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Short communication

Immunohistochemical identification of *Toxoplasma gondii* in tissues from Modified Agglutination Test positive sheep

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ABSTRACT

Toxoplasma gondii is a zoonotic agent of great importance in veterinary and public health. The aim of this study was to identify *T. gondii* by IHC (immunohistochemistry) in different sheep tissues and to determine if an association exists between the results obtained by this method and those obtained by the Modified Agglutination Test (MAT). Tissue specimens of twenty-six sheep seroreactive for *T. gondii* were selected for histopathological evaluation. The presence of *T. gondii* was investigated in brain, liver and heart samples by IHC and a possible anti-*T. gondii* antibody cross reactions with other parasites. McNemar's, Chi-square and Fisher's Exact Tests were applied for the statistical analysis of the results. The analysed tissues showed at least one of the following histopathological changes: mild-to-moderate congestion, focal polymorphonuclear inflammatory infiltrate and multifocal or focal mononuclear inflammatory infiltrate. *Sarcocystis* spp. were identified in the histological sections from both the heart and diaphragm tissues of 88.5% (23/26) of the animals. A total of 46.2% (12/26) of the *T. gondii* seroreactive sheep was also positive for *T. gondii* by IHC in at least one organ (brain, liver or heart). The liver IHC-positivity for *T. gondii* was statistically equivalent to the global individual IHC-positivity, according to McNemar's test. In addition, IHC allowed the detection of *T. gondii* in infected animals regardless of the titration observed in the MAT. The statistical difference observed between the three organs when comparing the low titration group, suggested that the heart might be the most suitable organ to detect *T. gondii* infection by IHC. The IHC results in this study revealed that almost half of MAT positive animals could serve as potential sources of infection for humans because bradyzoites were identified in different tissues, regardless of the MAT titration.

1. Introduction

Toxoplasma gondii is one of the most studied parasites because of its impact on veterinary and public health (Tenter et al., 2000). Seropositivity in humans has been reported in more than 80 countries (Dubey, 2010) and the prevalence ranges from 4% in Korea (Ryu et al., 1996) to 92% in the Mato Grosso State of Brazil (Figueiró-Filho et al., 2005). The major route of toxoplasmosis transmission to human is the consumption of contaminated food, especially undercooked meat containing bradyzoites cysts (Villena et al., 2012).

T. gondii infection occurs in sheep world wide, but the prevalence depends on the region (Dubey, 2010). In Brazil,

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the sheep seroprevalence of antibodies against this parasite has been evaluated by many studies, and the State of Paraná had the highest prevalence of *T. gondii* in sheep in the country 51.5% (158/305) (Romanelli et al., 2007).

The MAT has been used for the detection of antibodies against *T. gondii* in many animal species, including sheep (Raeghi et al., 2011; Villena et al., 2012). The animals present positive MAT titres for *T. gondii*, suggesting that they are an important source of toxoplasmosis for humans (Alvarado-Esquivel et al., 2012).

Histopathological examination by IHC is widely employed in the diagnosis of *T. gondii* infection (Pereira-Bueno et al., 2004). Nevertheless, there have been few studies to date describing the immunohistochemical detection of *T. gondii* in sheep (Pereira-Bueno et al., 2004; Motta et al., 2008). Considering that the consumption of ovine meat occurs in different countries around the world, the aim of this study was to identify *T. gondii* by IHC in different sheep tissues and to determine if an association exists between the results obtained by this method and those obtained by the MAT.

2. Materials and methods

2.1. Animal ethics approval

This study was approved by the Ethics Committee of Animal Use (CEUA) from the Universidade Federal Fluminense (UFF) under protocol number 00111/09.

2.2. Samples

Tissue samples were collected from 26 seropositive sheep with different titres for *T. gondii* by MAT, after the slaughter of the animals. These sheep belonged to a larger group of 287 animals that had been previously tested for the parasite by MAT in despite of the titres that they presented. At the time of the study, only these 26 sheep were allowed by the owners to be slaughtered. The samples were submitted to histopathological evaluation and identification of the parasite by IHC.

2.3. Serological test

The serological analysis was performed with the MAT according to Dubey and Desmonts (1987). All samples with agglutinating activity at a dilution of 1:25 were considered positive (Sousa et al., 2009). These serum samples were subsequently titrated against reacting antigens using serial two-fold dilutions up to 1:3200.

2.4. Histopathology

Tissue specimens from liver, heart, brain, diaphragm, kidney and lung were collected from 26 *T. gondii*-seropositive sheep and fixed in neutral-buffered, 10% formalin. These specimens were routinely processed in paraffin for light microscopy and histological sections were produced for both haematoxylin–eosin (H&E) and IHC staining.

2.5. Immunohistochemistry

The presence of *T. gondii* tissue cysts was investigated in IHC-stained sections of the brain, heart and liver of 26 seropositive sheep. The histological sections were deparaffinised and hydrated, and the endogenous peroxidase was blocked with a 3% hydrogen peroxide solution. The sections were incubated in a 96 °C water bath for 30 min for antigen recovery. The nonspecific binding was blocked by incubating the sections in a solution of milk and 10% bovine serum albumin for 30 min. Subsequently, the sections were incubated for 30 min with primary rabbit anti-*T. gondii* antibody (Neomarkers, Fremont, CA, USA) diluted 1:200. The sections were treated with DAKO LSAB DAKO Corp. Carpinteria, CA, USA) as recommended by the manufacturer. Diaminobenzidine (DAB; DAKO Corporation, Carpinteria, CA, USA) was used as the chromogen to reveal the life cycle stages of the parasite, and all samples were counterstained with Harris haematoxylin. Histological sections of human brain positive for *T. gondii* were used as positive controls for the IHC technique as recommended by the manufacturer, and the primary antibody was omitted for negative controls. The samples were considered positive when bradyzoite pseudocysts were stained in brown by DAB. The animal was considered positive by IHC when at least one of the evaluated organs was positive. In addition, the positivity of the test was analysed in two ways: by the global animal status (the animal as a whole) as well as by the individual organ status (each separate organ of the animal). The tissue sections were also evaluated in order to search for possible anti-*T. gondii* antibody cross reactions with other parasites.

2.6. Statistical analysis

McNemar's test was used to compare the results obtained by IHC. The tissue samples from the liver, heart and brain of the evaluated animals (the individual organ status) were compared to the global animal statuses.

A Fisher's Exact Test was used to determine the association between the IHC positive and negative results in the different organs (liver, heart and brain) and the different *T. gondii* titres obtained by the MAT in all 26 seropositive animals. The animals were separated into two groups based on their titres: 1:25 to 1:50 and 1:100 to 1:3200. Fisher's Exact Test was used for the comparative analyses between the two titration groups (1:25 to 1:50 versus 1:100 to 1:3200) and the immunohistochemical detection of *T. gondii* (positive or negative) in the samples from the brain, liver and heart, in order to identify the most suitable organ to detect infected animals even presenting low titres.

In addition, the Chi-square test was used to compare the animals that tested positive by IHC with their respective titres obtained by the MAT. This test was used to determine if there was an association between the titration at which the animal was seropositive for *T. gondii* and positive by IHC.

The Statistical Package for Social Science (SPSS) version 12.0 software was used. Differences where $P < 0.05$ were considered significant.