Conclusion

The seroprevalence of Salmonella on small pig farms in Slovenia is low. The implementation of a national control program could be a long term solution for eradication of disease.

Acknowledgements

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References


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16. Toxoplasma gondii: serological study in pigs slaughtered in Portugal

Caiado, A.1, Müller, A.2,3, Gomes-Neves, E.2,3

Abstract

Toxoplasma gondii has a worldwide distribution and usually is not detected macroscopically by traditional meat inspection methods. The aim of this study was to assess the seroprevalence of Toxoplasma gondii in slaughter swine using a modified direct agglutination method (MAT) (Toxo-Screen DA, Biomérieux® SA). No positive results were obtained in sera from 337 animals from 12 intensive production farms tested in duplicate at the dilutions of 1:20 and 1:40. The estimated seroprevalence was 0%. The 95% confidence intervals ranged from 0 to 21.8% within batches, and 0 to 1.1% of all animals. In 10 of the 12 farms, Toxoplasma seroprevalence was below 7% (>99% confidence level). In the remaining two farms, the sampling fraction was too small to distinguish a population with prevalence of 7% from a disease-free population. Our findings suggest that Toxoplasma serorelevance in intensive pig farms is likely to be below 7%. However, selection of farms was not random, so further studies are required to allow making inferences for all intensive pig farms in the centre and north of Portugal.

Introduction

Toxoplasma gondii has a worldwide distribution and usually is not detected macroscopically by traditional meat inspection methods. Like other countries, Portugal does not have measures to control this parasite at various stages of the food chain neither the prevalence in swine is clearly known. Detection of Toxoplasma in meat samples intended for human consumption is crucial for the knowledge of the risk level to this source of infection for humans (Gamble, 1997; EFSA, 2011a, b). The aim of this study was to assess the seroprevalence of Toxoplasma gondii in slaughter pig farming coming from intensive pig farms.

Material and Methods

In May of 2014 and in January of 2015 blood samples were collected from 330 finishing pigs and 7 breeding sows from 12 intensive production farms in 3 different slaughterhouses, located in the north and center regions of Portugal. Blood samples were centrifuged at 3000 rpm during 20 minutes and serum was recovered and stored at -20ºC until testing. All animals presented for slaughter were approved in the ante mortem exam and did not have clinical signs compatible with toxoplasmosis.

The serological testing was carried out in the Laboratory of Molecular Pathology of the Biomedical Institute Abel Salazar of the University of Porto. All sera were tested in duplicate using a modified direct agglutination method (MAT) (Toxo-Screen DA, Biomérieux® SA) at the dilution of 1:20 and 1:40. Sera were considered positive if agglutination was observed according to the instructions of the kit.

The 95% confidence interval (95% CI) of the proportion of positive sera was estimated according to the Clopper-Pearson procedure using SPSS v20.0. The population freedom from disease using imperfect tests and allowing for small populations was analysed with the EpiTools calculator “FreeCalc” (http://epitools.ausvet.com.au/). The input values for population size were the number of submitted pigs from a particular farm. The sensitivity and specificity of MAT at a serum dilution of 1:20 were considered 83.4% and 90.2%, respectively.
A design prevalence of 7% was used based on a recent serosurvey of intensive and extensive pigs at slaughter in Portugal (Esteves et al., 2014).

Results

All 337 samples tested negative (table). The estimated seroprevalence was 0% and the 95% confidence intervals (Clopper-Pearson) of the different batches ranged between 0 and 21.8% and for all farms together between 0 and 1.1%. These negative results were analysed incorporating sensitivity and specificity of the MAT test (83.4% and 90.2%, respectively) to demonstrate population freedom from disease. In 10 of the 12 farms, we are very confident (>99% confidence level) that toxoplasmosis is below 7%. In the remaining two farms, the sampling fraction was too small to distinguish a population with prevalence of 7% from a disease-free population.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Abattoir</th>
<th>Sample size (n)</th>
<th>Batches of pigs submitted (n) (“population”)</th>
<th>Seroprevalence (Clopper-Pearson 95% confidence interval)</th>
<th>Confidence level for population freedom at a seroprevalence of 7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>15</td>
<td>130</td>
<td>0 (0-0.218)</td>
<td>Unlikely *)</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>29</td>
<td>155</td>
<td>0 (0-0.119)</td>
<td>99.23 %</td>
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<tr>
<td>C</td>
<td>2</td>
<td>30</td>
<td>101</td>
<td>0 (0-0.116)</td>
<td>99.38 %</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>35</td>
<td>119</td>
<td>0 (0-0.100)</td>
<td>99.72 %</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>30</td>
<td>103</td>
<td>0 (0-0.116)</td>
<td>99.36 %</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>18</td>
<td>76</td>
<td>0 (0-0.185)</td>
<td>Unlikely *)</td>
</tr>
<tr>
<td>G</td>
<td>3</td>
<td>30</td>
<td>48</td>
<td>0 (0-0.116)</td>
<td>99.49 %</td>
</tr>
<tr>
<td>H</td>
<td>3</td>
<td>30</td>
<td>90</td>
<td>0 (0-0.116)</td>
<td>99.36 %</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>30</td>
<td>40</td>
<td>0 (0-0.116)</td>
<td>99.75 %</td>
</tr>
<tr>
<td>J</td>
<td>3</td>
<td>30</td>
<td>90</td>
<td>0 (0-0.116)</td>
<td>99.36 %</td>
</tr>
<tr>
<td>K</td>
<td>3</td>
<td>30</td>
<td>120</td>
<td>0 (0-0.116)</td>
<td>99.3 %</td>
</tr>
<tr>
<td>L</td>
<td>3</td>
<td>30</td>
<td>110</td>
<td>0 (0-0.116)</td>
<td>99.43 %</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>337</td>
<td>1182</td>
<td>0 (0-0.011)</td>
<td>100%</td>
</tr>
</tbody>
</table>

*) The sample size was too small to distinguish a population with prevalence of 7% from a disease-free population.

Discussion

We observed 100% negative results for toxoplasma antibodies using the MAT in 337 pig sera at slaughter. Our results differed from recent studies carried out in Portugal which found seroprevalence ranging between 5.2% and 15.6% (Esteves et al., 2014; Lopes et al., 2013; Sousa et al., 2006; Valadas et al., 2006).

This difference may be explained by the fact that we analysed samples only from intensive pig production whereas others included free range pigs.

The negative serological results presented here were obtained using an “imperfect test”. Sensitivity and specificity (83.4% and 90.2%, respectively) were taken into account in the freedom of disease testing. We estimated that 10 of the 12 farms were “free” of toxoplasmosis at an expected prevalence of 7% with >99% confidence. The question arises if 7% or which other level of seroprevalence of slaughter pigs would be acceptable for public health protection in Portugal.

Previous studies used the MAT in pig sera at 1:20 or 1:40 dilution, hampering the direct comparison of results (Coelho et al., 2014; Garcia et al., 2006, Sousa et al., 2006). Here we obtained 100% agreement of the results with both serum dilutions, but are aware that sample size is insufficient to allow generalizations regarding the “ideal” serum dilution to be used. An international harmonization of diagnostic test methodology for risk assessment of toxoplasma in pig sera would be desirable for the implementation of surveillance and monitoring programs in live animals as part of the FCI reaching the abattoir and thus enabling the correct implementation of risk-based meat inspection.

Conclusions

We found no serological evidence of Toxoplasma gondii in 337 slaughter pigs coming from 12 intensive pig farms. We obtained negative test results at both serum dilutions, 1:20 and 1:40. Our findings suggest that toxoplasmosis seroprevalence in intensive pig farms is likely to be below 7%. However, selection of farms was not random, so further studies are required to allow making inferences for intensive pig farms in Portugal.

Acknowledgements

The provision of one diagnostic kit by Biomérieux® is greatly acknowledged.

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• European Food Safety Authority, 2011b. Technical specifications on harmonized epidemiological indicators for public health hazards to be covered by meat inspection of swine. EFSA Journal, 9(10):2371.
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