Use of Protein AG in an Enzyme-Linked Immunosorbent Assay for Serodiagnosis of *Toxoplasma gondii* Infection in Four Species of Animals

Dongling Zhang,1,2 Zhengsong Wang,1,2 Rui Fang,1,2 Hao Nie,1,2 Huihui Feng,1,2 Yanqin Zhou,2,3* and Junlong Zhao1,2*

State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, Hubei, People’s Republic of China; College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, People’s Republic of China; and Key Laboratory of Preventive Veterinary Medicine of Hubei Province, Huazhong Agricultural University, Wuhan 430070, Hubei, People’s Republic of China

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An enzyme-linked immunosorbent assay (ELISA) employing protein AG (AG-ELISA) as a conjugate was developed to detect anti-*Toxoplasma gondii* antibodies (Ab) in experimentally infected pigs and naturally infected pigs, goats, dogs, and cats. The results indicate that AG-ELISA can be a useful method for serological diagnosis of *T. gondii* infection in these four species of animals.

Infections with *Toxoplasma gondii* are prevalent in human beings and animals worldwide (1). Numerous studies of *T. gondii* infections in pigs and other animals have demonstrated that enzyme-linked immunosorbent assay (ELISA) is the most sensitive method for diagnosing *T. gondii* infection (2, 4, 8). However, ELISA needs species-specific secondary antibodies for testing each species of animals. This requirement increases cost and makes manipulation more complicated when samples from different animal species are examined. Since the chimeric protein AG can strongly bind to the IgG of many mammalian species, it has been a powerful tool in immunological studies (5, 6). So far, there has been no report on the utility of chimeric AG in the immunological diagnosis of *T. gondii* infection. In this study, chimeric protein AG was employed to develop a simple protein AG enzyme-linked immunosorbent assay (AG-ELISA) to detect *T. gondii* antibodies from different animal species.

For establishing the AG-ELISA method, recombinant MIC3, which was highly expressed in *Toxoplasma gondii* SLC12, was used as the coating antigen. Flat-bottomed 96-well polystyrene micro-titration plates were coated with 0.1 ml of the antigens (2.5 mg/liter) diluted in 0.05 M carbonate buffer (pH 9.6) by incubation overnight at 4°C and were blocked with carbonate buffer–1% ovalbumin for 1 h at 37°C. The control and test sera were diluted 1:160 in phosphate-buffered saline–Tween (PBST) containing 0.1% ovalbumin, added to the microtiter plate at 0.1 ml per well, and incubated for 1 h at 37°C. Next, peroxidase-labeled chimeric protein AG (1:4,000; Pierce, Rockford, IL) was added at 0.1 ml per well and incubated for 30 min at 37°C. Peroxidase activity was revealed by adding 0.1 ml of tetramethylbenzidine (TMB) solution (100 mg TMB/liter of phosphate citrate buffer [pH 6.0] and 200 ul of H2O2) for 10 min at room temperature. The reaction was stopped by adding 0.05 ml of 0.25% hydrofluoric acid (HF), and the optical density (OD) was read at 630 nm in an ELISA microplate reader. A serum sample was considered to be positive when the OD of the sample/the OD of the negative control was ≥2.3.

Twelve mixed-breed pigs between 6 and 8 weeks old were purchased from one pig farm and randomly allocated to separate pens. The animals were acclimatized for 7 days before use. All pigs tested negative for the presence of *T. gondii* antibodies by AG-ELISA (OD < 0.16) and modified agglutination test (MAT) (titer < 1:16). At day 0, eight pigs were inoculated with 2 × 106 of viable tachyzoites of the RH strain by the subcutaneous route; the other four pigs were used as controls. Serum samples were collected from all groups, including the control group, on days −7, 0, 7, 10, 14, 21, 28, 35, 42, 50, and 57 after infection, and AG-ELISA was performed. MAT (3) and a validated commercial ELISA kit (SafePath Laboratories, Carlsbad, CA) were used as controls. The ELISA kit was able to detect that all animals were positive on day 7 after challenge, whereas AG-ELISA detected positive results on day 10 and MAT on day 14. The average antibody level was highest on day 28 for the ELISA kit and on day 35 for AG-ELISA and MAT. The reason for these differences may be that the ELISA kit uses formalin-fixed whole tachyzoites as the antigen and detects antibodies to surface antigen, whereas AG-ELISA uses MIC3 as the coating antigen and measures antibodies to secretory antigen. These results also indicate that ELISA methods are more sensitive than MAT. Agreement among these three serologic tests was calculated by Kappa statistics (7), and good agreement was observed with AG-ELISA versus MAT (κ = 0.86, *P* < 0.001) and AG-ELISA versus ELISA (κ = 0.83, *P* < 0.001).

A total of 304 serum samples were collected from 2006 to 2008 at the clinic of the animal hospital of our university from...
Table 1. Comparison of numbers of Toxoplasma gondii-positive results and prevalences of T. gondii infection in clinical serum samples from pigs, goats, dogs, and cats determined by AG-ELISA and MAT

| Animal species | Total no. of serum samples | AG-ELISA | MAT |
|---------------|---------------------------|----------|-----|
|               | No. of positive samples   | Prevalence (%) | No. of positive samples | Prevalence (%) |
| Pig           | 197                       | 81       | 41.1 | 72   | 36.5 |
| Goat          | 60                        | 19       | 31.7 | 16   | 26.7 |
| Dog           | 24                        | 11       | 45.8 | 11   | 45.8 |
| Cat           | 13                        | 5        | 38.5 | 5    | 38.5 |
| Total         | 304                       | 116      | 38.1 | 104  | 34.2 |

In conclusion, an ELISA using protein AG as the conjugate was developed and could be a useful method for serological detection of T. gondii infection in pigs, goats, dogs, and cats. Continued work using AG-ELISA to screen a large number of samples from other species of animals, such as rabbits, mice, and cattle, is in progress.

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