Research in Veterinary Science 100 (2015) 161–164

Contents lists available at ScienceDirect

Research in Veterinary Science

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

Evaluation of an indirect ELISA using recombinant granule antigen GRA1, GRA7 and soluble antigens for serodiagnosis of *Toxoplasma gondii* infection in chickens

Xuefei Sun [a,1], Zedong Wang [b,1], Jiping Li [a], Feng Wei [a,*], Quan Liu [b,**]

[a] College of Life Science, Jilin Agricultural University, 2888 Xincheng Street, Changchun 130118, Jilin Province, China
[b] Military Veterinary Institute, Academy of Military Medical Sciences, Changchun 130122, Jilin Province, China

ARTICLE INFO

Article history:
Received 16 November 2014
Accepted 13 April 2015

Keywords:
Toxoplasma gondii
GRA1
GRA7
Diagnostic marker
ELISA
Chickens

ABSTRACT

In the present study, recombinant granule antigen proteins GRA1, GRA7 and *Toxoplasma* soluble antigens (TSA) were evaluated as potential diagnostic markers for *T. gondii* infection in chickens by an indirect enzyme-linked immunosorbent assay (ELISA), showing a seroprevalence of 16.4% by GRA1-ELISA, and 15.5% by both GRA7- and TSA-ELISA in chickens. No significant difference was observed in the inconsistent results ($P > 0.05$), and a substantial agreement was found in the consistent results of the 3 tests (92.7–97.3%). Compared with the reference test Western blot, GRA7-ELISA showed the highest co-positivity and co-negativity rates. Receiver operating characteristic (ROC) analysis revealed a largest area under curve (AUC) of 0.99 (95% CI, 98–1.0), and a highest relative sensitivity (100%) and specificity (97.9%) for a cut-off value of 0.34 in GRA7-ELISA. These results of the present study showed that GRA7 is a potential diagnostic marker for the detection of *T. gondii* infection in chickens.

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite that infects virtually all warm-blooded animals, including mammals and birds. It is estimated that approximately 25% of the population is infected with the parasite in the world (Petersen, 2007). The infection can be obtained by eating raw or undercooked meat containing cysts, or consuming food or water contaminated with sporulated oocysts (Zhou et al., 2011). Chicken is one of the major meat-producing animal species in China and other countries, and plays an important role in the zoonotic transmission of *T. gondii* infection (Dubey, 2010). Raw or undercooked chicken meat is an important infection source for humans and other animals (Dubey, 2010; Zhao et al., 2012). Additionally, free-range chickens are the good indicator for soil contamination with *T. gondii* oocysts as they feed from the ground (Dubey et al., 2005). Thus, understanding the prevalence of *T. gondii* infection in chickens, especially in free-range chickens, represents considerable public health significance.

Toxoplasma gondii infection can be confirmed by the isolation of *T. gondii* from tissues or body fluids, detection of *T. gondii* specific nucleic acids or antibodies. Among these methods, detection of specific antibodies in serum samples has become a routinely diagnostic strategy of toxoplasmosis by use of various serological tests, including the Sabin–Feldman dye test (DT), indirect hemagglutination (IHA), modified agglutination test (MAT), latex agglutination test (LAT), indirect fluorescent antibody test (IFAT), and enzyme-linked immunosorbent assay (ELISA). DT cannot be used for the detection of *T. gondii* in chickens (Dubey, 2010). Previous studies have shown that both LAT and IHA are insensitive in detecting *T. gondii* antibodies in chickens (Dubey et al., 1993). Detection of infection in chicken is often performed by MAT, due to its high sensitivity and specificity in the animal species (Dubey, 2010). Comparison of the diagnostic performance of different detection methods in chickens have shown higher sensitivity and specificity of ELISA with *Toxoplasma* soluble antigens (TSA) than that of MAT (Hamidinejat et al., 2014). As TSA-based ELISA is difficult to standardize, the ideal approach to improving the test is to use recombinant proteins, with the advantages of the precise antigen composition, easy preparing composite antigens and standardization (Holec-Gasior, 2013).

T. gondii dense granule antigen proteins (GRAs) are important secretory proteins expressed by both tachyzoites and bradyzoites, which contribute to intracellular survival and replication of the parasites by modification of the environment within the parasitophorous

* Corresponding author. College of Life Science, Jilin Agricultural University, 2888 Xincheng Street, Changchun 130118, Jilin Province, China. Tel.: +86 431 86985981; fax: +86 431 86985889.
E-mail address: jlccwf@126.com (F. Wei).
** Corresponding author. Military Veterinary Institute, Academy of Military Medical Sciences, Changchun 130122, Jilin Province, China. Tel.: +86 431 86985981; fax: +86 431 86985889.
E-mail address: liuquan1974@hotmail.com (Q. Liu).
[1] These authors contributed equally to this study and are regarded as joint first authors.

http://dx.doi.org/10.1016/j.rvsc.2015.04.011
0034-5288/

vacuole (Ahn et al., 2006; Nam, 2009). GRAs are good serological markers for the diagnosis of toxoplasmosis in humans and animals. GRA1-based ELISA presents a sensitivity of 60–70%, and a specificity of 98% in humans (Ferrandiz et al., 2004; Lecordier et al., 2000). GRA7 based-ELISA shows a specificity of 98–100% and sensitivity of 81–96.4% in humans and cattle (Jacobs et al., 1999; Wang et al., 2014b). In the present study, recombinant proteins GRA1 and GRA7 were evaluated for serodiagnosis of *T. gondii* infection in free-range chickens by indirect ELISA.

2. Materials and methods

2.1. Serum samples

Blood samples were collected from free-range chickens in Jilin Province, northeastern China, and sera were separated by centrifugation at 1500 × *g* for 5 min and stored at –20 °C until use.

2.2. Antigens

TSA was prepared by sonicating the purified tachyzoites of *T. gondii* GT-1 strain (Liu et al., 2010). Recombinant granule antigens GRA1 and GRA7 were expressed in *Escherichia coli* BL21 (DE3), and purified using a Ni–NTA purification system (Qiagen, Hilden, Germany) as described before (Wang et al., 2014a). Briefly, the GRA1- and GRA7-encoding genes were amplified by RT-PCR and cloned into the expression vector pET-28a to generate recombinant plasmids pET28-GRA1 and pET28-GRA7, which were confirmed by restriction enzymes and sequencing, and further processed for the expression of recombinant products in *E. coli* BL21 (DE3) according to the standard techniques. The recombinant GRA1 and GRA7 were purified using a Ni–NTA purification system.

2.3. Western blot

The chicken serum samples were classified into anti-*T. gondii* antibodies positive and negative groups by Western blot as previously described (Zhu et al., 2008). Briefly, TSA was resolved in a 12% SDS–PAGE gel and transferred to a polyvinylidene fluoride membrane. The membrane was cut into strips and incubated with the serum samples. The membrane strips were further incubated with anti-chicken IgY (IgG) peroxidase-labeled conjugate antibodies (Thermo Scientific, USA) diluted at 1:5000. After washing, the strips were incubated with DAB substrate solution to visualize the protein bands that were recognized by the specific antibodies. A sample was considered positive if the protein bands were observed.

2.4. ELISAs

The ELISA assays were conducted to survey anti-*Toxoplasma gondii* antibodies in chickens as described by Wang et al. (2014a) with minor modification (Wang et al., 2014a). Briefly, microplates were coated with 50 μl TSA (10 μg/ml), GRA1 (5 μg/ml), or GRA7 (5 μg/ml), respectively. After washing, 100 μl chicken serum diluted at 1:50 was added to each well, and incubated for another 3 h at 37 °C, then 100 μl horseradish peroxidase-conjugated anti-chicken IgY antibodies diluted at 1:20,000 was added. The color was developed by the addition of a substrate solution TMB. The optical density (OD) was measured at 450 nm in a microplate reader. ELISA results were determined for each serum sample in duplicate. The cut-off point of a positive sample was set to be at least two times higher than that of the negative sample.

2.5. Data analysis

The significance of association between the results of different tests was analyzed using the McNemar chi-square test. The degree of agreement between the detection results was analyzed using kappa statistics. The accuracy of the ELISAs was evaluated in comparison with the results by Western blot. The expected ELISA performance at different cut-off points was determined using the receiver operating characteristic (ROC) curves (Kawada, 2012).

3. Results

3.1. Chicken serum samples classified by Western blot

The chicken serum samples were classified into anti-*T. gondii* antibodies positive and negative groups by Western blot. The positive sample showed specific Western blot bands, while no band was observed in the negative sample (Fig. 1). Of the 110 chicken serum samples, 16 were detected positive for anti-*T. gondii* antibodies, and 94 were detected negative.

3.2. Evaluation of ELISAs based on GRA1, GRA7 and TSA

To evaluate the potential of recombinant protein for serodiagnosis of *T. gondii* infection in chickens, the classified chicken serum samples were tested by indirect ELISAs using GRA1, GRA7 and TSA as coating antigens. As shown in Table 1, there were 18 positive and 92 negative samples tested by GRA1-ELISA, 17 positive and 93 negative samples by GRA7-ELISA, 17 positive and 93 negative samples

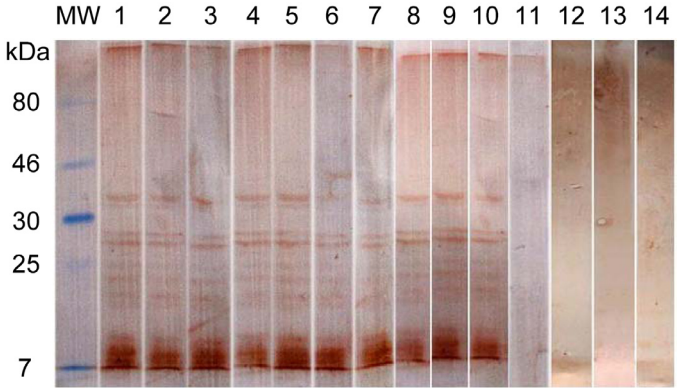

Fig. 1. The representative positive and negative samples confirmed by Western blot. MW, molecular weight; lanes 1–10, the positive samples showing reaction with TSA; lanes 11–14, the negative samples showing no reaction with TSA.

Table 1
Detection results of *T. gondii* infection in chickens by different serological methods.

	Western blot		
	Positive	Negative	Total
GRA1-ELISA			
Positive	13	5	18
Negative	3	89	92
Total	16	94	110
GRA7-ELISA			
Positive	16	1	17
Negative	0	93	93
Total	16	94	110
TSA-ELISA			
Positive	15	2	17
Negative	1	92	93
Total	16	94	110