Characterization and Evaluation of a Recombinant Multiepitope Peptide Antigen MAG in the Serological Diagnosis of *Toxoplasma Gondii* Infection in Pigs

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Abstract

Background

Toxoplasmosis caused by *toxoplasma gondii* (*T. gondii*) is a serious disease threatening human and animal health. People could be infected with *T. gondii* by ingesting raw pig meat contaminated with cysts or oocysts. Serological test is a sensitive and specific method usually used for large-scale diagnosis of *T. gondii* infection in humans and animals (such as pigs). Since commercial pig toxoplasma antibody ELISA diagnostic kits are too expensive, it is difficult to use them widely, moreover, the native antigen composition used in these diagnostic kits is still unclear and difficult to standardize. The multiepitope peptide antigen is novel diagnostic marker, and it has the potential to be developed into more accurate and inexpensive diagnostic kits.

Methods

The synthetic multiepitope antigen (MAG) gene encoding a protein with epitopes from 5 *T. gondii* dominant antigen (SAG1, GRA1, ROP2, GRA4, and MIC3) was designed, synthesized, and expressed in *Escherichia coli BL21* (DE3) strain. The recombinant protein was detected through western blot with pig anti-*T. gondii* positive and negative serum, then IgG enzyme-linked immunosorbent assay (ELISA) named MAG-ELISA was designed. The MAG-ELISA was evaluated in terms of specificity, sensitivity, and stability. The MAG-ELISA was also compared with a commercial PrioCHECK® *Toxoplasma* Ab porcine ELISA (PrioCHECK ELISA). Finally, the trend of pig anti-*T. gondii* IgG level after artificially infection with RH tachyzoites was evaluated through MAG-ELISA and two other ELISA methods (rMIC3-ELISA and PrioCHECK ELISA).

Results

MAG antigen could be specifically recognized by pig anti-*T. gondii* positive but not negative serum. MAG-ELISA possessed a high diagnostic performance in terms of specificity and sensitivity. The overall coincidence rate between MAG-ELISA and a commercial PrioCHECK Toxoplasma antibody ELISA was 78.47%. MAG-ELISA could be used for detecting anti-*T. gondii* IgG in the early stage of *T. gondii* infection in pigs (at least 7 days after artificial infection).

Conclusions

Our results suggest that MAG antigen could be applied to specifically recognize anti-*T. gondii* IgG in pig, and MAG-ELISA has the potential for large-scale diagnosis of *T. gondii* infection in pig farms and intensive industries.

Background

Toxoplasma gondii is an apicomplexan intracellular protozoan parasite, and it can infect any warm-blooded vertebrates including humans and domestic animals [1]. Toxoplasmosis caused by *T. gondii* infection threatens human and animal health especially for pregnant and in immunocompromised individuals [2, 3]. Human could be infected with *T. gondii* by ingesting food and raw pork meat contaminated with cysts or oocysts [4, 5]. Pork is the main meat source in many countries, such as China. Many epidemiological investigations show that pig farms and intensive industries possess high prevalence and parasite load through PCR detection and serological test, but the detection of *T. gondii* in pigs is usually not taken seriously in many pig farms and intensive industries for expensive diagnosis cost and high error rate [6, 7, 8]. Therefore, the development of simple, inexpensive, and sensitive diagnostic tests for *T. gondii* detection in pigs is crucial to reduce the risk of toxoplasmosis in humans and pigs.

The diagnosis approach of toxoplasmosis has been constantly evolving, including traditional techniques (etiology, immunology, and imaging diagnosis) and many emerging molecular techniques. The etiological diagnosis of toxoplasmosis is relatively time-consuming since it involves the isolation of numerous disease materials and requires considerable skills to get reliable results. Thus, it is impossible to apply etiological diagnosis for large-scale clinical test in pig farms and intensive industries. Imaging diagnosis is mainly applied to cerebral and ocular toxoplasmosis through large medical equipment including computed tomography (CT), magnetic resonance imaging (MRI), nuclear imaging and ultrasonography (US), but imaging diagnostic results might not be reliable, and it requires expert interpretation [9]. Molecular techniques are widely applied to the epidemiological survey and clinical diagnosis of toxoplasmosis for their accuracy and sensitivity [10]. Molecular technique used for toxoplasmosis diagnosis is a high-sensitivity nucleic acid detection method of parasites in biological samples, and it overcomes the limitations of the serological tests, in addition, molecular technique mainly includes PCR, nested PCR, Real-time PCR, loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA) assay [11, 12, 13]. However, parasite nucleic acid detection involving DNA extraction tends to be expensive, and it is only accessible to the laboratory. Immunological detection is common method to determine the immune status of host by examining the change patterns of several different specific antibodies (IgA, IgM, IgG and IgE) after *T. gondii* infection [1, 14]. The common immunological method of toxoplasmosis diagnosis includes enzyme-linked immunosorbent assays (ELISA), modified agglutination test (MAT), and others [15, 16, 17].

ELISA is a serological detection that can be easily performed on a large scale, and many commercial kits are available to detect specific immunoglobulins (Igs) after *T. gondii* infection. The solid phase antigen used for ELISA includes crude tachyzoite antigen, *Escherichia coli* recombinant antigen, and chimeric peptide antigen. Although toxoplasma lysate antigen (TLA) possesses high levels of sensitivity and specificity in ELISA, there are the problems with TLA such as false positive results, standardization difficulty, unclear antigen composition, and complex and expensive TLA preparation [18, 19]. It is impossible to detect all serologically positive individuals by using one or several *Escherichia coli*

recombinant antigens, because the expression patterns of genes from different *T. gondii* strains are diverse during different infection stages [20]. Synthetic multiepitope antigen also known as chimeric antigen is a new generation of recombinant product for ELISA, and it contains multiple immunoreactive epitopes from several dominant antigens of *T. gondii*. Multiepitope antigens have been widely used for toxoplasmosis diagnosis, for example, synthetic multiepitope antigens are applied to detect anti-*T. gondii* IgG and IgM, and AMA1-SAG2-GRA1-ROP1 chimeric antigens are used for detecting specific antibodies of human and mouse in the early and chronic *T. gondii* infection [21]. The chimeric antigen technology has been developed for the serological diagnosis of the *Trypanosoma cruzi* infection caused by another protozoan parasite, the cutaneous anthrax caused by *Bacillus anthracis*, the human T-lymphotropic virus type I (HTLV-1) infection,and others [22, 23, 24, 25]. However, few studies have been conducted to evaluate chimeric antigens for serodiagnosis of *T. gondii* in pigs and to design an ELISA kit using synthetic antigens for the large-scale diagnosis of toxoplasmosis in pig farms and intensive industries.

Many *T. gondii* proteins are mainly secreted outside through three specific organelles (rhoptry, dense granule, and microneme), some of which could well activate host immune system. *T. gondii* surface antigen 1 (SAG1), as a highly immunogenic protein, is mainly distributed on the tachyzoite surface by glycosyl-phosphatidylinositol anchoring [26, 27]. Dense granule protein 1 and 4 (GRA1 and GRA4) secreted by *T. gondii* have good antigenicity [28, 29, 30, 31]. Rhoptry protein 2 (ROP2) belonging to ROP2-protein family is expressed in three stages (tachyzoites, bradyzoites and sporozoites) of *T. gondii* life cycle, and this protein induces a strong antibody response in mice and humans [32, 33]. Microneme protein 3 (MIC3), as an adhesion molecule, expressed in *T. gondii* could be recognized by anti-*T. gondii* positive serum. Mice immunized with recombinant pseudorabies viruses expressing MIC3 can produce high level of anti-*T. gondii* IgG to provide effective protection against *T. gondii* challenge in BALB/c mouse model [34]. Though these antigens (SAG1, GRA1, ROP2, GRA4 and MIC3) have been well documented to stimulate host immunity, little work has been done to determine whether the chimeric antigen with their T cell and (or) B cell epitopes is a good diagnostic marker for toxoplasmosis in pig farms and intensive industries.

To develop an efficient and low-cost ELISA kit for toxoplasmosis diagnosis in pigs, our study synthesized a multiepitope antigen (MAG) gene from 5 *T. gondii* dominant antigen genes (SAG1, GRA1, ROP2, GRA4 and MIC3) and evaluated the chimeric protein expressed by this MAG. A multiepitope protein encoded by MAG was designed, expressed, and purified from *Escherichia coli BL21* (DE3) strain. The reactivity of MAG antigen was determined through western blot, and MAG protein was strongly recognized by pig anti-*T. gondii* positive serum, but not by negative serum. Horseradish peroxidase (HRP)-conjugated recombinant protein A/G was applied as secondary antibody in our ELISA since this recombinant protein has strong IgG binding ability, and it is less pH-dependent than Protein A or Protein G alone, thus performing better at pH 5–8 [35, 36]. The optimized MAG-ELISA was applied to detect 209 pig serum samples. The overall coincidence rate between MAG-ELISA and a commercial PrioCHECK ELISA was 78.47%. Furthermore, MAG-ELISA could diagnose positive IgG at 7 days post pig artificial peritoneal infection with RH tachyzoites with diagnosis results obtained earlier than those obtained with the

commercial ELISA kit. Therefore, our MAG-ELISA has the potential for large-scale diagnosis of *T. gondii* infection in pig farms and intensive industries.

Methods

Ethics statement

Our study was authorized by the animal ethical committee from Huazhong agricultural university in China with the number HZAUSW-2012-001. Pig serum samples were provided by the animal parasite laboratory of Huazhong agricultural university. Pigs for *T. gondii* artificial infection were provided by the pig farm of Huazhong agricultural university. Finally, all pigs were sacrificed through euthanasia after *T. gondii* infection experiment.

MAG recombinant chimeric antigen

The MAG gene used in the indirect ELISA was synthesized (TSINGKE Biological Technology), then the synthetic gene was cloned into the pGEX-KG vector, and expressed in *Escherichia coli BL21(DE3)* strain. MAG recombinant protein fused with glutathione S-transferase (GST) was induced with IPTG (isopropyl β-D-1-thiogalactopyranoside), and then purified by glutathione-based affinity chromatography (GE Healthcare Life Sciences, USA). Finally, the purified MAG antigens were quantified by enhanced BCA protein assay kit (Beyotime Biotechnology, China).

Western immunoblot analysis

The purified MAG protein was separated by SDS-PAGE to identify the protein molecular weight through protein molecular weight marker (ThermoFisher scientific), and analyzed by western blot. The Immobilon-PSQ PVDF membrane (0.2 μm pore size, Millipore, USA) onto which purified protein was transferred was incubated with pig anti-*T. gondii* positive or negative serum, and then detected with HRP-conjugated rabbit anti-swine IgG (H + L) (Frdbio bioscience & technology, China).

Pig artificial infection with tachyzoite

“Chang xin” binary miscellaneous commodity pigs (any gender, weight of 15-20kg) bred by the pig farm of Huazhong agricultural university were applied to perform RH (type I strain) tachyzoite artificial infection by intraperitoneal injection. Four pigs were randomly assigned into two groups with two pigs in treatment group and control group. Two pigs in treatment group were intraperitoneally injected with 5 million tachyzoites for *T. gondii* infection. The 2 pigs in control group were intraperitoneally injected with equal volume of sterile 0.9% NaCl. Serum samples were all collected from treatment group and control group at 0, 2, 4, 7, 14, 21, 28, 35, 42, and 49 day. The serum sample at 3 day before *T. gondii* infection was also collected for subsequent detection.

Optimization of ELISA procedure

ELISA was performed as described preciously [20]. The 96-well flat bottom microtiter plates (BIOFIL, China) were coated overnight at 4℃ with 100 ul per well of coating buffer (25mM carbonate buffer, PH9.6) containing MAG purified antigen. The 96 well plate was washed 3 times with wash buffer (PBS with 0.05% Tween 20). Blocking buffer (BSA in PBS) was added, then the plate was incubated for a period of time at 37℃. After three times rewash as described above, 100ul of pig serum diluted with serum dilution buffer was added to each well, and the plates were incubated for a period of time at 37℃. After being washed as described above, 100ul of HRP conjugated recombinant protein A/G diluted in blocking buffer was added to each well, and the plates were incubated for a period of time at 37℃. After being washed as described above, color was developed for a period of time at 37℃ in dark room after the addition of 100ul per well of the substrate solution containing 3, 3', 5, 5'- tetramethylbenzidine (TMB, Sigma-Aldrich, USA) and H_2O_2. Finally, the OD630 value was measured with microplate reader Bio-Tek ELx-800 (BioTek Instruments, USA). All the tests were performed in duplicate wells.

To obtain the optimal dilution ratio of MAG-coated antigen and pig serum, the cross-titration was performed at serial dilution ratios of MAG antigen (1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200), *T. gondii* positive serum, and negative pig serum samples (1:20, 1:40, 1:80, 1:160, 1:320 and 1:640). The OD630 was measured and the ratio of P/N (positive/negative serum) was calculated at different dilution ratios. The dilution ratios of MAG antigen and pig serum with the maximum value of P/N was designated to be their optimal dilution ratio.

The optimal blocking buffer concentration (0.5%, 1.0%, 1.5%, and 2.0% BSA) and the optimal blocking time (30, 40, 50, and 60 mins) were determined as described above. The optimal serum dilution buffer (0.1% BSA, 0.5% BSA, and PBS containing 0.05% Tween 20) was determined by the maximum value of P/N. The optimal coating time of pig serum (30, 40, 50, and 60 mins) was determined by the maximum value of P/N under the above optimal conditions. The optimal dilution ratio (1:3000, 1:4000, 1:5000, 1:6000, 1:7000 and 1:8000) and the optimal reaction time (30, 40, 50 and 60 mins) of HRP-conjugated recombinant protein A/G were also determined through cross-titration. The optimal reaction time of TMB substrate solution (5, 7.5, 10 and 12.5 mins) was determined by the maximum value of P/N. The cut-off value of MAG-ELISA was determined referring to the OD630 mean value of 25 *T. gondii* negative pig sera under the optimal conditions (Cut-off = mean + 3SD).

Evaluation of MAG-ELISA

The specificity of MAG-ELISA was assessed by the diagnosis of 5 pig common virus sera (swine fever virus (SFV), porcine reproductive and respiratory disease virus (PRRSV), pseudorabies virus (PrV), porcine circovirus (PCV), and foot-and-mouth disease virus (FMDV)), and the control group including pig anti-*T. gondii* positive and negative sera. The sensitivity of MAG-ELISA was determined by measuring OD630 at serial dilution concentrations of pig anti-*T. gondii* positive sera. The coefficient of variation (CV) of repeated tests within batch and between batches was calculated and confirmed respectively through 6 samples (one anti-*T. gondii* positive and five negative pig sera). The stability was tested by the destruction experiments in which MAG-coated plates were placed at 37℃ for 12, 24, 36, 48, 60, and 72h, respectively.

Coincidence test

The 209 pig serum samples were tested by MAG-ELISA and a commercial PrioCHECK® *Toxoplasma* Ab porcine ELISA (Prionics, Switzerland), respectively. The coincidence of MAG-ELISA versus PrioCHECK ELISA was assessed according to two ELISA results.

Procedure of rMIC3-ELISA

The rMIC3-ELISA was performed in a previously reported ELISA method for detecting pig toxoplasmosis [37]. Recombinant entire *T. gondii* microneme protein 3 (rMIC3) fused with GST tag was expressed and purified as coated antigen. The 96-well plates were coated with rMIC3 at 3.40ug/ml, and pig serum samples were diluted at 1:160. The cut-off value of rMIC3-ELISA was defined as 0.40. The OD630 value was measured as described above.

Results

Characterization of recombinant multiepitope antigen and Optimization of MAG-ELISA procedure

Our study selected 6 epitopes from 5 reported *T. gondii* dominant antigen genes (SAG1, GRA1, ROP2, GRA4, and MIC3) and designed a new chimeric recombinant multiepitope antigen (MAG) gene by concatenating peptides containing T cell and (or) B cell epitopes (Table 1, Fig. 1A). MAG was synthesized and cloned into pGEX-KG vector for prokaryotic expression. The MAG protein with GST tag (43.3KD) was successfully expressed in *Escherichia coli BL21* (DE3) and purified through glutathione-based affinity chromatography. Western blot results showed the MAG protein was recognized specifically by pig anti-*T. gondii* positive serum but not by negative serum (Fig. 1B).

Table 1
Sources of chimeric antigen MAG

Gene	Epitope	Strain	Sequence	Position
SAG1	S1	GT1	TCPDKKSTA	59–67
	S2	GT1	ILPKLTENPWQ	246–256
GRA1	G1	GT1	DTMKSMQRDED	104–114
ROP2	R2	GT1	PGDVVIEELFNRIPETSV	197–214
GRA4	G4	GT1	SGLTGVKDSSS	235–245
MIC3	M3	GT1	KRTGCHAFRE…SCKCDNGYSG	233–310

To improve the performance of MAG-ELISA, we attempted to optimize the procedures related to MAG antigen, pig serum, buffer, secondary antibody, and ELISA substrate, and others. The cross-titration showed that the optimal dilution ratio of MAG-coated antigen was 1:100 (0.91 ug/well) and that of pig

serum was 1:160 (Fig. 2A). The maximum P/N was obtained when MAG-coated plate was incubated with 1.0% BSA for 30 mins under the optimal condition described above (Fig. 2B). We also found 0.5% BSA was optimal serum dilution buffer, compared to 0.1% BSA and phosphate solution containing 0.05% Tween 20 (Fig. 2C). The optimal pig serum incubation time was determined to be 40 mins (Fig. 2D). HRP-conjugated recombinant protein A/G exhibited optimal performance when the plates were incubated at 1:8000 for 60 mins (Fig. 2E). The optimal color development was obtained after TMB substrate was incubated for 10 mins (Fig. 2F). The cut-off value of MAG-ELISA was set as 0.25 after testing 25 *T. gondii* negative pig sera under the optimal conditions described above (Fig. 2G). The subsequent MAG-ELISA was performed according to the above-mentioned optimal procedures.

Evaluation of MAG-ELISA

To evaluate the feasibility of our MAG-ELISA, we also examined its specificity, sensitivity, stability, and others. The OD630 values of the 5 common pig viruses (SFV, PRRSV, PrV, PCV and FMDV) were significantly lower than the cut-off value of MAG-ELISA (Fig. 3A), indicating our ELISA did not exhibit obvious cross reaction with these 5 common pig viruses, which confirmed the good specificity of MAG-ELISA. By measuring OD630 at serial dilutions of *T. gondii* positive pig serum, we found that pig anti-*T. gondii* positive serum was still determined as positive even at the dilution ratio of 1:320, indicating the sensitivity of MAG-ELISA was adequate (Fig. 3B). The CV of repeated tests within batches and between batches was all below 10%, suggesting the good stability of our MAG-ELISA (Fig. 3C). After placing MAG-coated plates at 37℃ for 72h, the OD630 only dropped by less than 20%. (Fig. 3D). We further compared MAG-ELISA with PrioCHECK ELISA by testing 209 pig serum samples simultaneously, and found their coincidence rate was 66.67% for *T. gondii* positive results, 91.84% for *T. gondii* negative results, and the total coincidence rate was 78.47% (Table 2).

Table 2
Coincidence between MAG-ELISA and PrioCHECK ELISA for detecting *T. gondii* infection

		PrioCHECK ELISA		
		Positive	Negative	Total
MAG-ELISA	Positive	74	8	82
	Negative	37	90	127
	Total	111	98	209

MAG-ELISA detection of Pig artificial infection with type I tachyzoite

MAG-ELISA and 2 other ELISA methods (rMIC3-ELISA and PrioCHECK ELISA) were applied to detect pig anti-*T. gondii* IgG level after artificial infection with RH tachyzoites. We collected pig serum samples from infection group and control group at day 0, day 2, day 4, day 7, day 14, day 21, day 28, day 35, day 42 and

day 49 after infection and at day 3 before infection, respectively. MAG-ELISA detected anti-*T. gondii* positive IgG in the early stage of pig infection (at least 7 days), which was earlier than the results obtained by rMIC3-ELISA and PrioCHECK ELISA (Fig. 4). Two weeks after artificial infection, our MAG-ELISA indicated that the overall level of anti-*T. gondii* IgG was gradually decreased, and rMIC3-ELISA and PrioCHECK ELISA showed that the level of anti-*T. gondii* IgG reached their maximum value at day 35 post infection. Therefore, MAG-ELISA had the potential to be applied for early detection of *T. gondii* infection in pig farms and intensive industries.

Discussion

Humans can be infected by *toxoplasma gondii* through digesting raw or uncooked meats (such as pork). Infection with *toxoplasma gondii* can lead to reproductive disorders in sows (such as miscarriage, stillbirths, and weak fetuses). Poor meat quality would threaten human health and cause serious economic losses to the livestock industry. *T. gondii* could survive in many pig tissues such as heart, lungs, and brain for a long time in the form of cyst. Pork meat is considered to be one of the main sources of human toxoplasmosis [4, 38]. The high prevalence of *T. gondii* is found in pig farms, especially organic farms and intensive pig industries [39, 40]. Therefore, it is necessary to develop a highly sensitive, specific, and inexpensive kit for the large-scale diagnosis of pig toxoplasmosis in farms and intensive industries.

ELISA is a common method for the large-scale detection of infectious disease mainly caused by pathogenic bacteria, viruses, and parasites. The specificity and sensitivity of ELISA depend mainly on the coated antigen. All the common coated antigens such as TLA and recombinant antigen have their own limitations to some extent. For example, the composition of TLA is poorly understood, and TLA preparation procedure is complex and infectious to operator. Although recombinant protein antigen of *T. gondii* is very useful for serodiagnosis of toxoplasmosis, one recombinant antigen could detect only one type of anti-*T. gondii* IgG, and the diagnosis result may be affected by the expression level of antigen gene[41]. Thus, the application of these antigens in ELISA may cause high error rate in large-scale detection. The chimeric antigen encoded by synthetic multiepitope antigen gene is a new generation of recombinant products, and it has competitive advantages since it contains more immunoreactive epitopes from several dominant antigens of *T. gondii* than conventional recombinant antigen. Chimeric antigen with the epitope from no more than 3 *T. gondii* genes used in ELISA has been reported to perform well in toxoplasmosis diagnosis [19]. To further improve the performance of ELISA, our MAG-ELISA used a chimeric antigen containing 6 epitopes of T and B cells from 5 *T. gondii* dominant antigens (SAG1, GRA1, ROP2, GRA4, and MIC3). SAG1 is only highly expressed in acute infection, but GRA1, ROP2, ROP4, and MIC3 are expressed in acute and chronic *T. gondii* infection, endowing MAG antigen with the potential to detect pig anti-*T. gondii* IgG in two different infection periods. Our results showed that MAG-ELISA possessed high specificity, sensitivity, and repeatability. The test of 209 pig serum samples indicated that the total coincidence rate of MAG-ELISA and PrioCHECK ELISA was only 78.47% with a coincidence of 66.67% for *T. gondii* positive results and a coincidence of 91.84% for *T. gondii* negative results (Table 2). The difference in the seroprevalence of *T. gondii* infection between MAG-ELISA and

PrioCHECK ELISA was significant in pigs. The low total IgG level in MAG-ELISA might be attributed to the inconsistence in the levels of anti-*T. gondii* individual IgGs against these 6 epitopes at different infection stages. Furthermore, false positive results of the PrioCHECK ELISA might be caused in the case that some sample test results were close to cut-off value due to the unclear component of TLA antigen.

We further compared the obtained levels of anti-*T. gondii* IgGs after artificial infection with RH tachyzoites through 3 different ELISA (MAG-ELISA, rMIC3-ELISA, and PrioCHECK ELISA). Our result showed that MAG-ELISA could detect *T. gondii* infection at day 7 after infection, which was earlier than the other 2 ELISA methods. However, the level of anti-*T. gondii* IgG obtained through MAG-ELISA was inconsistent with those through rMIC3-ELISA and PrioCHECK (Fig. 4). The reasons for the above-mentioned inconsistence might be as follows. First, pig anti-*T. gondii* IgGs against the epitope of SAG1, GRA1, ROP2, GRA4, and MIC3 respectively possessed inconsistent levels after RH artificial infection. Second, the IgG trend in MAG-ELISA could represent the overall level of several anti-*T. gondii* IgGs. Thus, our MAG-ELISA should be more reliable than the ELISA using only one recombinant antigen. However, the MAG-ELISA could detect anti-*T. gondii* IgG only within 1–7 weeks after artificial infection with detection time span shorter than that of other 2 ELISA methods, which might be due to the low expression of the antigen genes corresponding to some epitopes. Previous studied also indicated that antibody responses to GRA1 and MIC3 were very weak, or even absent six weeks post infection [42].

MAG could specifically recognize pig anti-*T. gondii* IgG, thus MAG-ELISA has a potential for diagnosing toxoplasma infection in pigs. The MAG-ELISA would be conducive to improving the accuracy and reducing cost of toxoplasmosis diagnosis in pig farms and intensive industries. MAG-ELISA can monitor the change trend of anti-*T. gondii* IgG after pig artificial infection, which increases our knowledge about the *T. gondii* infection in pigs. Further work is also needed to further optimize the performance of the MAG-ELISA and verify its diagnosis effect in detecting *T. gondii* acute and chronic infection in pigs and other animals (such as pets).

Conclusions

We reported here MAG, as a synthetic multiepitope antigen, could recognize pig anti-*T. gondii* positive but not negative serum. MAG-ELISA possessed a high diagnostic performance in terms of specificity and sensitivity. The overall coincidence rate between MAG-ELISA and a commercial PrioCHECK ELISA was good. MAG-ELISA has the potential for large-scale diagnosis of *T. gondii* infection in pig farms and intensive industries.

Abbreviations

T. gondii: *toxoplasma gondii*; MAG: synthetic multiepitope antigen; ELISA: enzyme-linked immunosorbent assay; PrioCHECK ELISA: PrioCHECK® *Toxoplasma* Ab porcine ELISA; TLA: toxoplasma lysate antigen; SAG1: *T. gondii* surface antigen 1; GRA1: dense granule protein 1; GRA4: dense granule protein 4; ROP2: rhoptry protein 2; MIC3: microneme protein 3; HRP: horseradish peroxidase; GST: glutathione S-

transferase; TMB: 3, 3', 5, 5'- tetramethylbenzidine; P/N: positive/negative serum; SFV: swine fever virus; PRRSV: porcine reproductive and respiratory disease virus; PrV: pseudorabies virus; PCV: porcine circovirus; FMDV: foot-and-mouth disease virus; CV: coefficient of variation; rMIC3: recombinant entire *T. gondii* microneme protein 3.

Declarations

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Authors' contributions

YQZ conceived this project, designed the experiment and reviewed the manuscript. YLS, YJZ and KP performed the experiment, analyzed and interpreted the data and drafted the manuscript. BS, RF and MH participated in designing the experiment and reviewing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All relevant data are within the manuscript and its Supporting Information files.

Ethics approval and consent to participate

Our study was authorized by the animal ethical committee from Huazhong agricultural university in China with the number HZAUSW-2012-001.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

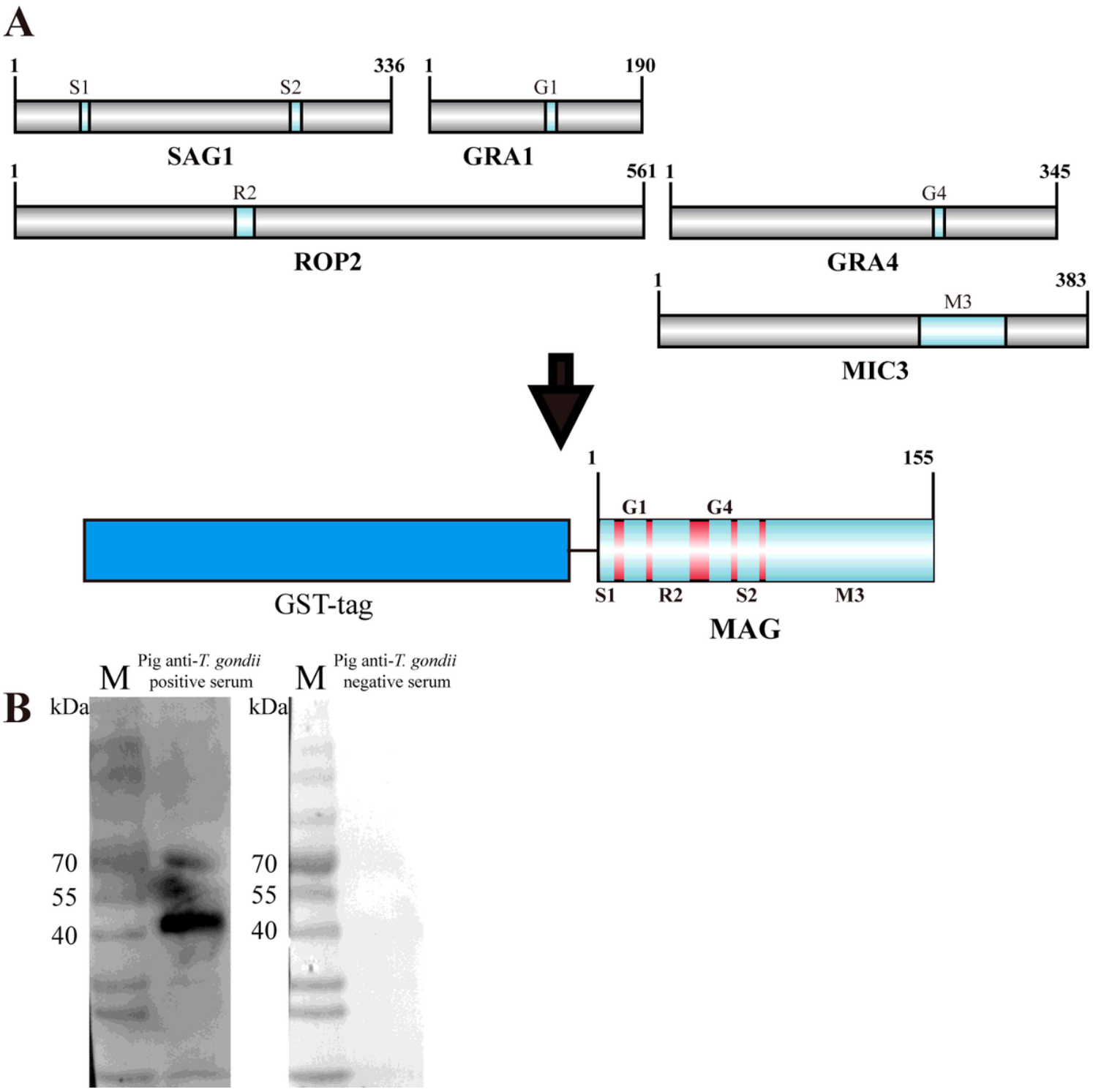

Figure 1

MAG construction and antigen identification (A) The construction of chimeric antigen MAG. (B) Western blot to identify MAG antigenicity.

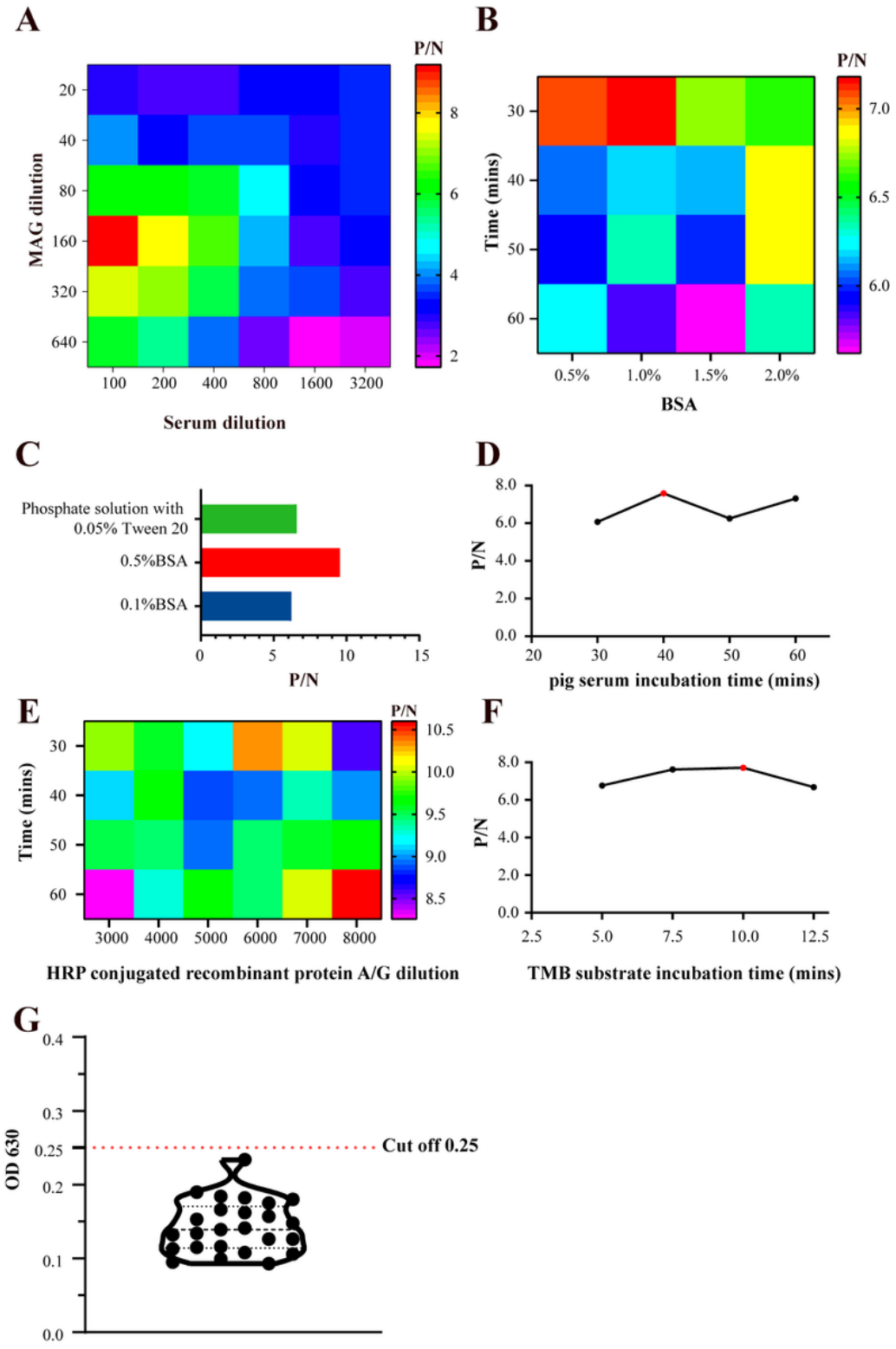

Figure 2

Optimization of MAG-ELISA (A) The optimal dilution ratio of MAG antigen and pig serum. (B) The blocking buffer optimization of BSA concentration and incubation time. (C) The optimization of pig serum dilution buffer. (D) The optimization of pig serum incubation time. (E) The optimization of HRP conjugated recombinant protein A/G dilution ratio and incubation time. (F) The optimization of TMB

substrate incubation time. (G) The cut-off value of pig anti-T. gondii negative serum (Cut-off = mean + 3SD).

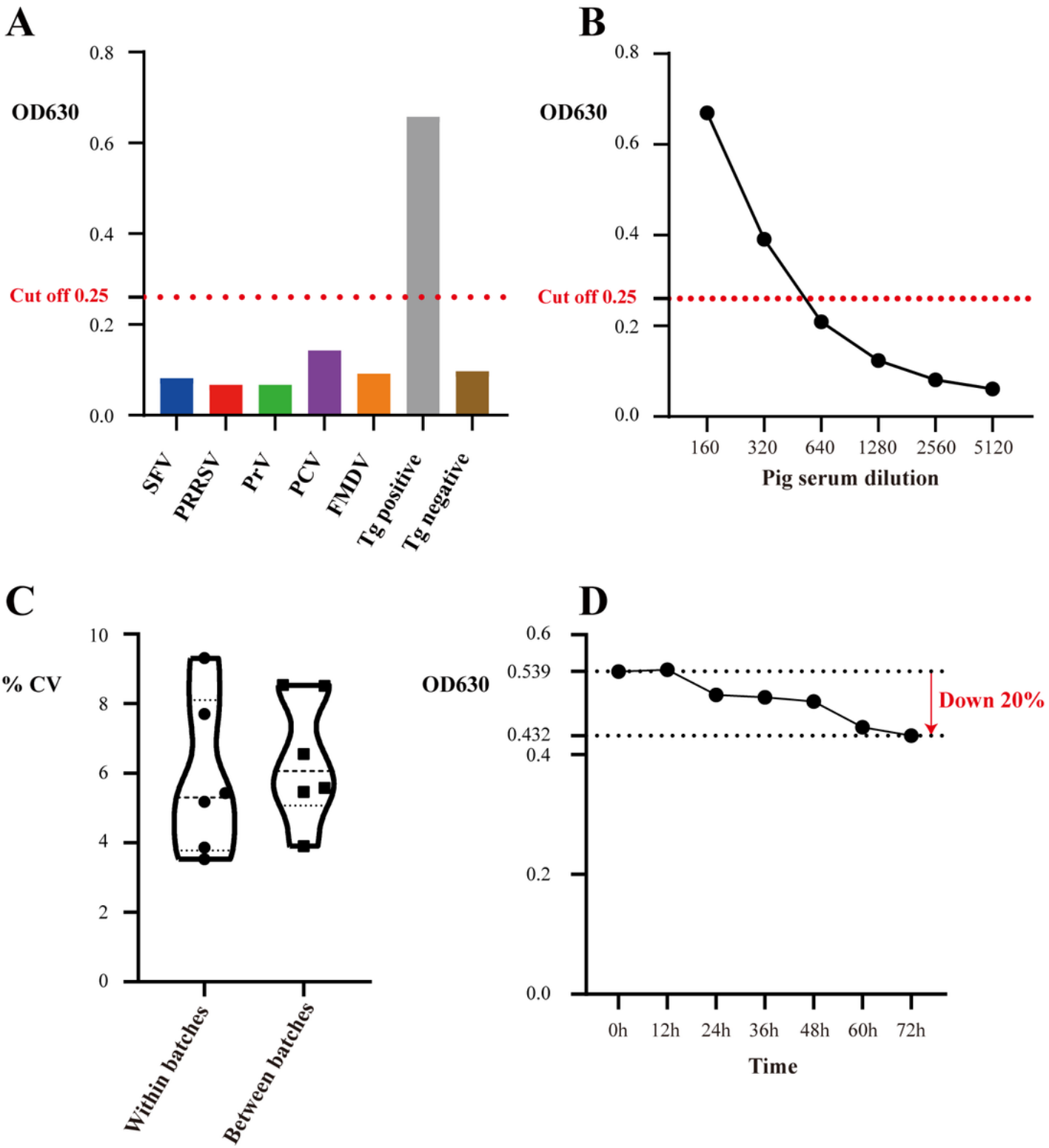

Figure 3

Evaluation of MAG-ELISA (A) The specificity of MAG-ELISA was assessed by the diagnosis of 5 pig common virus (SFV, PRRSV, PrV, PCV, and FMDV). (B) The sensitivity of MAG-ELISA was determined by serial dilutions of pig anti-T. gondii positive serum. (C) The CV of repeated tests within batches and

between batches. (D) The MAG-ELISA stability was tested by the destruction experiment at 37℃ for different time.

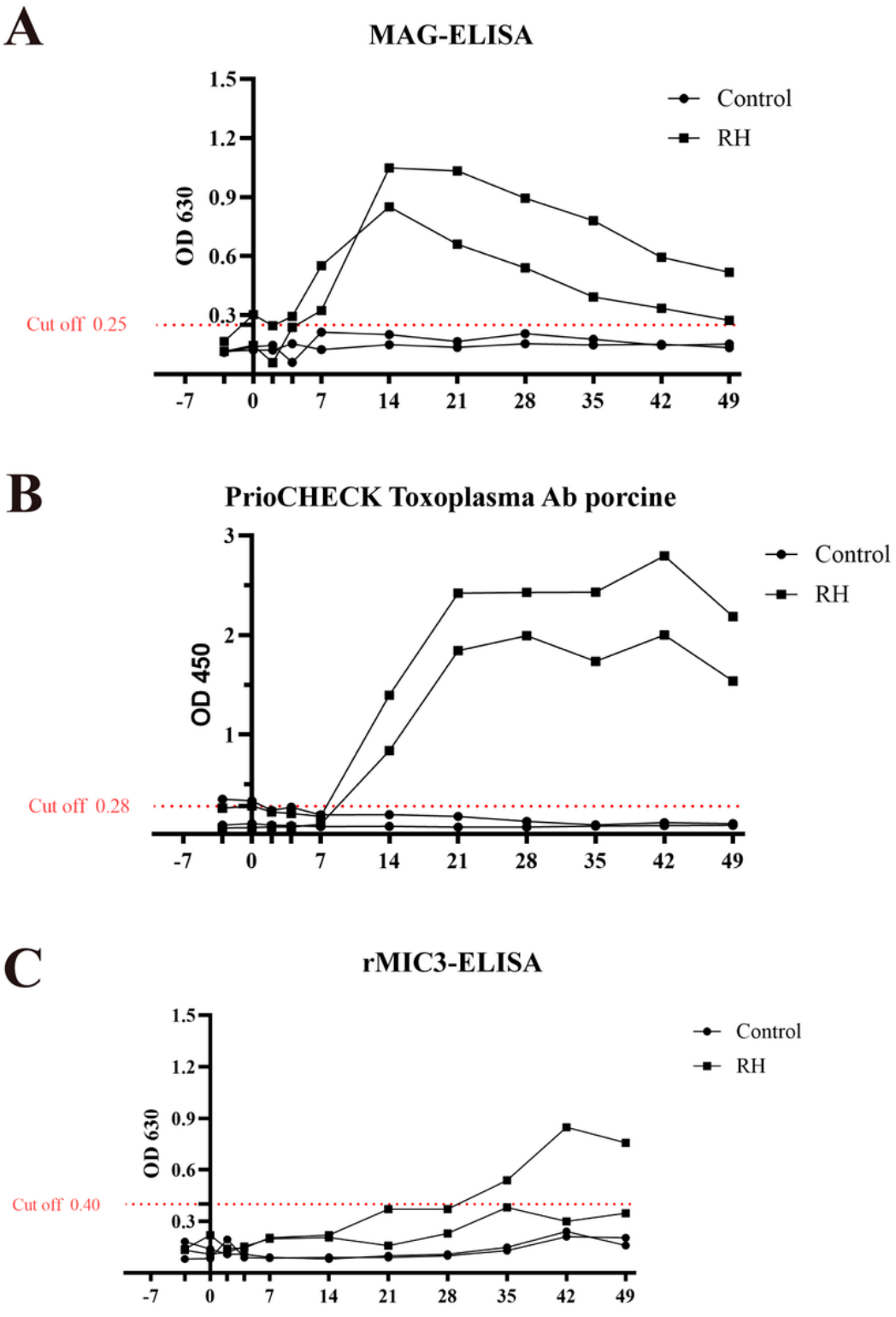

Figure 4

Trend of pig anti-T. gondii IgG level after artificially infection with RH tachyzoites in MAG-ELISA and two other ELISA methods

Supplementary Files

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