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TOXOPLASMA GONDII IN PORTUGUESE SLAUGHTER SWINE

1.INTRODUCTION AND PURPOSE

Toxoplasmosis is an important worldwide zoonosis and a foodborne hazard. The consumption of raw or undercooked pork contaminated with *T. gondii* cysts has been related with the transfer to humans. *T. gondii* is identified by an EFSA's qualitative risk assessment as a relevant biological hazard in the context of meat inspection of swine. This zoonotic parasitic disease does not cause visible and palpable lesions and is not reflected in clinical signs in the live animal, thus going unnoticed at the farm as well as the abattoir. The recent trends of zoonoses have led to a major change in the meat inspection system in the EU. Classical inspection methods, based on palpation and incision, are no longer considered effective to detect these zoonotic infections at slaughter, giving way to new methodology, the so called risk based or visual meat inspection (EU regulations 218/2014 and 219/2014). This approach places more weight onto the Food Chain Information (FCI), aiming to minimize the routine use of palpations and incisions of carcasses and reducing cross contaminations at the abattoir. The quality of current FCI reaching the abattoirs with each lot of pigs is clearly lacking relevant information on the presence and/or prevalence of zoonotic infections, hampering the correct implementation of risk based meat inspection.
The aim of the present study is to investigate occurrence of *Toxoplasma gondii* antibodies in sera from slaughtered swine from intensive production farms.

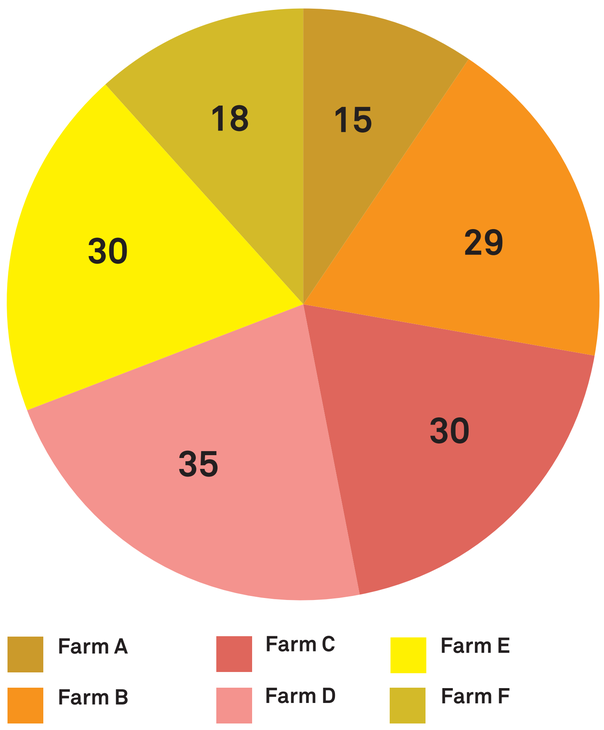
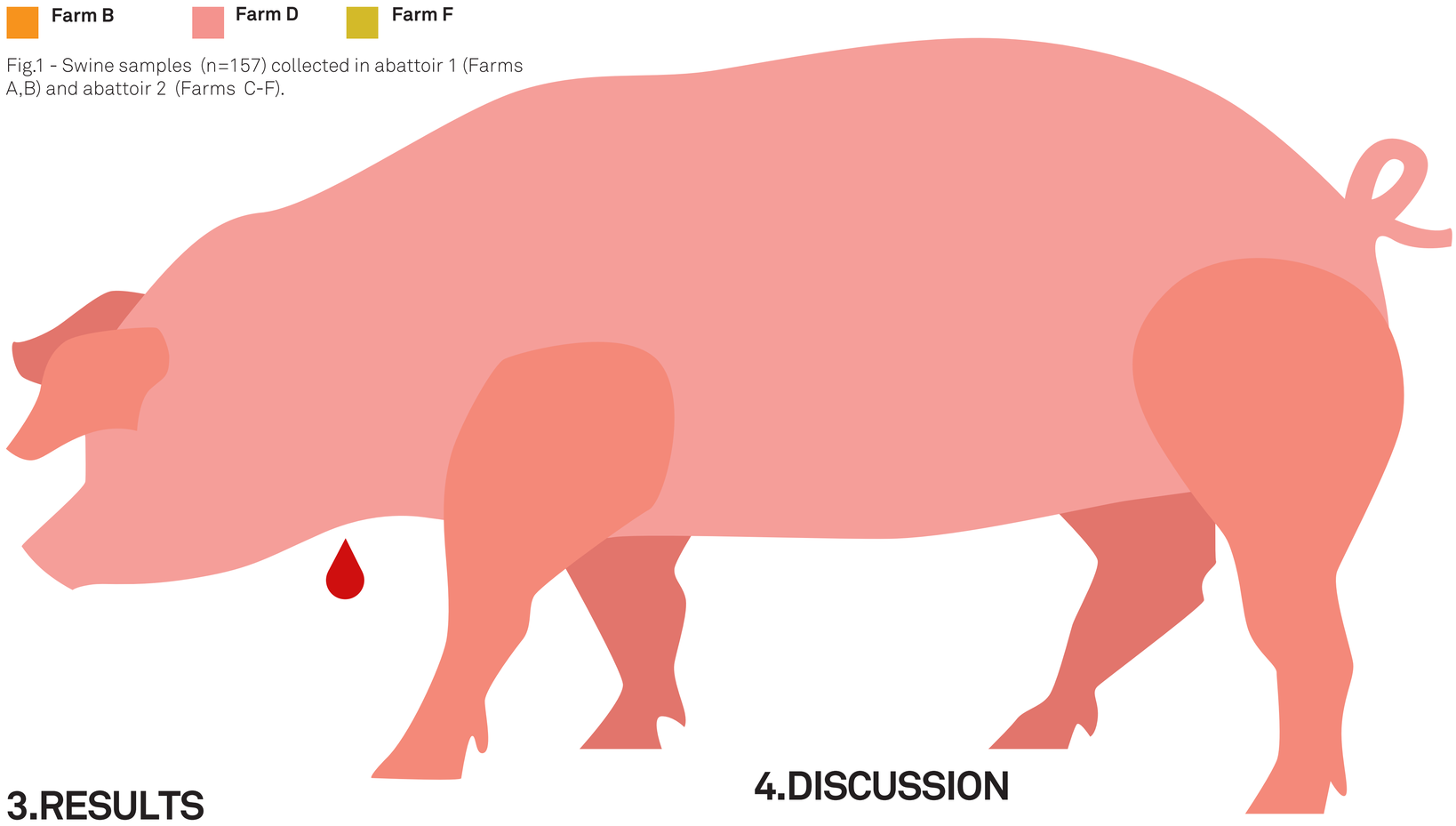


Fig.1 - Swine samples (n=157) collected in abattoir 1 (Farms A,B) and abattoir 2 (Farms C-F).

Table 1. Seroprevalence of *T. gondii* by abattoir and farm.

Farm	Abattoir	Sample Size (n)	Pigs submitted (n) ("population")	Seroplevalence (Clopper-Pearson 95% confidence interval)	Confidence level for population freedom at a seroprevalence of 7%
A	1	15	130	0 (0-0.218)	Unlikely*)
B	1	29	155	0 (0-0.119)	99.23%
C	2	30	101	0 (0-0.116)	99.38%
D	2	35	119	0 (0-0.100	99.72%
E	2	30	103	0 (0-0.116)	99.36%
F	2	18	76	0 (0-0.185)	Unlikely*)
TOTAL		**157**	**684**	**0 (0.023)**	

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



2.METHODS

A total of 157 slaughter pigs aged 5-6 months from six intensive production farms (A-F) was sampled. Blood samples were collected in May 2014 during slaughter in two abattoirs (1,2) from the north of Portugal (Fig.1). Blood was centrifuged and serum was recovered and stored at –20ºC. Serum samples were tested for immunoglobulin G (IgG) antibodies against T. *gondii*, by the modified agglutination test (MAT) using a commercial kit (Toxo-ScreenDA®, bioMérieux, Lyon, France), with whole formalin-fixed tachyzoites as antigen.

All samples were tested in duplicate at the dilutions of 1:20 and 1:40. 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. Data were analysed for each farm by the modified hypergeometric exact method.

3.RESULTS

All 157 samples tested negative by the commercial MAT (Table 1, Fig.2) in both serum dilutions. The estimated 95%CI for the toxoplasma seroprevalence for the individual farms ranged between 0 and 21.9% and for all farms together between 0 and 2.3%.
These negative results were analysed incorporating sensitivity and specificity of the MAT test to demonstrate population freedom from disease. In four of the six farms, we are very confident (>99% confidence level) that toxoplasma seroprevalence 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.

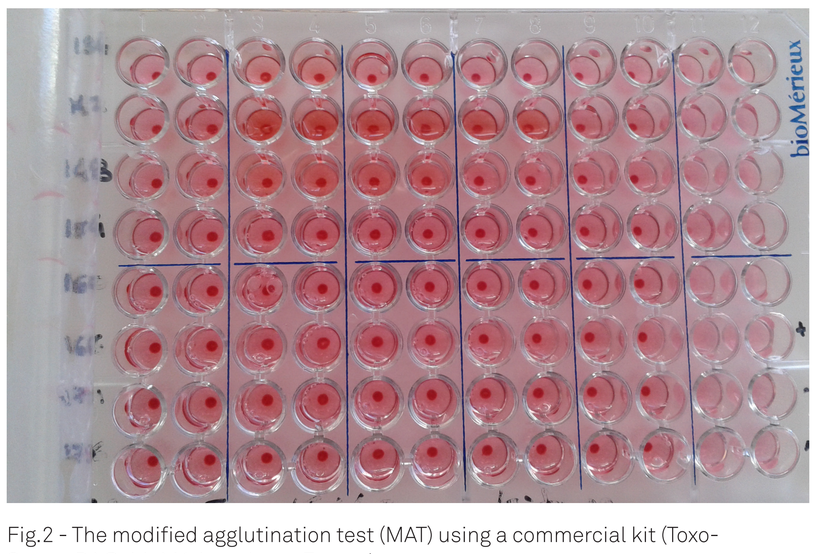


Fig.2 - The modified agglutination test (MAT) using a commercial kit (Toxo-ScreenDA®, bioMérieux, Lyon, France).

4.DISCUSSION

We observed 100% negative results for toxoplasma antibodies using the MAT in 157 pig sera at slaughter. Our results differed from recent studies carried on in Portugal which found a low proportion of positive samples. We analysed samples from intensive pig production whereas others included free range pigs, which may explain the differences. 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 four of the six farms are "free" of toxoplasmosis at an expected prevalence of 7% with > 99% confidence. The question arises which 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. 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.**