Acquired and Congenital Ocular Toxoplasmosis Experimentally Induced in *Calomys callosus* (Rodentia, Cricetidae)

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An experimental model for acquired and congenital ocular toxoplasmosis as well as a model to induce experimental autoimmune uveitis (EAU) was investigated in Calomys callosus. Toxoplasma gondii, *ME-49 strain, was used to infect males and pregnant- and not pregnant-females while S-antigen, a major glycoprotein of the retinal photoreceptor cell, was used to induce EAU. The ocular lesions elicited by* T. gondii *were characterized by the presence of cysts, free tachyzoites and inflammatory cells in the retina or related tissues. In the congenital form, 40% of the fetus presented ocular lesions, i.e., presence of cysts in the retina, vitreous, and extra-retinal tissues. In the acquired form, 75% of the females and 50% of the males presented unilateral ocular cysts both at 21 and 47 days post-infection. It was also demonstrated that S-antigen was not uveitogenic in the* C. callosus *model. No lesion was observed in the animals exclusively immunized with this retinal component, even when jacalin was used as additional adjuvant for polyclonal response to the retinal antigen. It can be concluded that* C. callosus *may constitute in a promising model for study both acquired and congenital ocular toxoplasmosis, particularly when it is important to make sure that a non autoimmune process is involved in the genesis of the ocular infection.*

Key words: C*alomys callosus* - *Toxoplasma gondii* - acquired and congenital ocular toxoplasmosis - S-antigen

Toxoplasma gondii, an obligate intracellular parasite found in many animal species throughout the world, causes a variety of clinical syndromes in humans and animals. The infection is frequently assymptomatic and most cases of acquired *Toxoplasma* infection are subclinical. However, there are two groups of high-risk individuals: the human fetuses and the immunosuppressed patients (Favoreto-Jr et al. 1998). The prevalence of *T. gondii*-specific antibodies in adults increases with advancing age but differs widely with the geographic location (Lynfield & Guerina 1997).

When congenital ocular toxoplasmosis is present in childhood, the infection may have interfered with the development of the eye (Dutton 1989). Uveitis is initiated in every instance by some form of tissue injury as, for example, the penetration of a single cell by an organism such as *Toxoplasma* (O'Connor 1983).

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Uveitis comprises a complex group of diseases and the mechanisms that lead to the initiation of this ocular inflammatory disease have been the subject of many investigations. An uveitogenic retinal agent (S-antigen) has been postulated to be involved in this disease. This soluble antigen presents a molecular weight of approximately 50 kDa and has been purified from the photoreceptor region of bovine, human, rabbit, and guinea-pig retinas. An immunizing dose as low as 5 mg of purified S-antigen injected at a distant site will induce a severe ocular inflammatory response in most laboratory animals tested (Nussenblatt et al. 1980).

Previous studies have shown that *Calomys callosus* (Rodentia, Cricetidae), a characteristic rodent in central Brazil, is an excellent experimental model for some parasitic infections (Mello 1981) and it was proposed as an alternative model to study acute phase of experimental toxoplasmosis (Favoreto-Jr et al. 1998).

The aim of this study was to assess the accomplishment of *C. callosus* as a new experimental model of acquired and congenital ocular toxoplasmosis. The maternal-fetal transmission of *T. gondii* was observed following experimental infection during the pregnancy and the transmission of the

acquired disease was observed in males and pregnant and non-pregnant females. In addition, we evaluated if the uveitis could be induced either by antigenic similarity between *T. gondii* and S-antigen or by the exposition of the retinal antigen which occurred subsequently to the *Toxoplasma* infection.

MATERIALS AND METHODS

Animals - *C. callosus* (Canabrava strain) were obtained from a resident colony housed at the Laboratory of Histology from Universidade Federal de Uberlândia, MG, Brazil. The animals were approximately 2-3 months old males and females, and were kept on a 10 hr light: 14 hr dark cycle in a controlled room temperature (25ºC), with food and water *ad libitum* and supplemented with corn and sunflower seeds.

Parasites - *T. gondii* ME-49 strain parasites were obtained from brains of previously infected BALB/c mice according to Brown et al. (1995). Brains were removed and washed with sterile saline, macerated and homogenized with needle and syringe, and submitted to the centrifugation at 1,000 g for 10 min. A volume of 40 ml of the suspension was placed on a glass microscope slide and the number of cysts was determined microscopically. In order to adapt the *T. gondii* ME-49 strain to *C. callosus*, animals were orally infected with 20 cysts obtained from brains of BALB/c mice inoculated at least 1 month earlier.

Mating - Three uninfected virgin females of *C. callosus* were housed in a cage with one male. The animals were inspected daily for the presence of vaginal plugs and the day of this evidence was designated as day 1.

Pregnant female infection - In order to evaluate the development of acquired and congenital forms of toxoplasmosis in *C. callosus*, females from 5 to 7 days of pregnancy were orally infected with 20 cysts of *T. gondii*, ME-49 strain, obtained from brains of the animals used for strain adaptation. The females and their respective fetuses were killed at 15, 16, 17, 18, 19 and 21 days of pregnancy. The mothers were anesthetized by ether inhalation and killed by cervical displacement after heart puncture. The fetuses were removed by laparatomy and decapitated. The maternal and fetal enucleated eyes were fixed in 0.01M phosphate buffered saline (PBS) pH 7.2 plus 10% formalin solution for 24 hr, and subsequently conditioned in 70% alcohol until the inclusion.

Infection of males and non pregnant females - For evaluation of the evolution of acquired toxoplasmosis, 8 males and 4 virgin females were housed in separated cages and orally infected with 20 cysts of *T. gondii*, ME-49 strain. The eyes of the animals were collected after 21 (4 males) or 47 (4 males and 4 females) days following infection as previously described.

Bovine S-antigen - S-antigen was obtained from bovine eyes, according to Wacker et al. (1977), with some modifications. These specimens were kindly provided by Frigorífico Triângulo, Uberlândia. The anterior portion of the ocular globe was removed by circumcorneal incision and the vitreous body and lens were dissected. A total of 100 retinas was separated from the choroid and epithelial pigment in a partially darkroom and conditioned in a beaker under ice-bath. A 20% suspension (w/v) of bovine retinas was prepared in extraction buffer (0.1M NaCl plus 0.05M potassium phosphate, pH 7.2), homogenized and centrifuged at 48,000 g for 3 hr 30 min. Proteins in the supernatant were precipitated by a half-saturated ammonium sulfate solution overnight at 4ºC. The precipitate was sedimented by centrifugation and dissolved in extraction buffer, dialyzed several times and submitted to gel filtration chromatography.

Gel filtration chromatography and SDS gel electrophoresis - A Sephadex G-100 column (2.5 x 100 cm) was previously calibrated with extraction buffer and the retinal extract was applied, subsequently eluted with the same buffer and 3.5 ml fractions were collected at a flow rate of 0.35 ml/min. The protein content was measured spectrophotometrically at 280 nm and the protein fractions were pooled and concentrated to a volume of 5 ml. After being dialyzed, the samples of each fraction were diluted (v/v) at 10 mg/ml in sample buffer (0.1M TRIS pH 6.8, 4% SDS - sodium dodecyl sulphate, 0.2% bromophenol blue and 20% glycerol), boiled for 3 min at 100ºC and submitted to the SDS gel electrophoresis at 12% (Laemmli 1970) and further stained by silver (Zigler-Jr et al. 1984).

Experimental autoimmune uveitis (EAU) - In attempt to induce EAU, *C. callosus* specimens were divided in 10 groups and inoculated with S-antigen by intradermal or intramuscular routes and/or cysts of *T. gondii* ME-49 strain orally, with or without adjuvants, as follows: (1) 20 cysts of *T. gondii* ME-49 strain; (2) 50 mg of S-antigen; (3) 50 mg of S-antigen + CFA (Complete Freund's Adjuvant - Difco Laboratories, Detroit, MI, USA); (4) 50 mg of S-antigen + 100 mg of jacalin (a soluble extract of *Artocarpus integrifolia*, as described by Roque-Barreira and Campos-Neto 1985); (5) 50 mg of S-antigen + CFA + 100 mg of jacalin; (6) 50 mg of S-antigen + 20 cysts of *T. gondii* ME-49 strain; (7) CFA; (8) 100 mg of jacalin; (9) CFA + 100 mg of jacalin; (10) PBS.

After 21 days of the immunization and/or infection, the animals were anesthetized by ether in-

halation, bled by heart puncture and killed by cervical displacement and the eyes were enucleated and fixed as previously described.

Histopathological assays - The eyes were processed and included in historesin (glycol methacrylate, LKB 702218-500, Sweden), according to the manufacturer instructions. Sections of 2 mm thickness were made and collected at each 10 mm. This procedure was adopted until approximately half of the ocular globe. The slides were stained with toluidin blue containing 1% borax and 1% toluidin for 30 sec and preserved in entellan (Merck, Darmstadt, Germany) covered by cover-slips. Finally, the material was analyzed for the presence of cysts or free tachyzoites or inflammatory cells by three independent observers who did not know in advance the identification of the groups from this study.

Serological assays - Enzyme-linked immunosorbent assay (ELISA) was carried out for detection of antibodies to S-antigen and *T. gondii*, as described (Mineo et al. 1980), with some modifications. Microtiter plates (Interlab Diagnóstica SA) were coated either with a soluble extract of *T. gondii* RH strain tachyzoites (2.5 mg/ml) or S-antigen (10 mg/ml) in 0.06M sodium carbonate buffer pH 9.6 overnight at 4ºC. The plates were washed three times with PBS containing 0.1% Tween-20 (PBST) and incubated with the *C. callosus* serum samples at dilution of 1:32 in PBST for 45 min at 37ºC. Positive and negative serum controls were included on each plate. After repeated washing, it was added peroxidase-labeled rabbit IgG anti-*C. callosus* gama-globulin conjugate which was prepared as described by Wilson and Nakane (1978). This reagent was used at 1:1000 dilution in PBST and incubated for 45 min at 37ºC. After final wash, it was added the substrate, consisting of hydrogen peroxide and o-phenylenediamine (Merck, Germany) in 0.1M citrate-Na_2HPO_4 buffer (pH 5.5) for 15 min at room temperature. The enzymatic reaction was stopped with 2N H_2SO_4 and the absorbance was determined at 492 nm using a microplate reader (Titertek Multiskan Plus, Flow, USA). The cut-off values were arbitrarily determined as those exceeding the mean absorbance plus three standard deviations obtained for the negative controls.

Western-blotting (WB) - In order to detect antigenic bands from *T. gondii* and S-antigen recognized by the *C. callosus* serum samples, the WB technique was carried out according to Towbin et al. (1979). The respective soluble antigenic extracts and molecular weight standards (Sigma Chem. Co., St. Louis, MO, USA) were submitted to electrophoresis in SDS-PAGE at 12% (Laemmli 1970). After SDS-PAGE, gels were transferred to a nitrocellulose membrane (Sigma Chem. Co.) and the protein-binding sites were blocked with 5% nonfat milk in 0.05% Tween 20-PBS for 2 hr at room temperature. Strips were incubated with *C. callosus* serum samples diluted at 1:50 in 1% nonfat milk-0.05% Tween 20-PBS (dilution buffer) overnight at 4ºC. After several washings with dilution buffer, the strips were incubated for 2 hr with peroxidase-rabbit IgG anti-*C. callosus* gama-globulin conjugate diluted at 1:500 in dilution buffer. The strips were washed and revealed by hydrogen peroxide plus 3'3' tetra-diamino-benzidine tetrahydrochloride (DAB) in PBS. The reaction was stopped by washing with H_2O.

RESULTS

Bovine S-antigen purification - Samples from each step of the retinal extract purification process by gel filtration were applied in SDS-PAGE and stained by silver. The fraction containing S-antigen was shown as a single band of 50 kDa, approximately (lane 9, Fig. 1).

Induction of experimental uveitis - After S-antigen purification, 50 mg doses were inoculated with or without various adjuvants by intradermal or intramuscular routes in attempt to induce ocular lesion in different groups of animals. As shown in Figs 2 and 3, no histopathologic features that could indicate the presence of EAU were seen in these respective groups. In contrast, the animals orally infected with *T. gondii* ME-49 strain cysts presented retinal cyst and cones- and rods-layer alterations which are attributes of toxoplasmic uveitis (Figs 4, 5).

Acquired and congenital ocular toxoplasmosis in pregnant females - The ocular lesion caused by *T. gondii* was characterized by the presence of parasitic cysts, free tachyzoites and/or inflammatory cells in the retina or sustentation layer (Table I). The presence of inflammatory cells was a rare event in most of the animals (fetuses and adults) with ocular cysts, or even in those with free tachyzoites in the retina. From a total of 25 fetuses that had been studied, 10 (40%) presented ocular lesion characterized by presence of cysts in: retina (16%), vitreous (8%), retina external tissues (8%), optic nerve (4%) and lens (4%) (Figs 6-11). The 15 remaining fetuses (60%) did not present ocular lesion. Samples from six pregnant females that have been examined, 3 (50%) presented ocular lesion and among these, 1 (33%) presented retinitis with destruction of the cones- and rods-layer (Figs 12, 13).

Acquired ocular toxoplasmosis in males and non pregnant females - Males and non pregnant females after 21 or 47 days of infection were examined for the presence of the acquired form of the disease, without influence of pregnancy fac-

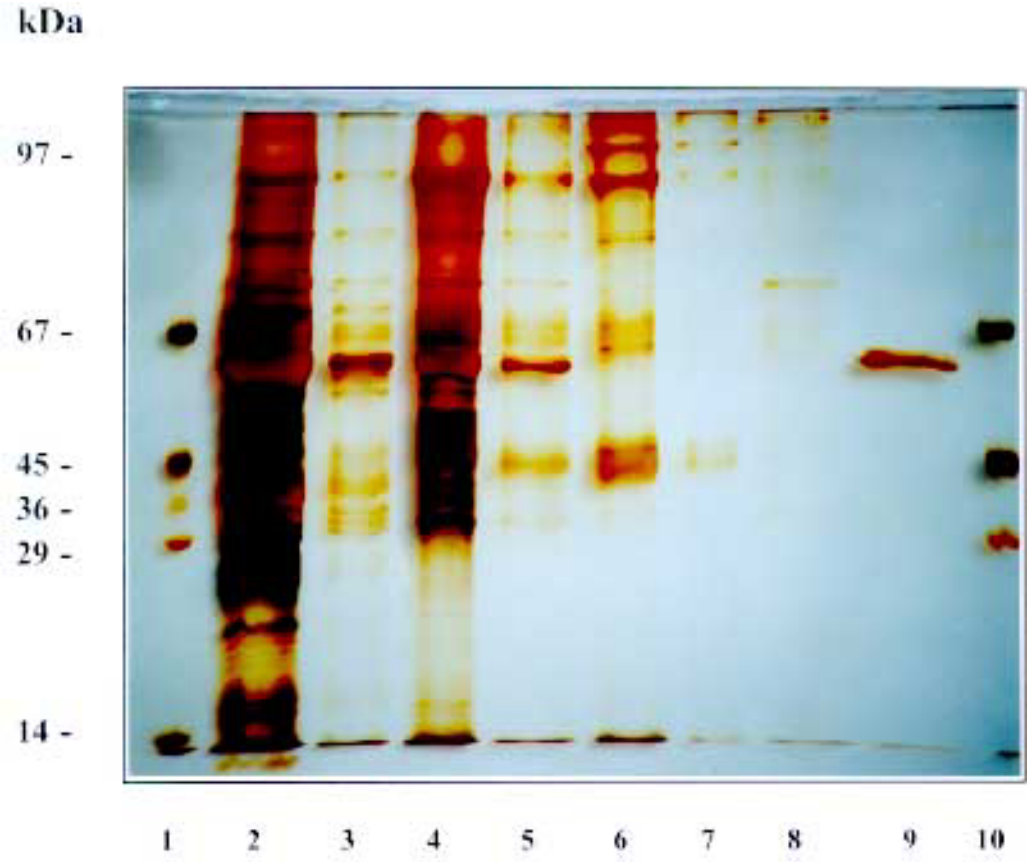


Fig. 1: silver-stained SDS gel electrophoresis (12%) of retinal extracts before and after purification in Sephadex G-100. Lane 1, low molecular weight standards; lane 2, crude retinal extract (50 mg); lane 3, crude retinal extract (10 mg); lane 4, half-saturated ammonium sulfate precipitated extract (20 mg); lane 5, half-saturated ammonium sulfate precipitated extract (5 mg); lane 6 - fraction 1 (25 mg); lane 7, fraction 1 (5 mg); lane 8, fraction 2 (5 mg); lane 9, fraction 3 (5 mg); lane 10, high molecular weight standards.

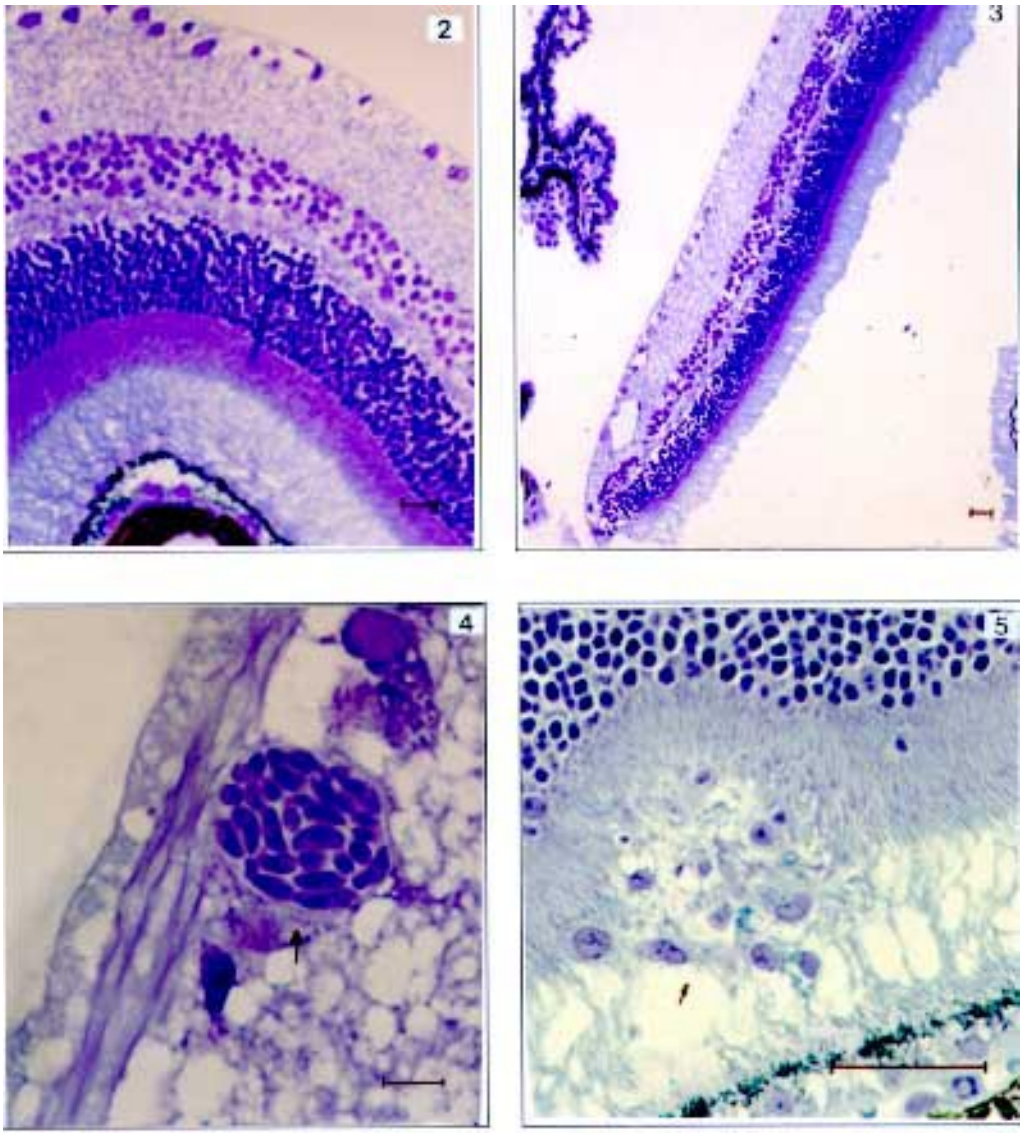
tors (Table II). Among the males with 21 days post-infection, 50% presented retinal cysts in the absence of inflammatory cells (Figs 14, 15). Animals with 47 days post-infection presented cysts and/or inflammatory cells in the retina, vessels and sustentation layer. It was estimated that 75% of the females presented unilateral lesion (cysts) and 25% of the males presented unilateral lesion (cysts), while 25% of them presented bilateral lesion (cysts in an eye and inflammatory process in contralateral eye) (Figs 16-19).

Serological evaluation and WB - Antibodies against *T. gondii* and S-antigen were examined in serum samples at dilution of 1:32. Table III shows the reactivity of various groups of animals orally infected with cysts of *T. gondii* ME-49 strain or immunized with S-antigen, associated or not with

TABLE I

Acquired and congenital ocular toxoplasmosis in *Calomys callosus* infected orally with 20 cysts of the *Toxoplasma gondii* ME-49 strain at 5 or 7 days of pregnancy

Pregnancy time (days)		Presence of ocular lesion	
At infection	At analysis	Pregnant females	Fetus
5	15	-	1 / 4
7	16	-	3 / 5
5	17	+	0 / 3
5	18	+	2 / 6
7	19	-	2 / 3
7	21	+	2 / 4



Micrographs of eye section, stained by toluidin blue, from *Calomys callosus*, adult male, after 21 days of being treated with one of the following conditions. Fig. 2: immunization with S-antigen. Fig. 3: immunization with S-antigen plus jacalin. Figs 4, 5: immunization with S-antigen plus infection with 20 cysts of *Toxoplasma gondii*. Bar = 10 mm.

different adjuvants. It was observed a great dispersion of the values from different groups. However, in general the antibodies were detected in all homologue groups. It was observed that the presence of antibodies directed to the retinal antigen was not related with the presence of the ocular lesion. To assess the specificity of the antibody detection by ELISA, it was carried out the western blot reaction with those samples that presented unclear results.

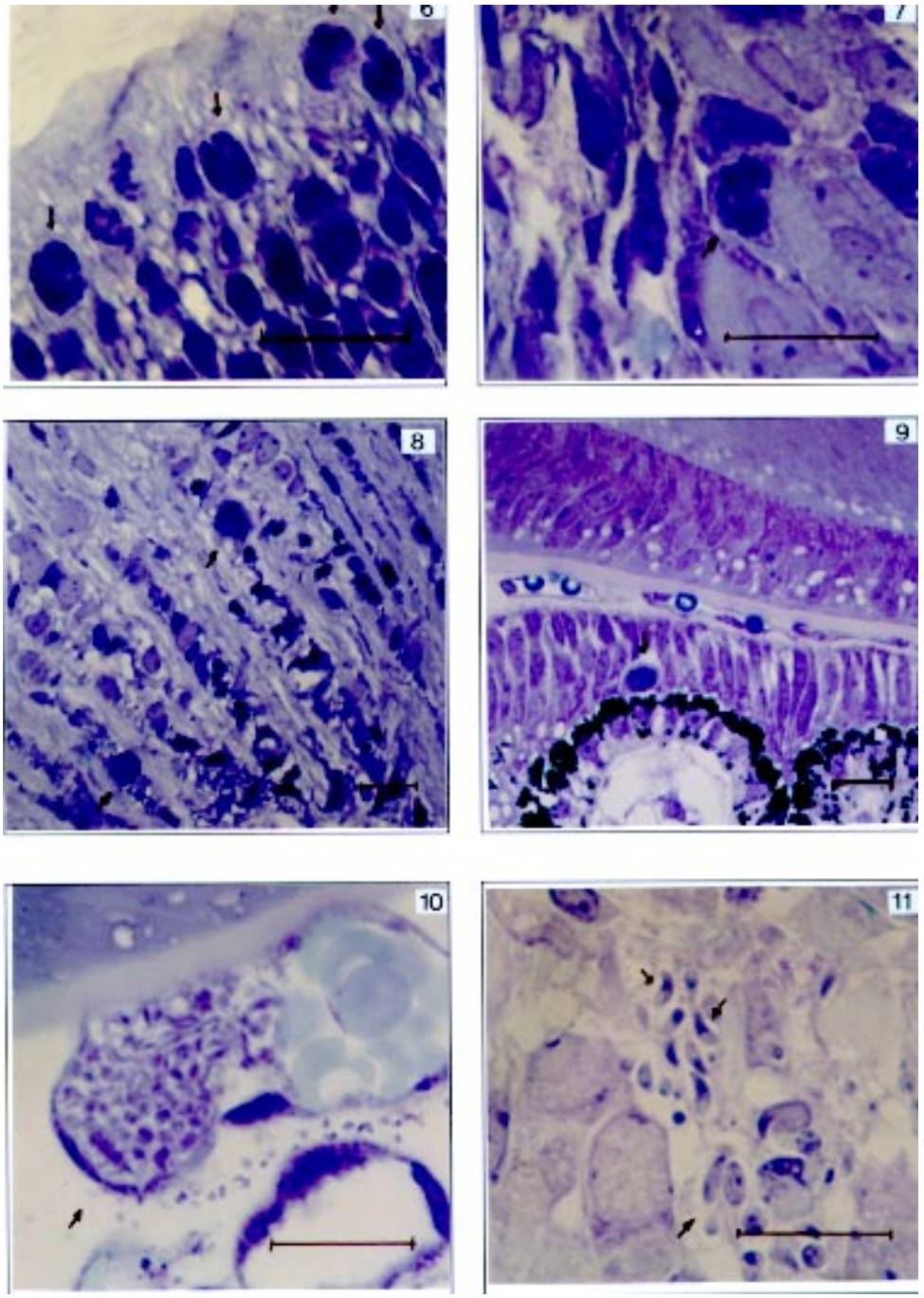
The WB results obtained from serum samples of *C. callosus* of the different groups when S-anti-

TABLE II

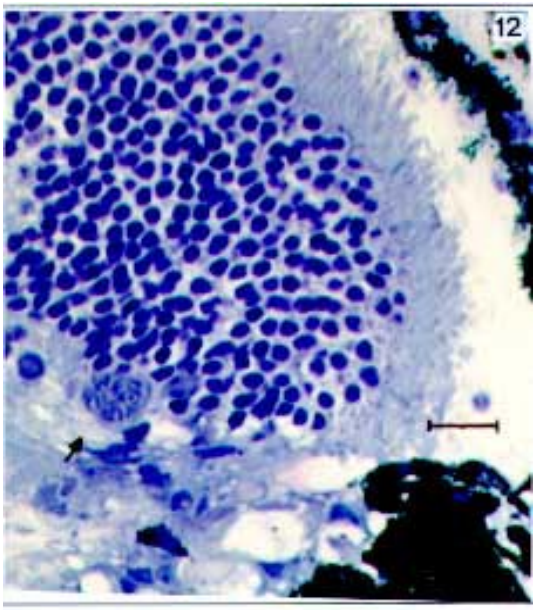
Acquired ocular toxoplasmosis in *Calomys callosus* infected orally with 20 cysts of the *Toxoplasma gondii* ME-49 strain

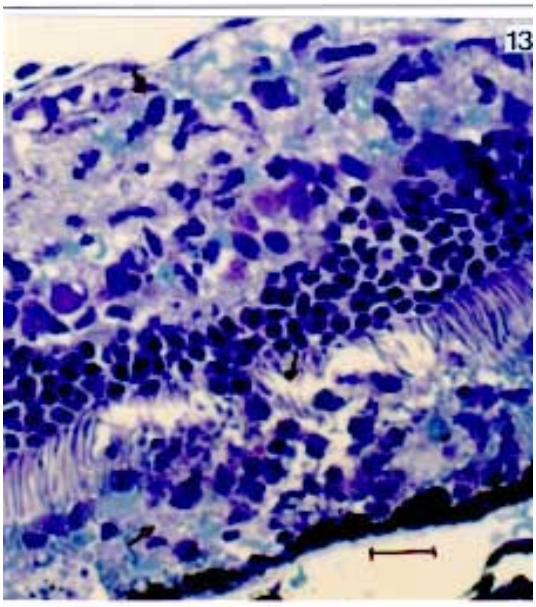
Sex	Time Post-infection (days)	Presence of ocular lesion among the infected animals	Type of ocular lesion
Male	21	2/4	Unilateral
Male	47	2/4[a]	Unilateral
Female	47	3/4	Unilateral

a: an animal presented bilateral ocular lesion.

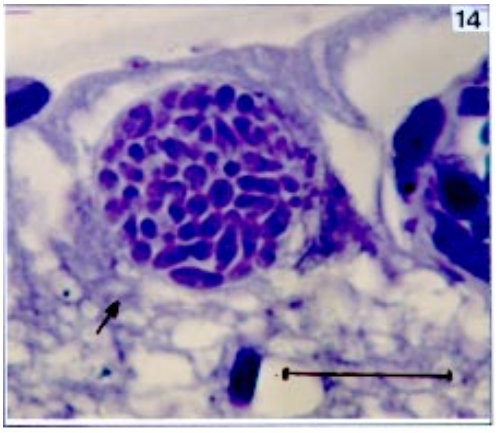


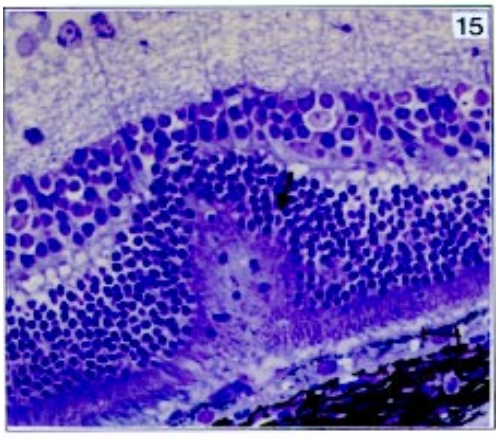
Micrographs of eye section, stained by toluidin blue, from fetuses of *Calomys callosus* which mothers had been infected perorally with 20 cysts of *Toxoplasma gondii*. Fig. 6: lens from fetus presenting 16 days of development and 9 days of infection; arrow indicates cysts. Fig. 7: retina adjacent tissues from fetus presenting 16 days of development and 9 days of infection; arrow indicates cyst. Fig. 8: optic nerve from fetus presenting 18 days of development and 13 days of infection; arrows indicate cysts. Fig. 9: retina from fetus presenting 21 days of development and 14 days of infection; arrow indicates cyst. Fig. 10: vitreous of fetus presenting 19 days of development and 12 days of infection, without inflammatory reaction; arrow indicates cysts nearby a blood vessel. Fig. 11: retina from fetus presenting 21 days of development and 14 days of infection; arrows indicate free tachyzoites, without inflammatory reaction. Bar = 10 mm.





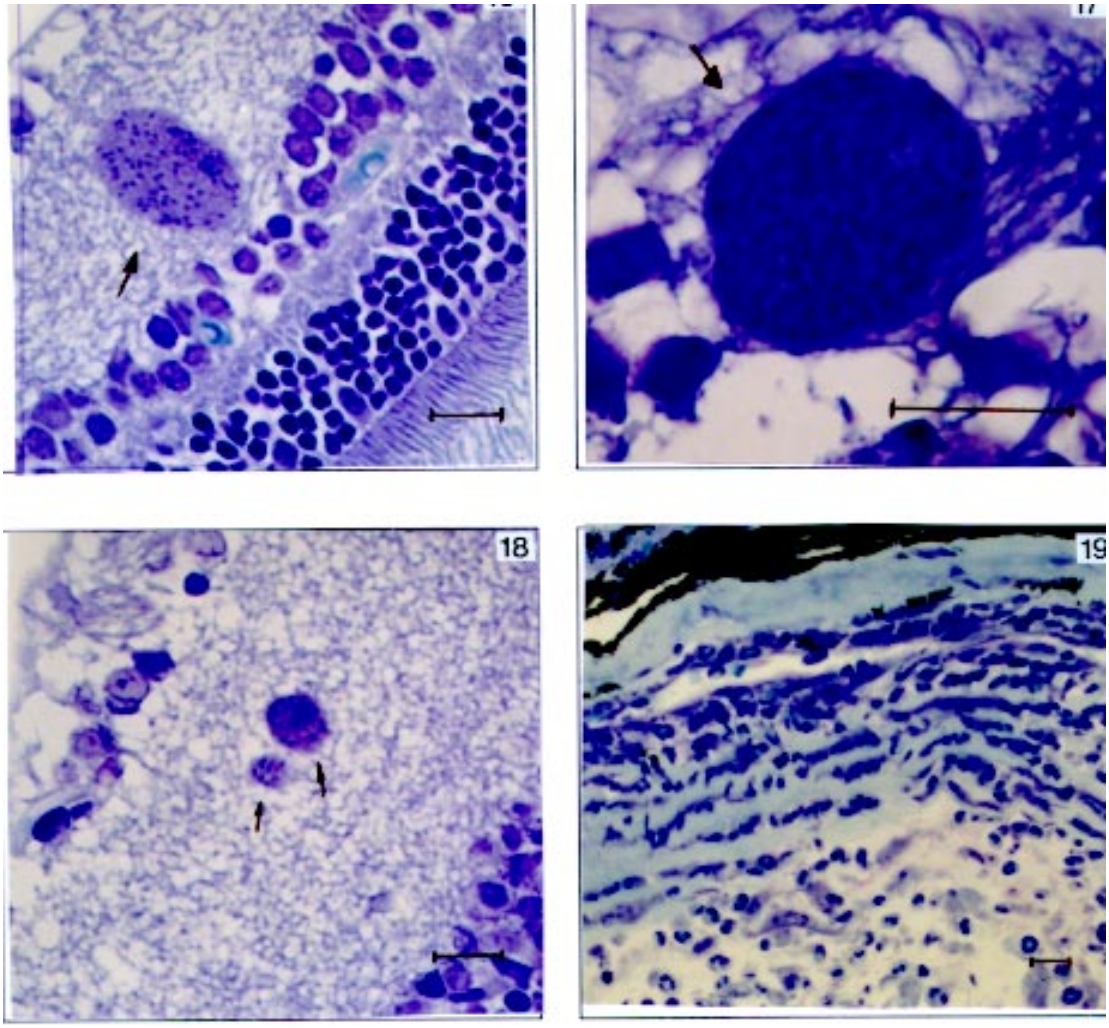
Micrographs of eye section, stained by toluidin blue, from *Calomys callosus*, pregnant females, infected orally with 20 cysts of *Toxoplasma gondii*. Fig. 12: retina of animal with 21 days of pregnancy and 12 days post-infection; arrow indicates cyst. Fig. 13: retina of animal with 17 days of pregnancy and 12 days post-infection; arrows indicate presence of inflammatory cells (neuthrophils). Bar = 10 mm.





Micrographs of eye section, stained by toluidin blue, from *Calomys callosus*, adult male, infected orally 21 days before with 20 cysts of *Toxoplasma gondii*. Fig. 14: retina without inflammatory reaction; arrow indicates cyst of *T. gondii*. Fig. 15: retina presenting morphological changes; arrow indicates alterations in the retinal layers. Bar = 10 mm.

gen was immobilized in the nitrocellulose membrane are showed in Fig. 20. The animals immunized with S-antigen only showed a weak intensity of antibody response to this antigen (lane 1), while those immunized with S-antigen + CFA associated or not with jacalin revealed a strong reactivity to S-antigen (lanes 2 and 4, respectively). It was also observed reactivity for other two bands > 67 kDa (lanes 2 and 4). There was no detection of antibody synthesis to S-antigen in the animals immunized with either S-antigen + jacalin, or jacalin or CFA only or jacalin + CFA (lanes 3, 5, 6 and 7, respectively). In contrast, it was observed a weak antibody response to S-antigen when the animals had been infected with *T. gondii* ME-49 strain, even following 47 days of infection (lane 8) while a strong reactivity was observed in the animals that had been immunized with ME-49 strain + jacalin at 21 days post-infection (lane 9). Serum samples of *C. callosus* inoculated with the *T. gondii* RH strain as well as sera of uninfected animals showed no reactivity to S-antigen (lanes 10 and 11).



Micrographs of eye section, stained by toluidin blue, from *Calomys callosus,* at 47 days post-infection with 20 cysts of *Toxoplasma gondii*, perorally. Figs 16, 17: retina from non-pregnant female; arrows indicate presence of retinal cysts without inflammatory reaction. Fig. 18: retina from adult male; arrows indicate presence of retinal cysts without inflammatory reaction. Fig. 19: retinal adjacent tissues from adult male; presence of intense inflammatory process. Bar = 10 mm.

TABLE III

Reactivity for *Toxoplasma gondii* and S-antigen determined by ELISA in serum samples from groups of *Calomys callosus* infected or immunized with various immunogens

Groups of animals infected or inoculated with various immunogens	Detection of antibodies in serum samples at dilution of 1/32 (mean absorbance ± standard deviations)	
	Anti-*T. gondii*	Anti-S-antigen
T. gondii ME-49	.447 (± .078)	.310 (± .145)
S-antigen	.313 (± .249)	.382 (± .125)
S-antigen + CFA	.279 (± .151)	.638 (± .209)
S-antigen + jacalin	.264 (± .125)	.395 (± .246)
S-antigen + CFA + jacalin	.190 (± .136)	.515 (± .220)
S-antigen + ME-49 + CFA	.385 (± .032)	.312 (± .112)
CFA	.242 (± .081)	.255 (± .052)
Jacalin	.225 (± .164)	.245 (± .096)
CFA + jacalin	.489 (± .210)	.439 (± .176)
PBS (Control)	.116 (± .089)	.288 (± .137)

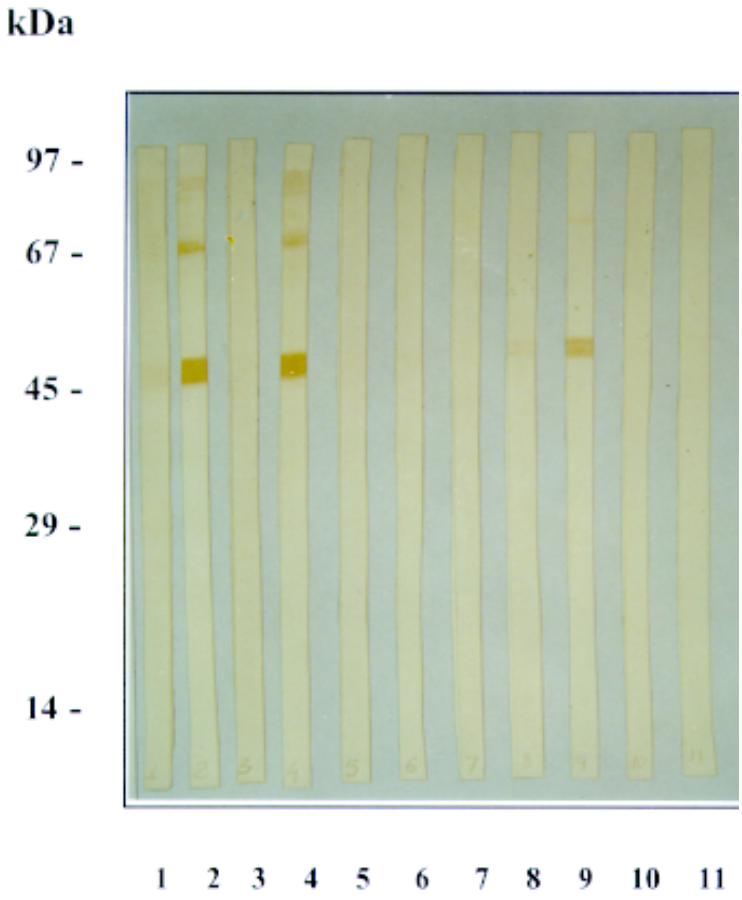


Fig. 20: Western-blot of S-antigen, purified from bovine retina, recognized by serum samples of *Calomys callosus*, immunized with: lane 1, S-Antigen (50 mg); lane 2, S-Antigen (50 mg) and CFA (1 mg/ml); lane 3, S-Antigen (50 mg) and jacalin (100 mg); lane 4, S-Antigen (50 mg), jacalin (100 mg) and CFA (1 mg/ml); lane 5, jacalin (100 mg); lane 6, CFA (1 mg/ml); lane 7, CFA (1 mg/ml) and jacalin (100 mg); lane 8, 20 cysts of *Toxoplasma gondii*, strain ME-49,by oral route, after 47 days of infection; lane 9, 20 cysts of *T. gondii*, strain ME-49, by oral route, after 21 days of infection and jacalin (100 mg); lane 10, positive serum sample control; lane 11, negative serum sample control.

Fig. 21 shows the WB results when *T. gondii* antigen was immobilized in the nitrocellulose membrane. In all studied groups, that is, animals immunized with only S-antigen and/or S-antigen associated to CFA or jacalin as adjuvants, it was not possible to detect antibodies anti-*T. gondii* (lanes 1 to 7). On the other hand, the animals infected with *T. gondii* ME-49 strain showed a characteristic profile of antibodies to the major antigens of this parasite (lanes 8 and 9), in consonance to the profile obtained with sera of animals inoculated with *T. gondii* RH strain (lane 10) and absence of antibodies in non-inoculated animals (lane 11).

DISCUSSION

The utilization of experimental models to study congenital and acquired ocular toxoplasmosis appears to be mandatory to understand the different factors influencing ocular lesion induced by *T. gondii*. C57BL/6 mice infected with the ME-49 strain of this parasite develop a progressive meningoencephalitis, which is initially characterized by the presence of occasional cysts and scattered neuronal necrotic foci with microglial and lymphocytic infiltrates (Gazzinelli et al. 1994).

C. callosus has demonstrated to be an animal of easy handle in laboratory allowing a gestational phase following-up for the fetal study. It survives to infection with the *T. gondii* ME-49 strain for several months without treatment and demonstrating no clinical signs of the disease.

In human and murine models, vertical transmission of *T. gondii* occurs from acutely infected

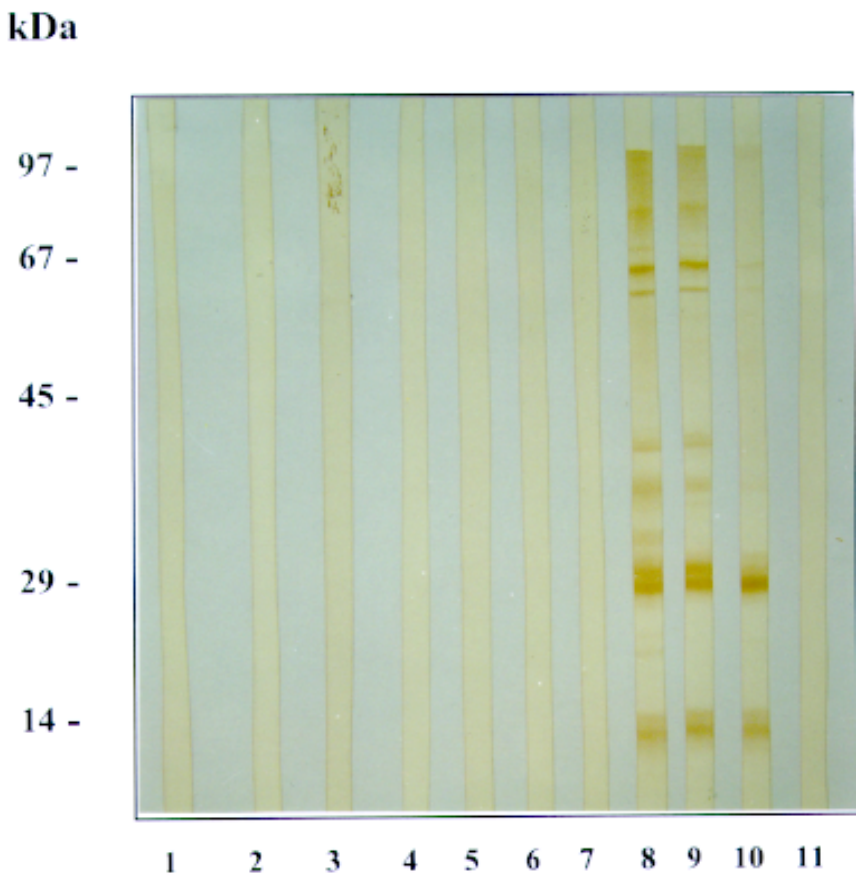


Fig. 21: Western-blot of *Toxoplasma gondii* antigen-specific markers recognized by serum samples of *Calomys callosus*, immunized with: lane 1, S-Antigen (50 mg); lane 2, S-Antigen (50 mg) and CFA (1 mg/ml); lane 3, S-Antigen (50 mg) and jacalin (100 mg); lane 4, S-Antigen (50 mg), jacalin (100 mg) and CFA (1 mg/ml); lane 5, Jacalin (100 mg); lane 6, CFA (1 mg/ml); lane 7, CFA (1 mg/ml) and jacalin (100 mg); lane 8, 20 cysts of *T. gondii*, strain ME-49,by oral route, after 47 days of infection; lane 9, 20 cysts of *T. gondii*, strain ME-49,by oral route, after 21 days of infection and jacalin (100 mg); lane 10, positive serum sample control: lane 11, negative serum sample control.

mothers to her fetus (Lynfield et al. 1997). Transmission rates appear to correlate well with placental blood flow: the risk of fetal infection may be as low as 1% or less when maternal infection occurs in the periconception period and as high as 90% or greater when maternal infection occurs near term. The average transmission rate is approximately 15% for the first trimester, 30% for the second trimester, and 60% for the third trimester (Lynfield et al. 1997). Since the reproductive cycle of *C. callosus* is approximately three weeks, it might be considered the first pregnancy week as corresponding to first gestational trimester in humans. In the present study the occurrence of ocular toxoplasmosis was of 40% in the fetuses from mothers infected at 5 or 7 days of pregnancy. Probably, the remaining fetuses could contain the parasite in other organs but the eye. It was possible to verify the presence of parasites in the fetal placentas of this study (data not showed). Thus, it seems that ocular toxoplasmosis in those fetuses could occur in a later period.

According to Gonçalves and Yamamoto (1997), the retina is the primary site of *Toxoplasma* infection. *Toxoplasma* antigens probably reach the eye by bloodstream as would occur during the congenital or acquired infection (Newman et al. 1982). Thus, the majority of intra-retinal cases of cyst formation probably occurs as a result of pre-or postnatal parasitaemia. These cysts may remain dormant within the retina and other tissues. It has also proposed that the parasites might gain access to the eye by passage along the optic nerve (Dutton 1989).

It was observed in the present study that the most of the animals presented unilateral cysts in

the retina. In the congenital form, 16% of the fetuses presented cysts in the retina and the remaining in another areas such as lens, vitreous, optic nerve and tissues from sustentation layers. In some fetuses, the presence of cyst was verified adjacent to a vessel in the vitreous. Perhaps, these cysts have developed in the parenquimal cells during the embryonic development, but further studies are necessary to clarify this subject.

In *C. callosus*, the fetal retina showed little differentiation in the layers, with significant vascularization as it occurs with the embryonic retina from humans which does not present individualization of the layers, becoming avascular during the fetal period (Moore 1990).

Rothova et al. (1986) suggest that ocular involvement has rarely occurred in cases of acquired toxoplasmosis in humans. Nogueira et al. (1996) reported that during the acquired toxoplasmosis, generally unilateral retinochoroiditis could occur in 1-2% of the human cases. However, it has been observed that the incidence of acquired ocular toxoplasmosis may be high in some geographical areas. The Erechim, RS, Brasil is a region with the highest incidence of this form of ocular toxoplasmosis in the world and it has been suggested that this might be related to the popular habit of ingesting raw meat (Silveira et al. 1988). In this population, it was proposed that the probability of the acquired toxoplasmosis inducing ocular disease is similar to that observed for congenital toxoplasmosis (Martins et al. 1990). In the present study, it was observed that the presence of retinal cysts occurs in a high percentage of the animals with the acquired form of the disease, that is, 50% of the pregnant females, 75% of the non pregnant females and 50% of the males presented retinal cysts. Thus, *C. callosus* has demonstrated to be a good experimental model for both acquired and congenital ocular toxoplasmosis.

In contrast, *C. callosus* had showed to be an unsuitable experimental model for induction of autoimmune uveitis. In the present study, after purification of S-antigen, the electrophoretic profile analyzed by SDS-PAGE and stained by silver showed an unique band of approximately 50 kDa, as described by Zigler-Jr et al. (1984). It was observed that the S-antigen did not demonstrate to be uveitogenic in none of the experimental groups of animals. There are reports at the literature demonstrating that, from 7 to 21 days after immunization with small doses of S-antigen, it is possible to induce EAU (Wacker et al. 1977, Rao et al. 1979, Li et al. 1994). Intradermal or subcutaneous routes of purified S-antigen cause an organ-specific uveoretinitis in about 14 days in the majority of the species so far studied and the inflammatory disease is induced in a dose-dependent way (Mahlberg 1989).

In the present study, no success was obtained even when several doses (6, 12, 25 and 50 mg) of purified S-antigen + CFA were previously tested in attempt to induce EAU by intradermal route (data not showed). Further, inoculation of purified S-antigen (50 mg) with or without different adjuvants by intradermal or intramuscular routes were also tested. At 21 days post-immunization, the presence of ocular lesion was not verified by histopathological analysis. Three hypothesis may be raised: (1) S-antigen obtained in the described conditions is not uveitogenically active; (2) the immunization protocol was not appropriate to induce autoimmune uveitis or (3) *C. callosus* is not a susceptible animal to present uveitis induced by systemic contact with S-antigen.

The antibody titers detected for S-antigen by ELISA showed no correlation with activity of the disease, as already described by Forrester et al. (1989). Antibodies to S-antigen detected later on in animals infected with *T. gondii* demonstrated that the ocular lesions caused by this parasite might induce S-antigen exposition and then stimulate the production of antibodies against this retinal component.

By WB analysis, it was verified that the presence of CFA appears to be necessary for production of antibodies anti-S-antigen and that jacalin may not constitute as a good reinforcement for S-antigen or CFA. Thus, the adjuvants tested together with S-antigen did not demonstrate enough sensitivity to induce autoimmune uveitis in *C. callosus*. On the other hand, *T. gondii* can induce autoimmune uveitis in the present experimental model, probably by the exposition of retinal antigens as a result of the inflammatory response to the parasitic antigens.

It can be concluded that *C. callosus* may constitute in a promising model for study both congenital and acquired ocular toxoplasmosis, particularly when it is important to make sure that a non autoimmune process is involved in the genesis of the ocular infection.

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