532	3·4	64	Silva *et al*. (1982/1983)
Unknown	LA	440	6·0	64	Nishikawa *et al*. (1984)
São Paulo					
Jaboticabal	IFA	204	32·3	64	Costa *et al*. (1978)
Jaboticabal	IFA	50	18·0	64	Costa *et al*. (2011*b*)
Taquarituba	ELISA[1]	200	11·0	1·0	Meireles *et al*. (2003)
São Paulo and Minas Gerais	IFA	600	47·1	64	Costa *et al*. (2001*a*)
WATER BUFFALO (*Bubalus bubalis*)					
Bahia					
Recôncavo region	LA[1]	104	3·9	64	Gondim *et al*. (1999)
Pará					
13 counties	IFA	374	1·1	64	Silva *et al*. (2010)
Rio Grande do Sul					
Porto Alegre	IHA	34	0	64	Braccini *et al*. (1992)
São Paulo					
Vale do Ribeira region	IFA	222	3·2	64	Fujii *et al*. (2001)
12 counties	IFA	411	49·9	64	de Souza *et al*. (2001)
RABBIT (*Oryctolagus cuniculus*)					
Minas Gerais					
Metalúrgica region	MAT	21	9·5	10	Dubey *et al*. (2011)
São Paulo					
São Paulo city	DT	20	60·0	2	Sogorb *et al*. (1972)
EQUIDS *(Equus caballus, E. asinus, E. mulus)*					
Bahia					
Jacobina , Jequié	IFA	343	1·4	64	Mendonça *et al*. (2001)[a]

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Table 13. (*Cont.*)

SPECIES, state, city or area	Test	No. tested	% positive	Cut- off titre	Reference
Mato Grosso do Sul					
Statewide	IFA	750	32·8	16	Larangeira *et al*. (1985)
Minas Gerais					
Uberlândia	IFA	117	12·8	16	Naves *et al*. (2005)
Paraná					
Jaguapitã	IFA	173	12·1	16	Garcia *et al*. (1999*a*)
Apucarana[b]	IFA	561	31·5	16	Vidotto *et al*. (1997)
Pernambuco					
Fernando de Noronha island	MAT	16	43·7	25	Costa *et al*. (2012*a*)
Rio de Janeiro					
12 counties	IFA	430	4·4	64	Gazêta *et al*. (1997)
Rio Grande do Sul					
Porto Alegre	IHA	100	8·0	16	Silva *et al*. (1981*a*)
Porto Alegre	IHA	98	2·0	63	Braccini *et al*. (1992)
Unknown	LA	551	4·7	16	Nishikawa *et al*. (1984)
São Paulo					
São Paulo city Jockey club	MAT	101	16·0	16	Dubey *et al*. (1999)
São Paulo city	IFA	327	70·0	16	Ishizuka *et al*. (1975)
São Paulo city	DT	77	68·0	16	Macruz *et al*. (1975)
13 counties	IFA	900	25·0	16	Costa *et al*. (1986)
São Paulo and Rio Grande do Sul					
Unknown	IHA	23	17·4	64	Spósito Filha *et al*. (1986)

[a] Five of 343 (124 *E. caballus*, 197 *E. asinus*, 22 *E. mulus)* animals were positive both by IFA and MAT.
[b] The horses were killed for meat in a slaughter house in Apucarana but originated in other states. Seroprevalences were: 41·1% of 233 from SP, 23·3% of 131 from PR, 21·3% of 120 from MS, and 13·7% of 77 from MT.

(Meireles *et al*. 2003) and 80 chickens in Espírito Santo (Beltrame *et al*. 2012).

Transmission by eating meat of other animals. Ingestion of undercooked meat of rabbits, horse, capybaras, and other game animals can be a source of infection. Antibodies to *T. gondii* were found in many species of wildlife in Brazil (Table S8, online version only) and viable *T. gondii* were isolated from some of them (Table 10). Congenital toxoplasmosis was diagnosed in a child born to a 24-year-old French woman who had eaten uncooked horse meat imported from Brazil (Pomares *et al*. 2011).

CLINICAL TOXOPLASMOSIS IN OTHER ANIMALS

Dogs

Primary toxoplasmosis is rarely clinical in dogs (Dubey, 2010*a*). In most cases clinical toxoplasmosis is seen in immunosuppressed dogs, often with distemper virus infection (Dubey and Beattie, 1988). Earlier reports of canine toxoplasmosis are summarized in Table S9 (online version only). We are not aware of clinical canine toxoplasmosis reports from Brazil in the last 30 years.

Sheep and goats

Toxoplasmosis is a leading cause of ovine and caprine abortions in many countries and this has been known since the 1950s (see Dubey and Beattie, 1988; Dubey, 2009*a*). However, *T. gondii* was only recently reported in goat and ovine fetuses in Brazil (Pescador *et al*. 2007; de Moraes *et al*. 2011). Pescador *et al*. (2007) examined 6 aborted fetuses, stillborn and weak newborn goats in Rio Grande do Sul. *Toxoplasma gondii* was demonstrated by immunohistochemical methods in several tissues of 1 fetus that had degenerative lesions. In the 5 other cases, *T. gondii* DNA but not parasites, was found in caprine tissues. The 6 dams had IFA titres of 1:512 to 1: 2048.

Presumptive evidence of toxoplasmosis abortion was found in 5 of 35 fetuses from 30 ewes from 5 farms in the state of Pernambuco. *Toxoplasma gondii* DNA was detected in several organs and placentas by nested PCR, and the placentas had necrotic lesions. There is no mention of finding intact *T. gondii* in aborted fetuses or their fetal membranes or search for other abortifacients. Silva and de la Rue (2006) also reported possible congenital transmission of *T. gondii* in lambs on a Rio Grande do Sul farm but did not report any abortion. There is need for a comprehensive study to determine the causes of abortion in sheep and goats in Brazil.

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Table 14. Genotyping of 363 *Toxoplasma gondii* isolates from Brazil

ToxoDB PCR-RFLP genotype #	Fr.[a]	PCR-RFLP markers											*T. gondii* isolate designation	References
		SAG1	5′ – 3′ SAG2	alt. SAG2	SAG3	BTUB	GRA6	c22–8	c29–2	L358	PK1	Apico		
1 (Type II)	1	II or III	II	II	II	II	II	II	II	II	II	II	TgNmBr1	Dubey *et al*. (2011)
2 (Type III)	12	II or III	III	III	III	III	III	III	III	III	III	III	TgCkBr31, 56, TgCkBr158, 161, 164, TgCkBr231, TgDgBr11, TgCpBr2, 4, 5,6, 7	Dubey *et al*. (2007*b*); Dubey *et al*. (2007*a*); Dubey *et al*. (2008); Yai *et al*. (2009); Dubey *et al*. (2010)
3 (Type II variant)	7	II or III	II	II	II	II	II	II	II	II	II	I	TgCkBr221, 225, 226, 228, 230, TgOvBr2, 5	Dubey *et al*. (2010); da Silva *et al*. (2011)
6 (Type BrI)	40	I	I	I	III	I	II	u-1	I	I	I	I	TgCkBr10, 55, 79, 86, 87, 98, 101, 102, 104, 123, 124, 144,201, 203, 207, TgCatBr2, 12, 17, 21, 30, 42, 47, 53, 54, 55, 62, 71, 75, TgDgBr3, 7, TgShBr8, 9, 10, 11, TgGtBr2, 3, 4, 9, TgBrCp14, 534N	Su *et al*. (2006); Dubey *et al*. (2007*a*); Pena *et al*. (2008); Ragozo *et al*. (2010); Soares *et al*. (2011*b*); Ferreira *et al*. (2011)
7	4	I	III	III	III	III	III	III	III	III	III	I	TgCkBr111, 112, TgCkBr182, TgCkBr196	Dubey *et al*. (2007*b*); Dubey *et al*. (2008); Soares *et al*. (2011*b*)
8 (Type BrIII)	23	I	III	III	III	III	III	II	III	III	III	III	TgCatBr3, 4, TgDgBr4, 12, TgCkBr11, 7, 17, 131,132, 133,134,194,195, TgCatBr58, 59, 60, 73, 74, TgShBr15, TgCpBr17, 18, 20, 36	Su *et al*. (2006); Pena *et al*. (2008); Dubey *et al*. (2008); Yai *et al*. (2009); Ragozo *et al*. (2010); Soares *et al*. (2011*b*)
9	1	u-1	II	II	III	III	II	II	nd	II	II	I	TgCkBr116	Dubey *et al*. (2008)
10 (Type I)	1	I	I	I	I	I	I	I	I	I	I	I	TgCkBr146	Dubey *et al*. (2008)
11 (Type BrII)	20	I	I	II	III	III	III	I	III	I	II	III	TgCkBr57, 64, 97, TgCpBr11, 23, 24, TgOvBr3, TgRabbitBr2, TgCatBr1, 7, 39, 51, 52, 56, 61, 68, 77, 78, TgDgBr1, 13	Dubey *et al*. (2007*a*); Pena *et al*. (2008); Dubey *et al*. (2008); Yai *et al*. (2009); da Silva *et al*. (2011); Dubey *et al*. (2011)
13	12	I	I	I	I	I	III	II	III	III	I	III	TgCkBr165, 167, 170,174,176, 179, 180, 183, 184, 185, TgGtBr10, TgRhHmBr1	Dubey *et al*. (2008); Ragozo *et al*. (2010); Pena *et al*. (2011)
14	5	I	III	III	III	III	III	III	I	III	III	III	TgCkBr82, 90, 153, TgCatBr15, TgDgBr19	Su *et al*. ((2006)); Dubey *et al*. (2007*a*); Dubey *et al*. (2008)
15	7	u-1	I	II	III	III	III	III	I	I	III	I	TgCkBr119, 120,122,129,135, 137,140	Dubey *et al*. (2008)
17 (Type BrIV)	11	u-1	I	II	III	III	III	u-1	I	I	III	I	TgCkBr81, TgCkBr147, 148, 151, 154, 160, 162, 163,	Dubey *et al*. (2007*b*); Dubey *et al*. (2008)
19	16	I	III	III	III	III	III	I	I	I	u-1	I	TgCatBr5, 11, 16, TgCkBr28, 33, 50, 52, 58, TgCatBr84, TgCpBr10, 31, TgOvBr4, TgRabbitBr1, TgMmBr03, TgCkBr205, 209	Su *et al*. (2006); Dubey *et al*. (2008); Pena *et al*. (2008); Yai *et al*. (2009); Dubey *et al*. (2010); Araújo *et al*. (2010); Dubey *et al*. (2011). Soares *et al*. (2011); da Silva *et al*. (2011*b*)
21	10	I	III	III	III	III	III	I	I	I	III	III	TgCatBr10, 22, 23, 28, 31, 32, 37, TgCkBr95, TgCpBr29, TgRrBr09	Su *et al*. (2006); Dubey *et al*. (2008); Yai *et al*. (2009); Araújo *et al*. (2010)

22	8	u-1	I	II	III	III	III	u-1	I	III	III	III	TgCkBr38, 27, 44, 51, 65, 66, 78, 80	Dubey *et al*. (2008)
25	1	I	III	III	I	III	III	III	III	III	I	I	TgCkBr110	Dubey *et al*. (2007*b*)
26	6	II or III	III	III	III	III	III	I	III	III	I	III	TgCkBr149, 150, 152, 157, TgCatBr65, 66	Dubey *et al*. (2007*b*); Pena *et al*. (2008)
28	3	I	I	I	I	I	I	II	I	III	I	III	TgCkBr115, 142, 145	Dubey *et al*. (2007*b*)
29	1	I	I	II	III	I	III	II	I	III	III	I	TgCkBr114	Dubey *et al*. (2007*b*)
30	1	I	III	III	I	III	III	III	III	III	I	III	TgCkBr113	Dubey *et al*. (2007*b*)
32	3	I	III	III	III	III	II	I	I	I	I	I	TgDgBr8, 9, 10	Dubey *et al*. (2007*a*)
33	7	u-1	I	II	III	I	III	u-1	I	I	I	I	TgCkBr41, 42, 49, 60, 62, TgCpBr34, 35	Dubey *et al*. (2008); Yai *et al*. (2009)
34	8	u-1	I	II	III	III	III	II	I	I	u-1	I	TgCatBr44, 48, 69, 70, TgDgBr5, TgCpBr8, 13, 15	Dubey *et al*. (2007*a*); Pena *et al*. (2008); Yai *et al*. (2009)
36	4	I	I	I	III	I	III	II	I	III	I	III	TgCkBr59, 30, 34, 67	Dubey *et al*. (2008)
37	4	I	II	II	III	III	III	u-1	I	I	III	I	TgCkBr36, 32, 84, 85	Dubey *et al*. (2008)
40	3	u-1	I	II	III	III	III	III	III	I	III	I	TgCkBr75, 76, 92	Dubey *et al*. (2008)
41	5	I	I	I	III	I	II	I	I	I	I	I	TgCkBr136, 138, 139, TgCpBr9, 12	Dubey *et al*. (2008); Yai *et al*. (2009)
42	2	I	I	I	III	III	II	I	I	I	u-1	I	TgCatBr9, 19	Su *et al*. (2006)
45	3	I	III	III	III	I	II	II	III	I	I	III	TgCkBr126, 127, 117	Dubey *et al*. (2008)
47	3	I	III	III	III	III	II	u-1	I	I	II	I	TgCatBr25, TgCkBr99, 100	Su *et al*. (2006); Dubey *et al*. (2008)
48	1	I	III	III	III	III	III	III	III	III	III	III	TgCkBr181	Dubey *et al*. (2008)
51	3	u-1	I	II	III	I	III	II	I	I	I	I	TgCkBr46, TgDgBr6, 17	Dubey *et al*. (2007*a*); Dubey *et al*. (2008)
53	6	u-1	I	II	III	III	III	II	I	I	III	I	TgCkBr96, TgDgBr14, 15, TgCpBr19, 21, 22	Dubey *et al*. (2007*a*); Dubey *et al*. (2008); Yai *et al*. (2009)
55	2	I	I	I	I	III	I	u-1	I	I	I	I	TgCatBr79, 80	Pena *et al*. (2008)
56	2	I	I	I	III	I	II	u-1	I	III	I	I	TgCatBr45, 46	Dubey *et al*. (2008)
57	2	I	I	I	III	I	II	u-1	I	III	II	III	TgCkBr171, 172	Dubey *et al*. (2008)
58	2	I	I	I	III	I	III	u-1	III	III	I	I	TgCatBr83, TgDgBr2	Dubey *et al*. (2007*a*); Pena *et al*. (2008)
59	2	I	I	I	III	III	II	u-1	I	I	I	I	TgCkBr40, 47	Dubey *et al*. (2008)
63	2	I	I	II	III	III	III	I	III	I	II	I	TgCkBr13, 23	Dubey *et al*. (2008)
64	2	I	I	II	III	III	III	u-1	I	I	u-2	I	TgCkBr19, 24	Dubey *et al*. (2008)
65	2	I	I	II	III	III	III	u-1	I	I	III	I	TgCatBr82, TgCkBr89	Pena *et al*. (2008); Dubey *et al*. (2008)
67	3	I	III	III	III	I	III	I	III	III	u-1	III	TgCatBr76, TgDgBr16, TgCpBr33	Dubey *et al*. (2007*a*); Pena *et al*. (2008); Yai *et al*. (2009);
69	2	I	III	III	III	III	II	I	III	I	II	I	TgCkBr93, 94	Dubey *et al*. (2008)
70	2	I	III	III	III	III	II	u-1	I	I	I	III	TgCkBr107, 108	Dubey *et al*. (2007*b*)
71	3	I	III	III	III	III	III	II	I	III	III	I	TgCkBr26, 69, 180N	Dubey *et al*. (2008); Ferreira *et al*. (2011)
75	2	u-1	I	II	III	III	III	II	I	I	III	III	TgCkBr48, 88	Dubey *et al*. (2008)
76	2	u-1	III	III	III	III	III	u-1	I	I	III	I	TgCkBr155, 159	Dubey *et al*. (2007*b*)
77	1	I	I	I	I	I	I	u-1	I	I	III	III	TgCkBr141	Dubey *et al*. (2007*b*)
78	1	I	I	I	I	I	III	II	I	III	I	III	TgCkBr169	Dubey *et al*. (2008)
80	1	I	I	I	III	I	II	I	I	I	u-1	I	TgCatBr26	Su *et al*. (2006)
81	1	I	I	I	III	I	II	u-1	I	I	III	III	TgCkBr173	Dubey *et al*. (2008)
82	1	I	I	I	III	I	III	II	I	I	I	III	TgCkBr54	Dubey *et al*. (2008)
85	1	I	I	I	III	III	II	u-1	I	I	II	I	TgCatBr72	Pena *et al*. (2008)
86	1	I	I	I	III	III	II	u-1	I	I	III	I	TgCatBr50	Pena *et al*. (2008)
87	1	I	I	I	III	III	III	I	I	III	I	III	TgCkBr156	Dubey *et al*. (2007*b*)
88	1	I	I	I	III	III	III	II	I	III	I	III	TgCkBr186	Dubey *et al*. (2008)

Table 14. (*Cont.*)

ToxoDB PCR-RFLP genotype #	Fr.[a]	PCR-RFLP markers											*T. gondii* isolate designation	References
		SAG1	5′ – 3′ SAG2	alt. SAG2	SAG3	BTUB	GRA6	c22–8	c29–2	L358	PK1	Apico		
92	1	I	I	II	I	III	II	II	I	I	II	I	TgCatBr40	Pena *et al*. (2008)
93	1	I	I	II	I	III	II	u-1	I	I	III	I	TgCkBr61	Dubey *et al*. (2008)
94	1	I	I	II	I	III	III	I	I	I	II	I	TgCkBr16	Dubey *et al*. (2008)
96	1	I	I	II	I	III	III	II	III	I	III	III	TgCkBr109	Dubey *et al*. (2007*b*)
104	1	I	I	II	III	I	III	u-1	I	I	III	I	TgCatBr34	Su *et al*. (2006)
105	1	I	I	II	III	III	II	u-1	III	III	III	I	TgCkBr143	Dubey *et al*. (2008)
106	1	I	I	II	III	III	II	u-1	I	I	II	I	TgDgBr18	Dubey *et al*. (2007*a*)
107	1	I	I	II	III	III	II	u-1	I	I	III	I	TgCkBr37	Dubey *et al*. (2008)
108	1	I	I	II	III	III	III	II	I	I	III	I	TgCatBr57	Pena *et al*. (2008)
109	1	I	I	II	III	III	III	III	I	III	III	III	TgCkBr177	Dubey *et al*. (2008)
111	6	I	I	u-1	III	III	III	u-1	I	III	III	I	TgCatBr64, TgShBr6, 7, 16, TgOvBr12, TgRtBr1	Pena *et al*. (2008); Ragozo *et al*. (2010); da Silva *et al*. (2011); Muradian *et al*. (2012)
114	1	I	III	III	I	III	III	III	I	III	I	I	TgCkBr166	Dubey *et al*. (2008)
116	1	I	III	III	III	I	III	II	III	III	III	III	TgCkBr130	Dubey *et al*. (2008)
117	1	I	III	III	III	I	III	u-1	I	I	u-1	III	TgCatBr41	Pena *et al*. (2008)
119	1	I	III	III	III	III	II	u-1	I	I	u-1	I	TgCatBr18	Su *et al*. (2006)
120	1	I	III	III	III	III	III	I	I	III	III	III	TgCatBr20	Su *et al*. (2006)
121	1	I	III	III	III	III	III	I	III	I	III	III	TgCatBr67	Pena *et al*. (2008)
124	1	I	III	III	III	III	III	II	III	I	u-1	I	TgCatBr81	Pena *et al*. (2008)
125	1	I	III	III	III	III	III	II	III	III	u-2	III	TgCkBr8	Dubey *et al*. (2008)
126	1	I	no data	I	III	III	II	u-1	I	I	u-1	I	TgCatBr6–20	Su *et al*. (2006)
129	1	II or III	II	II	II	III	II	II	II	II	II	II	TgCkBr168	Dubey *et al*. (2008)
134	1	u-1	I	II	III	I	III	II	III	III	I	III	TgCkBr178	Dubey *et al*. (2008)
135	2	u-1	I	II	III	III	III	II	I	III	III	I	TgCkBr45, TgGtBr8	Dubey *et al*. (2008); Ragozo *et al*. (2010)
136	1	u-1	I	u-1	III	III	III	II	I	I	III	I	TgCatBr38	Pena *et al*. (2008)
138	1	u-1	III	III	III	III	III	III	I	III	III	III	TgCkBr74	Dubey *et al*. (2008)
142	1	I	I	I	I	I	II	II	II	II	I	I	TgCkBr222	Dubey *et al*. (2010)
144	2	I	I	I	III	I	III	u-1	III	I	I	I	TgShBr1, 2	Ragozo *et al*. (2010)
145	1	I	I	I	III	I	II	I	I	I	I	III	TgOpBr1	Pena *et al*. (2011)
146	15	I	I	I	III	II	II	I	III	III	II	III	TgCkBr210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 223, 224, 227, 229, 233	Dubey *et al*. (2010)
148	1	I	I	II	III	I	III	II	I	I	III	I	TgCpBr26	Yai *et al*. (2009)
149	4	I	I	II	III	III	II	II	I	I	I	I	TgGtBr1, 6, 7, 12	Ragozo *et al*. (2010)
150	3	I	I	II	III	III	II	u-1	III	I	I	I	TgShBr3, 4, TgGtBr5	Ragozo *et al*. (2010)
152	3	I	I	u-1	III	III	III	II	I	I	III	I	TgShBr12, 13, 14	Ragozo *et al*. (2010)
153	1	I	II	I	III	I	III	I	III	III	II	III	TgCkBr232	Dubey *et al*. (2010)
157	2	I	III	III	III	I	III	I	III	III	III	III	TgCkBr202, 204	Soares *et al*. (2011*b*)
158	1	I	III	III	III	I	III	u-1	III	III	III	I	TgCkBr206	Soares *et al*. (2011*b*)
159	1	I	III	III	III	III	I	I	III	I	III	III	TgCkBr200	Soares *et al*. (2011*b*)
160	1	I	III	III	III	III	III	I	III	III	u-1	III	TgShBr5	Ragozo *et al*. (2010)
161	1	I	III	III	III	III	III	I	I	III	III	I	TgCkBr199	Soares *et al*. (2011*b*)
162	1	I	III	III	III	III	III	II	I	I	III	I	TgCpBr1	Yai *et al*. (2009)

163	1	I	III	III	III	II	I	III	III	III	TgCkBr220			Dubey *et al.* (2010)
164	1	I	III	III	III	III	I	I	III	I	TgCkBr208			Soares *et al.* (2011*b*)
165	1	I	III	III	III	u-1	I	I	III	III	TgCpBr27			Yai *et al.* (2009)
166	1	I	III	III	III	u-1	I	III	III	III	TgJagBr1			Pena *et al.* (2011)
171	2	u-1	I	I	III	u-1	III	III	I	III	TgOvBr7, 8			da Silva *et al.* (2011)
172	6	u-1	I	II	I	u-1	III	III	III	I	TgCkBr188, 189, 190, 191, 192, 193			Soares *et al.* (2011*b*)
173	1	u-1	I	II	III	u-1	I	I	III	I	TgCpBr3			Yai *et al.* (2009)
174	1	u-1	I	II	III	u-1	III	II	III	I	TgCkBr197			Soares *et al.* (2011*b*)
175	2	u-1	I	II	III	III	I	I	u-1	I	TgCpBr25, 30			Yai *et al.* (2009)
106 types	**363**													

[a] Frequency.

Pigs

Fatal toxoplasmosis was reported once in a 28-day-old piglet from a herd in Belo Horizonte, MG (Lamas da Silva, 1959). The piglet was one of 5 siblings, 4 had died earlier but the aetiology was not investigated. The piglet investigated had diarrhoea, dyspnoea and fever. *Toxoplasma gondii* was identified histologically in sections of lung, heart, liver, and mesenteric lymph nodes.

Non-human primates

New World monkeys, in general, are highly susceptible to clinical toxoplasmosis, whereas Old World primates are resistant to clinical toxoplasmosis. Nery-Guimarães *et al*. (1971) first reported clinical toxoplasmosis in a Rhesus monkey (*Macacca mulatta*, an Old World species) and a tufted capuchin (*Cebus apella*, a New World species) in Brazil. The *M. mulatta* was captive in the laboratory at Oswald Cruz Institute, Rio de Janeiro. The *C. apella* was a pet in suburban Rio de Janeiro. Both animals died after a short illness and tachyzoites were found in their tissues. Of interest is the observation that the *Cebus* was routinely fed raw meat. The private owner also had 2 black-striped capuchins (*Cebus libidinosus*) that were not ill but had dye test titres of 1:64 (Nery-Guimarães *et al*. 1971).

Little is known of clinical toxoplasmosis in New World primates in the wild. In nature, these animals are herbivores and live in treetops and thus are unlikely to be exposed to *T. gondii*. The finding of *T. gondii* antibodies in some species of capuchin and howler monkeys in the wild indicates that some of these New World primates survive *T. gondii* exposure (Table S7). However, several other species of New World primates are highly susceptible to clinical toxoplasmosis experimentally and there are many worldwide reports of clinical toxoplasmosis in these animals in captivity (Dubey and Beattie, 1988; Dubey, 2010*a*). In Brazil, disseminated toxoplasmosis was diagnosed in 3 squirrel monkeys (*Saimiri sciureus*), 7 golden-headed lion tamarins (*Leontopithecus chrysomelas*), 3 emperor marmosets (*Saguinus imperator*), 1 golden-handed marmoset (*Saguinus midus*), 1 black marmoset (*Saguinus niger*), 5 wooly monkeys (*Lagothrix lagotricha*), 1 black tifted ear marmoset (*Callithrix penicillata*), 1 night monkey (*Aotus triviragatus*), 1 black lion tamarin (*Leontopithecus chrysopygus*), 2 golden lion tamarins (*Leontopithecus rosalia*), 6 brown howler monkeys (*Alouatta fusca*), and 2 white ear-tufted marmosets (*Callithrix jacchus)* that were examined post-mortem during 1991 to 2001 (Epiphanio *et al*. 1999, 2000, 2001, 2003); half of these animals died peracutely without any clinical signs. Pneumonitis and hepatitis were the main lesions (Epiphanio *et al*. 2003). Fatal toxoplasmosis was also observed in

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Table 15. Basic statistics of 6 *Toxoplasma gondii* populations from São Paulo state, Brazil

Host	Dog	Cat	Chicken	Capybara	Sheep	Goat
No. of isolates	19	44	10	33	17	9
No. of haplotypes	12	20	6	16	7	3
No. of loci	10	10	10	10	10	10
No. of polym. loci	9	10	9	9	8	4
Gene diversity (haplotype)	0·953 +/-0·028	0·921 +/- 0·023	0·889 +/-0·075	0·945 +/-0·018	0·875 +/-0·044	0·667 +/-0·105
Mean number of pairwise difference	4·836 +/-2·459	4·865 +/-2·414	4·378 +/-2·342	4·632 +/-2·325	3·941 +/-2·067	1·889 +/-1·176

3 squirrel monkeys from a captive colony in Rio de Janeiro (Andrade *et al*. 2007), and a black-headed night monkey (*Aotus nigriceps*) from a zoo in Mato Grosso (Antoniassi *et al*. 2011). Disseminated toxoplasmosis was reported in 3 adult captive (Túry *et al*. 1999) and 1 free-living (Maluenda *et al*. 2009) *Lagothrix lagotrica*.

Wild birds

Fatal toxoplasmosis has been reported in pigeons (*Columba livia*), sometimes in epizootic form (Carini, 1911; Pires and Santos, 1934; Reis and Nóbrega, 1936; Nóbrega and Reis, 1942; Springer, 1942; Dubey, 2002). Affected pigeons were anorexic, dull, emaciated, and had conjuctivitis with demonstrable organisms in ocular exudate and convulsions towards the time of death (Carini, 1911; Reis and Nóbrega, 1936). In pigeons that died, *T. gondii* was found in many tissues, especially in the lungs and spleen.

Marine mammals

Bandoli and de Oliveira (1977) reported *T. gondii* tachyzoites and tissue cysts in histological sections of lymph nodes of a wild Tucuxi dolphin (*Sotalia fluviaatilis guinensis*) that was found dead at the beach in Rio de Janeiro.

GENETIC DIVERSITY AND MOLECULAR EPIDEMIOLOGY

There is an intense debate as to whether virulence of the parasite contributes to the severity of disease in humans or animals in nature (Dubey, 2010*a*). Prior to the development of genetic markers, *T. gondii* isolates were grouped by their virulence to outbred mice. During the 1980s and 1990s methods were developed to recognize genetic differences among *T. gondii* isolates from humans and animals. Based on restriction fragment length polymorphism (RFLP), Howe and Sibley (1995) classified *T. gondii* into 3 genetic Types (I, II, III) and linked mouse virulence to genetic type. They proposed that Type I isolates were 100% lethal to mice, irrespective of the dose, and that Types II and III generally were avirulent for mice.

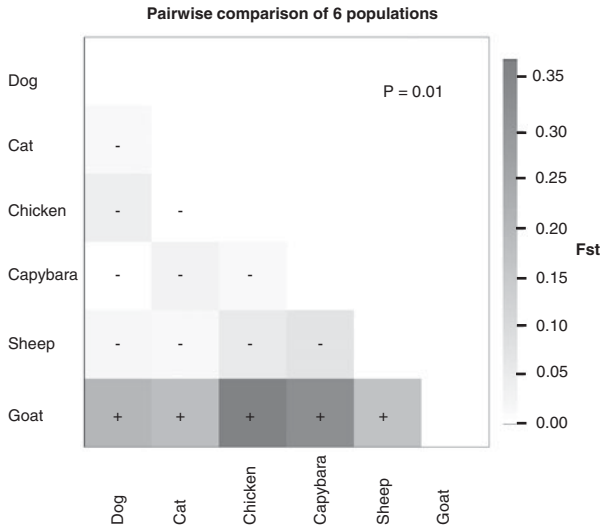

Fig. 2. Pairwise Fst of six *Toxoplasma gondii* populations from different hosts from São Paulo state, Brazil. Comparison of the populations was conducted using Arlequin ver 3.5. Statistical significance is determined at $P = 0{\cdot}01$. The '+' sign indicates significant difference between two populations, whereas '−' indicates insignificance. The heat map indicates the Fst value.

Lehmann *et al*. (2006), using microsatellite markers recognized geographical differences among *T. gondii* isolates, with some isolates confined to Brazil whereas others were worldwide in distribution.

In 2002, we initiated a study on the population structure of *T. gondii* in Brazil based on viable isolates of the parasite (Dubey and Su, 2009). *Toxoplasma gondii* isolates from a variety of animals from different geographical areas in Brazil (Fig. 1) were intensively studied and high diversity was revealed. Most samples were isolated from chickens, cats, dogs, goats, sheep and capybaras, with a few from other animals; we have listed sources of these isolates from different counties and states of Brazil in Table 14 and in Fig. 1. Most samples were obtained from the eastern parts of the country and São Paulo State.

Here, we summarized recent genotyping results of 363 samples in Table 14. All samples were typed using 10 PCR-RFLP markers developed recently (Su *et al*. 2010). From these samples, 106 unique genotypes were identified and each genotype was

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designated with a ToxoDB PCR-RFLP genotype number. The three most common genotypes were #6, #8 and #11 which accounted for 11·0% (40/363), 3% (23/363) and 5·5% (20/363), respectively. These major genotypes were previously designated as type BrI, BrIII and BrII, respectively (Pena *et al*. 2008). The Paraná waterborne outbreak was epidemiologically linked to a BrI type strain that was prevalent in cats and chickens in the local area (Vaudaux *et al*. 2010). In our studies only one Type I strain (#10, Table 14) was identified in a chicken from Brazil, and this strain was lost during revival. Two other strains (OH3 from a human and S11 from a pig) are listed in www.ToxoDB.org; however, their isolation history is not clear. We found 1 Type II strain (TgNmBr1) from a feral guinea fowl (Dubey *et al*. 2011), and 7 Type II variant strains (Type I allele at locus Apico) from chickens from the Fernando Noronha island, off the coast of Brazil (Dubey *et al*. 2010) and from sheep in the inland of Brazil (da Silva *et al*. 2011). The Type II (including Type II variant) strains that are dominant in Europe, North America and Africa were identified in Brazil with a relative low frequency. Type III strains were also relatively infrequent (12 of 363). Thus, most strains from Brazil were different from those found in other countries.

Overall, there is a lack of a dominant *T. gondii* genotype, and many genotypes were only identified from a single isolate. These results indicate that existing data identified only a small portion of the overall diversity of *T. gondii* in animals from Brazil.

We analysed 6 *T. gondii* populations from animals in São Paulo state (Table 15, Fig. 2). *Toxoplasma gondii* populations from dog, cat, chicken, capybara, and sheep all have high within-population diversity (gene diversity $\sim = 0{\cdot}9$, mean number of pairwise differences $\sim = 5$). Parasite populations of goat have a slightly lower diversity (gene diversity $\sim = 0{\cdot}7$, mean number of pairwise differences $\sim = 2$). Pairwise comparisons (Fst tests) suggested that there was no significant difference ($P = 0{\cdot}01$) among the dog, cat, chicken, capybara and sheep populations, except the goat population was different from all the others. Since there were only 9 *T. gondii* isolates from the goat population, the conclusion is not definitive. Therefore, overall there is no clear host preference of parasite genotypes. Many genotypes can infect different animal hosts.

Information concerning *T. gondii* strain diversity from human infection is very limited. Only a few studies have been performed using multilocus genetic markers and the data are fragmented due to use of different markers (de Melo Ferreira *et al*. 2004, 2006; Khan *et al*. 2006; Belfort-Neto *et al*. 2007;Vaudaux *et al*. 2010; Ferreira *et al*. 2011; Frazão-Teixeira *et al*. 2011*a*). Direct PCR-RFLP analysis of 62 tissue samples from patients with toxoplasmosis in São Paulo state was able to genotype 20 samples which belonged to 3 genotypes (#6, #65 and #71, Ferreira *et al*. 2011). Interestingly, 18 of these 20 samples were genotype #65, suggesting a possible association of this genotype to human toxoplasmosis. However, without isolation of *T. gondii* strains from patients, this result is only suggestive, and further study on isolated strains is needed to confirm the result. A question of interest is whether *T. gondii* genotypes are associated with disease phenotypes in human patients. To address this question, a large-scale genetic study of human isolates is necessary. To our knowledge, there is no report of genotyping based on DNA recovered from viable *T. gondii* isolates from sick or asymptomatic humans in Brazil. Currently, linking the higher burden of toxoplasmosis in congenitally infected children in Brazil to parasite genotype is only a hypothesis. Severe clinical toxoplasmosis in adult immunocompetent people reported from the neighbouring country French Guiana (Demar *et al*. 2011) has not been recognized in Brazil.

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