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Short Communication

Isolation and pathogenicity of *Toxoplasma gondii* in naturally infected (rustic farm) pigs in southern Brazil

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ABSTRACT

This study reported a serological test for *Toxoplasma gondii* infection in 100 pigs from 58 rural farms in the state of Rio Grande do Sul, Brazil. Thirty-six pigs were seropositive (IFAT ≥ 1:64). Bioassays were performed for all 36 seropositive pigs, and 17 isolates were obtained (47.2%). Seven of these isolates (41.2%) were highly pathogenic to mice, as clinical signs of acute infection were observed, and tachyzoites were found in the peritoneal exudates, livers, and lungs. The remaining 10 isolates were able to establish a chronic infection in mice, therefore, they were not highly virulent. The results of this study indicate that pork is a potential source of *T. gondii* transmission to humans.

1. Introduction

The parasite *Toxoplasma gondii* is an obligate intracellular protozoan with a worldwide distribution, and it infects both humans and animals. The two principal routes of transmission for this organism are the ingestion of oocysts from cat feces in contaminated food or water and the ingestion of viable cysts found in raw or undercooked meat (Dubey, 2010).

In several countries, including Brazil, pigs are considered the most important reservoir among food animals of *T. gondii* that is transmitted to humans (Dubey, 2009; da Silva et al., 2010). In Rio Grande do Sul, State in southern Brazil, the consumption of pork and handmade sausages is high, and they are therefore important sources of protozoan infection (Dias et al., 2005). The prevalence of the parasite in the population is high, reaching 74.5% in some areas (Spalding et al., 2005; Cademartori et al., 2008).

Modern pig production systems, particularly those in which intensive management has been adapted, have decreased *T. gondii* infection in many countries (Dubey, 2009). However, in some regions of Brazil, the incidence of parasitic infection is high due to the practice of rearing pigs outdoors. These animals can be infected by *T. gondii* through the ingestion of sporulated oocysts from contaminated environments or water or through the ingestion of cysts contained in the tissues of infected animals, such as rodents and birds (Cavalcante et al., 2006; de Azevedo et al., 2010; Dubey, 2010).

T. gondii isolates from Brazil are biologically and genetically different from those in North America and Europe (Dubey et al., 2007, 2008, 2012; Belfort-Neto et al., 2007; Velmurugan et al., 2009; Bezerra et al., 2012). Previous studies of isolates from Brazil and other South American countries have demonstrated high genetic variability, with the exception of Chile, where genotypes II and III are

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dominant (Lehmann et al., 2006; Su et al., 2006; Pena et al., 2008; Rajendran et al., 2012). Previous studies performed using the tissues of naturally infected pigs in Brazil revealed that isolation rates varied between 8.7% and 50.0% (Dias et al., 2005; Frazão-Teixeira et al., 2006). Additionally, genotypes I and III as well as atypical *T. gondii* have been identified (Belfort-Neto et al., 2007; Velmurugan et al., 2009; Bezerra et al., 2012). Therefore, it is necessary to perform additional studies in other regions to demonstrate the importance of this etiological agent to public health.

The aims of this study were to isolate *T. gondii* from naturally infected pigs intended for human consumption and to evaluate the pathogenicity of the parasite using bioassays in mice.

2. Materials and methods

2.1. Naturally infected pigs

Blood and tissue samples (heart and brain) of 100 pigs that were slaughtered for human consumption were collected. The study area spanned five municipalities in Rio Grande do Sul in southern Brazil, including Piratini, São Lourenço do Sul, Morro Redondo, Capão do Leão and Cerrito, which are located between latitudes 31°21" and 31°46" S and meridians 53°06" and 51°58" W. The examined animals originated from 58 farms, and at most, two pigs from each farm were collected.

2.2. Serological examination

The sera were examined for *T. gondii* IgG antibodies using an indirect fluorescence antibody test (IFAT) according to the technique described by Camargo (1974) using an anti-pig IgG conjugate antibody (Sigma Chemical®) and a commercial antigen obtained from WAMA Diagnostic® (ME 49 strain of *T. gondii*). The cut-off used was 1:64, and positive samples were tested until the maximum dilution titer was reached. Positive and negative control pig sera, maintained in the Parasitology Laboratory, were included in all reactions.

2.3. Bioassay of pig tissue homogenates in mice

The brains and hearts of pigs that were serologically positive for *T. gondii* were minced, homogenized and prepared using peptic digestion, as described by Dubey (1998).

For the bioassays, each pig brain (25 g) and heart (25 g) was ground (total of 50 g) and homogenized in 125 vol (v/v) of aqueous 0.85% NaCl (saline). This homogenate was then mixed with 5 vol of acidic pepsin, and the mixture was incubated in a shaking water bath for 1 h at 37 °C. The homogenate was then filtered through two layers of gauze and centrifuged at 1200 × *g* for 10 min. After centrifugation, the supernatant was discarded, and a neutralizing solution (1.2% sodium bicarbonate, pH ~ 8.3) was added to the sediment. The material was centrifuged again, and the supernatant was discarded. The sediment was resuspended in 5 mL of an antibiotic solution that contained 1000 IU of penicillin and 100 μg streptomycin per mL of sterile saline. This product was intraperitoneally inoculated in five Swiss Webster (25–35 g) mice at a dose of 1 mL per mouse; mice were given a second identical injection 24 h after the first inoculation.

Table 1
Toxoplasma gondii antibodies (IFAT) in naturally infected pigs from different municipalities in southern Brazil.

Municipality	No. of farms	No. of pigs examined	IFAT positive (%)
São Lourenço do Sul	39	73	16 (21.9)
Morro Redondo	4	4	3 (75.0)
Piratini	9	16	11 (68.5)
Capão do Leão	5	5	4 (80.0)
Cerrito	1	2	2 (100.0)
Total	58	100	36 (36.0)

Swiss Webster female mice with an average age of two months were obtained from the Central Animal Facility of the Faculty of Veterinary Medicine at the Federal University of Pelotas. The animals were observed daily, and tissues sections of the lung, brain and liver of the mice that died were examined for the presence of *T. gondii* tachyzoites and/or tissue cysts (Dubey and Beattie, 1988). The survivors were bled on day 45 post-inoculation (pi), and a 1:16 dilution of serum from each mouse was tested for *T. gondii* antibodies by IFAT (Camargo, 1974). The mice were sacrificed at day 60 p.i., and heart, brain and liver smears were examined microscopically for tissue cysts (Dubey and Beattie, 1988). Furthermore, portions of the brain, lung and liver of these specimens were frozen at −70 °C for future DNA extraction and genetic characterization of the *T. gondii* isolates. The mice were considered to be infected with *T. gondii* when tachyzoites or tissue cysts were found in their tissues.

2.4. Statistical analyses

Epi-Info (version 3.5.3) was used for the statistical analyses, and Fisher's exact test was used to compare antibody titers and mortality among the inoculated mice. Differences were considered statistically significant when $p \leq 0.05$.

2.5. Ethical consideration

This research was conducted with the approval of the Ethics Committee on Animal Experimentation at the Federal University of Pelotas, as granted by Opinion No. 4632/2011.

3. Results

Antibodies to *T. gondii* were found in 36 of 100 (36.0%) pigs from 58 small farms (Table 1). *T. gondii* was isolated from tissues of 17 of the 36 (47.2%) pigs with IFAT titers of 1:64 or higher (Table 2).

We observed that 41.2% (7/17) of the isolates were highly pathogenic in the inoculated mice. These animals became ill between days 8 and 13 p.i. and died by days 10–17 p.i. (Table 3). Tachyzoites were observed in the peritoneal exudates, lungs and livers of all dead mice beginning at day 10 p.i. Most of the visceral impressions demonstrated extracellular parasites, which could be observed alone or in