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Toxoplasma gondii Serology in Slaughter Pigs from Intensive Production

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Abstract

Toxoplasma gondii is a foodborne zoonotic pathogen that has a worldwide distribution. It cannot be detected macroscopically by traditional meat inspection methods. Instead, serology can be used to assess exposure of pigs to this agent. The aim of this study was to assess the seroprevalence of *T. gondii* in slaughter swine reared in intensive indoor production systems in Portugal. A total of 337 sera from 12 farms tested negative by a modified direct agglutination method (MAT). The apparent seroprevalence was 0% and the upper 95% confidence intervals ranged from 1.1% considering all animals, and 10 to 21.8% considering the individual sampled batches. Given the negative test results, the estimated maximum possible apparent seroprevalence was 0.76% for all 337 samples, and between 7.5% to 17.7% for the individual batches. We conclude that despite negative test results, our findings do not prove absence of infection, but instead suggest the possibility of a maximum *Toxoplasma* seroprevalence of 7% or higher in all batches. We underline the importance of regular monitoring of the serological status of pigs at farm level regarding risk-based meat inspection.

Introduction

Toxoplasma gondii has a worldwide distribution and infection is not detected macroscopically by traditional meat inspection methods. In the European Union, based on a qualitative risk assessment, *Toxoplasma gondii* is considered a hazard of medium relevance in meat inspection of pigs [1,2]. Similar to other countries, Portugal does not have specific measures in place to control this parasite at the various stages of the food chain Detection of *T. gondii* in meat samples intended for human consumption is considered useful for the accurate assessment of the risk level to consumers [1-4]. However currently there is no legal requirement for carcass testing and this is due to, among others, methodological limitations [1,3,5]. So currently, detection of exposure of pigs to *T. gondii* is mostly based on serological testing [6,7]. Previous studies in Portugal found a seroprevalence between 5.2% and 15.6% in pigs [8-11]. These studies included also free-ranging pigs, where exposure is considered to be higher compared to pigs raised in indoor production systems. In other EU countries, namely Italy, Denmark, France and Spain, the prevalence in intensive production ranged from 0.5% to 24.5% and 13.4% to 33.7%

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respectively, in fattening pigs and sows [12-15]. In Portugal, to our knowledge, there is a lack in the characterization of the pig intensive production farms regarding the *T. gondii* status. The aim of this study was to assess the seroprevalence of *Toxoplasma gondii* in slaughter pigs from commercial pig farms with intensive husbandry and to test any seropositive pigs for the presence of *T. gondii* by PCR analysis of heart samples.

Material and methods

Sampling

Porcine blood samples were collected at three different slaughterhouses, located in the north and centre of Portugal. A total of 330 finishing pigs and 7 breeding sows from 12 intensive production farms were randomly sampled at two time points, once in May of 2014 and then in January of 2015. Sample size to estimate true prevalence was calculated with the online calculator EpiTools [16]. Assuming a true prevalence of 2%, a sensitivity of 83.4% and a specificity 90.2% for the MAT at a serum dilution of 1:20 [12,17], a desired precision of 5% and 95% confidence, a sample size of 293 was determined. This value was considered as total number of sera required, suggesting a sample of 24 sera from each batch of pigs sent to slaughter from 12 farms. To account for eventual problems with the sera, e.g. haemolysis, it was determined to sample 30 pigs per batch whenever possible. All sampled pigs were asymptomatic and approved by *ante-mortem* examination. Additionally, on the slaughter line, heart samples (minimum 200 g) of each serum-sampled pig were collected in sterile plastic containers labelled with animal identification code for traceability purposes. All samples were transported in cooled boxes at +4°C within 12 h of slaughtering to the Laboratory of Molecular Pathology of the Biomedical Institute Abel Salazar of the University of Porto. Once in the laboratory, the heart samples were stored at +4°C until availability of the serological results.

Serology

Blood samples were centrifuged at 3000 rpm during 20 minutes and serum was separated and stored at -20°C until testing. All sera were tested in duplicate using a commercial 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.

Analysis

Incorporating the above mentioned values of sensitivity and specificity for the MAT, the Epitools Surveillance utilities were used to calculate the Clopper-Pearson exact 95% confidence intervals (95% CI) for apparent and true prevalence, and the Epitools Diagnostic test evaluation and comparison utilities were used to estimate the probability of infection in test negative samples [16,17]. The maximum possible prevalence given negative test results was calculated with Win Episcope 2.0 [18]. As values of MAT sensitivity and specificity are not incorporated these refer to maximum apparent prevalence. The input values for population size were the number of pigs in each slaughter batch and for sample size were the number of pigs sampled from each batch.

Results

All 337 serum samples tested negative for *T.gondii* antibodies at both serum dilutions, 1:20 and 1:40 (Table 1). The apparent point seroprevalence for the total number of pigs as well as for all batches was 0%. The respective upper 95% confidence limits ranged between 1.1% for the total number of pigs, and 10% to 21.8% in the individual batches. The calculations of the true point prevalence and confidence intervals yielded estimate values <0. These are not consistent with the assumed sensitivity and specificity values, and are therefore not shown. The maximum possible apparent seroprevalence for all 337 samples was 0.76%, and considering individual batches 7.5% to 17.69%. Assuming a seroprevalence of 2%, the probability of infection in the negative samples was 0.72 considering the total number of pigs and between 5% and 12% in the batches. As no seropositive samples were obtained, the heart samples were not further tested for the presence of the *T. gondii* by molecular methods, and the material was safely disposed of.

Table 1: Negative serological results to Toxoplasma gondii in slaughter pigs from intensive production systems in Portugal.

Farm	Abattoir	Number of pigs sampled (Sample size)	Number of pigs in batch (Population size)	Apparent seroprevalence (95% confidence interval)	Maximum possible Apparent seroprevalence (%)	Probability of infection in negative sample from population with 2% prevalence[a]
A	1	15	130	0 (0-0.218)	17.69	0.05
B	1	29	155	0 (0-0.119)	9.03	0.10
C	2	30	101	0 (0-0.116)	8.91	0.11
D	2	35	119	0 (0-0.100)	7.56	0.12
E	2	30	103	0 (0-0.116)	8.74	0.11
F	2	18	76	0 (0-0.185)	14.47	0.07
G	3	30	48	0 (0-0.116)	8.33	0.11
H	3	30	90	0 (0-0.116)	8.89	0.11
I	3	30	40	0 (0-0.116)	7.5	0.11
J	3	30	90	0 (0-0.116)	8.89	0.11
K	3	30	120	0 (0-0.116)	9.17	0.11
L	3	30	110	0 (0-0.116)	9.09	0.11
TOTAL		337	1182	0 (0-0.011)	0.76	0.72

Discussion

All 337 slaughter pig sera of the 12 batches tested negative for toxoplasma antibodies using the MAT. This apparent seroprevalence of 0% seems to be different from previous studies in Portugal which found point prevalence values up to 15% [8-10,19]. We highlight that the negative serological point prevalence in our study does not prove absence of infection in the sampled slaughter pig batches. The upper 95% confidence limits of the apparent 0% prevalence suggests a possible seroprevalence of up to 0.1% for all pigs and up to 21.8% in individual batches. The disadvantage of using the upper confidence interval is that it takes into consideration only the sample size but not the population size, i.e. the size of the batch it was taken from. We consider the estimation of the maximum possible prevalence given negative test results more adequate, as it takes into consideration both, the sample and the population sizes. Accordingly, the highest possible seroprevalence was estimated as 17.6% for the batch of Farm A, and the lowest as 7.5% for the batch of Farm I. These estimates mirror the sampling fractions of each batch. However they do not allow to make any inferences about the level of exposure to *T. gondii* at the farm. If all 337 samples are taken together, a maximum apparent seroprevalence in slaughter pigs in Portugal is estimated as 0.8%. Due to differences in exposure according to farm management and biosecurity practices the results per batch at slaughter are possibly more meaningful for public health purposes than the overall total of all samples [20-22].

Despite previous serological studies in Portugal describing seroprevalence values higher than 7%, a design prevalence of 2% was selected, because a lower prevalence was expected in pigs reared under intensive farming practices [5,10,23]. The sample size was calculated for the total number of pigs needed to be sampled to detect infection. To find zero positive reactions was rather unexpected and led to the decision of cancelling the molecular analyses in the heart samples. Given the negative serological results as well as the design prevalence of 2%, the probability of infection in the test negative samples ranged from 5% in the smaller samples to 12% in the larger samples. This illustrates that sample size calculations should be made for subpopulations such as whole farms or slaughter batches, if inferences are to be made from them with a certain precision.

Regarding the serological test methodology, the MAT is relatively easy to perform, not requiring sophisticated equipment. It could be suitable for testing a few samples, for example, in the abattoir. Results are available within a few hours and the reading of the results is carried out visually. A disadvantage is that different serum dilutions have to be evaluated, adding cumbersome extra pipetting steps. For larger sample sizes, the use of ELISA tests could be more appropriate, as automated systems allow large sample throughput [12,13,24,25]. As ELISA tests require specific equipment, testing would best be carried out in laboratories, and results sent back to the abattoir.

Currently, EFSA proposes to consider harmonized epidemiological indicators (HEI) when adaptations in meat inspection methods may be relevant and to carry out risk analysis to support decisions regarding meat safety [1,2]. The use of HEI is particularly relevant to help categorise farms/herds and slaughterhouses according to the risk related to the hazards as well as setting appropriate targets for final chilled carcases in the pork safety assurance framework [1-3]. As *Trichinella* spp., *T. gondii* is considered a medium relevance hazard in pigs [1]. However, *Trichinella* control in farms or at slaughter is a legal requirement and positive carcasses are considered unfit for human consumption [26]. Regarding *T. gondii*, no comparable surveillance is in place and therefore no measures at the farms or abattoirs are legally established. For pork-borne hazards which are closely associated with pigs on-farm contamination as *T. gondii,* the main control measures are applied during farm-to-chilled carcass stages and in the case of pigs from high-risk farms the carcasses should undergo an effective treatment, namely freezing (e.g. −12°C/2 days) [3,4,27].

An international harmonization of diagnostic test methodology for risk assessment of *T. gondii* in pig sera would be necessary to implement surveillance and monitoring programs in live animals as part of the food chain information reaching the abattoir and thus enabling an efficient implementation of risk-based meat inspection [2,3,28,29]. Since current meat inspection at the slaughterhouse cannot detect the presence of *T. gondii*, the implementation of specific management procedures to reduce the risk of infection of pigs can help to prevent the transmission of the pathogen to humans through pork consumption.

Conclusions

We found no serological evidence of *T. gondii* in 337 slaughter pigs sampled from 12 batches sent by intensive pig farms. Despite 0% seroprevalence, our study was unable to prove absence of infection. The maximum apparent seroprevalence ranged between 7.5% and up to 17.7% of the different batches. Representative sampling and larger sampling sizes are required to better characterize intensive pig farms in Portugal. Currently, the detection of *T. gondii* is not possible by meat inspection, but serological tests can be useful to categorize pig farms as a strategy to identify potentially infected pork and could be used as an effective control tool by the meat industry.

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