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Cross sectional study of *Toxoplasma gondii* infection in pig farms in England

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3 **1 Cross sectional study of *Toxoplasma gondii* infection in pig farms in England**

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3 **13 Abstract**

4 **14** Ingestion of undercooked meat has been proposed as an important source of human *T. gondii*
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6 **15** infection. To ascertain the contribution of meat consumption to the risk of human infection, estimates
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8 **16** of the prevalence of infection in meat-producing animals are required. A cross sectional study was
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10 **17** conducted to assess *T. gondii* infection in pigs raised in England, to identify risk factors for infection
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12 **18** and to compare performance of two serological tests: modified agglutination test (MAT) and enzyme-
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14 **19** linked immunosorbent assay (ELISA).

15 **20** Blood samples from 2071 slaughter pigs originating from 131 farms were collected and 75 (3.6%)
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17 **21** were found positive by MAT. Positive pigs originated from 24 farms. A subset of samples (n=492)
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19 **22** were tested using ELISA, and a significant disagreement (p<0.001) was found between the two tests.
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21 **23** An empirical Bayes approach was used to estimate the farm-level prevalence and the probability of
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23 **24** each individual farm having at least one positive animal considering the uncertainty arising from the
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25 **25** sampling strategy and the imperfect test performance. The adjusted farm-level prevalence was 11.5%
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27 **26** (95% credible interval of positive farms 8.4%-16.0%). Two different criteria were used for classifying
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29 **27** farms as infected: (i)≥50% probability of having at least one infected pig (n=5, 6.8%); (ii)≥10%
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31 **28** probability (n=15, 20.5%). Data on putative risk factors was obtained for 73 farms. Using a 10% cut-
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33 **29** off, the relative risk (RR) of infection was higher on farms where cats have direct access to pigs' feed
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35 **30** (RR=2.6; p=0.04), pigs have outdoor access (RR=3.0; p=0.04) and farms keeping ≤200 pigs (RR=3.9;
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37 **31** p=0.02), with strong collinearity between the three variables.

38 **32** The findings suggest a low level of *T. gondii* infection in the farms studied, most of which are likely
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40 **33** to send to slaughter batches composed of 100% uninfected pigs. These results provide key inputs to
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42 **34** quantitatively assess the *T. gondii* risk posed by pork to consumers.
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38 Introduction

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40 Toxoplasmosis is a worldwide distributed zoonosis caused by the protozoan parasite *Toxoplasma*
41 *gondii* (*T. gondii*). Most warm-blooded animals can be infected and act as intermediate hosts in the
42 life-cycle of the parasite. Felines are the definitive host and the only species able to excrete sporulated
43 oocysts in faeces potentially contaminating the environment, soil and crops (Montoya and Liesenfeld,
44 2004).

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46 Humans can become infected via three main routes: (i) congenital, (ii) ingestion of sporulated oocysts
47 present in cats' litter trays or contaminated soil, water and vegetables and (iii) consumption of raw or
48 undercooked meat containing *T. gondii* bradyzoites clustered in tissue cysts ('infective cysts')
49 (Andreoletti et al., 2007; Tenter et al., 2000). The latter has been considered the most important route
50 of infection in developed countries by the World Health Organization (WHO, 2015). It is estimated
51 that up to a third of the world's population is currently infected with *T. gondii* with important
52 differences between and within countries (Pappas et al., 2009; Tenter et al., 2000). In recent years,
53 *Toxoplasmosis* has been ranked as posing the highest disease burden among foodborne pathogens in
54 Europe (Havelaar et al., 2012; WHO, 2015), and consumption of pork has been ranked second among
55 the top 10 pathogen-food combinations in the US (Batz et al., 2011). Estimates of the overall
56 incidence of human toxoplasmosis in England are lacking, as records of the number of confirmed
57 cases (on average 330 cases per year) represent a small proportion of the total number of cases in the
58 population given the asymptomatic nature of the infection in healthy individuals (PHE, 2015, 2016).
59 On the contrary, immunocompromised people can become seriously ill, whilst infection during
60 pregnancy could result in lifelong complications for the offspring (Andreoletti et al., 2007).

61

62 Pigs rarely show clinical signs when infected with *T. gondii* and detection of *T. gondii* cysts during
63 meat inspection is not feasible given their microscopic size. Numerous techniques are available for
64 antibody detection and a fairly good correlation has been reported in pigs between seropositivity and
65 presence of cysts (Dubey et al., 2002; Gamble et al., 2005; Hill et al., 2006). Therefore presence of
66 antibodies can be used as an indicator for the potential presence of infective cysts in pork. Among the
67 serological tests available, the modified agglutination test (MAT) has the highest sensitivity and
68 specificity (based on isolation of viable *T. gondii* from tissues of experimentally-infected pigs as gold
69 standard) having the advantage of not being affected by cross-reactivity with other parasites (Dubey,
70 1997; Dubey et al., 1996; Dubey et al., 1997). In field conditions however, the limited number of
71 studies have reported inconsistent results. A study conducted in naturally infected sows found higher
72 sensitivity and specificity in MAT compared with enzyme-linked immunosorbent assays (ELISA)
73 (Dubey et al., 1995); whilst the contrary was found in a study conducted in finishing pigs (Gamble et
74 al., 2005).

75

76 The prevalence of toxoplasmosis in pigs varies between countries and is mainly associated with the
77 presence of cats and contamination of pigs' feed with cat faeces with differences in risk found
78 depending on the type of housing and production system (Assadi-Rad et al., 1995; Garcia-Bocanegra
79 et al., 2010a; Garcia-Bocanegra et al., 2010b; Guo et al., 2016; Kijlstra et al., 2004; Klun et al., 2006;
80 Ortega-Pacheco et al., 2013; Tao et al., 2011; van der Giessen et al., 2007; Weigel et al., 1995). It has
81 been hypothesized that recent trends in consumer habits in developed countries, with a shift towards
82 the consumption of free range and organic pork, where animals have a higher risk of exposure to *T.*
83 *gondii* from the environment, may result in a higher risk of consumer exposure to *T. gondii* (Kijlstra
84 et al., 2009; van der Giessen et al., 2007).

85

86 Policies to mitigate the risk of foodborne exposure to *T. gondii* should be based on scientific risk
87 assessment and best available data. Lack of information regarding prevalence and risk factors for *T.*
88 *gondii* infection of pigs reared in the UK have been highlighted as important data gaps for the
89 assessment of the risk of pork to human infection (AMCSF, 2012). A recent UK survey in slaughtered
90 pigs (Powell et al., 2016) found that 7.7% of pigs were sero-positive by Sabin-Feldman Dye test (a
91 test that detect *T. gondii* IgG antibodies); potential risk factors for *T. gondii* infection were not
92 assessed. Ideally, prevalence estimation should take into account the imperfect performance of the
93 test and the sampling strategy used.

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95 The objectives of this study were (i) to assess, by means of an empirical Bayes estimation, the
96 probability of *T. gondii* infection in selected commercial farms in England, (ii) to identify factors
97 associated with a higher risk of *T. gondii* infection at farm level and (iii) to compare the performance
98 of the reference serological test for *T. gondii* in pigs (MAT), with a commercially available ELISA.

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100 **Material and Methods**

101

102 *Study design*

103 A cross sectional study was conducted in England between January and July 2015 with the pig batch
104 as the unit of interest. A batch was defined as a group of pigs received in the abattoir from the same
105 herd and on a given day. A note explaining the aim of the study was published in the British Pig
106 Executive (BPEX) newsletter in December 2014 and five commercial slaughterhouses volunteered to
107 take part in the study; they varied in size and throughput from 40 to >10,000 pigs processed per week.
108 Farmers regularly sending pigs to these slaughterhouses were contacted and invited to participate.

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110 The target sample size was calculated as 129 batches in order to be able to estimate prevalence at the
111 level of the batch (expected to be 25%) with 7.5% precision and 95% confidence. In the absence of

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3 112 farm-level prevalence estimates in England, values reported in other European countries were used as
4 113 reference (Steinparzer et al., 2015; van der Giessen et al., 2007). Within each batch, the number of
5 114 pigs needed to be sampled to classify, with 90% confidence, the study batches in 3 groups based on
6 115 within-batch prevalence (<7.5%; 7.5-25%; >25%) was estimated as 25 pigs.
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9 116
10 117 The study received ethical approval from the Royal Veterinary College Ethics and Welfare
11 118 Committee under the reference URN 2015-1328
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15 120 *Samples and data collection*

16 121 Each slaughterhouse was visited up to five times. On the day of the visit, batches of pigs from farmers
17 122 who agreed to participate were included (in later visits farms already sampled were excluded). From
18 123 each batch, blood samples were collected from individual pigs during routine slaughter at the point of
19 124 bleeding (sticking). Nine ml of blood was collected from each pig using pre-labelled vacutainer tubes.
20 125 For large batches, every third animal was sampled until the required sample of 25 pigs was achieved,
21 126 whilst for small batches (less than 25 pigs) all pigs in the batch were sampled. Date of sampling and
22 127 sex were recorded.
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29 129 Information on farm characteristics, management practices and biosecurity were gathered using a
30 130 standardised questionnaire designed based on a putative risk factors identified in a literature review
31 131 (Opsteegh et al., 2016). The questionnaire was either sent by post (with a pre-paid envelope to be
32 132 posted back) or handed directly to farmers at the slaughterhouse. Copies of the questionnaire are
33 133 available from the corresponding author upon request.
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37 134

38 135 *Serology*

39 136 Blood samples were centrifuged to separate sera from blood cells and sera samples were stored at
40 137 -20°C until testing using MAT for the detection of *T. gondii* specific immunoglobulin (IgG). Testing
41 138 was performed at the French Agency for Food, Environmental and Occupational Health and Safety in
42 139 Reims, France, as previously described (Dubey and Desmonts, 1987). A sample was considered
43 140 positive if the titre was $\geq 1:25$ (Dubey, 1997). Titres between 1:1 and 1:10 were classified as
44 141 suspicious.
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50 143 All MAT-positive and suspicious samples from which sera were available (n=152), plus a subset of
51 144 340 samples randomly selected among all the negative (n=1916) with maximum three negative
52 145 samples per farm, were tested in duplicate by a commercially available ELISA (ID Screen®
53 146 toxoplasmosis indirect multi-species) according to manufacturer's instructions. The optical density
54 147 (OD) readings for the sample were used to calculate percentage seropositivity (SP) as described by the
55 148 manufacturer. A sample with an SP value of $\geq 50\%$ was considered positive, $\leq 40\%$ was a negative
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3 149 result and between 40% and 50% was considered doubtful. Testing was repeated (also in duplicate)
4 150 for those samples which had contradictory results during the first ELISA test (i.e. one well classified
5 151 as positive and one negative or doubtful). If the repeated test results were also contradictory the
6 152 sample was considered inconclusive.
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10 154 McNemar's Chi-squared test for paired data was used to assess whether there was a significant
11 155 difference in the proportion positive between MAT and ELISA excluding inconclusive results.
12 156 Repeatability between ELISA results was measured using the coefficient of variation (CV). Low
13 157 values indicate high precision while the opposite is true for high values. A CV up to 0.20 can be
14 158 expected due to random variation (Reed et al 2002) and considered acceptable. The CV of each
15 159 sample was calculated for all the replicate values and then averaged across all 492 samples.
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21 161 *Data analysis*

22 162 Descriptive statistics were obtained at animal level for all pigs sampled (n=2071) and at farm level for
23 163 farms which completed the questionnaire (n=73).
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27 165 The extent to which sex was associated with infection was determined using a logistic regression
28 166 model including farm as a random effect. Animals with sera titres $\geq 1:25$ were considered positive and
29 167 suspicious results were considered negative.
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33 169 Intra-farm correlation (ICC) for positive status of individual pigs was estimated using the farm
34 170 variance (σ) from the mixed effect model considering the farm as a random effect (Wu et al., 2012).
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$$36 \quad ICC = \frac{\sigma^2}{\sigma^2 + \pi^2/3}$$

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40 172 An empirical Bayes model was used to estimate the farm-level prevalence (Beauvais et al., 2016).
41 173 Briefly, the probability of each farm having at least one true positive pig was estimated after taking
42 174 into account the number of pigs tested, how many of them were found to be positive, the imperfect
43 175 sensitivity and specificity of the test, the uncertainty arising from sampling only a proportion of
44 176 animals on each farm and "prior" information about the within-farm prevalence probability
45 177 distribution. The within-farm prevalence probability distribution was generated empirically from this
46 178 study and does not therefore rely on prior knowledge about the distribution of the disease. For each
47 179 iteration of the model, based on the probabilities of each farm being positive, we simulated the overall
48 180 farm-level prevalence. The results for each iteration were combined to create an uncertainty
49 181 distribution for the true farm-level prevalence. The median value of this uncertainty distribution was
50 182 taken as the adjusted farm-level prevalence. Sera titres $\geq 1:25$ were considered positive. MAT
51 183 sensitivity and specificity of 86% and 95% respectively, were used as inputs (Gamble et al., 2005).
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184 Model results were used to classify farms as positive or negative using two cut-offs: positive farms for
185 which the probability of having at least one true positive pig was ≥ 0.50 (cut-off 1) or those for which
186 the probability was ≥ 0.10 (cut-off 2).

187 In addition, to explore whether there was a difference on the number of farms deemed positive
188 depending on the serological test used, the probability of a farm having at least one true positive pig
189 was estimated using results from the subset of samples tested in duplicate by MAT and ELISA.
190 ELISA sensitivity and specificity of 89% and 98% respectively were used (Gamble et al., 2005).

191
192 Putative predictors of exposure to *T. gondii* within a farm were categorised on the basis of answers
193 given in the questionnaire and risk factors previously identified in the literature. The re-categorisation
194 of variables is described in Table 1.

195
196 Crude associations between predictor variables (table 1) and farm status were tested by Fisher's exact
197 or Pearson's Chi squared test as appropriate; relative risk (RR) was calculated as a measure of
198 strength of association. Collinearity was assessed between all predictor variables for which $p \leq 0.05$ in
199 the univariate analysis and when present ($p < 0.1$) only one of the variables was kept in the model for
200 further multivariable analysis. Logistic regression was used to assess the relationship between the
201 individual predictor variables and the outcome, accounting for the potential confounding effect of
202 other variables. Odds ratios (OR) obtained from the logistic regression were converted to Relative
203 Risk: $RR = OR / (1 - p_0 + (p_0 * OR))$, where p_0 was the baseline risk (i.e. the risk of being positive in the
204 control group) (Grant, 2014). Note that risk factors were collected retrospectively and therefore,
205 exposure to a given risk factor might have happened after infection. In that cases the relative risk
206 would have been overestimated.

207

208 Statistical analyses was performed in R 3.0 (R Development Core Team, 2015) using packages
209 epicalc (Chongsuvivatwong, 2010) and lme4 (Bates et al., 2013).

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211

212 **Results**

213

214 A total of 2071 pigs from 131 farms were sampled; including 1101 females (53.6%) and 953 (46.3%)
215 males (sex was not recorded for 17 pigs). Antibodies against *T. gondii* by MAT were found in 155
216 pigs (7.5%) but only 75 pigs (3.6%) had titres $\geq 1:25$ (Figure 1). Sex was not significantly associated
217 with *T. gondii* sero-status ($p = 0.14$).

218

219 A higher number of samples were classified as positive using MAT (73 samples were positive by
220 MAT and 37 by ELISA) and the difference was statistically significant ($p < 0.001$) (Table 2; Figure

221 S1.1 supplementary material), suggesting serious disagreement between the two tests. For repeated
222 samples, the mean CV values for ELISA were 0.62, therefore there was substantial variation and low
223 precision of the test.

224
225 The proportion of farms deemed positive (i.e. farm-level prevalence) was 1.5% higher using results
226 given by ELISA when considering a $\geq 50\%$ cut-off. However, the opposite happened when
227 considering a 10% cut-off, with more farms deemed positive using results given by MAT (Table S2.1
228 and S2.2 supplementary material).

229
230 Twenty four farms out of 131 sampled had at least 1 animal positive (apparent prevalence 18.3%)
231 (Table 3). The adjusted farm-level prevalence was 11.5% (95% credible interval 8.4%-16.0%) after
232 adjusting for the number of pigs tested per farm and the imperfect sensitivity and specificity of the
233 test; the credible interval refers to the sample estimate rather than a population estimate. The between-
234 farm variance was 21.38, giving an intra-farm correlation of 0.99.

235
236 Seventy three farms (55.7%) returned a completed questionnaire. The median number of pigs in the
237 farm at the time of sampling was 220 (1st and 3rd quartiles 31 and 2217 pigs). In almost half of the
238 farms (48%) pigs had outdoor access for some stage of the production cycle. Twenty seven farms
239 (37%) had cats on the site and 62% considered it was possible for cats not belonging to the site to
240 have access to the farm (Table 4)

241
242 Out of those farms that returned a completed questionnaire (n=73), only two were deemed positive
243 using a cut-off of $\geq 90\%$ probability of having at least one infected animal; four farms were deemed
244 positive using $\geq 80\%$ cut-off and five farms using a cut-off of $\geq 50\%$ (Figure 2). There were no
245 statistically significant associations ($p \leq 0.05$) between farm status and any of the putative risk or
246 protective factors explored (Table 4). This could be due to the lack of statistical power given the small
247 number of positive farms (16% and 28% power of identifying a risk factor with $OR \geq 2.5$ and ≥ 3.5
248 respectively with 5 positive farms). Fifteen farms were deemed positive considering a lower cut-off:
249 $\geq 10\%$ probability of having at least one true positive, increasing the power to 30% (for $OR \geq 2.5$) and
250 50% (for $OR \geq 3.5$). Three farm characteristics were statistically significant from the univariate
251 analysis; having outdoor access (RR=3.0; $p=0.04$), holding up to 200 pigs (RR=3.9; $p=0.02$) and cats
252 having direct access to feed (RR=2.6; $p=0.04$). These 3 variables exhibited strong collinearity ($p < 0.1$)
253 and therefore, the three univariate models were kept. Overall 17 (23.3%) of the farms had the three
254 characteristics (small herds, outdoor access and allowed cats have access to pigs' feed), of which 7
255 farms (41.2%) were positive ($\geq 10\%$ probability).

256

Discussion

257
258
259 A low proportion of pigs tested positive in the current study (3.6%) with the majority of these having
260 a low MAT titre. Some of the animals tested could have been sows or boars which may have
261 increased the number of animals that tested positive. This suggests a low level of *T. gondii* infection
262 in the farms studied, most of which are likely to send to slaughter batches composed of 100%
263 uninfected pigs. Crucially, positive pigs came from a small number of farms (24 farms out of 131) and
264 a very high intra-farm correlation was found, suggesting that the risk of *T. gondii* infection in pigs is
265 largely driven by farm-level factors. In a previous study in the UK, 7.4% of pigs tested positive for *T.*
266 *gondii* antibodies (Powell et al., 2016). Although important geographical overlap exists between
267 studies, our study only included farms in England where 82% of the UK pig production is located
268 (PHWC, 2015). The results are not directly comparable given the differences of study design and the
269 test used.

270

271 Although the five collaborating slaughterhouses reflect the diversity of abattoirs in the country in
272 terms of throughput, specialisation and type of farms (PHWC, 2015), voluntary participation of
273 slaughterhouses and farms is a limitation of this study. However, one of the collaborating abattoirs is
274 among the few in the country that slaughters finishing pigs only and has one of the highest
275 throughputs. The remaining four slaughterhouses handle other species and two of them also slaughter
276 boars and sows. Similarly, the farms in the study reflect the variability of pig production in England
277 (PHWC, 2015).

278

279 Studies comparing the sensitivity and specificity of MAT and ELISA in naturally infected pigs, are
280 scarce and results are contradictory (Dubey et al., 1995; Gamble et al., 2005). Variation of test results
281 could be due to the *T. gondii* strain and time elapsed between infection and sampling (Dubey et al.,
282 1997). Antibodies are detected by MAT 3 weeks post infection, peaking at week 6 and then
283 decreasing but maintained permanently. Titres $\geq 1:320$ are indicative of recent infection (Dubey et al.,
284 1996). In this study a higher number of samples were classified as positive using MAT ($p < 0.001$),
285 which is aligned with results elsewhere (Steinparzer et al., 2015). MAT has been shown to have better
286 precision and accuracy under experimental conditions, but it is time consuming, expensive and not
287 commercially available. Conversely, ELISA is cheap, easy to conduct and commercially available, yet
288 its accuracy is low. For surveillance purposes, ELISA could be used as a routine screening test, while
289 MAT should be the test of preference if regional or national farm-level prevalence estimates are
290 required.

291

292 Once adjusted for the number of animals tested per batch and the sensitivity and specificity of MAT,
293 the farm-level prevalence was 11.5% (95% credible interval 8.4%-16.0%). Although extrapolations

294 and comparisons should be made with caution given the non-probabilistic selection of farms and
295 different survey methodologies applied in different countries, the level of *T. gondii* infection appears
296 to be lower than that reported by studies in Germany (69.1%) (Damriyasa et al., 2004), Italy (42.3%)
297 (Villari et al., 2009), Spain (85.0%) Greece (26.2%) (Papatsiros et al., 2016) and Austria (23.3%)
298 (Steinparzer et al., 2015). It is important to note that prevalence estimates reported in these studies
299 were not adjusted for test sensitivity and specificity and the criteria for classification of positive farms
300 varied.

301

302 Regional differences within some European countries have been reported. Farms located in regions
303 with high temperatures and moderate rainfall in Spain had higher risk of infection than those located
304 in regions below or above the average rain fall, and a similar pattern was reported outside Europe
305 (Alvarado-Esquivel et al., 2014; Alvarado-Esquivel et al., 2015). Comparisons between areas on the
306 basis of climatic conditions should be made with caution as there are likely to be other potential
307 confounding effects, such as farm characteristics or management practices. However, it has been
308 hypothesised that survival of oocysts might increase with humidity, while sporulation time might be
309 shortened with higher temperatures (Dubey, 2010; Opsteegh et al., 2016). Although further studies are
310 needed to explore the role of climatic conditions on the survival of *T. gondii* oocysts, English climatic
311 conditions could potentially limit oocyst survival and therefore reduce the level of exposure and
312 infection in pigs, compared to other climates.

313

314 Smaller herds (≤ 200 pigs) had a higher risk of infection (RR=3.0; $p=0.02$) which is in accordance
315 with studies elsewhere (Villari et al., 2009; Zimmerman et al., 1990). Herd size is often related to
316 other management practices and should not be considered as an isolated factor. In this study, farms
317 with smaller herds were more likely to keep other livestock species, have a continuous cycle, allow
318 outdoor access to pigs and have an open food storage.

319

320 Having outdoor access, presence of cats in the farm and feed stored with the possibility for
321 contamination with cats' faeces, have been previously reported as risk factors for *T. gondii* infection
322 (Assadi-Rad et al., 1995; Garcia-Bocanegra et al., 2010a; Garcia-Bocanegra et al., 2010b; Gebreyes et
323 al., 2008; Guo et al., 2016; Kijlstra et al., 2004; Klun et al., 2006; Ortega-Pacheco et al., 2013; Tao et
324 al., 2011; Weigel et al., 1995). In our study the relative risk of infection was higher on those farms
325 where pigs had outdoor access at any production stage (RR=3.0; $p=0.04$). Keeping cats in the farm or
326 cats from outside being able to access the farm were not significantly associated with *T. gondii*
327 infection. However, cats having direct access to pigs' feed increased the risk of infection 2.6 fold and
328 was significant ($p=0.04$) when a 10% cut-off was considered. Recommendations to farmers should
329 emphasise the importance of ensuring cats do not have access to pigs' feed. Such recommendations
330 should reduce the level of exposure to sporulated oocysts and therefore, the level of infection

331 regardless of the herd size and level of confinement. At EU level, requirements for controlled housing
332 (Anonymous, 2015) could be amended to include mandatory feed storage in closed silos or containers
333 impenetrable to cats, in order to distinguish between low and high biosecurity herds for *T. gondii*.

334

335 The true incidence of human toxoplasmosis in England is unknown as a result of underreporting; an
336 enhanced surveillance programme in England and Wales introduced in 2008 (Halsby et al., 2014)
337 identified 1824 confirmed cases during its first five years, with over a third of them coming from the
338 London area. A previous study had reported a sero-prevalence of 17% among pregnant women in
339 London, with African, Afro-Caribbean, Middle Eastern and mixed race ethnic origins and
340 consumption of undercooked meat as the main risk factors (Flatt and Shetty, 2013). Lower sero-
341 prevalence (9.9%) was reported in studies conducted in Northern England (Zadik et al., 1995) and
342 Southern England (7.7%) (Allain et al., 1998) fifteen years previously. Both studies tested women
343 during the antenatal screening, but risk factors were not reported.

344

345 The foodborne route has been considered as the most important route for human *T. gondii* infection in
346 a recent WHO expert elicitation (WHO, 2015). Furthermore, consumption of undercooked meat
347 (pork, beef and lamb) has repeatedly been found as a risk factor for *T. gondii* infection (Baril et al.,
348 1999; Bobic et al., 2007; Cook et al., 2000; Flatt and Shetty, 2012; Jones et al., 2009; Kapperud et al.,
349 1996), however the type of meat reported varies across countries. Ascertainment of the relative
350 contribution of pork and other animal products to the risk of human *T. gondii* infection and of the
351 effect of farm-level measures warrants a formal risk assessment in which risk mitigation measures
352 along different stages of meat production chain are assessed by probabilistic risk modelling.

353

354 **Conclusions**

355

356 This study provides an approximation to the level of *T. gondii* infection in pigs raised in commercial
357 farms in England using a novel method for prevalence estimation. It also investigates farm
358 characteristics and management practices which may increase the risk of pigs becoming infected.
359 Most of the batches included in this study were likely to contain 100% of uninfected pigs, with a
360 small number of batches accounting for a large proportion of the positive pigs, which indicates that
361 the risk of *T. gondii* infection is largely driven by farm-level factors. At pre-harvest level, mitigation
362 of the risk of exposure to toxoplasmosis via consumption of pork should target farms with outdoor
363 access and/or open feed storage. The study fills some of the data gaps previously identified by the UK
364 Food Standard Agency (AMCSF, 2012) and provides inputs that could be used to populate
365 probabilistic assessments of human foodborne exposure.

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Table 1. Variables considered in the standardised questionnaire to assess potential risk factors for *T. gondii* status in commercial pigs in England. Information collected between January and July 2015 (n=73)

Variable description and question asked in the questionnaire	Categories / options provided in the questionnaire	Variable re-grouped for analysis	
PRODUCTION CYCLE			
<i>Which of the following describe the production cycle in the farm?</i>	Farrow to finish	Complete cycle	
	Breeding to weaning	Part of the cycle	
	Weaning to finishing		
	Grower to finishing		
SOURCE OF PIGS			
<i>If weaning to finishing or grower to finishing, where did you get the pigs from the last batch sent to the slaughterhouse?</i>	From a unit placed in another site but part of the same farm (same owner)	Same owner	
	From another farm (different owner)	Another farm(s) different owner	
	From different farms		
	Other (please specify)		
FARM HOLDINGS			
<i>Do you keep pigs in more than one site/holding?</i>	Yes	Yes	
	No	No	
PRODUCTION SYSTEM			
<i>What is the production system in the farm?</i>	All in all out	All in all out	
	By farm		
	By site		
	By building		
	By pen		
	Continuous	Continuous	
	Other (Please specify)		
OUTDOOR ACCESS			
<i>Using the definitions provided below, please complete the table by ticking the box that best describes the way animals are kept in the farm</i> <i>Indoors is defined as keeping pigs in enclosed buildings (i.e. delimited by solid walls) and pigs are not able to go outside the building.</i> <i>Outdoors is defined as kept in the field within defined boundaries where they are free to roam and are provided with food, water and shelter.</i>	Asked per production stage and 3 possible options (keep outdoor all the time, keep indoor all the time and keep part of the time outdoor and part indoor)	Have outdoor access at any production stage	
		Yes	
		No	
	dry sows lactating sows boar piglets weaners growers finishers	} outdoor / indoor / part outdoor part indoor	
	NUMBER OF ANIMALS		
<i>Please fill in the table below indicating the total number of pigs for each production stage at this moment</i>	Number of pigs held in each production stage in the farm	Total number of pigs (continuous)	
		1-220 pigs; >220pigs	
OTHER LIVESTOCK SPECIES			
<i>Are there other livestock species (apart from pigs) in this site?</i>	Yes	Yes	
	No	No	
FOOD STORAGE			
<i>Where is the animal feed stored? Tick all that apply</i>	Open silo	Open storage (Yes/No)	
	Open storage		
	Closed silo		
	Closed storage		
	Bags for food		
	Other (Please specify)		
TYPE OF FEEDERS			
<i>Which types of feeders are used in this site? Tick all that apply</i>	None (floor)	On the floor (Yes/No)	•Off the floor only •Either all on the floor or some on the floor and some off floor
	Dump feeders		
	Individual feeders	Off the floor (Yes/No)	
	Bowl		
	Pipeline		
	Other (Please specify)		

Variable description and question asked in the questionnaire	Categories / options provided in the questionnaire	Variable re-grouped for analysis									
PIGS' DRINKING WATER <i>Where does the pigs' drinking water come from? Tick all that apply</i>	-Main supply (community tap water) -Local canal / stream -Well -Other (Please specify)	Main supply Other (local canal/stream, well or bore)									
CLEANING BETWEEN BATCHES <i>Is it common practice to clean between batches?</i>	-Yes, it is always cleaned between batches -Yes, most of the times it is cleaned between batches -Rarely -NA (Continuous system)	Yes No									
DISINFECT BETWEEN BATCHES <i>Is it common practice to disinfect between batches?</i>	-Yes, it is always cleaned between batches -Yes, most of the times is cleaned between batches -Rarely -NA (Continuous system)	Yes No									
STAFF <i>Are staff designated to work exclusively in certain areas of this site?</i>	-Yes -No	Yes No									
KEEP CATS <i>Do you keep cats in this site?</i>	-Yes -No	Yes No									
CATS NO BELONGING TO THE FARM <i>Is it possible that cats not belonging to this site get into the site?</i>	-Yes -Not sure -No	Possible No									
CATS – CONTACT WITH PIGS <i>Is it possible that cats come into direct contact with the pigs?</i>	-Yes, cats definitely come into direct contact with pigs / pigs' food / pigs' drinking water	Possible									
CATS – CONTACT WITH PIGS' FOOD <i>Is it possible that cats come into contact with pigs' food?</i>	-Yes, it is very likely that cats come into contact with pigs/ pigs' food / pigs' drinking water -Not sure										
CATS – CONTACT WITH PIGS' DRINKING WATER <i>Is it possible that cats come into contact with pigs' drinking water?</i>	-No, cats cannot come into contact with pigs/ pig's food / pigs' drinking water	No possible									
DE-WORMING <i>Please complete the table below concerning the routine de-worming used on the farm</i>	<table border="0"> <tr> <td>Asked per production stage</td> <td rowspan="7">} product used and frequency</td> </tr> <tr> <td>dry sows</td> </tr> <tr> <td>lactating sows</td> </tr> <tr> <td>boar</td> </tr> <tr> <td>piglets</td> </tr> <tr> <td>weaners</td> </tr> <tr> <td>growers</td> </tr> <tr> <td>finishers</td> </tr> </table>	Asked per production stage	} product used and frequency	dry sows	lactating sows	boar	piglets	weaners	growers	finishers	Yes / No
Asked per production stage	} product used and frequency										
dry sows											
lactating sows											
boar											
piglets											
weaners											
growers											
finishers											

Table 2. MAT titres and ELISA results for serum samples tested for *T. gondii* (n=492). Samples collected between January and July 2015 from commercial pigs in England. Results in this table are not adjusted for the Sensitivity and Specificity of the test

MAT Status	Titre	ELISA			TOTAL
		Positive	Inconclusive	Negative	
<i>Positive</i>	1:25	2	2	6	10
	1:50	8	5	11	24
	*1:100	5	1	5	11
	1:200	7	1	6	14
	1:400	5	0	1	6
	1:800	3	1	0	4
	1:1600	2	2	0	4
	*1:3200	0	0	0	0
Total		32 (4.9%)	12 (2.4%)	29 (5.9%)	73
<i>Suspicious</i>	*1:1	1	0	31	32
	1:3	0	3	21	24
	1:6	1	2	13	16
	1:10	0	4	3	7
	Total	2 (0.41%)	9 (1.8%)	68 (13.8%)	79
<i>Negative</i>	0	3 (0.61%)	3 (0.61%)	334 (67.9%)	

*There was no serum left for three serum sample to be tested by ELISA – one sample with titre 1:10; one sample with titre 1:100 and one sample with titre 1:3200.

Table 3. Apparent batch-level prevalence for *T. gondii* in commercial pigs in England. Serum samples tested by MAT. Samples collected between January and July 2015 (n=131).

Apparent batch-level prevalence *	Number of farms	Herd size Median (1 st – 3 rd quartile)
0%	107	260 (32 - 2624)†
0.1 – 10%	11	
10.1 – 20%	4	
20.1 – 30%	1	
30.1 – 40%	2	
40.1 – 50%	1	
50.1 – 60%	1	66 (11 - 960)‡
60.1 – 70%	1	
70.1 – 80%	1	
80.1 – 90%	0	
90.1 – 100%	2	

*Results in this table are not adjusted for the number of pigs tested per batch/farm and MAT sensitivity and specificity. The number of animals included in a batch ranged from 1 to 235 pigs

‡Nine out of the 13 farms with >10% apparent within-herd prevalence returned a completed questionnaire. In 5 out of 13 farms (55.6%) pigs had outdoor access and in 5 farms (55.6%) cats had access to pigs' food.

†Sixty four out of the 118 farms with ≤10% apparent within-herd prevalence returned a completed questionnaire. In 30 out of 64 farms (46.9%) pigs had outdoor access and in 22 farms (34.4%) cats had access to pigs' food.

Table 4. Distribution of potential risk factors for *T. gondii* positive and negative pig farms in England following univariate analysis.

Risk factor	≥50 probability of being a positive farm				≥10 probability of being a positive farm			
	No. negative (%)	No. positive (%)	<i>p</i>	Relative Risk	No. negative (%)	No. positive (%)	<i>p</i>	Relative Risk
Production cycle								
• Complete cycle	45 (66.2)	2 (40.0)	0.34	2.7	37 (63.8)	10 (66.7)	1	0.9
• Part of the cycle	23 (33.8)	3 (60.0)			21 (36.2)	5 (33.3)		
Source								
• Same owner	51 (25.0)	2 (40.0)	0.12	4.0	15 (25.9)	5 (33.3)	0.56	0.75
• Different owner	17 (75.0)	3 (60.0)			43 (74.1)	10 (66.7)		
Farm holdings								
• More than one site	18 (26.5)	1 (20.0)	1	1.4	40 (69.0)	14 (93.3)	0.10	0.2
• One site	50 (73.5)	4 (80.0)			18 (31.0)	1 (6.7)		
Production system								
• All in all out	26 (38.8)	3 (60.0)	0.39	2.2	25 (43.9)	4 (26.7)	0.26	1.6
• Continuous	41 (61.2)	2 (40.0)			32 (56.1)	11 (73.3)		
Outdoor access (at any production stage)								
• No	36 (52.9)	2 (40.0)	0.67	1.6	34 (58.6)	4 (26.6)	0.04	3.0
• Yes	32 (47.1)	3 (60.0)			24 (41.4)	11 (73.3)		
Farm size								
• Large herds (>200 pigs)	34 (50.0)	3 (60.0)	1	1.2	33 (56.9)	3 (20.0)	0.02	3.9
• Small herds (1-200 pigs)	34 (50.0)	2 (40.0)			25 (43.1)	12 (80.0)		
Hold other livestock species in the farm								
• No	31 (45.6)	1 (20.0)	0.38	3.1	28 (48.3)	4 (26.7)	0.16	2.2
• Yes	37 (54.4)	4 (80.0)			30 (51.7)	11 (73.3)		
Food and water								
Food storage open								
• No	66 (97.0)	4 (20.0)	0.19	5.8	56 (96.6)	14 (93.3)	0.50	1.7
• Yes	2 (3.0)	1 (80.0)			2 (3.4)	1 (6.7)		
Type of feeders								
• On floor (some or all)	31 (45.6)	1 (20.0)	0.37	3.1	33 (56.9)	8 (53.3)	0.80	1.1
• Off floor only	37 (54.4)	4 (80.0)			25 (43.1)	7 (46.7)		
Pigs drinking water: stream well or bore								
• No	49 (26.5)	3 (60.0)	0.62	1.6	39 (67.2)	13 (86.7)	0.20	0.4
• Yes	19 (73.5)	2 (40.0)			19 (32.8)	2 (13.3)		
Biosecurity								
Cleaning between batches								
• Yes	28 (41.2)	3 (60.0)	0.65	2.0	31 (53.4)	11 (73.3)	0.24	0.5
• No	40 (58.8)	2 (40.0)			27 (46.6)	4 (26.7)		
Disinfect between batches								
• Yes	29 (42.6)	3 (60.0)	0.65	1.9	30 (51.7)	11 (73.3)	0.16	0.5
• No	39 (57.4)	2 (40.0)			28 (48.3)	4 (26.7)		
Staff working exclusively in certain areas								
• Yes	10 (14.7)	2 (40.0)	0.18	3.4	49 (84.5)	12 (80.0)	0.70	1.3
• No	58 (85.3)	3 (60.0)			9 (15.5)	3 (20.0)		
Keep cats in the farm								
• No	44 (64.7)	2 (40.0)	0.35	2.6	38 (65.5)	8 (53.3)	0.38	1.5
• Yes	24 (35.3)	3 (60.0)			20 (34.5)	7 (46.7)		
Cats not belonging to the farm get into the site								
• No	27 (39.7)	1 (20.0)	0.64	2.5	25 (43.1)	3 (20.0)	0.14	2.5
• Possible	41 (60.3)	4 (80.0)			33 (56.9)	12 (80.0)		
Cats can get in contact with pigs								
• No	29 (42.6)	2 (40.0)	1	1.1	27 (46.6)	4 (26.7)	0.24	2.0
• Possible	39 (57.4)	3 (60.0)			31 (53.4)	11 (73.3)		
Cats can get in contact with pigs' food								
• No	44 (64.7)	2 (40.0)	0.35	2.6	40 (69.1)	6 (40.0)	0.04	2.6
• Possible	24 (35.3)	3 (60.0)			18 (31.0)	9 (60.0)		
Cats can get in contact with pigs' drinking water								
• No	44 (64.7)	3 (60.0)	1	1.2	40 (69.0)	7 (46.7)	0.11	2.1
• Possible	24 (35.3)	2 (40.0)			18 (31.0)	8 (53.3)		
Preventive medicine								
Deworm in at least one production stage								
• No	30 (44.1)	4 (80.0)	0.18	0.2	27 (46.6)	7 (46.7)	0.99	1.0
• Yes	38 (55.9)	1 (20.0)			31 (53.4)	8 (53.3)		



Figure 1. Number of suspicious (titre between 1:1 and 1:10) and positive (titre \geq 1:25) pigs in England to *T. gondii* by MAT in each titre band. Samples collected between January and July 2015. Results in this figure are not adjusted for the sensitivity and specificity of the test.

Figure 1
160x76mm (150 x 150 DPI)

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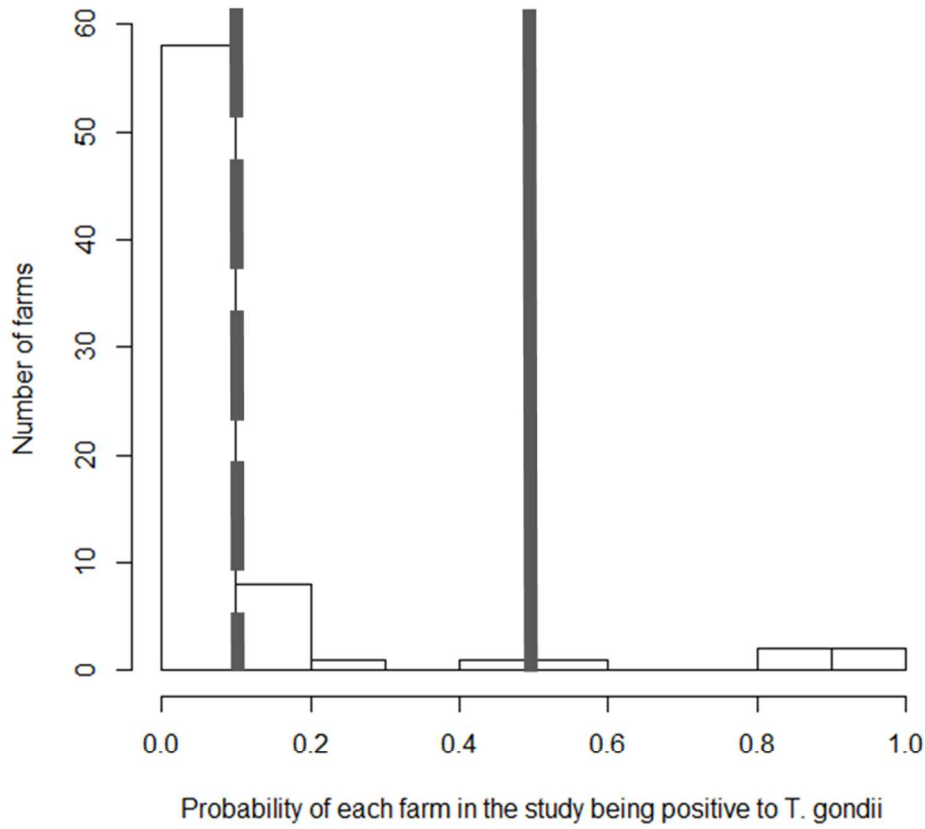


Figure 2. Frequency distribution of the probability of each English pig farm in the study being positive to *T. gondii* after adjusting for test sensitivity and specificity and proportion of animals sampled in each batch. Cut-off used to consider farms positive or negative are illustrated with a dashed line ($\geq 10\%$) and a solid line ($\geq 50\%$).

Figure 2
 160x154mm (150 x 150 DPI)

Distribution

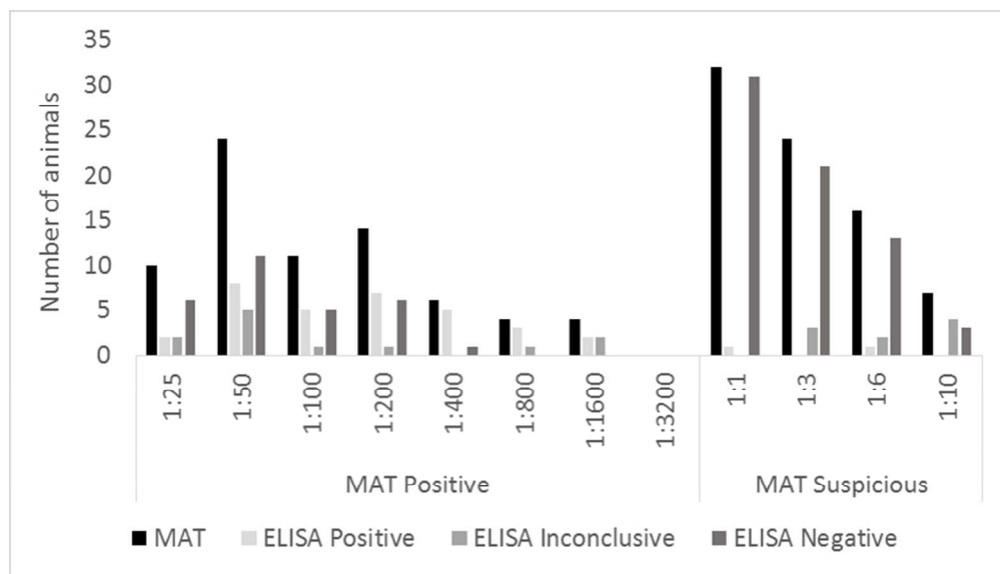


Figure S1. MAT titres and ELISA results for serum samples tested for *T. gondii* (n=492). Samples collected between January and July 2015 from commercial pigs in England. Results in this figure are not adjusted for the Sensitivity and Specificity of the test

Figure S1

142x81mm (150 x 150 DPI)

Table S2.1 Number of farms deemed positive to *T. gondii* after adjusting for MAT and ELISA sensitivity and specificity and proportion of pigs sampled in each batch. A farm was considered positive if the probability of having at least one pig positive was $\geq 50\%$.

MAT	ELISA		Total	P value†
	Positive	Negative		
Positive	6	2	8	
Negative	4	118	122	
Total	10	120	130	0.41

† McNemar's Chi-squared test

Table S2.2 Number of farms deemed positive to *T. gondii* after adjusting for MAT and ELISA sensitivity and specificity and proportion of pigs sampled in each batch. A farm was considered positive if the probability of having at least one pig positive was $\geq 10\%$

MAT	ELISA		Total	P value†
	Positive	Negative		
Positive	14	11	25	
Negative	3	102	105	
Total	17	113	130	0.03

† McNemar's Chi-squared test

Figures captions

Figure 1. Number of suspicious (titre between 1:1 and 1:10) and positive (titre \geq 1:25) pigs in England to *T. gondii* by MAT in each titre band. Samples collected between January and July 2015. Results in this figure are not adjusted for the sensitivity and specificity of the test.

Figure 2. Frequency distribution of the probability of each English pig farm in the study being positive to *T. gondii* after adjusting for test sensitivity and specificity and proportion of animals sampled in each batch. Cut-off used to consider farms positive or negative are illustrated with a dashed line (\geq 10%) and a solid line (\geq 50%).

Supplementary material

Figure S1. MAT titres and ELISA results for serum samples tested for *T. gondii* (n=492). Samples collected between January and July 2015 from commercial pigs in England. Results in this figure are not adjusted for the Sensitivity and Specificity of the test