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NOTE

Parasitology

Development and evaluation of an enzyme-linked immunosorbent assay based on recombinant TgSRS2 for serodiagnosis of *Toxoplasma gondii* infection in cats

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ABSTRACT. An enzyme-linked immunosorbent assay (ELISA) based on recombinant SAG1-related sequence 2 of *Toxoplasma gondii* (rTgSRS2) was developed to detect toxoplasmosis in cats. The specificity and sensitivity of rTgSRS2 ELISA were confirmed using a series of serum samples from *T. gondii*-experimentally infected mice. A total of 76 field samples from cats were examined by the developed ELISA. The rTgSRS2 ELISA showed a good diagnostic performance characterized by high concordance (88.16) and kappa value (0.76) with latex agglutination test (LAT). The sensitivity and specificity of the test were 92.68% and 82.86%, respectively. These results suggest that the ELISA based on rTgSRS2 could be a useful tool for serodiagnosis of *T. gondii* infection in cats.

KEY WORDS: cat, enzyme-linked immunosorbent assay, serodiagnosis, SAG1-related sequence 2 of *Toxoplasma gondii*, toxoplasmosis

Toxoplasma gondii, an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa, can infect nearly all warm-blooded animals, including humans, livestock, companion animals, and wildlife throughout the world. Members of family Felidae (such as cats) are the definitive hosts of this parasite [2]. Any of the following routes can infect both the definitive hosts and intermediate hosts: ingestion of raw or undercooked meat that contains encysted bradyzoites; accidental consumption of feed or water that contains oocysts; contact with contaminated soil; or congenital transmission [4, 13]. Toxoplasmosis is the disease caused by *T. gondii*. Toxoplasmosis symptoms are rarely observed in healthy adults as the immune system usually controls *T. gondii* infection. However, toxoplasmosis is life-threatening in immunocompromised human (such as in immunosuppressive therapies patients). In pregnant women, infection with the parasite can cause stillbirth, neonatal malformations, or abortion [5, 13]. Toxoplasmosis in animals has a significant reproductive and economic impact, especially in sheep and goats [1].

The development of accurate diagnostic tools is crucial for proper management and control of *T. gondii* infection in humans and animals. Several diagnostic methods have been developed, including histological demonstration and isolation of the organism. These methods are less sensitive, time-consuming, and requires an experienced technician [11]. On the other hand, serologic tests may be more practical and a more suitable method for serodiagnosis of *T. gondii* infection. The latex agglutination test (LAT) is considered a standard method for detecting *T. gondii* infection but is costly [11]. Enzyme-linked immunosorbent assays (ELISAs) seem to be the most suitable for routine mass diagnosis, such as in seroepidemiological investigations, because of its cost-effectiveness [11].

Several recombinant proteins hold potential for *T. gondii* infection diagnosis in cats and humans, and among them, major surface antigens (TgSAGs) and dense granule antigens (TgGRAs) are good candidates [8]. SAG1-related superfamily (TgSRS) proteins, expressed in a stage-specific manner, are essential for *T. gondii* to enter into the host cell, and regulate host immunity to promote chronic infection [6]. SRS29B (TgSAG1) and SRS34A (TgSAG2A) are TgSRS proteins that have been used in indirect ELISAs for serodiagnosis of feline toxoplasmosis [3, 7]. SAG1-related sequence 2 of *T. gondii* (TgSRS2) is a major tachyzoite surface antigen and has been identified to be expressed on the surface of *Toxoplasma* tachyzoites [10]. TgSRS2 is also an important

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negative regulator of acute virulence in mice model [14], however, its antigenicity is unknown. In the present study, we describe the performance of TgSRS2 for application in serological surveys.

The *T. gondii* (PLK strain) and *Neospora caninum* (Nc-1 strain) tachyzoites were grown *in vitro* on human foreskin fibroblast (HFF) cell monolayers. Total RNA of *T. gondii* PLK was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) from 5 × 10^7 parasites and then reverse transcribed to cDNA using SuperScript III First Strand Synthesis Kit (Invitrogen) according to the manufacturer's protocol. The coding sequence for *T. gondii* SRS2 amino acids 1 to 372 (GenBank accession number AF012276) was amplified from *T. gondii* cDNA by PCR using the primers 5′-CGAGCTCATGGCGACGCGTGCGTCTT-3′ and 5′-ATAAGAATGCGGCCGCTCAATAGGCAAGTGCCGTC-3′. The amplified SRS2 gene was digested with the *Sac*I and *Not*I restriction enzymes and then ligated into the glutathione S-transferase (GST) of the pGEX-4T-1 vector. Recombinant protein TgSRS2 was expressed as GST fusion protein in the *Escherichia coli* (*E. coli*) BL21 strain. The purification of recombinant proteins was performed according to a protocol described previously [3]. The molecular masses of purified rTgSRS2 and GST proteins were estimated to be 67 kDa and 26 kDa, respectively, as expected (Fig. 1A). The concentration of the proteins was measured by bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Inc., Rockford, IL, USA) according to the manufacturer's protocol.

To prepare *T. gondii*- and *N. caninum*-infected mice sera, ICR mice (6–7 weeks old, the IACUC approval ID No. for mouse experiments is: 18-104.) were intraperitoneally inoculated with 10^3 tachyzoites of *T. gondii* or 10^4 *N. caninum* tachyzoites. Sera were collected every week until the 12th week post-infection. The purified rTgSRS2 could be recognized by sera from mice experimentally infected with the PLK strain in Western blot analysis (Fig. 1B). An ELISA based on rTgSRS2 was developed using a previously established protocol [3]. In detail, MaxiSorp plates (Nunc, Roskilde, Denmark) were coated with each recombinant antigen (TgSRS2, GST) at a final concentration of 1 μg/m*l* and used for testing the mouse sera. Examined sera were diluted at 1:100, and horseradish peroxidase (HRP)-conjugated goat anti-feline/mouse IgG (Thermo Fisher Scientific) were used in the assay at 1: 5,000 dilution. Each sample was examined at least twice. The ELISA based on rTgSRS2 succeeded in clearly differentiating between *T. gondii*-infected and non-infected mouse sera or *N. caninum*-infected sera which were seropositive for *N. caninum* (Fig. 2A). Furthermore, to determine the sensitivity of the developed ELISA, *T. gondii*-infected mouse sera were serially sampled post-infection. The rTgSRS2-based ELISA could detect specific antibodies starting from 3rd week after infection (Fig. 2B).

To evaluate the potential of rTgSRS2 for the serodiagnosis of *T. gondii* infection in cats, the developed ELISA was tested and compared to the commercial LAT using cat serum samples. A total of 76 field serum samples and 89 serum samples obtained from cats which tested negative for toxoplasmosis (confirmed by LAT) were obtained from the Clinical Laboratory Department of Malpi−Lifetech Inc. in Osaka, Japan. The cutoff value for cat sera was calculated as the average OD of the 89 negative cat serum samples plus 3 standard deviations. LAT was performed according to manufacturer's instructions (Toxocheck-MT, Eiken Chemical, Tokyo, Japan). Samples were considered positive when agglutination was observed at a dilution of 1:32 or greater. The results of ELISA and LAT for tested samples were used to evaluate sensitivity, specificity, percentage of agreement, and the kappa values of the new diagnostic test (http://vassarstats.net/). Among the 76 clinical cat samples, the rTgSRS2-based ELISA detected *T. gondii*-specific antibodies in 44 (57.89%) samples (Table 1). The performance of the ELISA was compared to LAT (Table 1), while the reactivity of rTgSRS2 with cat samples is presented in Fig. 3.

The sensitivity and specificity of the developed ELISA were 92.68% and 82.86%, respectively. Moreover, the ELISA based on rTgSRS2 showed high concordance (88.16) and high kappa value 0.76 with the results of LAT.

In the present study, the performance of an ELISA based on rTgSRS2 was evaluated using sera of *T. gondii* experimentally-

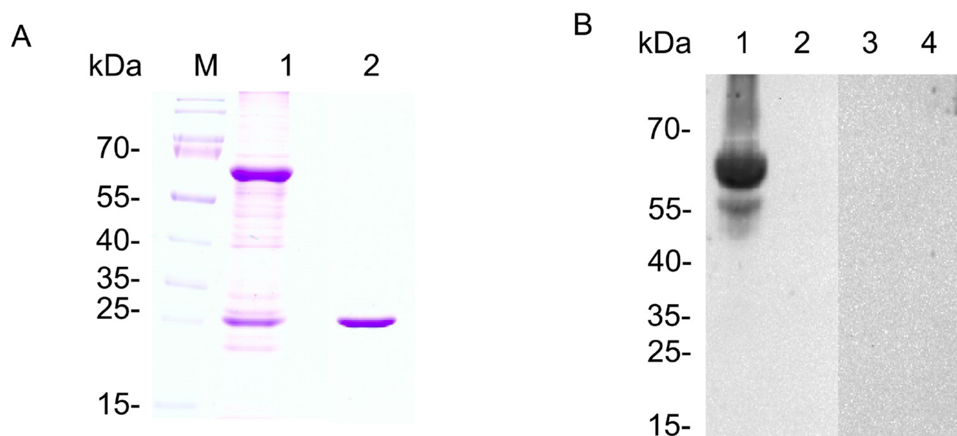

Fig. 1. Western blot analysis of rTgSRS2 using serum collected from a mouse experimentally infected with *Toxoplasma gondii*. (A) SDS-PAGE of expression of recombinant proteins in *Escherichia coli.* M, molecular marker; Lane 1, rTgSRS2; Lane 2, GST. (B) Western blot analysis of rTgSRS2 protein. Lanes 1 and 2, rTgSRS2 and GST incubated with a serum from mouse experimentally infected with *T. gondii*. Line 3 and 4, rTgSRS2 and GST incubated with a serum from healthy mouse.

Table 1. Comparison of the results for *Toxoplasma gondii* antibodies in cat samples examined by enzyme-linked immunosorbent assay (ELISA) and latex agglutination (LAT)

	ELISA+	ELISA−	Total
LAT+	38 (50%)	3 (3.95%)	41 (53.95%)
LAT−	6 (7.89%)	29 (38.16%)	35 (46.05%)
Total	44 (57.89%)	32 (42.11%)	76 (100%)

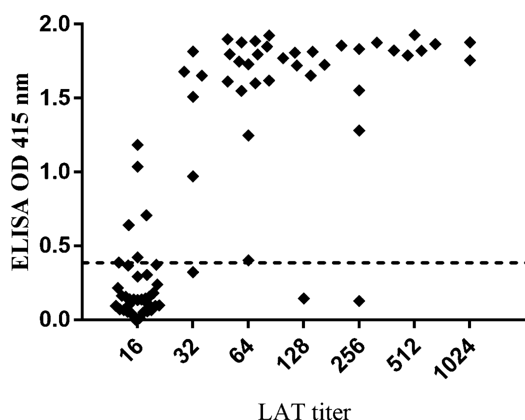

Fig. 3. Comparison of ELISA and LAT in the detection of antibodies against *Toxoplasma gondii* in domestic cats. The cutoff point in ELISA is shown by a dotted line.

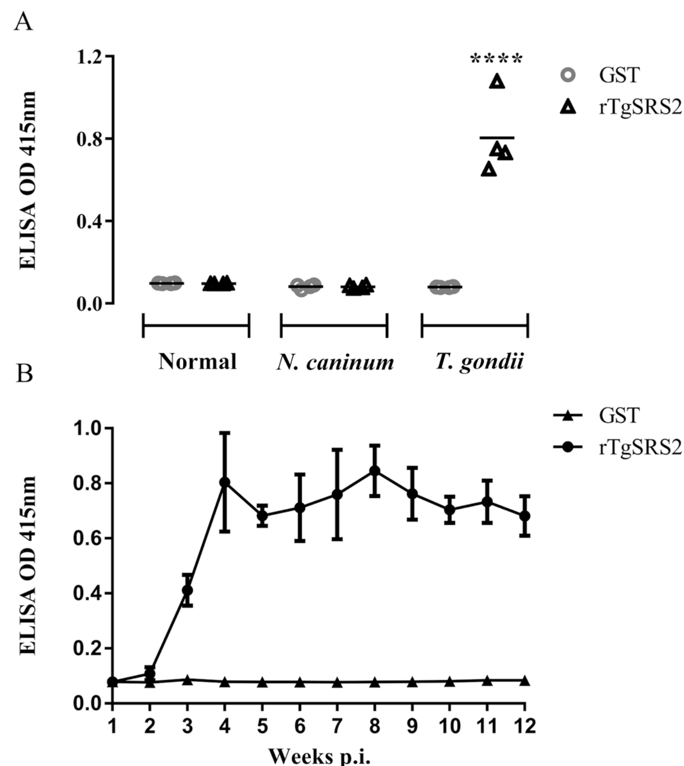

Fig. 2. Detection of antibodies to *Toxoplasma gondii* by ELISA using recombinant antigens in the sera of experimentally infected mice. (A) The reactivities of rTgSRS2 and GST with sera from normal mice, *T. gondii* experimentally-infected mice and *Neospora caninum* experimentally-infected mice at the 4th week after infection were evaluated by ELISA (n=4). (B) The reactivities of recombinant antigens with experimentally *T. gondii*-infected mouse sera (n=4) collected weekly (1st–12th). Each asterisk indicates a significant difference by ANOVA test. Differences of $P<0.05$ were considered significant.

infected mice and clinical cats. The absence of reactivity in *N. caninum*-infected mouse sera indicates the specificity of our rTgSRS2-based ELISA. The developed assay successfully detected antibodies against *T. gondii* in the acute and chronic stages. Detection of *T. gondii* antibodies in the chronic stage in human patient may contribute to the diagnosis of disseminated toxoplasmosis that could be reactivated by immune suppression due to viral infection or cancer [7]. In the cat samples from the field, rTgSRS2-based ELISA demonstrated good diagnostic performance with high concordance (88.16) and kappa value (0.76) in comparison with the LAT. The antigenic properties of rTgSRS2 can be explained by the fact that this protein is an essential regulator of virulence and is capable of developing protective immunity [14].

Although LAT shows the advantage that it can be used in serum samples from various animals, it does not provide information on the stage of infection as it does not detect specific IgM to *T. gondii* in feline sera [9]. Antigens of bacterial recombinant proteins have many advantages in diagnosis, with low cost of production and better standardization of the tests [12]. In the current study, the recombinant protein TgSRS2 showed good antigenicity and reactivity with mice and cat serum samples when tested as an ELISA antigen. Our results suggest that ELISA with rTgSRS2 could be a useful diagnostic tool for the detection of *T. gondii* infection in animals. This is the first report of a TgSRS2-based ELISA to detect *T. gondii* antibodies in felines.

CONFLICT OF INTEREST. The authors declare that they have no conflict of interest.

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