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Toxoplasma gondii: Detection by mouse bioassay, histopathology, and polymerase chain reaction in tissues from experimentally infected pigs

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Abstract

In the present study, we evaluated three techniques, mouse bioassay, histopathology, and polymerase chain reaction (PCR) to detect *Toxoplasma gondii* infection in tissues from experimentally infected pigs. Twelve mixed breed pigs, seronegative for *T. gondii* using an indirect immunofluorescent antibody test (IFAT), were used. Ten pigs were infected with 4×10^4 VEG strain oocysts, and two were maintained as uninfected controls. Animals were killed 60 days pos infection. Muscle (heart, tongue, diaphragm, and masseter) and brain samples were collected to investigate the presence of *T. gondii* tissue cysts by the different assay methods. For the bioassay, samples of brain (50 g) and pool of muscle samples (12.5 g of tongue, masseter, diaphragm, and heart) were used. PCR was performed using Tox4 and Tox5 primers which amplified a 529 bp fragment. The DNA extraction and PCR were performed three times, and all tissue samples were tested individually (brain, tongue, masseter, diaphragm, and heart). For histopathology, fragments of tissues were fixed in 10% of buffered formal saline and stained with HE. Histopathological results were all negative. PCR showed 25/150 (16.6%) positive samples, being 17/120 (14.1%) and 8/30 (26.6%) from muscle, and brain tissues, respectively. Tissue cysts of *T. gondii* were identified by mouse bioassay in 54/98 (55.1%) samples, being 31/48 (64.6%) from muscle samples, and 23/50 (46.0%) from brain samples. *Toxoplasma gondii* isolation in muscle samples by mouse bioassay was higher than in PCR ($P < 0.01$). Results indicate that DNA from pig tissues interfered with 529-bp-PCR sensitivity, and mouse bioassay was better than PCR in detecting *T. gondii* in tissues from pigs.

Index Descriptors and Abbreviations: Toxoplasma gondii; Protozoa; Apicomplexa; Pigs; Mouse bioassay; Histopathology; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; HE, hematoxylin–eosin; IFA, indirect immunofluorescence assay; IM, intramuscular

1. Introduction

Toxoplasma gondii is a protozoan parasite that can infect humans and warm blooded animals. In spite of toxoplasmosis being generally clinically asymptomatic, a primary infection during pregnancy in women and animals may cause abortion, fetal abnormalities, or perinatal death (Gilbert et al., 2000). Humans can become infected by ingesting raw or undercooked meat containing tissue cysts (Garcia et al., 2005). Tissue cysts in pork can persist for more than two years (Dubey, 1988) and is one of the most

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important sources of *T. gondii* infections for humans (Dubey et al., 1991). Modern management has reduced the prevalence of *T. gondii* in young pigs in Europe and North America (Tenter et al., 2000). However, older pigs which have higher prevalence are used in manufacture of sausage, salami, and cured meats (Dubey, 2000).

Polymerase chain reaction (PCR) is widely used for detection of *T. gondii* in amniotic fluid, blood, and tissues (Chabbert et al., 2004). Mouse bioassay is the standard test to detect infection in tissues (Homan et al., 2000), but it is arduous, expensive, time consuming, and hazardous for the operator (Esteban-Redondo et al., 1999). There are situations in which diagnosis needs to be rapid and accurate (e.g., AIDS patients and fetal infection) to start treatment (Chabbert et al., 2004). Additionally, serology is very difficult to interpret in pregnant patients (Fuentes et al., 1996), and is not useful for diagnosis of cerebral abscesses in immunocompromised patients (Joseph et al., 2002). Jauregui et al. (2001) developed a real-time PCR assay to detect *T. gondii* in tissues from pigs, and they suggested that the test could supplement or replace current bioassay or indirect serology tests.

The aim of this study was to compare *T. gondii* infection detection by mouse bioassay, histopathology, and PCR in tissues from experimentally infected pigs.

2. Materials and methods

2.1. Toxoplasma gondii strains

Toxoplasma gondii VEG and RH strains were used in the experiment. VEG strain (Dubey, 1996) used for the pig infection was kindly provided by Dr. J.P. Dubey (USDA, Beltsville, Maryland, USA). The RH strain (Sabin, 1941) was used as the antigen in the indirect immunofluorescence antibody test. Tachyzoites of RH strain were obtained from peritoneal fluid of infected Swiss Webster mice. The material was passed three times through a 26 gauge needle washed twice with 10 mM phosphate-buffered saline (PBS, pH 7.5) and centrifuged. The pellet was resuspended and washed twice in PBS; after washing, tachyzoites were prepared at a concentration of 10^7 tachyzoites/ml.

2.2. Animals and infection

The maintenance and care of experimental animals complied with the National Institute of Health guidelines for the humane use of laboratory animals. Twelve mixed breed pigs (Large White × Landrace) between 15.5 and 16.5 weeks of age, including females and castrated males, were randomly allocated in separate stables. They received food and water ad libitum. All pigs were serum negative (title < 64) in the *T. gondii* indirect immunofluorescence assay (IFA). Ten pigs were infected with 4×10^4 oocysts of the VEG strain by oral route and two remained as uninfected controls. Animals were treated intramuscularly at day 5 after infection with sulfadiazine (3 mg/kg) and trimethoprim (15 mg/kg). All pigs were treated because this promotes encystation of parasites and interrupts clinical symptoms (Alexander and Hunter, 1998). Euthanasia of the animals was performed 60 days later. Muscles (heart, tongue, diaphragm, and masseter) and brain samples were collected to investigate *T. gondii* tissue cysts by mouse bioassay, histopathology, and PCR.

2.3. Indirect immunofluorescence assay

The presence of IgG antibodies against *T. gondii* in serum samples of pigs and mice were measured by IFA (Camargo, 1973) considering as positive pigs with title $\geqslant 64$ and mice with title $\geqslant 16$.

2.4. Bioassay of pig tissues for T. gondii

Brain (50 g) and pool of muscle (12.5 g of tongue, masseter, diaphragm, and heart) samples from each pig were processed for presence of *T. gondii* tissue cysts as described previously (Dubey, 1998). Briefly, each sample was homogenized in a blender for 30 s in 250 ml of saline solution (0.14 M NaCl). After homogenization 250 ml of pepsin solution was added and incubated at 37 C for 1 h. The homogenate was filtered through two layers gauze and centrifuged at 1180*g* for 10 min. The supernatant was discarded and the sediment was resuspended in 20 ml PBS (pH 7.2) and 15 ml 1.2% sodium bicarbonate (pH 8.3) was added and centrifuged at 1180*g* for 10 min. The supernatant was discarded and the sediment was resuspended in 5 ml of antibiotic saline solution (1000 U penicillin and 100 µl of streptomycin/ml of saline solution) and inoculated 1 ml subcutaneously into five Swiss Webster mice (weighting 25–35 g).

Impression smears of lung from the mice that died were fixed in methanol, stained with Giemsa, and examined microscopically. Blood samples were drawn from the mice that survived 60 days after inoculation, and the brain of each mouse was examined microscopically for *T. gondii* tissue cysts by squashing a portion of brain between a coverslip and a glass slide. Serum from each mouse was diluted at 1:16 and 1:64 and examined for *T. gondii* antibodies, using IFA.

2.5. DNA extraction from tissue samples

Fragments of muscle and brain samples were stored at −20 °C prior to the extraction of DNA. Approximately 15 g of each sample was cut into small pieces and homogenized in 10 ml of TE buffer (200 mM NaCl, 20 mM Tris, 50 mM EDTA, and pH 8.0) after being transferred to a 50 ml tube. Next, sterilized glass-beads were added and samples were homogenized for 3 min. The homogenized sample (500 µl) was transferred to a microtube with equal volume of extraction buffer (200 mM NaCl, 20 mM Tris, 50 mM EDTA, proteinase K 1 mg/ml, and 2% SDS) and incubated