**PRELIMINARY ASSESSMENT OF ELISA, MAT, AND LAT FOR DETECTING *TOXOPLASMA GONDII* ANTIBODIES IN PIGS**

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**Abstract**

Applicability and sensitivity of the commercial tests MAT (modified agglutination test), LAT (latex agglutination test), in comparison to in-house serologic assay (ELISA), and reference test (indirect fluorescence antibody test, IFAT) to detect specific antibodies against *Toxoplasma gondii* in pigs were investigated. One hundred and six blood samples were examined. The most specific test, as compared to IFAT, was MAT (100.0%) and the most sensitive test was ELISA (88.9%). LAT showed the least specificity and sensitivity at 91.4%, and 47.2%, respectively. After analysis of the tests with Cohen’s Kappa agreement, it was found that IFAT, ELISA and MAT were in high agreement, and LAT was moderately consistent with these tests. Similar results were achieved using Spearman’s rank correlation coefficient. Our study confirmed the usefulness of the ELISA and MAT, much greater than LAT, for the detecting of *T. gondii* antibodies in pigs.

**Key words:** pigs, *Toxoplasma gondii*, antibodies, MAT, LAT, ELISA, IFAT.

Toxoplamosis is one of the most frequent parasitic infections in humans and animals. It is especially dangerous in its congenital form and in people with immunologic deficiency. People from Poland are serologically positive up to 40%-60%, depending on age, habit of feeding, and residence. The epidemiologic studies carried out by various scientific centres from Europe and USA identified undercooked or raw pork as the major source of *Toxoplasma* infection for people (1, 10). It should be an important source of infection in Poland because of common pork consumption. The prevalence of *T. gondii* in pigs varies but generally exceeds 10%-20% in most countries. The prevalence of *T. gondii* in slaughter animals is being assessed on the basis of serological examination. In Poland, no sufficient data on *T. gondii* infections in slaughter animals are available. Serological examinations of pig populations carried out recently in Poznań and the Lublin regions showed significant percentage of positive reactions (10.5-13.2%). To evaluate the risk of infection by consumption of meat, appropriate and sensitive diagnostic methods are needed to identify infected animals.

In the present study, we compared the applicability and sensitivity of commercial tests MAT (modified agglutination test) and LAT (latex agglutination test) and in-house serological assay (ELISA) in the detection of specific antibodies against *Toxoplasma gondii* in pigs. As the reference standard, an indirect fluorescent antibody test (IFAT) was used.

**Material and Methods**

**Swine sera.** One hundred and six blood samples were analysed. Seventy-seven sera originated from 6-month-old pigs slaughtered at an abattoir; 12 sera were collected from pigs during routine veterinary examinations, and nine sera originated from a pig inoculated with RH strain of *T. gondii*. One serum sample from pigs subjected to the inoculation was collected before the immunisation and eight sera were collected after the immunisation, at 7-day intervals. After clotting, the sera were separated by centrifugation at 3,500 rpm for 10 min, and stored at \( -20^\circ C \) until the examination.

**Control sera.** As positive controls for IFAT and ELISA, blood samples collected from pig inoculated with *T. gondii* were used. Serum obtained from this pig before the inoculation was used as a negative control. To determine positive-negative threshold for ELISA, there were used 25 positive and 21 negative sera, earlier evaluated with IFAT. Each of these controls
was confirmed with Sabin-Feldman Dye Test (DT), recognised as the referential test. DT was performed according to methods described by Sabin and Feldman (13) and Kulasiri (11).

**Indirect ELISA.** The test was performed according to Lind et al., (12) with some modifications. Optimal dilutions for ELISA were established using checkerboard titrations of coating preparations, sera and conjugates. Polystyrene plates (PolySorp, Nunc) were coated with 100 µl of antigen/well (suspension of purified and sonificated tachyzoites of *T. gondii* RH strain, Fitzgerald Industries International, USA) diluted 1:3,000 in phosphate-buffered saline (PBS, pH 7.2) and then incubated overnight at 4°C. The plates were washed three times with PBS containing 0.01% Tween 80 (PBS-Tween), and blocked for 0.5 h at 37°C with carbonate buffer (pH 9.6) containing 10% non-fat dry milk (blocking buffer) in a humid chamber. After removing blocking solution, 100 µl of serum sample, diluted 1:100 in buffered milk, was added in duplicate and the plate was incubated for 1 h at 37°C. After washing as above, 100 µl of HRP (horseradish peroxidase)-labelled rabbit anti-swine conjugate (Sigma-Aldrich) diluted 1:3,000 in blocking buffer, pH 5.0, was added and the plate was left for 10 min at room temperature. The reaction was stopped with 100 µl per well of 0.5M sulphuric acid (in the case of positive reaction) or lack of agglutination (for negative sera) was observed. The addition of equal volume of 0.01% Evans blue to immunoglobulins helped in later visualisation of *Toxoplasma* fluorescence. The slides were incubated, washed and dried as above. Next, a drop of mounting medium Fluoprep (bioMérieux, France) was added and a cover glass was put on the slide. The slides were analysed using a fluorescence microscope at 400× magnification. Each slide contained a positive and a negative control serum and PBS as a control of conjugate. Visible fluorescence of cytoplasmic membrane contrasting with red dyed cytoplasm of parasite was regarded as positive. Positive sera were examined in higher dilutions to establish endpoint titre.

**Indirect fluorescent antibody test (IFAT).** Commercially available glass slides coated with formalinised tachyzoites (Toxo-Spot IF, bioMérieux, France) were used. The 20 µl of serum sample diluted 1:128 in PBS was placed on test spot (10 spots per slide), then the slides were incubated at 37°C for 30 min in a humid environment. After washing twice for 5 min in PBS and once in ddH₂O, the slides were dried. Then, 20 µl of FITC (fluorescein isothiocyanate)-labelled rabbit anti-swine conjugate (Sigma-Aldrich) diluted 1:128 in PBS was placed on test spot (10 spots per slide). The test was performed in microtitration plates with U-shaped wells. In the screening procedure, control and diagnostic sera were diluted 1:40 and 1:4,000. A positive reaction exhibits agglutination of the *Toxoplasma* in a mat covering about half of the well base. In the absence of specific antibodies, a solid button is obtained. A positive reaction in the screening test was confirmed by a quantitative test with additional dilutions from 1.60 to 1:1,620 or 1:6,000 to 1:162,000, depending on the results of screening. The minimal titre for a positive result in this kit was established as greater or equal to 1:40.

**Latex agglutination test (LAT).** Commercial kit Pastorex-Toxo (Bio-Rad, France) was used to determine antibodies of IgG and/or IgM class. The kit includes a positive and negative control serum, latex beads suspension and diluent (0.9% NaCl). The test was performed according to the manufacturer’s instructions. One drop of serum, diluent, and latex suspension were put on marked places on the paper card. Next, the reagents were mixed with a stick, and the card was shaken for 5 min. Agglutination and changing of colour (in the case of positive reaction) or lack of agglutination and change in colour (for negative sera) was observed.

**Statistical analysis.** The data were analysed by Cohen’s Kappa statistic and Spearman’s rank correlation coefficient with the use of Statistica for Windows v. 8.0 package (StatSoft Inc., USA).

**Results**

The results of pig sera examination for the presence of specific antibodies, obtained by the IFAT, MAT, ELISA and LAT tests are shown in Table 1. Sera examined with IFAT were positive in 36 cases (34.0%), 35 sera samples were found positive by ELISA (33.0%), and even fewer positive results were detected in MAT (28 cases - 26.4%) and LAT (23 cases - 21.7%). The percentages of positive results obtained in ELISA and IFAT were similar (33.0% vs. 34.0%). Between IFAT and LAT, a wide difference of percentages was found (34.0% vs. 21.7%). The most specific test as compared to IFAT was MAT (100.0%). The specificity of ELISA was established at 95.7% and that of LAT at 91.4%. In the group of sera negative in IFAT (n=70), three sera in ELISA and six sera in LAT were positive. The most sensitive test was ELISA (88.9%), MAT was sensitive at 77.8%, and LAT at 47.2%, only. In the group of positive sera in IFAT (n=36) four sera in ELISA, eight sera in MAT and nineteen in LAT were negative. The results were shown in Table 2.
The results of examination of eight sera from the pig inoculated with RH strain of *T. gondii* showed perfect level of agreement (100%) between ELISA and IFAT, as well as between MAT and IFAT. However, poor level of agreement (62.5%) was observed between results of LAT and IFAT. Medium positive results in ELISA (OD – 0.52), MAT (titre – 540), and high in IFAT (titre – 6,000) were obtained with serum collected on the 7th day post-inoculation (7 DPI). Samples collected weekly from 14 DPI to 56 DPI revealed high positive results in ELISA (OD>1.0), MAT, and IFAT (titre>2,000).

Using statistical analysis (Cohen’s Kappa statistic), significant agreement between qualitative results of ELISA, MAT, and IFAT was found. The results of LAT showed moderate agreement in the comparison to IFAT and to all remaining tests. The results of this analysis are shown in Fig. 1.

Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>Negative results (N)</th>
<th>Positive results</th>
<th></th>
<th></th>
<th></th>
<th>Total [N (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (N)</td>
<td>Medium (N)</td>
<td>High (N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFAT</td>
<td>70</td>
<td>17</td>
<td>9</td>
<td>10</td>
<td>36 (34.0)</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>71</td>
<td>12</td>
<td>8</td>
<td>15</td>
<td>35 (33.0)</td>
<td></td>
</tr>
<tr>
<td>MAT</td>
<td>78</td>
<td>15</td>
<td>3</td>
<td>10</td>
<td>28 (26.4)</td>
<td></td>
</tr>
<tr>
<td>LAT</td>
<td>83</td>
<td>10</td>
<td>5</td>
<td>8</td>
<td>23 (21.7)</td>
<td></td>
</tr>
</tbody>
</table>

N – number of serum samples

Table 2

<table>
<thead>
<tr>
<th></th>
<th>ELISA</th>
<th>MAT</th>
<th>LAT</th>
</tr>
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<tbody>
<tr>
<td>+ -</td>
<td>Total</td>
<td>+ -</td>
<td>Total</td>
</tr>
<tr>
<td>+</td>
<td>32</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>IFAT</td>
<td>-</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>71</td>
<td>106</td>
</tr>
<tr>
<td>Specificity</td>
<td>95.7</td>
<td>100.0</td>
<td>91.4</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>88.9</td>
<td>77.8</td>
<td>47.2</td>
</tr>
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</table>
Discussion

Toxoplasma infection in pigs is mainly considered as a public health problem but the infection may cause also reproductive disorders in animals and thus notable economic losses in productivity. Serology testing is an appropriate method for the detection of Toxoplasma seropositive pigs. These pigs constitute a potential risk for the humans as there was established the correlation between the antibody titre in the pigs and the potential risk for the humans as there was established the Toxoplasma testing is an appropriate method for the detection of thus notable economic losses in productivity. Serology may cause also reproductive disorders in animals and considered as a public health problem but the infection detection of bioassay, it can be performed in reference centres only.

A few of serological tests are used for the detection of Toxoplasma antibodies. The Sabin-Feldman dye test is still regarded as the test of choice, but as a bioassay, it can be performed in reference centres only. IFAT is considered as a gold standard in serodiagnostics of Toxoplasma infection in pigs (6). This test may detect antibodies that appear at the early stage of infection against components of the membrane of tachyzoites (8, 9). The test results are easy to evaluate. ELISA is another test used to detect T. gondii antibodies (7, 12, 14). Some authors used a whole-tachyzoite antigen and the obtained results were slightly better than the MAT in detecting antibodies to T. gondii in naturally infected pigs (88.6% sensitivity of ELISA and 85.7% sensitivity of MAT) (5). Dubey (1995) using naturally infected sows, reported a higher sensitivity with the MAT as compared with ELISA (3). An attempt to use recombinant P30 antigen was effective in detecting antibodies to T. gondii in experimentally infected pigs (4). However, Gamble et al. (5) in initial testing the sera from naturally infected pigs with P30 antigen found that the sensitivity of the test was reduced to an unacceptable level. The prozone phenomenon occurs in excess of specific antibodies in MAT and requires each serum sample to be examined at least at two dilutions. Almost all positive sera in which the effect occurred at the 1:40 dilution had titres at least 1,620. The MAT results are evaluated visually and subjectively, and depend on the skills of the technician. With regard to the long time needed to perform the MAT and the difficulty in interpretation of the results, the ELISA seems to be a more useful test for routine screening of pigs. The potential automatisation of ELISA is another advantage of this test.

Detection of Toxoplasma gondii IgM class antibodies in pigs is not being practised in routine diagnostics, because swine produce specific IgM antibodies only during a short, initial phase of the infection (2-4 weeks). Therefore, it may not be a good method to evaluate the chronic infection in animals (12). However, it could be a good diagnostic tool in case of reproduction problems.

In our study, we compared the sensitivities of three methods for the detection of T. gondii in naturally and experimentally infected swine. Two of them – commercial kits MAT and LAT are used by some laboratories in scientific and service activities. Based on the results of this study, the ELISA and MAT demonstrated similar, good correlation in the detection of serum antibodies to Toxoplasma in pigs (Spearman’s rank correlation coefficient R and Cohen’s Kappa statistic K above 0.8) in comparison to reference IFAT. LAT had poor specificity and sensitivity when compared with the remaining tests (both statistics R and K below 0.6). Among 28 samples, which were positive in IFAT, MAT and ELISA, only 16 samples (57%) were positive with LAT. In samples from immunised pigs, positive results in LAT were obtained mainly in the early phase of infection, which would confirm the special usefulness of this test for the detection of antibodies IgM class in pigs. Some authors also considered insensitivity of LAT in the detection of Toxoplasma antibodies in pigs (2, 3, 8).

Because ELISA and MAT enable the detection of the T. gondii IgG class antibodies only, whereas LAT enables detecting antibodies of both classes – IgG, and IgM, then hypothetically LAT should be optimal test to screening study. But the result of this study showed completely the opposite situation: LAT did not fulfil appropriate requirements for the detection of Toxoplasma antibodies in pigs. Our preliminary study showed that LAT used to detect Toxoplasma antibodies in sera of cats revealed better sensitivity and specificity (data not published). This indicates that commercial test LAT could be used for the examination of other species of animals.

Based on the results of this study, the ELISA is slightly better test than the MAT for the detection of serum antibodies to Toxoplasma in pigs. As the ELISA enables faster and easier interpretation of results, it may be more useful test for routine screening of pigs.

References


