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ORIGINAL PAPER

Toxoplasma gondii in Capybara (*Hydrochaeris hydrochaeris*) antibodies and DNA detected by IFAT and PCR

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Abstract Toxoplasmosis is considered nowadays as one of the most important foodborne diseases in the world. One of the emerging risks in acquiring infection with *Toxoplasma gondii* is the increasing popularity of wild animals and game meat. Capybara (*Hydrochaeris hydrochaeris*) is the world's largest extant rodent and is used for human consumption in many areas of South America, and in case it carries *T. gondii* cysts, it may act as a source of infection. In the present study, we detected infection with *T. gondii* in capybaras from the south of Brazil. Antibodies to *T. gondii* were assayed in the serum of capybaras using the indirect fluorescent antibody test (IFAT≥1:16). Blood, liver, heart, lymph nodes, and spleen tissues were collected and tested by polymerase chain reaction (PCR) for B1 gene and ITS1 region. The results showed that 61.5% (16/26) capybaras were seropositive to *T. gondii*. Titers of specific antibodies to *T. gondii* ranged from 1:16 to 1:512. Among the feral rodents studied, 7.7% (2/26) were PCR positive for B1 gene assay and 11.5% (3/26) were positive for ITS1 PCR assay; for both test, the prevalence was 15.4%. Liver, heart, and blood tissues were those which tested positive for the apicomplexan. Our findings show a high percentage of infection with *T. gondii* in asymptomatic capybaras. Based on those data, we hypothesize that the consumption of raw or undercooked capybara meat could be a source of infection for humans.

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Introduction

The protozoan *Toxoplasma gondii* is an obligate intracellular parasite that belongs to the Apicomplexa phylum. This tissue cyst-forming coccidium has a worldwide distribution and is able to infect all warm-blooded animals (mammals and birds) and humans and is one of the most polyxenous parasites known (Sibley 2003; Tenter et al. 2000). Toxoplasmosis is a widespread zoonosis of veterinary and medical importance because infection with *T. gondii* is frequently associated with congenital infection and abortion, with great morbidity and death reported in immunocompromised patients. It is estimated that about one third of the world's human population is infected with this apicomplexan parasite (Tenter et al. 2000; Kim and Weiss 2008).

Infection with *T. gondii* can occur pre- or postnatally. Before birth, humans and some animals can be infected vertically by transplacental transmission of tachyzoites. After birth, intermediate and definitive hosts may acquire a *T. gondii* infection horizontally by oral ingestion of infectious oocysts in soil, food (raw vegetables or fruit),

or water contaminated with definitive hosts feces or by ingestion of tissue cysts in uncooked or undercooked meat or viscera of intermediate hosts (Dubey and Jones 2008; Kijlstra and Jongert 2008a).

An emerging risk in acquiring toxoplasmosis is the increasing popularity of wild and game meat. *T. gondii* tissue cysts in meat of wild animals are a potential source of infection for humans, mainly for hunters and their families, who can be considered a risk group for the acute disease (EFSA 2007; Kijlstra and Jongert 2008b). Several outbreaks of toxoplasmosis have been reported after the consumption of raw or undercooked wild meat worldwide (Dubey and Jones 2008; Kijlstra and Jongert 2008a). Kangaroo meat, for instance, was implicated as the cause of an outbreak of toxoplasmosis in Australia (Robson et al. 1995). Other meat of wild animals can be implicated as a potential source of infection for humans (McDonald et al. 1990; Cook et al. 2000; Ross et al. 2001; Carme et al. 2002; Dubey and Jones 2008). Our concern is about the possibility of the capybara meat being a source of infection for humans.

The capybara (*Hydrochaeris hydrochaeris*) is used for human consumption in certain populations and areas of South America. They are vegetarians, eating grass on dry land as well as aquatic vegetation, and can be found in areas with permanent standing or running water and inhabit marshes or estuaries and live along rivers and streams (Redford and Eisenberg 1992; Burnie and Wilson 2001). It is a typical and well-known wild animal in Brazil, where it is present throughout the country, particularly at the Brazilian Pantanal. There are reports of its presence from Panama—in Central America—down south into northern Argentina. Although the capybara's original range may have been more extensive, its current range has been severely affected by hunting (Montiani-Ferreira et al. 2008). In the present study, we investigate the infection with *T. gondii* in capybaras from the south of Brazil by serological and molecular methods.

Materials and methods

Area of study and the capybaras' population

The rodents were all from the Tingui Park, located in the city of Curitiba, state of Paraná, south of Brazil (25°39′ S and 49°3′ W). At this specific location, the animals live among the park's fresh-water islands for exhibition to the public and have total access to all areas of the Park, and therefore, in direct contact with both domestic and wild animals, including dogs. The estimated population of capybaras in that area is about 250 animals. A total of 26 young adult capybaras were used for investigation (15 females and 11 males ranging from 2 to 4 years of age).

Sample collection

Chemical restraint

Twenty-six capybaras were trapped live, then chemically restrained to immobilize the animals. Based on estimated weights of each animal, the following drug dosages were each calculated and administered concurrently: (1) 3 mg/kg of a fixed-ratio combination of zolazepam/tiletamine (Zoletil®, Virbac, Carros, France); (2) 0.3 mg/kg of morphine (Dimorf®, Cristalia, Itapira, SP, Brazil); (3) 1.2 mg/kg of Azaperone (Destress®, Serra, ES, Brazil). The drugs were all mixed in the same dart and then administered intramuscularly by a blow gun. The body weights of the immobilized animals varied from 37 to 63 kg, with the majority weighing approximately 50 kg.

Liver biopsy and blood collection

Rodents were individually submitted to liver biopsy. In order to avoid peritonitis and cross contaminations between samples, sterile Tru-cut needles (OM14100 OMNI-RAM, AGORAM®, Mirandola, Italy) were used for each animal. Liver biopsy was guided by ultrasound; the image was obtained from the lateral right abdomen immediately after the last right rib, with the capybara positioned in left lateral recumbency. Subsequently, the biopsy needle was introduced percutaneously until it was inside the right liver lobe. Samples measured about 1 cm by 0.2 cm and were kept in single vials in sterile water at 6–8°C for 2 to 4 h until delivery to the Parasitology Laboratory of the UFPR, where they were immediately frozen at −20°C. Blood samples were collected from the saphena vein (10 mL). From these samples, 5 mL were dispensed in BD Vacutainer® spray-coated K2EDTA tubes for DNA extraction of whole blood, and 5 mL were kept in BD Vacutainer® tubes to collect the sera for serological assay by indirect immunofluorescent antibody test.

Necropsy

From the above selected 26 capybaras, three (one female and two males) died of unknown reasons during the study. Therefore, in addition to the liver biopsy and blood sample collection, these three animals were sent for necropsy for additional sample collection. Fragments of liver, lymph nodes, spleen, and heart were collected from those animals and stored at −20ºC until DNA extraction.

Indirect immunofluorescent antibody test

Prevalence of anti-*T. gondii* antibodies was evaluated using the indirect immunofluorescent antibody test. The indirect immunofluorescent antibody test (IFAT) was carried out

using *T. gondii* RH strain tachyzoites as the antigen (prepared in our laboratory). The conjugate was anti-capybara antibody (IgG-fraction) from sheep, conjugated to fluorescein isothiocyanate, and diluted to 1:100 (purchased from University of Sao Paulo, Experimental Epidemiology and Zoonoses Department, Brazil). *T. gondii* positive and negative dog sera were used as controls. All samples were screened at a 1:16 dilution (positive cutoff value) in PBS, pH7.2, and the positives were titrated using double dilutions. Sera giving a uniform fluorescence reaction only to tachyzoites were considered positive. The data was statistically analyzed using the Fisher exact test to determine differences in seroprevalence between sex groups. A value of $P \leq 0.05$ was considered statistically significant.

DNA extraction and polymerase chain reaction

Liver, lymph node, spleen, and heart tissues were submitted to DNA extraction with the magnetic beads technology using the ChargeSwitch® gDNA Mini Tissue Kit (Invitrogen™, Carlsbad, CA, USA) following the manufacturer's protocol. To homogenize the samples before extraction, approximately 15 mg of liver tissue, obtained by biopsy (n=26) and about 25 mg of lymph node, spleen, and heart tissues, obtained from necropsy (n=3), were submitted to two cycles of ultrasound. Each cycle consisted of 70 Hz for 30 s in an ice bath, and then another ice bath for an additional 30 s followed, with a 1-min interval between the cycles. Positive control was obtained by DNA extraction from *T. gondii* (RH strain) tachyzoites culture. Contamination between samples was avoided by using sterile instruments with each individual sample. DNA extraction from blood samples (n=26) was performed using the Illustra blood genomicPrep Mini Spin Kit (GE Healthcare UK Limited™, Little Chalfont, Buckinghamshire, UK), according to the manufacturer's recommendations.

Subsequently, blood, liver, spleen, lymph nodes, and heart DNA samples were assayed for the presence of *T. gondii* by a single PCR program directed at two different targets: B1 gene and ITS1 region. The primers used to flank the gene B1 target, which is highly specific to *T. gondii*, were T1 5′ GGGTGAACCGAGGGAGTTG3′ and T2 5′GTGCGT CCAATCCTGTAAC3′ as described by Pujol-Riqué et al. (1999). The polymerase chain reaction (PCR) amplification reactions were performed in a total volume of 50 μL, with the primary mixture containing 5.0 μL of 10× DNA polymerase buffer (100 mM Tris–HCl, pH8.3, and 500 mM KCl); 1.5 mM $MgCl_2$; 0.2 mM each deoxyribonucleotide triphosphate (dNTPs), 25 pmol of each primer; 0.1% de Triton-100; 1.25 U of *Taq* DNA polymerase (Invitrogen™), and 80 to 100 ng of template genomic DNA. Reaction cycles comprised an initial denaturing step at 94°C for 5 min, followed by 30 cycles of amplification, each cycle consisting of: 94°C for 1 min, 58°C for 1 min, and 74°C for 2 min and 30 s, with a final extension step at 72°C for 5 min.

The DNA samples were also submitted to a single PCR of the ITS-1 region using the primers ITS5 5′GGAAG TAAAAGTCGTAACAAGG3′ and ITS2 5′GCTGC GTTCTTCATCGATGC3′ described by White et al. (1990). The PCR amplification reactions were performed in a total volume of 50 μL, with the primary mixture containing 5.0 μL of 10× DNA polymerase buffer (100 mM Tris–HCl, pH 8.3, and 500 mM KCl); 1.5 mM $MgCl_2$; 0.2 mM each dNTPs, 30.0 pmol of each primer; 0.1% de Triton-100; 1.25 U of *Taq* DNA polymerase (Invitrogen), and 80 to 100 ng of template genomic DNA. Reaction cycles comprised an initial denaturing step at 94°C for 5 min, followed by 30 cycles of amplification, each cycle consisting of: 94°C for 20 s, 57°C for 45 s, and 74°C for 45 s, with a final extension step at 72°C for 2 min.

Samples containing *T. gondii* DNA and samples without DNA (i.e., sterile water as template) were also included in all the PCR reactions (as controls). PCR reactions were performed in a heated-lid Hibayd GeneAmp thermocycler. The amplification products were electrophoresed in a 1.6% (w/v) agarose gel in TBE buffer at 60 V for 2 h. After staining with ethidium bromide (0.5 μg/mL) for 20 min, the gels were illuminated under UV and photographed with Life Technologies™ Gibco BRL UV transilluminator documentation system.

Results

IFAT

Antibodies against *T. gondii* were detected in sera from 16 of the 26 (61.5%) capybaras analyzed (Fig. 1). The seroprevalence in female capybaras (7/15) was not significantly different from that of male capybaras (9/11) (P=0.287).

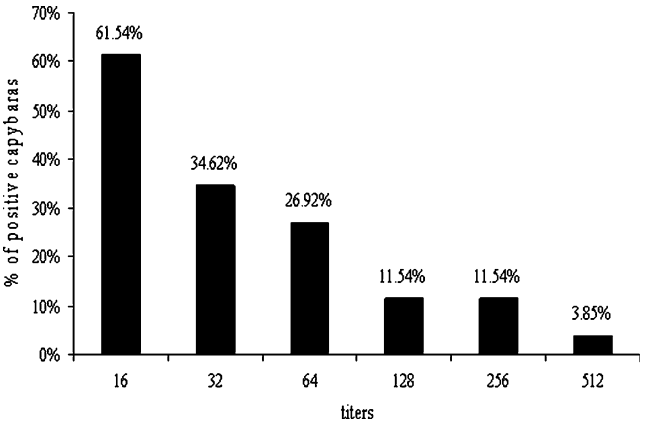

Fig. 1 The prevalence of *T. gondii* seropositive capybaras obtained by IFAT, correlated with different serum dilutions (titration of 1:16 to 1:512)

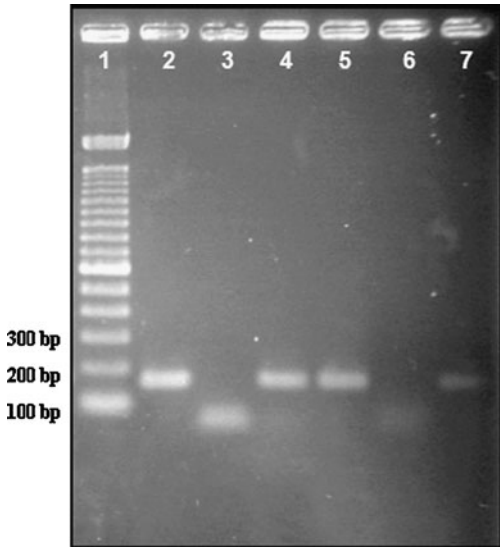

Fig. 2 A representative figure indicating the presence (*4*, *5*, *7*) or absence (*6*) of *T. gondii* DNA (B1 gene) amplified from distinct capybara tissues. *1* DNA Ladder 100 bp Sigma™, *2* positive control—RH strain of *T. gondii*, *3* negative control, *4–7* CAPY 33M liver, CAPY 31F liver, CAPY 36M lymph node, and CAPY 31F blood, respectively. The amplified fragment is 155 bp

PCR

In PCR assay using B1 primer, two capybaras (7.7%) were positive for *T. gondii* DNA. In one animal, PCR was positive in liver DNA sample; and in the other one, PCR tested positive in both liver and blood samples. Additionally, all spleen, lymph nodes, and heart samples showed negative results for B1 gene PCR.

Considering the ITS1 region, positive PCR results were obtained in three animals (11.5%). In the present assay, *T. gondii* DNA was detected in the heart of one rodent and in the liver of the other two animals. All blood, lymph nodes, and spleen DNA samples showed negative results for ITS1 PCR. Figure 2 shows representative PCR results from positive animals as compared to the *T. gondii* reference strain (positive control) and negative control.

Analyzing the two tests, *T. gondii* DNA was detected in 3.8% (1/26) in both B1 gene and ITS1 PCR, and in 15.4% (4/26) in either B1 gene or ITS1 (Table 1). All PCR positive capybaras presented occurrence of antibodies anti-*T. gondii* as detected by IFAT. The animal identified as CAPY 31F, which had the highest titer in IFAT (1:512), was also positive in both B1 gene and ITS1 PCR.

Discussion

The results from this work show that capybaras from southern Brazil are infected with *T. gondii*, as antibodies were detected in sera and *T. gondii* DNA was detected in the blood and tissue samples. Sera from 61.5% of capybaras were shown to contain antibodies against T. gondii by IFAT. There are only a few reports in the literature about *T. gondii* infection in this wild animal and they show similar or slightly higher seroprevalences. Our results corroborate the two studies performed in the state of São Paulo, where 69.8% to 76.6% of the capybaras analyzed presented antibodies to *T. gondii* (Cañon-Franco et al. 2003; Yai et al. 2008, 2009). In São Paulo, it was also used based on IFAT with a positive cutoff of 1:16. The high percentage of infection by *T. gondii* in capybaras from Brazil suggests a widespread contamination of their environment with oocysts (Dubey and Beattie 1988).

Through PCR, we have found *T. gondii* DNA in the heart, liver, and blood of 15.4% of the capybaras. This result is much lower than the 61.5% obtained by IFAT. A positive IFAT with negative PCR may be obtained with the following cases: When the capybara was only exposed to the parasite in the past or if the parasite was in the chronic phase of infection and not present in the blood or in the samples analyzed. In addition, the PCR could give a false negative result if the tissue fragments obtained by biopsy were insufficient. All animals positive on the PCR assays had antibodies against *T. gondii*, confirming the specificity of the molecular diagnosis.

The capybaras from the Tingui Park are probably being infected by the ingestion of oocysts shed by the definitive hosts (felids). Considering the habits of capybaras, it is possible to raise at least four reasons that could be contributing to the high prevalence of infection in these rodents. (1) They are gregarious animals; therefore, they are exposed to the same sources of infection. (2) They are

Table 1 The prevalence of *T. gondii*-positive capybaras obtained by PCR directed at the B1 gene and the ITS1 region

Target	Number of animals positive/total					Positive animals/total (percentage)
	Blood	Liver	Spleen	Heart	Lymph node	
B1 gene	1/26	2/26	0/26	0/26	0/26	2/26 (7.7%)
ITS1	0/26	2/26	0/26	1/26	0/26	3/26 (11.5%)
B1 and ITS1	0/26	1/26	0/26	0/26	0/26	1/26 (3.8%)
B1 or ITS1	1/26	3/26	0/26	1/26	0/26	4/26 (15.4%)

herbivores, prefer short pasture, and spend several hours of the day in the water, thus, they can get infected by several sources, including soil, food, or water. (3) They can migrate to other parks, which contain a high density of both felids and capybaras, facilitating the dispersion of the parasite. (4) The capybara's high prolificity, associated with possible transplacental transmission of tachyzoites, could also contribute to the high prevalence of the parasite. One finding, suggesting transplacental transmission among the capybaras involved in this study, is that *T. gondii* DNA was found in the blood and liver of one female capybara with high titer on IFAT (1:512). The occurrence of acute infection with T. gondii and parasitemia is associated with the transplacental transmission in other species, including rodents (Remington and Desmonts 1990; Owen and Trees 1998; Ebbesen 2000; Marshall et al. 2004). Capybaras' habits and position in the food chain are factors that contribute to the importance of this animal in the life cycle of *T. gondii* in Latin America. Besides being a possible protein source for humans, this rodent is also consumed by wild canids and felids, thus it could be a source of infection for humans and animals. Generally, the most important routes of infection to humans are horizontal, with only a small percentage acquired vertically (Kijlstra and Jongert 2008a). It is still unknown which route of postnatal transmission is more important epidemiologically since sources of infection vary depending on the cultural habits of people among different ethnic groups and geographical locations (Cook et al. 2000; Tenter et al. 2000). It is believed that the majority of postnatal transmissions to humans are caused by the ingestion of raw or undercooked meat or viscera infected with parasite-containing tissue cysts (Baril et al. 1999; Kapperud et al. 1996; Kijlstra and Jongert 2008a). In fact, outbreaks of acute toxoplasmosis have been described following the consumption of uncooked or undercooked meat worldwide (Choi et al. 1997; Ross et al. 2001; Carme et al. 2002).

Hunting of capybaras is not allowed in Brazil; nonetheless, these animals are consumed by the population either from illegal hunting or from animals raised in captivity. Future outbreaks could be previewed in and outside of the country if hunting becomes allowed and the meat exported. Therefore, to prevent possible toxoplasmosis outbreaks, the population and the scientific community, including epidemiologists, should be aware of the risks associated with the consumption of raw or undercooked capybara meat.

In summary, the results obtained in the present study indicate that free-living capybaras are infected with *T. gondii*. Most importantly, their meat could be a source of infection for humans and felids; therefore, this rodent should be included in epidemiological studies, especially those carried out during toxoplasmosis outbreaks. As these animals are widely distributed in South and Central America and have been found infected with *T. gondii*, we support the hypothesis that capybaras could act as important reservoirs of *T. gondii* and play a role in the life cycle of this apicomplexan parasite.

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Ethical standards All the experiments conducted in the present study comply with the current Brazilian laws.

Conflict of interest Research funder: CAPES
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The authors declare that they have no conflict of interest.

References

Baril L, Ancelle T, Goulet V, Thulliez P, Tirard-Fleury V, Carme B (1999) Risk factors for *Toxoplasma* infection in pregnancy: a case control study in France. Scand J Infect Dis 31:305–309

Burnie D, Wilson DE (2001) Animal. Dorling Kindersley, London

Cañon-Franco WA, Yai L, Joppert AM, Souza CE, D'Auria SR, Dubey JP, Gennari SM (2003) Seroprevalence of *Toxoplasma gondii* antibodies in the rodent capybara (*Hidrochoeris hidrochoeris*) from Brazil. J Parasitol 89:850

Carme B, Bissuel F, Ajzenberg D, Bouyne R, Aznar C, Demar M, Bichat S, Louvel D, Bourbigot AM, Peneau C, Neron P, Dardé ML (2002) Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. J Clin Microbiol 40:4037–4044

Choi WY, Nam HW, Kwak NH, Huh W, Kim YR, Kang MW, Cho SY, Dubey JP (1997) Foodborne outbreaks of human toxoplasmosis. J Infect Dis 175:1280–1282

Cook AJC, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE, Dunn DT (2000) Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. Br Med J 321:142–147

Dubey JP, Beattie CP (1988) Toxoplasmosis of animals and man. CRC Press, Boca Raton, Florida

Dubey JP, Jones JL (2008) *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol 38:1257–1278

Ebbesen P (2000) Placenta physiology. In: Ambroise-Thomas P, Petersen E (eds) Congenital toxoplasmosis: scientific background, clinical management and control. Springer, Paris, pp 27–35

EFSA (2007) Monitoring of toxoplasma in humans, food and animals. Scientific opinion of the panel on biological hazards. EFSA J 583:1–64

Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A, Eng J (1996) Risk factors for *Toxoplasma gondii* infection in pregnancy—results of a prospective case-control study in Norway. Am J Epidemiol 144:405–412

Kijlstra A, Jongert E (2008a) Control of the risk of human toxoplasmosis transmitted by meat. Int J Parasitol 38:1359–1370

Kijlstra A, Jongert E (2008b) *Toxoplasma* safe meat: close to reality? Trends Parasitol 781:1–5

Kim K, Weiss LM (2008) *Toxoplasma*: the next 100 years. Microb Infect 10:978–984

Marshall PA, Hughes JM, Williams RH, Smith JE, Murphy RG, Hide G (2004) Detection of high levels of congenital transmission of *Toxoplasma gondii* in natural urban populations of *Mus domesticus*. Parasitol 128:39–42

McDonald JC, Gyorkos TW, Alberton B, MacLean JD, Richer G, Juranek D (1990) An outbreak of toxoplasmosis in pregnant women in northern Quebec. J Infect Dis 161:769–774

Owen MR, Trees AJ (1998) Vertical transmission of *Toxoplasma gondii* from chronically infected house (Mus musculus) and field (*Apodemus sylvaticus*) mice determined by polymerase chain reaction. Parasitol 16:299–304

Pujol-Riqué M, Derouin F, Garcia-Quintanilla A, Vails ME, Miro JM, de Anta MTJ (1999) Design of a one-tube heminested PCR for detection of *Toxoplasma gondii* and comparison of three DNA purification methods. J Med Microbiol 48:857–862

Redford KH, Eisenberg JF (1992) Mammals of the neotropics. The Southern Cone. The University of Chicago Press, Chicago, IL

Remington JS, Desmonts G (1990) Toxoplasmosis. In: Remington JS, Klein JO (eds) Infectious diseases of the fetus and newborn infant, vol. 3. WB Saunders, Philadelphia, pp 89–195

Robson JMB, Wood RN, Sullivan JJ, Nicolaides N, Lewis BR (1995) A probable foodborne outbreak of toxoplasmosis. Comm Dis Intelligence 19:517–522

Ross RD, Stec LA, Werner JC, Blumenkranz MS, Glazer L, Williams GA (2001) Presumed acquired ocular toxoplasmosis in deer hunters. Retina-J Retin Vitreous Dis 21:226–229

Sibley lD (2003) Recent origins among ancient parasites. Vet Parasitol 115:185–198

Tenter AM, Heckerotha AR, Weiss LM (2000) *Toxoplasma gondii*: from animals to humans. Int J Parasitol 30:1217–1258

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols, a guide for methods and applications. Academic Press, pp 315–322

Yai LE, Ragozo AM, Aguiar DM, Damaceno JT, Oliveira LN, Dubey JP, Gennari SM (2008) Isolation of *Toxoplasma gondii* from capybaras (*Hydrochaeris hydrochaeris*) from São Paulo State, Brazil. J Parasitol 94:1060–1063

Yai LE, Ragozo AM, Soares RM, Pena HF, Su C, Gennari SM (2009) Genetic diversity among capybara (*Hydrochaeris hydrochaeris*) isolates of *Toxoplasma gondii* from Brazil. Vet Parasitol 162:332–337