Experimental Parasitology 172 (2017) 1–4

Contents lists available at ScienceDirect

Experimental Parasitology

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

Full length article

Evaluation of recombinant antigens in combination and single formula for diagnosis of feline toxoplasmosis

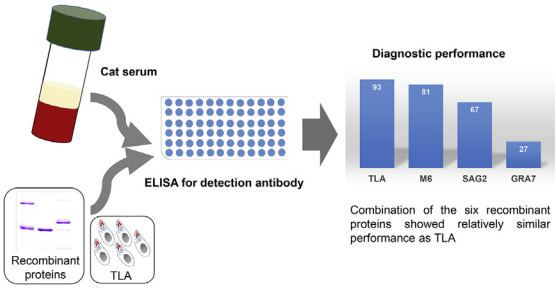
Abdelbaset Eweda Abdelbaset [a, b], Hend Alhasan [a], Doaa Salman [c], Mohamed Hassan Karram [b], Mahmoud Abd Ellah Rushdi [b], Xuan Xuenan [a], Makoto Igarashi [a, *]

[a] National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, 2-13 Inada-cho, Obihiro, Hokkaido 080-8555, Japan
[b] Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt
[c] Department of Animal Medicine, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt

HIGHLIGHTS

- Accurate diagnosis of feline toxoplasmosis is essential for control.
- ELISAs with recombinant antigens offer promising tools for diagnosis.
- Combination formula of antigens improves sensitivity and specificity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form
1 November 2016
Accepted 18 November 2016
Available online 19 November 2016

Keywords:
ELISA
Recombinant proteins
Serodiagnosis
Toxoplasmosis

ABSTRACT

Cats are the only definitive hosts of *Toxoplasma gondii* and constitute an essential source of infection to all warm blooded animals and humans. Diagnosis of *T. gondii* infection in cats is fundamental for proper management and control of infection in humans and animals. In the current study, we have evaluated the diagnostic performance of tachyzoite lysate antigen (TLA) and different *T. gondii* recombinant antigens including surface antigen 2 (SAG2), dense granule proteins 2, 6, 7, 15 (GRA2, GRA6, GRA7, GRA15) and microneme 10 protein (MIC10) in immunoglobulin G enzyme linked-immunosorbent assay (IgG ELISA) using cat serum samples, with reference to latex agglutination test (LAT). Remarkably, TLA showed better performance than other recombinant antigens in IgG ELISAs as compared to LAT, with concordance and Kappa values of 94.27% and 0.93, respectively. Furthermore, to improve the reactivity of the recombinant antigens, we have developed IgG ELISAs using different combinations with these recombinant antigens. Strikingly, a combination of SAG2 and GRAs has relatively similar performance as TLA evidenced by concordance and Kappa values of 94.27% and 0.81, respectively. The developed ELISA with a combination of recombinant antigens can be used as a promising diagnostic tool for routine testing of *T. gondii* infection and mass screening in cats. The major advantages of this assay are the high sensitivity and specificity, lower cost, safer production and easiness of standardization in various laboratories worldwide.

* Corresponding author.
E-mail address: makoto@obihiro.ac.jp (M. Igarashi).

http://dx.doi.org/10.1016/j.exppara.2016.11.003

1. Introduction

Toxoplasmosis is globally prevalent zoonotic disease caused by the intracellular protozoan parasite *Toxoplasma gondii* (*T. gondii*) (Tenter et al., 2000). Prevalence of infection is often highest in regions of the world that have hot, humid climates and lower altitudes. Felids are the definitive hosts of this parasite and mostly all warm-blooded mammals including humans serve as intermediate hosts (Dabritz and Conrad, 2010; Elmore et al., 2010). Transmission to humans and animals occurs via ingestion of food and water contaminated with infectious oocysts shed in cat feces (Dubey and Beattie, 1988). Postnatal infection causes fatal encephalitis in immunocompromised patients such as those with AIDS. Congenital infection may cause abortion, neonatal deaths, or foetal abnormalities result in blindness and mental retardation in pregnant woman (Jones et al., 2003; Luft et al., 1984). In animals, it causes abortion and stillbirth in all types of livestock, especially in sheep and goats resulting in significant reproductive and economic losses (Buxton, 1998).

Little attention has been paid to feline toxoplasmosis, because *T. gondii* infection in cats is usually asymptomatic and latent infections are the most common. Development of accurate diagnostic tests in cats is crucial for proper management and control of *T. gondii* infection. Several assays have been developed for diagnosis of *T. gondii* infection in humans and animals. Serological techniques seem to be the most suitable for routine mass screening of samples (Montoya, 2002). TLA has been traditionally used in serological detection of *T. gondii* infection, however, the recombinant proteins offer better test standardization with less production costs. Although number of immunodominant antigens has been used for serodiagnosis of feline toxoplasmosis (Cai et al., 2015; Hosseininejad, 2012; Huang et al., 2002; Kimbita et al., 2001), none of these showed all criteria required to replace the native antigen of *T. gondii* in serological tests. Therefore, further research is required to develop an accurate serodiagnostic test in cats. In the present study, we have evaluated the diagnostic performance of recombinant protein antigens and different combinations for the serodiagnosis of feline toxoplasmosis. Our results indicate that a combination of SAG2, GRA2, GRA6, GRA7, and GRA15 can be used as a promising tool for detection of *T. gondii* infection in cats using ELISA. This is the first report demonstrating the usefulness of combination formula antigen in diagnosis of feline toxoplasmosis.

2. Materials and methods

2.1. Preparation of T. gondii lysate antigen (TLA)

Tachyzoites were maintained in human foreskin fibroblast (HFF) cells cultured in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO, Grand Island, NE, U.S.A.) supplemented with 7.5% heat-inactivated fetal bovine serum (FBS). TLA preparation was based on the standard procedure, by three times freezing and thawing of tachyzoites obtained from *T. gondii* (ME49 strains).

2.2. Cloning of T. gondii genes

The cloning of *T. gondii* genes encoding SAG2 (from position 79 to 516 bp), GRA2 (from position 1 to 558 bp), GRA6 (from position 1 to 690 bp), GRA7 (from position 91 to 711 bp), GRA15 (from position 1 to 1650 bp), MIC10 (from Position 148 to 597 bp) proteins was conducted. The RNA was extracted from the purified tachyzoites (RH strain) using a commercial RNeasy mini kit (QIAGEN) and reverse transcribed using One step RNA PCR kit for reverse transcription (One step RNA PCR kit, Takara, Japan) and then used as a template to amplify the target genes. Oligonucleotide primers used for amplification of *T. gondii* genes were shown in Table 1. The amplified cDNAs of *SAG2, GRA6,* and *GRA7* were double digested with *Bam*HI and *Eco*RI whereas that of *GRA2*, *GRA15* and *MIC10* were double digested with *Bam*HI and *Xho*I, and subcloned into the identical restriction sites of pGEX-5X-1 or pGEX-6P2 (GE Healthcare UK Ltd.). Plasmids were transformed into *Escherichia coli* DH5α competent cells. Cycle sequencing reactions were carried out using a BigDye Terminator Cycle Sequencing kit Ver. 3.1 according to the manufacturer's protocol (Applied Biosystems, USA), and each sample was analyzed using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA).

2.3. Expression and purification of recombinant fusion proteins

The resulting plasmids were transfected in *E. coli* strain BL21 (DE3) pLysS cells. Then, the GST-fused proteins were induced with isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM with mild shaking at 23 °C overnight. The cells were centrifuged at 5000×*g* for 20 min and the bacterial pellet was resuspended with 20 ml pre-chilled STE buffer (150 mM NaCl, 50 mM Tris-HCl [pH 9.5] and 1 mM EDTA [pH 8.0]), then stored at −20 °C. After thawing, the cells were disrupted by sonication on ice for 10 min, and 20% (w/v) Triton X-100 in 1 × PBS was added to the samples to be a final concentration of 1% (w/v) Triton X-100. Total proteins in the soluble fraction were affinity purified by glutathione-Sepharose beads according to the manufacturer's protocols (Pharmacia biotech, Uppsala, Sweden). The eluted fractions were dialysed against PBS and the amount of recombinant protein was measured using both SDS-PAGE and the Coomassie protein assay reagent kit using BSA as a calibration standard according to the manufacturer's protocol (Pierce Biotechnology, Inc., USA). All recombinant proteins remained soluble during purification and conservation. Apparent recombinant antigens on SDS-PAGE match the expected size of each protein (data not shown).

2.4. Cat serum samples

A total of 419 serum samples were obtained from cats visiting animal hospitals in Tokachi prefecture. The serum samples were then prepared and stored at −30 °C for future use.

2.5. IgG ELISA

MaxiSorp plates (Nunc, Denmark) were coated overnight at 4 °C with either single recombinant antigen, mixture of recombinant proteins, or TLA at a final concentration of 1 μg/ml of each antigen in a coating buffer (50 mM carbonate, pH 9.6) and were incubated. Plates were washed and blocked with 3% skim milk in phosphate buffer saline (PBS-SM) for 1 h at room temperature (RT). 50 μl cat

Table 1
Primers used for cloning of *T. gondii* genes.

Genes	Primer sequence	Enzyme site
sag2	5′-ttttggatcctccaccaccgagacgcca-3′	*Bam*H1
	5′-ttttgaattcttacttgcccgtgagaga-3′	*Eco*R1
gra2	5′-ttggatccatgttcgccgtaaaacat-3′	*Bam*H1
	5′-ttctcgagttactgcgaaaagtctgg-3′	*Xho*1
gra6	5′-ttttggatccatggcacacggtggcatccatctga-3′	*Bam*H1
	5′-ttttgaattcataatcaaacacattcacacgttcc-3′	*Eco*R1
gra7	5′-ttttggatccgatgacgaactgatgagt-3′	*Bam*H1
	5′-ttttgaattcctactggcgggcatcctccccatctt-3′	*Eco*R1
gra15	5′-ttttggatccatggtgacaacaaccacgccaacgc-3′	*Bam*H1
	5′-ttttctcgagtggagttaccgctgattgtgtgtcc-3′	*Xho*1
mic10	5′-ttggatccagtctccaggcgtctattgg-3′	*Bam*H1
	5′-ttctcgagctacattgatttcctgcgtc-3′	*Xho*1