Veterinary Parasitology 171 (2010) 53–57

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Detection of *Toxoplasma gondii* oocysts in environmental samples from public schools

Thaís Rabelo dos Santos[a,*], Cáris Maroni Nunes[b], Maria Cecília Rui Luvizotto[c], Anderson Barbosa de Moura[d], Welber Daniel Zanetti Lopes[a], Alvimar José da Costa[a], Katia Denise Saraiva Bresciani[b]

[a] *Department of Animal Pathology, CPPAR, FCAV, UNESP - Jaboticabal, Access road Prof. Paul Donato Castellane s/n, Jaboticabal, SP 14884-900, Brazil*
[b] *Department of Production and Animal Health, FMVA, UNESP - Araçatuba, SP, Brazil*
[c] *Department of Clinic, Surgery and Animal Reproduction, FMVA, UNESP - Araçatuba, SP, Brazil*
[d] *Department of Veterinary Medicine, CAV, DESC, Lages, SC, Brazil*

ARTICLE INFO

Article history:
Received 26 October 2009
Received in revised form 24 February 2010
Accepted 25 February 2010

Keywords:
Toxoplasma gondii
Oocysts
Bioassay in mice
IFAT
Immunohistochemistry

ABSTRACT

The number of *Toxoplasma gondii* oocysts that can be found in random environmental samples is probably low; in addition, these cysts may be confused with *Hammondia* spp. and *Neospora* spp. oocysts. The aim of the present work was to evaluate the presence of *T. gondii* oocysts in the soil of public elementary schools in the northwest area of the state of São Paulo, Brazil using mouse bioassays. A comparison was made between the different available bioassay techniques, such as squash, histopathology, immunohistochemistry and indirect fluorescent antibody test (IFAT). *T. gondii* was isolated by bioassay in mice (squash brain samples) from 22.58% (7/31) of the school playgrounds. Immunohistochemistry and IFAT showed positive results in 32.26% (10/31) and 25.80% (8/31) of samples, respectively. The sensitivity and specificity of the immunohistochemistry method were 85.71% and 83.33%, respectively. The IFAT results showed 100% sensitivity and 95.83% specificity. The presence of *T. gondii* was not detected in histopathological examinations. The results of the present study strongly suggest that *T. gondii* oocysts are widely distributed in elementary public schools in the region that was evaluated, likely constituting the main contamination source for these children. Educational programs directed at reducing environmental contamination with *T. gondii* would eventually lower the cost of treating humans for clinical toxoplasmosis. It is also possible to conclude that the use of IFAT in mouse bioassays can be recommended without the need for brain cysts research, which is extremely difficult and laborious.

1. Introduction

Toxoplasmosis is an infection that usually remains asymptomatic but can cause severe illness when the organism is reactivated in immunosuppressed persons or when it is contracted congenitally.

Transmission of toxoplasmosis occurs by consumption of undercooked or raw meat containing tissue cysts, or by ingestion of resistant oocysts from environmental sources (soil, water, fruits and vegetables). The relative importance of each transmission route in toxoplasmosis cannot be determined because it is not yet possible to discriminate infections due to oocysts from those induced by cysts using serological assessment (Boothroyd and Grigg, 2002; Dubey, 2004).

Oocysts that are shed into the environment have caused several outbreaks of this disease in humans (Teutsch et al.,

* Corresponding author. Tel.: +55 1632092605.
E-mail address: rabelo.vet@pop.com.br (T.R.d. Santos).

0304-4017/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2010.02.045

1979; Benenson et al., 1982; Bowie et al., 1997; de Moura et al., 2006). Direct contact with felines, on the other hand, had little epidemic consequence, although the presence of those infected animals indicates a contaminated environment, posing a risk to the human population and other animals (Santos et al., 2009).

Assessing environmental contamination with *T. gondii* oocysts is technically difficult. Domestic cats normally choose to bury their feces in soft and moist soil, but cat feces can be found on street pavement, grass, grain or hay. Little is known about the oocyst sporulation and survival rate of oocysts when openly exposed to the sun and other environmental conditions.

T. gondii was isolated for the first time from drinking water stored in small water tanks on the roofs of school buildings; these tanks were served by the reservoir that was epidemiologically linked to the Brazilian outbreak (de Moura et al., 2006).

The number of oocysts that can be found in random environmental samples is probably low, and these oocysts could be confused with those of *Hammondia* spp. and *Neospora* spp., two closely related species of coccidia that can occur in the environment (Dubey et al., 2002).

Debris in samples further complicates the detection of oocysts. Microscopy and bioassays in mice are often unsuited to sensitive and simple detection. The development of new detection methods is therefore necessary. Specific and sensitive methods exist for other protozoa but they have not yet been developed for detection of *T. gondii* oocysts in environmental samples (Quintero-Betancourt et al., 2002). However, PCR is becoming a favored technique for detection of *T. gondii* oocysts in water (Kourenti and Karanis, 2004, 2006; Sroka et al., 2006) over the conventional mouse bioassay (Villena et al., 2004), as it reduces the detection time from weeks to 1–2 days.

There are several unresolved issues regarding the effectiveness of PCR detection of *T. gondii* oocysts in water. The most readily available method for isolation of *T. gondii* oocysts from water samples is flocculation or sucrose floatation prior to DNA extraction (Kourenti and Karanis, 2004, 2006; Sroka et al., 2006). Borchardt et al. (2009) recently reported a continuous separation channel centrifugation for concentrating *T. gondii* and *Cyclospora cayetanensis* oocysts from surface water and drinking water.

The work presented herein aims to evaluate the presence of *T. gondii* oocysts in the soil of elementary public schools from the northwest area of the state of São Paulo using mouse bioassays. A comparison between different bioassay techniques such as squash, histopathology, immunohistochemistry and indirect fluorescent antibody test (IFAT) was performed.

2. Materials and methods

The soil specimens used for the isolation of *T. gondii* oocysts were obtained from 31 elementary public school playgrounds from the northwest area of the state of São Paulo in Brazil. The samples were collected from a depth of 5 cm, at the rate of one sample (1 kg)/20 m^2 and processed according to the method proposed by Ito et al. (1975).

The sugar flotation technique (Sheather, 1923) was performed by mixing 5 mL of suspension (soil sample in water) with 45 mL of a sugar solution (density 1.208) and centrifuging the mix at 1000 × *g* for 10 min (Dubey et al., 1970; Dubey and Beattie, 1988). After sporulation in 2% sulfuric acid at ambient temperature for two weeks, oocysts were stored at 4 °C until use (Dubey, 1995). The suspension was neutralized with an equal volume of 3.3% sodium hydroxide and resuspended in saline. After neutralization, the suspensions of samples (1 mL) were inoculated orally into two mice (Dubey and Frenkel, 1973).

Albino mice (20–25 g) were checked twice daily for clinical signs of toxoplasmosis for 60 days or until death. The half of the brain from each mouse was examined for *T. gondii* tissue cysts as a squash preparation which is considered the gold standard in diagnosis (Dubey and Beattie, 1988) and the other half of the brain was used for histopathology and immunohistochemistry.

For the histological study, brains were trimmed and dehydrated with graded alcohols before being embedded in paraffin wax using routine procedures. From each paraffin block, four to six 5 μm thick sections were cut semi-serially, deparaffinized, rehydrated and stained with hematoxylin–eosin and examined by light microscopy. Tissues were examined for *T. gondii* organisms by immunohistochemistry (Guesdon et al., 1979) by staining with a species-specific, monoclonal antibody-based system.

Mice were bled from the retro-orbital plexus and killed 8 weeks after the oocyst inoculation. Blood samples were taken on the first and last days of the experiment to assess for the seroconversion of infected animals. To search for anti-*Toxoplasma gondii* antibodies, IFAT was performed (Camargo, 1964). To obtain the antigens, the "RH" strain was used, as described by Camargo (1974). The sera were diluted in a saline buffer solution containing 0.1 M phosphate, pH 7.2 (PBS), with cut-off points ≥16. Positive samples, were processed until the final titer was obtained. A commercial conjugate (Sigma Chemical: mouse-F4018) was used, and positive and negative controls were used for each slide. Reactions in which the tachyzoites presented total peripheral fluorescence were defined as positive.

The results of the mouse bioassay were compared using the exact Fisher test, at $\alpha = 0.05$, with a 95% confidence interval. The concordance between the techniques was determined by calculating the *Kappa* index using the Epi-Info program (CDC, version 6.04).

3. Results

T. gondii was isolated by bioassay in mice (squash brain samples) from 22.58% (7/31) of the playgrounds of elementary public schools from the northwest area of the State of São Paulo (Table 1). The immunohistochemistry and IFAT results were positive in 32.26% (10/31) and 25.80% (8/31) of the samples, respectively.

The sensitivity and specificity of the immunohistochemistry method were 85.71% and 83.33%, respectively (Table 2). The sensitivity of the IFAT was 100% and its specificity was assessed at 95.83%. The differences observed between the diagnostic methods applied (immunohistochemistry and IFAT) were statistically significant ($P \leq 0.05$).