食肉検査における豚トキソプラズマ症診断のためのPCR試験と豚主要急性相タンパク質(Pig-MAP)測定の有用性
Usefulness of the assay of pig major acute-phase protein (pig-MAP) and polymerase chain reaction (PCR) test for diagnosis of swine toxoplasmosis in meat inspection

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Summary

The lymph nodes and sera of pigs with suspected toxoplasmosis during meat inspection at an abattoir were investigated using the PCR for the B1 gene of *Toxoplasma gondii* (PCR test), anti-*T. gondii* antibody tests (antibody test) and assays for pig major acute-phase protein (Pig-MAP), haptoglobin (Hp) and alpha1-acid glycoprotein (AGP), adding to the microscopic examination of an acridine orange-strained stamp-smear samples from lesions (AOS test). High levels of Pig-MAP in sera were observed in cases that were positive for AOS test, PCR and antibody tests. These findings suggest that the utilizations of Pig-MAP and PCR test provide supplementary information for diagnosis of toxoplasmosis at the time of meat inspection.

Key words: pig major acute-phase protein, toxoplasmosis, meat inspection.

Introduction

The protozoan *Toxoplasma gondii* (*T. gondii*) causes toxoplasmosis, which presents a significant health risk to the developing fetus and immunocompromised individuals. Porcine meat has been considered one of the major sources responsible for oral transmission to humans⁶. In Japan, the incidence of toxoplasmosis in pigs determined by meat inspection at abattoirs has decreased dramatically to almost one seventh of that.
in the last twenty years. However, the number of cases of porcine toxoplasmosis in Okinawa Prefecture is certainly not inconsiderable relative to other prefectures in Japan, several tens of pigs per year having been condemned due to the disease in Okinawa.

At abattoirs, porcine toxoplasmosis is confirmed on the basis of typical macroscopic features, which include hemorrhagic or necrotic lesions of the lymph nodes (mainly mesenteric). Carcasses suspected to harbor toxoplasmosis are subjected to the microscopic examination of an acridine orange-stained smear samples from lesions (AOS test). If the \textit{T. gondii} tachyzoite is recognized in the AOS test, the carcass is condemned under the Abattoir Law. The AOS test is important for meat inspection, and is the only method for the final diagnosis of swine toxoplasmosis. However, even if the typical macroscopic features of this disease are recognized, the \textit{T. gondii} tachyzoite is often not revealed in the AOS test. Therefore, development of methods other than the AOS test has been anticipated, because of the lack of an in vitro system for culture of \textit{T. gondii}.

We have previously reported PCR-based discrimination of \textit{T. gondii} from pigs at abattoirs in Okinawa\textsuperscript{18}, and suggested that the PCR method is useful for detection of \textit{T. gondii} at the time of meat inspection. We have also reported that the serum level of Pig-MAP, one of the acute phase proteins (APPs), increases in wasting pigs that are brought to the abattoir\textsuperscript{17}. APP levels increase following infection, inflammation or trauma, and their quantification in plasma can provide valuable diagnostic information for disease monitoring\textsuperscript{5,12}. Accordingly, it has been suggested that estimation of APPs during meat inspection would be helpful for improving food safety\textsuperscript{19}. However, the reports about the assay of APPs in meat inspection of the food animals brought to the abattoirs are limited.

In this study, in order to clarify the usefulness of PCR and assay of APPs for diagnosis of toxoplasmosis in meat inspections, we carried out PCR for the B1 gene of \textit{T. gondii} and determined the presence of representative APPs, Pig-MAP, haptoglobin (Hp) and alpha 1-acid glycoprotein (AGP) in pigs with suspected toxoplasmosis.

### Materials and methods

(1) Lymph nodes and sera

The lymph nodes (mainly mesenteric) and sera of pigs with suspected toxoplasmosis during meat inspection at an abattoir were used for the PCR and determination of APPs. Forty lymph nodes from normal pigs were also collected for the PCR. Blood samples were collected mainly from the mesenteric vein or heart, then the serum was separated from each sample by centrifugation (3,000 rpm, 10 min) and stored at -80°C until use.

(2) PCR procedure

The PCR using three primer pairs for the B1 gene of \textit{T. gondii} were investigated. The following primers were used in these reactions: \textit{Z500F/R} (5'-GGAACGTGCATCCGGTCATGAG-3' and 5'-CACAGCAATCAACGGAACTG-3'); \textit{B22123} (5'-AACGGGCGAGTGACACCTGAGGAGA-3' and 5'-TGGGTCTAGCGTGGCATGACACAC-3'); and \textit{JW58/59} (5'-AAGGGCTGACTCGAACCAGATGT-3' and 5'-GGGCGGACCTCTTTGGCTCTCG-3'). The condition used for these reactions were as indicated in previous reports\textsuperscript{1,11,10}. Parasite DNA was extracted and purified (final volume: 100 μl of TE buffer) from 30 mg of the lymph node using a Get Pure DNA Kit - Cell, Tissue (Dojindo Molecular Technologies, Inc.), and this extract was used as a template of the PCR. Brain homogenate of mice infected with \textit{T. gondii} was used as a positive control for PCR procedure.

(3) Titration of anti-\textit{T. gondii} antibody and determination of Pig-MAP, Hp and AGP.

Anti-\textit{T. gondii} antibody (Toxo-check MT, Eiken Co. Ltd., Japan) were measured by immunoprecipitation, Pig-MAP (PigCHAMP Pro Europa S.A., Segovia, Spain), by ELISA. Hp and AAG (Saikin-Kagaku Institute Co. Ltd., Japan) were measured by radial immunodiffusion. A titration value of over 64 was considered to indicate positivity in the anti- \textit{T. gondii} antibody test.

### Results and discussion

No PCR amplification was observed with the primer pairs in forty lymph nodes from normal pig (data not shown). Twenty-two lymph nodes suspected to harbor
T. gondii were classified into nine tachyzoite-positive and thirteen AOS test-negative in the AOS test (Table 1). In all tachyzoite-positive samples, the PCR test was positive for one of the three primer pairs used. These results indicated that the PCR method was useful for detection of T. gondii. However, twelve of thirteen samples that were negative in the AOS test gave a positive PCR result with one of the primer pairs used. This result might be dependent on the number of tachyzoites in each microscopic preparation, so that pigs with a negative result in the AOS test cannot be condemned for toxoplasmosis even if they show a positive PCR result.

In eight of the pigs suspected to have toxoplasmosis, we performed the AOS test, PCR, and the anti-T. gondii antibody test, assayed Pig-MAP, Hp and AAG levels in sera, and investigated the relationships among those results we obtained. As shown in Table 2, five specimens for the AOS test, six for the PCR test and three for the antibody test were positive. Normal concentrations of Pig-MAP in the finishing period of pig fattening were 0.7-0.8 mg/ml, and those of Hp were around 0.9 mg/ml. The levels in samples Nos. 4 and 8 were 600 and 521 µg/ml, respectively, which were considered abnormal. As shown for Nos. 1 and 2 in Table 2, when the PCR and antibody tests gave positive results, high levels of Pig-MAP were observed, and as shown in No. 6, even if the AOS test result was negative, a high level of Pig-MAP was observed when the results of both the PCR and antibody tests were positive. These findings indicated that Pig-MAP would probably be useful for diagnosis of toxoplasmosis. Therefore, we examined the relationship between the total number of cases positive (TTS; toxoplasmosis test score) in the AOS, PCR and antibody tests, and the

Table 1. Results of the PCR test with three primer pairs for Bl gene of Toxoplasma gondii in the lymph nodes of pigs with suspected toxoplasmosis.

<table>
<thead>
<tr>
<th>Primer names</th>
<th>AOS test (+)* (n=9)</th>
<th>AOS test (+)* (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z500F/R</td>
<td>8 1 9 4</td>
<td></td>
</tr>
<tr>
<td>BW23/23</td>
<td>7 2 12 1</td>
<td></td>
</tr>
<tr>
<td>JW58/59</td>
<td>9 0 11 2</td>
<td></td>
</tr>
<tr>
<td>Total**</td>
<td>9 0 12 1</td>
<td></td>
</tr>
</tbody>
</table>

* (+) or (-) indicates whether T. gondii tachyzoite is observed on microscopic examination of an acridine orange-stained (AOS) stamp-smear preparation, or not.

** The number of this line indicates the total number of individuals reacted to one of three primer pairs in PCR test.

Table 2. Results of acridine orange-stained (AOS). PCR and anti-Toxoplasma gondii (T. gondii) antibody tests, concentrations of pig major acute-phase protein (pig-MAP), haptoglobin (Hp) and alpha 1-acid glycoprotein (AGP) and macroscopic features in pigs suspected to have toxoplasmosis during meat inspection.

<table>
<thead>
<tr>
<th>Number of specimen</th>
<th>AOS test</th>
<th>PCR test</th>
<th>Antibody test</th>
<th>Pig-MAP mg/ml</th>
<th>Hb mg/ml</th>
<th>AGP µg/ml</th>
<th>Macroscopic feature except toxoplasmosis lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P²</td>
<td>P</td>
<td>P</td>
<td>3.48</td>
<td>3.83</td>
<td>192</td>
<td>Not observed</td>
</tr>
<tr>
<td>2</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>2.54</td>
<td>0.77</td>
<td>91</td>
<td>Pleurisy, pericarditis, peritonitis</td>
</tr>
<tr>
<td>3</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td>1.75</td>
<td>2.72</td>
<td>238</td>
<td>Pleurisy, pericarditis, peritonitis, Myco.*</td>
</tr>
<tr>
<td>4</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td>1.03</td>
<td>2.13</td>
<td>600</td>
<td>Myco.</td>
</tr>
<tr>
<td>5</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>0.51</td>
<td>2.11</td>
<td>247</td>
<td>Hemorrhagic colitis</td>
</tr>
<tr>
<td>6</td>
<td>N³</td>
<td>P</td>
<td>P</td>
<td>2.02</td>
<td>4.02</td>
<td>468</td>
<td>Pleurisy, pericarditis, peritonitis</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>0.56</td>
<td>2.70</td>
<td>163</td>
<td>Myco.</td>
</tr>
<tr>
<td>8</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>0.50</td>
<td>1.45</td>
<td>521</td>
<td>Pleurisy, pericarditis, peritonitis</td>
</tr>
</tbody>
</table>

¹ Microscopic examination of T. gondii tachyzoites in an AOS stamp-smear preparation.
² PCR for the Bl gene of T. gondii.
³ Anti-T. gondii antibody test.
⁴ Positive (P) and negative (N) decisions in each test of toxoplasmosis.
* Cheesy or invasive lesion in lymph node with suspected mycobacteriosis.
concentrations of Pig-MAP, Hp and AGP (Fig 1). The concentration of Pig-MAP increased in accordance with TTS (Fig. 1A). Those of Hp also tended to increase in the same way as for Pig-MAP, but the former showed a wider distribution of values (Fig. 1B).

The serum level of Hp has frequently been evaluated clinically as an index of inflammation and is also reported to be elevated in pigs with toxoplasmosis. Pig-MAP is a novel APP in pigs that was found in 1994 and -like Hp - is currently being evaluated as an index of inflammation. Pig-MAP and Hp might be correlated with each other although a wider distribution of Hp compared with Pig-MAP has been observed in pigs without evident clinical signs of disease. Our finding that elevated Pig-MAP was more closely correlated with Hp in pigs with suspected toxoplasmosis suggests that the level of Pig-MAP increases as a result of Toxoplasma gondii infection, and that determination of Pig-MAP might provide more helpful information to a meat inspector than Hp. AGP levels were not correlated with TTS in this study (Fig. 1C), suggesting that AGP assay for pigs with suspected toxoplasmosis is not useful.

Elevated serum levels of AGP are also observed in pigs with infectious diseases such as pneumonia and meningitis. However, the kinetics of AGP production differs from those of Pig-MAP and Hp. As described above, a recent study of apparently healthy pigs has demonstrated a significant correlation between plasma levels of Hp and Pig-MAP (r=0.57), whereas that of AGP was not correlated with Hp (r=0.04) or Pig-MAP (r=-0.11). In the present study, the coefficient of correlation (r) between Hp and Pig-MAP was 0.37, that between Hp and AGP was 0.08, and that between Pig-MAP and AGP was -0.38 (data not shown). In pigs suspected to have toxoplasmosis, correlations similar to those of healthy pigs tended to be recognized.

Pleurisy, pericarditis, peritonitis, hemorrhagic colitis and cheesy or invasive lesion in lymph node with suspected mycobacteriosis on macroscopic examination except toxoplasmosis were observed in the present study (Table 2). The relation between these lesions and levels of the three APPs was not clear. However, it is of interest that elevated Pig-MAP level is observed in toxoplasmosis. These carcasses are condemned under the Abattoir Law. These results suggested that the measurement of Pig-MAP in meat inspection was useful. However, it was difficult to say this suggestion, since the numbers of samples in this study were only eight, which were insufficient to conclude the usefulness of the assay of the pig-MAP for meat inspection.

** Significant correlation (P<0.01).

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**Figure 1.** Correlations between the three acute phase proteins, pig major acute-phase protein (Pig-MAP) (A), haptoglobin (Hp) (B) and alpha 1-acid glycoprotein (AGP) (C), and toxoplasmosis test score (TTS) in pigs with suspected toxoplasmosis. TTS indicates the total number of cases positive in microscopic observation of *Toxoplasma gondii* tachyzoite, anti-*T. gondii* antibody test and PCR test for B1 gene of *T. gondii*. TTS is three in a case that is positive for the three tests, and is zero in a case that is negative for all tests.
establish serum levels of Pig-MAP in meat inspection, further studies are required in the slaughtered pigs.

In conclusion, it is suggested that in meat inspections for toxoplasmosis, PCR for the B1 gene of T. gondii and measurement of Pig-MAP would give helpful diagnostic information supplementary to that obtained in the AOS test.

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References


3) CLAPPERTON, M., BISHOP, SC., PIÑEIRO, M., et al. (2007) The association between plasma levels of acute phase proteins, haptoglobin, alpha-1 acid glycoprotein (AGP), Pig-MAP, transthyretin and serum amyloid A (SAA) in Large White and Meishan pigs. Veterinary Immunology and Immunopathology. 119, 303-309.


要約
（食肉検査におけるトキソプラズマ症診断のためのPCR試験とPig-MAP測定の有用性）
トキソプラズマ症が疑われた豚のリンパ節と血清を用いて、アクリジンオレンジ染色による病変のスタンプ標本の鏡検（AOS test）に加えてToxoplasma gondii B1遺伝子のPCR検査（PCR test）、抗T. gondii抗体検査（antibody test）およびPig-MAP、ハプトグロビン、アルファ酵素性猪タンパクの測定を行った。AOS test、PCR test、antibody testが陽性の時、高いPig-MAP値がみとめられた。これらの成績からPig-MAPの測定とPCR検査は、とちく検査におけるトキソプラズマ症の診断時に補佐的な情報を提供してくれることが示唆された。

キーワード：豚主要急性相タンパク、トキソプラズマ症、食肉検査