Available online at www.sciencedirect.com
ScienceDirect
Research in Veterinary Science 84 (2008) 237–242
www.elsevier.com/locate/rvsc

Evaluation of IFA, MAT, ELISAs and immunoblotting for the detection of anti-*Toxoplasma gondii* antibodies in paired serum and aqueous humour samples from experimentally infected pigs

João Luis Garcia [a,*], Solange Maria Gennari [b], Italmar Teodorico Navarro [a], Rosângela Zacarias Machado [c], Selwyn Arligton Headley [d], Odilon Vidotto [a], José da Silva Guimarães Junior [a], Felipe Monteiro Bugni [a], Michelle Igarashi [a]

[a] *Laboratório de Protozoologia, Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina – UEL, P.O. Box 6001, 86050-970 Londrina, PR, Brazil*
[b] *Laboratório de Parasitologia, Departamento de Medicina Veterinária e Saúde Animal, Universidade de São Paulo – USP, Av. Prof. Orlando Marques de Paiva, 87, Cidade Universitária, 05508-000 São Paulo, SP, Brazil*
[c] *Immunoparasitology Laboratory, Departamento de Patologia Veterinária, Campus de Jaboticabal, Universidade Estadual de São Paulo-UNESP, Via de Acesso Prof. Paulo Donato Castellane Km 5 s/n, 14884-9000 Jaboticabal – SP, Brazil*
[d] *Section of Veterinary Pathology, Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland*

Accepted 26 April 2007

Abstract

The study evaluated the efficiency of diagnostic laboratory methods to detect anti-*Toxoplasma gondii* antibodies in paired serum and aqueous humour samples from experimentally infected pigs. 18-mixed breed pigs were used during the experiment; these were divided into two groups, G1 (infected group, $n = 10$) and G2 (uninfected group, $n = 8$). Infection was performed with 4×10^4 VEG strain oocysts at day 0 by the oral route in G1 animals. All pigs were euthanized at day 60, when retina, aqueous humour, and blood samples were collected. Anti-*T. gondii* antibody levels were assessed in serum (s) and aqueous humour (ah) by indirect immunofluorescence assay (IFA), modified agglutination test (MAT), m-ELISA (using crude membranes from *T. gondii* tachyzoites as antigen) and r-ELISA (using rhoptries from *T. gondii* tachyzoites as antigen). Polymerase chain reactions (PCR) of samples from the retina were performed by using Tox4 and Tox5 primers. Antibody titers of G1 animals ranged from 128 to 1024 and from 16 to 256 in serum and aqueous humour, respectively. There were differences in the correlation coefficients between IFA(s) × IFA (ah) ($r = 0.62$, $P = 0.05$), MAT(s) × MAT (ah) ($r = 0.97$, $P < 0.0001$); however, there was no significant difference between r-ELISA(s) × r-ELISA (ah) ($r = 0.14$, $P = 0.7$). Antibodies present in serum and aqueous humour recognized similar antigens. Samples of retina were positive by PCR in 30% (3/10) of infected pigs. G2 animals remained without antibody levels and were PCR negative throughout the experiment. These results suggest that the use of a combination of tests and immunoblotting for paired aqueous humour and serum samples could improve the sensitivity and specificity for the diagnosis of ocular toxoplasmosis.

Keywords: *Toxoplasma gondii*; PCR; ELISA; IFAT; MAT; Aqueous humour; Pigs

1. Introduction

Toxoplasma gondii is an intracellular parasite that infects a variety of cell types from a wide range of mammals and birds throughout the world, including humans. Usually, *T. gondii* does not produce clinic signals, but the

* Corresponding author. Tel./fax: +55 43 3371 4485.
E-mail address: jlgarcia@uel.br (J.L. Garcia).

0034-5288/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2007.04.014

primary infection during pregnancy in women and a few animal species may result in abortion, fetal abnormalities or perinatal death (Gilbert et al., 2000). Human infection occurs by two main routes, ingestion of oocysts and the ingestion of undercooked or raw meat containing tissue cysts of the parasite (Andrews et al., 1997).

T. gondii is one of the most important causes of uveitis in humans (Silveira et al., 2003). The prevalence of human ocular toxoplasmosis is extremely elevated in Southern Brazil, and was described as 17.7% (Glasner et al., 1992). Additionally, 21.9% (9/41) of patients with IgG anti-*T. gondii* antibodies demonstrated ocular toxoplasmosis from the State of Paraná (Garcia et al., 1999).

Despite the vast existing knowledge of *T. gondii* as a parasite, the diagnosis of ocular toxoplasmosis still depends on characteristic clinical findings that are considered as the gold standard for ophthalmologists (Garweg et al., 2004; Garweg, 2005). Consequently, this has created an active interest in the development of a reliable confirmatory laboratory test for the diagnosis of ocular toxoplasmosis (Garweg, 2005).

This study evaluated the results of an indirect immunofluorescence assay (IFA), modified agglutination test (MAT), enzyme linked immunosorbent assay (ELISA), and immunoblotting as laboratory test for the diagnosis of anti-*T. gondii* antibodies in paired serum and aqueous humour samples from experimentally infected pigs.

2. Material and methods

2.1. Toxoplasma gondii strains

VEG *T. gondii* strain genotype III (Dubey, 1996) was used in this experiment.

2.2. Infection of pigs

The pig infection procedures and the animals from group 1 (G1) and group 2 (G2) derived from a previous study (Garcia et al., 2006b).

2.3. Sampling and measurements

Clinical signs and body temperatures were recovered before and after challenge. The serum samples were collected at days −7, 0, 14, 21, 35, and at slaughter (day 60), and stored at −20 °C. At euthanasia, samples from the retina, serum, and aqueous humour were collected to investigate the presence of anti-*T. gondii* antibodies and tissue cysts.

2.4. IgG antibody evaluation

The presence of antibodies against *T. gondii* in serum and aqueous humour samples was measured by indirect immunofluorescence assay (IFA, Camargo, 1973), modified agglutination test (MAT, Dubey and Desmonts, 1987), and the enzyme linked immunosorbent assay (ELISA, Garcia et al., 2006a). Sample dilution was initiated at 1:16 during all tests. The antibody titer was determined as the reciprocal of the highest dilution shown positive in IFA and MAT, and whose absorbance was 2.5-fold greater than the absorbance of the sera of control pigs at the same dilutions.

2.5. Antigens used in ELISAs

We previously isolated membranes and rhoptries from *T. gondii* tachyzoites by isopycnic sucrose density gradient centrifugation (Garcia et al., 2004). Five visible fractions were observed: fractions 1 and 3 had membranes and rhoptries, respectively. Fraction 1 had a major antigen with 29 kDa, while fraction 3 demonstrated a major protein with 55 kDa. Herein, membrane-ELISA (m-ELISA) and rhoptry-ELISA (r-ELISA) were performed using fraction 1 and fraction 3 as antigens to coat plate wells, respectively. These ELISAs were performed as previously described (Garcia et al., 2006a).

2.6. SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Tachyzoites from *T. gondii* were lysed by boiling in Laemmli sample buffer, and separated on a 12% SDS-PAGE (Laemmli, 1970). After electrophoresis, the proteins were transferred onto a nitrocellulose membrane (Pharmacia) as previously described (Towbin et al., 1979). After transfer, the nitrocellulose membrane was inhibited in a blocking solution (7 mM Na_2HPO_4, 3 mM NaH_2PO_4, 140 mM NaCl and 5% dried skimmed milk) for 1 h at room temperature. After washing, strips of nitrocellulose membrane were incubated with pool of sera (diluted 1:100) and pool of aqueous humour (diluted 1:5) of the pigs from G1; while strips from G2 animals were incubated in washing buffer (7 mM Na_2HPO_4, 3 mM NaH_2PO_4, 140 mM NaCl, and 1% dried skimmed milk). After washing, strips were incubated for 1 h at room temperature with peroxidase-labeled anti-pig IgG antibody (diluted 1:2000 in washing buffer). The peroxidase activity was revealed using chromogenic substrate solution (diaminobenzidine and H_2O_2); reaction was then inhibited by washing in distilled water.

2.7. DNA extraction from retina samples

Fragments of retina were stored at −20 °C prior to DNA extraction. These fragments were cut into small pieces and homogenized in 1 ml of TE buffer (200 mM NaCl, 20 mM Tris, 50 mM EDTA, pH 8.0) after being transferred to a 1.5 ml tube. 500 μl of the homogenized solution were transferred to a microtube to which an equal volume of extraction buffer (200 mM NaCl, 20 mM Tris, 50 mM EDTA, proteinase K 1 mg/ml and 2% SDS) was added. This new solution was then incubated for 1 h at