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Development and evaluation of a modified agglutination test for diagnosis of *Toxoplasma* infection using tachyzoites cultivated in cell culture

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ABSTRACT

Toxoplasma gondii is a zoonotic protozoan parasite that can cause significant disease in most warm-blooded animals, including humans. Surveillance testing of human and animal populations is essential to estimate disease prevalence, assess food safety risks and establish control programmes. Serological tests are the most practical methods to detect the prevalence of infection in a broad range of host populations. The modified agglutination test (MAT) is a sensitive serological method to detect *Toxoplasma* IgG antibodies in livestock and wild animals. An in-house MAT was developed using tachyzoites produced by *in vitro* cultivation instead of traditional propagation in murine peritoneal cavity. The assay was evaluated using samples of serum and/or meat juice from pigs and cats experimentally infected with *T. gondii*. Samples were also tested by a commercially available MAT kit. Comparative analysis of test results from serum and meat juice samples showed excellent agreement between the in-house MAT and the commercial MAT. Serum and/or blood samples from naturally infected cats, sheep, and 20 wildlife host species were also tested by the in-house MAT, with overall results comparable to those obtained using the commercial MAT kit. Therefore, this new MAT is an efficient and convenient method for testing a variety of terrestrial and aquatic domestic or wild host species for *T. gondii*.

1. Introduction

Toxoplasma gondii is an important coccidian parasite that has a complex reproductive cycle involving two hosts. Members of the family Felidae are the only known definitive hosts, whereas humans and a wide range of domestic and wild animals are intermediate hosts for this parasite (Dubey, 2010). Infection with *T. gondii* can cause abortion or neurological symptoms in their intermediate hosts (Gilbert et al., 2000; Sushrut and Davis, 2012). Transmission occurs *via* consumption of meat or other animal tissues containing tissue cysts of this parasite, or of water or other materials contaminated with sporulated *Toxoplasma* oocysts.

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Several serological tests have been developed to detect antibodies to *T. gondii* in humans and animals for the detection of infection (Dubey et al., 1995). The modified agglutination test (MAT) is a sensitive and specific method for the detection of *T. gondii* in a wide range of host species, and is simple, rapid and of relatively low cost compared to other serological tests (Dubey, 1997; Hill et al., 2006; Dubey, 2010). However, the routine use of MAT for the diagnosis of *T. gondii* infection can be challenging because of the lack of readily available commercial kits and the tachyzoite-derived antigen. Typically, tachyzoites used for MAT antigen are generated by intraperitoneal propagation in mice (Desmonts and Remington, 1980). To reduce the use of live animals, antigen required for the assay in this study was prepared from tachyzoites grown in cell culture. Performance of the test was assessed using samples of serum, whole blood or meat juice from experimentally infected pigs and cats, and naturally infected cats, sheep and 20 species of wild animals.

2. Materials and methods

2.1. Samples

Samples of serum, whole blood (EDTA) and meat juice were assigned to 1 of 3 groups, based on origin of the samples (Table 1).

2.1.1. Experimentally-infected domestic animals group

A total of 74 serum and 39 meat juice samples previously generated from 13 pigs experimentally inoculated with *T. gondii* were used (Forbes et al., 2012). The *Toxoplasma-free* pigs had been inoculated with 1×10^2, 3×10^2, 5×10^2 or 1×10^3 sporulated oocysts, and three uninoculated pigs served as controls (Table 1). Serum samples were collected prior to inoculation and serially post-inoculation. Post-mortem samples of meat juice from each carcass were obtained from diaphragm, heart and sternomastoideaus muscle. An additional 36 serum samples (provided by Dr. Dolores Hill, USDA, Beltsville, Maryland, USA) were collected from nine pigs each experimentally infected with 1000 oocysts of *T. gondii per os* (Hill et al., 2006). Samples were collected prior to inoculation and at 11, 31 and 66 days post-inoculation (DPI). Thirty serum samples obtained from cats experimentally infected with *T. gondii via* various routes of inoculations and described in an earlier study were also used. The samples were collected from 11 SPF cats at pre-inoculation and 32 weeks post-inoculation. These cats were inoculated orally with mouse brain tissues containing tissue cysts of *T. gondii* (Al-Adhami and Gajadhar, 2014). An additional eight serum samples obtained from a previous study in which four SPF cats were fed brain or muscle tissues of seals experimentally infected with *T. gondii*. Samples were collected at pre- and post-inoculation (Gajadhar et al., 2004).

2.1.2. Naturally-infected domestic animals group

A total of 134 serum samples were obtained from pet cats in France (provided by Drs. Pascal Boireau and Radu Blaga), and 20 serum samples were collected from outdoor-raised sheep in Saskatoon, Canada (provided by Dr. Gita Malik-Dahiya).

2.1.3. Naturally-infected wildlife animals group

A total of 244 serum and 424 whole blood samples were collected from 20 wildlife species between 2008 and 2010 at various locations in the northern and arctic regions in Canada as part of an International Polar Year project (Fig 1, Table 1).

2.2. Serological tests

Serum and whole blood samples from all 3 groups were tested for antibodies to *T. gondii* using the commercial MAT kit (Toxo-Screen DA, Biomerieux, France) as the gold standard, and the in-house MAT. Kit controls and test samples were tested at 1:40 and 1:40,000 dilutions as per the kit manufacturer's instructions.

The in-house MAT was developed using *Toxoplasma* antigen produced from *in vitro* cultured tachyzoites. To produce the antigen, cryopreserved tachyzoites obtained from VEG, type III strain of *T. gondii* were propagated in tissue culture using MDBK cells and appropriate culture medium (Minimum Essential Medium-MEM-GIBCO-USA) supplemented with foetal bovine serum (FBS) and antibiotics (Lindsay et al., 1991). *Toxoplasma* tachyzoites were grown in conventional cell culture flasks (T75) and Falcon® cell culture multi-flasks (Corning-USA) to improve tachyzoites yield and productivity. Tachyzoites were harvested from the culture medium by washing in phosphate buffer saline (PBS), followed by filtration through PD-10-Sephadex column (GE-Healthcare-USA) to remove cells and other particulate materials. The viability of the purified tachyzoites was assessed by trypan blue dye exclusion assay (Elsheikha et al., 2006). Purified tachyzoites were fixed in 6% formaldehyde solution and stored at 4 °C overnight. After fixation, the formalin suspension was centrifuged, washed 3× in sterile filtered PBS and resuspended in alkaline borate buffer (pH 8.7) containing 0.4% bovine serum albumin (BSA/borate buffer) and 0.2% sodium azide to a final concentration of approximately 2×10^8/ml (antigen stock suspension), and stored at 4 °C until used. The test method described by Dubey (2010) was followed with modifications as described below. Agglutination was performed in round-bottom 96-well plates (Corning-USA). Test serum and blood samples were titrated at serial 2-fold dilutions from 1:25 to 1:200. Negative (pre-inoculation and uninoculated controls) and positive (terminal bleed) sera from various species experimentally infected with *T. gondii* were chosen as negative and positive controls, respectively. The positive control was required to have a minimum titre of 1:200. Meat juice samples were diluted at 1:10 and 1:25 prior to testing. Serum, whole blood, and meat juice samples were diluted with sterile filtered PBS. The antigen mixture for each plate was prepared by mixing 200 μl of formalin-fixed tachyzoites,