Knowledge gaps in host-parasite interaction preclude accurate assessment of meat-borne exposure to *Toxoplasma gondii*

Crotta M[a]*, G. Limon[a], DP. Blake[b], J.Guitian[a]

[a] Veterinary Epidemiology, Economics and Pubic Health Group. The Royal Veterinary College. Hawkshead Lane. North Mymms. AL9 7TA, Hatfield, UK
[b] Pathology and Pathogen Biology. The Royal Veterinary College. Hawkshead Lane. North Mymms. AL9 7TA, Hatfield, UK

*Corresponding author: Matteo Crotta, mcrotta4@rvc.ac.uk

Abstract

Toxoplasma gondii is recognized as a widely prevalent zoonotic parasite worldwide. Although several studies clearly identified meat products as an important source of T. gondii infections in humans, quantitative understanding of the risk posed to humans through the food chain is surprisingly scant. While probabilistic risk assessments for pathogens such as Campylobacter jejuni, Listeria monocytogenes or Escherichia coli have been well established, attempts to quantify the probability of human exposure to T. gondii through consumption of food products of animal origin are at early stages. The biological complexity of the life cycle of T. gondii and limited understanding of several fundamental aspects of the host/parasite interaction, require the adoption of numerous critical assumptions and significant simplifications. In this study, we present a hypothetical quantitative model for the assessment of human exposure to T. gondii through meat products. The model has been conceptualized to capture the dynamics leading to the presence of parasite in meat and, for illustrative purposes, used to estimate the probability of at least one viable cyst occurring in 100g of fresh pork meat in England. Available data, including the results of a serological survey in pigs raised in England were used as a starting point to implement a probabilistic model and assess the fate of the parasite along the food chain. Uncertainty distributions were included to describe and account for the lack of knowledge where necessary. To quantify the impact key model inputs, sensitivity and scenario analyses were performed. The overall probability of 100g of a hypothetical edible tissue containing at least 1 cyst was 7.5%. Sensitivity analysis indicated that the input parameters with high uncertainty (i.e. number of cysts per meat sample from an infected animal and number of bradyzoites per cyst), exerted the greatest effect on the output mean. Under the best and the worst scenarios, the probability of a single portion of fresh pork meat containing at least 1 viable cyst resulted 1.3% and 16.0% indicating that the uncertainty and lack of data surrounding key input parameters of the model preclude accurate estimation of T. gondii exposure through consumption of meat products. The hypothetical model conceptualized here is coherent with current knowledge of the biology of the parasite. Simulation outputs clearly identify the key gaps in our knowledge of the host-parasite interaction that, when filled, will support quantitative assessments and much needed accurate estimates of the risk of human exposure.

Keywords: Toxoplasma gondii; quantitative risk assessment; probabilistic assessment; food safety; zoonotic disease; meat, foodborne disease

1. INTRODUCTION

Toxoplasmosis is a worldwide distributed zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*. The life cycle of *T. gondii* includes felines as the definitive host and mammals and birds as the most common intermediate hosts. Oocysts produced in the definitive host are excreted in faeces and sporulate in the environment, before being ingested by an intermediate or another definitive host. Following ingestion, sporozoites are released from the oocyst, developing into tachyzoites in an intermediate host and primarily targeting muscle or neural tissues where the parasite develops into a tissue cyst, the site of bradyzoite replication (Dubey, 1998). The number of bradyzoites within a tissue cyst varies depending on the age and size of the cyst (Dubey et al., 1998). Except in instances of congenital or sexual transmission, blood transfusion or organ transplantation (Guerina et al., 1994), *T. gondii* is not passed from person to person. The ingestion of raw or undercooked meat containing viable cysts has been suggested to be one of the major sources of infection in Europe and North America (Guo et al., 2015a; Hoffmann et al., 2007; Scallan et al., 2011). In the United States, the US Centre for Disease Control and Prevention (CDC) has included toxoplasmosis in a group of five parasitic diseases (together with Chagas disease, cysticercosis, toxocariasis and trichomoniasis) that are considered public health priorities (CDC, 2016). At the global level, *T. gondii* was ranked in fourth position in a multicriteria-based risk ranking of foodborne parasites compiled by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) in 2014 (WHO/FAO, 2014). However, the ranking is the results of expert meetings and it should be considered as an overall "picture" that is representative of the information available at time, the criteria used for ranking and the weighting that were assigned to those criteria. Recently, a report published by the WHO on the global burden of foodborne diseases (WHO, 2015), ranked *T. gondii* in sixth position among 31 food-borne hazards with respect to the relative contribution of years of life lost due to premature mortality (YLL). Concern in relation to foodborne *T. gondii* infection has led agencies such as the UK Food Standards Agency (FSA) and the European Food Safety Authority (EFSA) to conduct consultations and issue scientific opinions on key aspects of the parasite-host interaction (FSA, 2012; Opsteegh et al., 2016). Information gained can be used to quantitatively estimate the probability of human exposure and/or infection through food consumption.

Increasingly, quantitative microbial risk assessment (QMRA) models that provide estimates of risk to human health (typically expressed as probability of infection and/or exposure) are used to support decisions on the management of food safety issues. The application of QMRA has improved the transparency and scientific basis of many regulatory decisions, including scenarios involving established or emerging food-borne pathogens (FAO/WHO, 1995). To date, four risk assessments of *T. gondii* exposure/infection have been published, two qualitative assessments (Guo et al., 2015a; Mie et al., 2008) and two QMRA (Guo et al., 2016; Opsteegh et al., 2011). In the first QMRA (Opsteegh et al., 2011), which aimed at exploring and quantifying the relative contribution of sheep, beef and pork meat products to *T. gondii* infections in

humans, the biological complexity of the system combined with some uncertainties and incomplete evidence resulted in an estimate reliant upon a number of implicit and critical assumptions. In particular, the model assumed that the level of *T. gondii* contamination (log_{10}-transformed bradyzoite/g) detected in sheep hearts by MC-PCR was the same as the level of contamination in sheep, beef and pig meat and that bradyzoites were homogeneously distributed throughout the meat of infected animals (as opposed to clustered into discrete tissue cysts). This QMRA also assumed that the overall probability of a meat portion being infected was the same as the proportion of animals with detectable antibodies (for pork and sheep products) or the proportion found positive by PCR (for beef). The infective dose for humans in this model was assumed to be the same as for mice. In the second study (Guo et al., 2016), *T. gondii* bradyzoite concentration in lamb muscle tissue was described based on a real-time PCR study of limb muscles from experimentally infected goats. As highlighted by the authors, bradyzoite concentration in muscle tissue from a naturally infected lamb might not be the same as that from experimentally infected goats. Although that model included a number of important improvements such as the inclusion of a formal human-derived dose-response model and detailed modelling of the steps along the food chain, the critical assumption of homogeneous dispersion of bradyzoites in meat was made by necessity. Recent experimental studies and epidemiological investigations have begun to fill some of these knowledge gaps, providing evidence on the relationship between the results of serological tests and presence of the parasite in meat in different meat-producing species, predilection tissues and the frequency of exposure in livestock animals across EU countries (Deng et al., 2016; Hosein et al., 2016; Opsteegh et al., 2016). Nonetheless, many key knowledge gaps surrounding assumptions highlighted in the previous QMRAs remain, continuing to undermine effective QMRA and limiting practical value for policy makers. In response, the development of a well-structured hypothetical model aimed at capturing the relevant biological parameters and defining the remaining knowledge gaps is now timely since the model can be used as the foundation for a formal QMRA as data for key parameters currently unknown become available. In the meantime, the relevance of the inputs involved and the impact of the uncertainty (i.e. lack of data/evidence) that characterizes some can be explicitly quantified by sensitivity analysis, with the subsequent possibility of ranking those inputs by their effect on the output. Therefore, while purely hypothetical, this process does allow decision makers and researchers to prioritize further research.

Following these considerations, the objectives of the present work were to: (i) conceptualize and develop a hypothetical model to estimate the probability of human exposure to at least one viable cyst of *T. gondii* in a 100g portion of pig meat, and (ii) parameterize the model with the latest evidence generated by experimental and epidemiological research and quantify the effects of remaining data gaps on the final estimate.

To this end, a probabilistic model for the assessment of human exposure to *T. gondii* from consumption of fresh pig meat was developed drawing upon recent data and evidence collected in England (Limon et al., Under revision).

2. MATERIAL AND METHODS

The flowchart in Figure 1 illustrates the steps and biological dynamics of the system that were reproduced in the stochastic model for the assessment of human exposure to *T. gondii* through a 100g portion of pig meat. Briefly, the model has been structured in four main steps. The first and the second steps aim at estimating the proportion of pigs destined for human consumption that are seropositive to *T. gondii* (step 1) and the proportion of seropositive and seronegative animals being infected with viable cysts (step 2). The model continues with the estimation, in animals carrying viable cysts, of the number of viable cysts in 100g of *T. gondii* election tissue (step 3). In the final step (step 4), the expected number of viable cysts in 100g of meat is inferred from the number in 100g of election tissue by using a ratio coefficient (γ) expressing the number of cysts in the edible tissue of interest *i* as a function of the number of cysts in the predilection tissue.

2.1. Step 1. Seroprevalence of *T. gondii* in pigs.

The outcome of this step is an estimate of the proportion of seropositive animals or seroprevalence (P_{SERA}^{+}) in the target population given the number of animals tested as part of a survey (*n*) and the number of animals found seropositive (*s*).

For the illustrative purpose of this model, the seroprevalence of *T. gondii* infection in pigs in England was estimated based upon the serological results of a cross-sectional study conducted between January and July 2015 (Limon et al., Under revision). In that study, a total of 2,071 pigs (*n*) were sampled and tested for antibodies (IgG) specific for *T. gondii.* The modified agglutination test (MAT) was used and animals were considered seropositive when above the cut-off titre of 1:10. A total of 155 samples (*s*) were found to be positive. The beta distribution: *Beta (s+1, n-s+1)* was used to describe the uncertainty in the proportion of seropositive pigs when estimated on the basis of the total number of animals tested (n) and the total number of them found positive (s).

2.2. Step 2. Proportion of animals with viable cysts.

The purpose of this second step was to estimate the proportion of pigs carrying viable cysts among those found seropositive and seronegative in the previous step. Although in pigs the presence of detectable antibodies is known to correlate well with the physical presence of the parasite in animal tissues, the correlation is not perfect (Opsteegh et al., 2016). For the purpose of a quantitative exposure assessment, it is important to discriminate the presence of viable and non-viable organisms. For this reason, in this step the probability of an animal being infected with viable cysts (PV_{cyst}) is calculated. To this end, results from

an experimental study aimed at comparing the performance of ELISA and MAT for detection of *Toxoplasma* infection in pigs were used (Gamble et al., 2005). In that study, the author reported the concordance between demonstration of viable *T. gondii* by bioassay and detection of antibodies in sera above different cut-off titres. The total number of samples with a MAT titre of at least 1:10 (the lowest titre that was considered) taken from bioassay-positive results was used to parameterize the Beta distributions describing the uncertainty in the conditional probabilities of observing viable cysts in seropositive and seronegative animals.

Of 70 bioassay-positive animals (*n*), 67 and 3 blood samples resulted positive (s^+) and negative (s^-) by MAT respectively. Thus, the Beta distributions describing the uncertainty in the conditional probabilities were parameterized as:

$$P(SERA^+|BIOASSAY^+) = Beta(s^+ + 1; n - s^+ + 1)$$
$$P(SERA^-|BIOASSAY^+) = Beta(s^- + 1; n - s^- + 1)$$

And the overall probability of an animal being infected with viable cysts was then estimated as:

$$P_{VCysts} = [P_{SERA^+} * P(SERA^+|BIOASSAY^+)] + [(1 - P_{SERA^+}) * P(SERA^-|BIOASSAY^+)]$$

Where $P(SERA^+|BIOASSAY^+)$ and $P(SERA^-|BIOASSAY^+)$ are the assumed probabilities of animals infected with viable cysts testing positive and negative by MAT.

2.3. Step 3 Number of *T. gondii* viable cysts in 100 g of predilection tissue in infected animals.

In this step, the number of *T. gondii* cysts in 100g of predilection tissue are estimated. An extensive literature review and a recent experimental study aimed at identifying predilection tissues of *T. gondii* (Juránková et al., 2014; Juránková et al., 2015; Opsteegh et al., 2016), have found the brain to be the organ where the parasite was most likely to be detected in pigs. Considering that quantitative PCR techniques to determine the number of bradyzoites in tissue samples are available (Opsteegh et al., 2010), and under the assumption that future (quantitative) investigations on *T. gondii* will involve organs where the parasite is more likely to be found; this step estimates the number of viable cysts (N_{cyst}) in a portion of *T. gondii*'s predilection tissue as a function of: the number of bradyzoites (N_{br}) detected and the current knowledge about the number of bradyzoites per cyst ($N_{br/cyst}$).

2.3.1. *Number of bradyzoites/100g of predilection tissue.*

An estimate of bradyzoite concentration in sheep hearts was described by Opsteegh et al. in a previous study (Opsteegh et al., 2011). In that work, the author fitted a distribution to the concentrations of bradyzoites observed in 35 sheep heart samples of 100g analysed with MC-PCR (Opsteegh et al., 2010). A *Betageneral* distribution with parameters α_1 of 6.5, α_2 of 5.7, minimum of 0 and maximum of 6.8 was found to adequately describe the $\log_{10}$-transformed number of bradyzoites for 100g samples (N_{br}). Without

quantitative estimation of the number of bradyzoites in a standard 100g portion of infected pig's brain and for the illustrative purpose of the model, the *Betageneral* distribution described by Opsteegh et al., was used in this step.

2.3.2. *Number of cysts/100g of predilection tissue.*

This step involves the probabilistic modelling of the number of *T. gondii* cysts in 100g of predilection tissue (N_{cyst}) as a function of: (i) the number of bradyzoites (N_{br}) and (ii) the estimated number of bradyzoites per cyst ($N_{br/cyst}$), with this last parameter related to cyst size.

Several studies have shown that tissue cysts vary in size as a function of their age (Ferguson and Hutchison, 1987; Hossein et al., 2009; Shaapan and Ghazy, 2007; Van der Waaij, 1959). Young tissue cysts may be as small as 5 µm in diameter, containing as few as two bradyzoites, while older cysts may contain hundreds of bradyzoites (Dubey et al., 1998). The size of a mature tissue cyst is variable; up to 70 µm in diameter for cysts of 16 weeks in mice (mean diameter of 100 cysts = 42 µm) as reported by Van der Waaij et al. (Van der Waaij, 1959). Recently, Hossein et al., observed diameters of 20 and -approximately due a not perfect circle- 100µm for cysts of the same age (Hossein et al., 2009). Accurate data about the size of mature cysts, together with the relative number of bradyzoites they may contain are still lacking, with available information characterized by very large variability and uncertainty. The presence of more than 1,000 bradyzoites was estimated by Dubey et al. in a highly flattened tissue cyst that initially passed through a 63-µm filter (Dubey et al., 1998), while in the mature tissue cyst described by Huskinson-Mark et al., about 990 bradyzoites were clearly visible but the size of the cyst was not reported (Huskinson-Mark et al., 1991). In 1958 Beverley reported the observation of up to 60,000 bradyzoites in one cyst (Beverley, 1958), however, the validity of this information has been questioned by Dubey et al. (Dubey et al., 1998).

In conclusion, there are no firm data on the number of bradyzoites in a tissue cyst. In addition, it is not possible to predict the age (and therefore the size) of the cysts in infected animals. Therefore, the number of cysts (N_{cyst}) was estimated as follows: assuming cysts in the brain are spherical (Dubey et al., 1998) with a 0.5 µm thick wall and diameters ranging from a minimum of 5 to a maximum of 100 µm, N_{cyst} was calculated as the total number of spherical cysts of a given diameter (Ø) that are necessary to match the total volume of the sampled bradyzoites (N_{br}). The diameter of the cysts in infected animals was described by the distribution:

$Ø$ = uniform ($Ø_{min}$; $Ø_{max}$)

Meaning that with no information on the cyst's age, the size is a random value ranging from the minimum ($Ø_{min}$ = 5 µm (Dubey et al., 1998)) for young cysts and the maximum ($Ø_{max}$) size observed for mature cysts. To describe the uncertainty surrounding $Ø_{max}$, the Pert distribution was used. This distribution is usually adopted to model expert opinions and its shape is determined by the minimum, maximum, and most likely

value of the parameter of interest. In our model, the parameters 'Minimum', 'Maximum' and 'Most likely' are equal to 20µm, 100µm and 60µm respectively with the minimum and the maximum values extrapolated from data reported in the published literature (Hossein et al., 2009; Van der Waaij, 1959), whilst the 'Most likely' value was obtained as simple mathematical mean. In each iteration, the sampled value of Ø was used to calculate the volume of the spherical cysts and, taking the size of a bradyzoite to be 7 x 1.5µm (Mehlhorn and Frenkel, 1980); the approximated maximum number of bradyzoites each cyst can contain ($N_{br/cyst}$) was estimated. Finally, the actual number of cysts in infected predilection tissue was obtained as:

$N_{cyst} = N_{br} / N_{br/cyst}$

Decimal results were conservatively rounded up and N_{cyst} was fixed to 1 if $N_{br} < N_{br/cyst.}$

Step 4. Number of *T. gondii* viable cysts in 100g of edible tissue in infected animals.

In this step, the number of *T. gondii* cysts in 100 g of edible tissue are estimated**.** As discussed in the previous step (step 3), the quantity of viable cysts that are present in the predilection tissue is expected to be different from that in edible tissue of the same animal (Esteban-Redondo and Innes, 1998; Guo et al., 2015a). However, in the absence of consistent quantitative indications/evidence about the extent to which cysts are more or less likely to be present in the different tissues of an infected pig, the ratio coefficient (γ) describing the relationship: N_{cyst_Ti}/N_{cyst} (i.e. number of cysts in the edible tissue of interest *i* given the number of cysts in the predilection tissue) remains unknown but was reasonably expected to be lower than 1, formally:

$$N_{cyst\_Ti} = \gamma * N_{cyst}$$

With: $\gamma = N_{cyst\_Ti} | N_{cyst} = Uniform(0{,}01; 0{,}99)$

Multiplying N_{cyst} by a fractional number (γ) might generate fractional results; the extreme combination that can be computed (i.e. N_{cyst} = 1 and γ = 0.01) would generate a predicted number of viable cysts in 100g (N_{cyst_Ti}) equal to 0.01 with the practical meaning that viable cysts are present but cannot be expected to be found in every portion of 100g of meat. The actual number of cysts was obtained following a three-step process: first the value of N_{cyst_Ti} was multiplied x100 to obtain a discrete value at any combination of γ and N_{cyst}. Secondly, considering that the value obtained is equivalent to the expected number of viable cysts in 10Kg of meat (100gx100), a multinomial process was used to randomly allocate the discrete number of cysts in 100 pieces (of 100g each). In fact, as the multinomial process can be considered an extension of the binomial process (where there are only two possible outcomes), it was used to describe how many

independent trials (cysts) randomly fall into each of several categories (100g portions). Finally, one of those 100 pieces was randomly sampled and the discrete value of N_{cyst_Ti} obtained.

Simulation and model's outputs.

As outlined in figure 1, the output of the stochastic model was the probability of exposure to at least one *T. gondii* viable cyst in 100g of a hypothetical edible tissue *i* (PV_{cyst_Ti}), this probability was estimated from the distribution describing the number of cysts in the simulated meat portions (N_{cyst_Ti}). Results were obtained as a mean of 1,000,000 Monte Carlo iterations. In order to quantify the impact of the uncertainty distributions included in the model, a sensitivity analysis was performed to rank the inputs involved by their effect on the output mean. The software @Risk (version 7.0.1 for Excel, Palisade Corporation, Newfield, NY) was used.

Moreover, in order to quantify the practical implications of the distributions representing the uncertainty and the lack of data on the model's output, results of two extreme scenarios were arbitrarily assessed:

- Best scenario: a scenario in which the uncertainty distributions describing P_{SERA}^{+}, $P(SERA^{+}|BIOASSAY^{+})$, $P(SERA^{-}|BIOASSAY^{+})$ and γ were kept fixed to the value corresponding to their 5th percentile while $\emptyset_{MAX}$ was kept to its 95th percentile.
- Worst scenario; a scenario in which the uncertainty distributions describing P_{SERA}^{+}, $P(SERA^{+}|BIOASSAY^{+})$, $P(SERA^{-}|BIOASSAY^{+})$ and γ were kept fixed to the value corresponding to their 95th percentile while $\emptyset_{MAX}$ to its 5th percentile.

Those combinations were expected to generate the minimum and maximum values for probability of exposure to at least one viable cyst (PV_{cyst_Ti}) together with the number of cysts in simulated meat portions (N_{cyst_Ti}).

3. RESULTS

After simulation, the distribution describing the overall probability of an animal being infected with viable cysts (PV_{cyst}) shown a median value of 11.8% (5th and 95th percentiles were 8.7% and 16.9% respectively) but that of 100g of a hypothetical edible tissue *i* containing at least 1 viable cyst (PV_{cyst_Ti}) was 7.5%. This difference is to be attributed to the steps modelling the number of viable cysts in edible tissue and the multinomial process describing whether a portion of 100g taken from an infected animal do contain viable cysts or not only by chance.

The cumulative distribution that was used to estimate PV_{cyst_Ti} and consequently, the probability of being exposed to a given number of viable cysts/100g of an edible tissue (N_{cysts_Ti}) is reported in Figure 2. This distribution was obtained plotting the results of the 1,000,000 iterations of the baseline model and it shows that the probabilities of the N_{cysts_Ti} being greater than zero and greater than one were 7.5% and 5.0% respectively.

Results of sensitivity analysis performed on the output (N_{cysts_Ti}) and the other two key inputs involved (i.e. PV_{cyst} and N_{cyst}) are presented in figures 3, 4 and 5 respectively as tornado charts with inputs ranked by their effect on the outcomes' means.

The tornado chart in figure 3 shows that the computed mean of N_{cyst_Ti} may range from 0.1 to 22.0 as a function of N_{cyst} when all the other variables are held at their baseline values. Similarly, γ ranked second and was able to displace the mean of N_{cyst_Ti} from 0.2 to 4.2 when all the other variables were held at their baseline values. The effect of the distribution describing the overall probability of an animal being infected with viable cysts ranked in third position.

To explore the effects of the inputs involved in the estimation of the number of cysts in 100g of predilection tissue (N_{cyst}), namely, the number of bradyzoites detected ($N_{br,}$) and the maximum diameter of the cysts ($Ø_{MAX}$), a dedicated sensitivity analysis was performed. The results of this analysis (tornado chart in figure 4) shown that both the inputs involved had a significant influence on the output means. When N_{br} was fixed to its baseline value, the uncertainty in $Ø_{MAX}$ was able to displace the mean of N_{cyst} from 10.2 to 117.6, similarly, the mean of N_{cyst} ranges 1.1 to 268.1 when $Ø_{MAX}$ is on its baseline value and the outcomes is driven by N_{br}. Similarly, a dedicated sensitivity analysis was performed to explore the effects of the inputs involved in the estimation of the overall probability of an animal being infected with viable cysts (PV_{cyst}); namely, the seroprevalence (P_{SERA}^{+}) and the conditional probabilities of animals infected with viable cysts resulting seropositive P($SERA^{+}|BIOASSAY^{+}$) or seronegative P($SERA^{-}|BIOASSAY^{+}$) by MAT. The results of this analysis (tornado chart in figure 5) identified the uncertainty in the probability of a seronegative animal having viable cysts as the input with the greater effect on PV_{cyst}. In fact, it was able to displace the mean of PV_{cyst} from 8.8% to 17.3%. On the other hand, the uncertainty in the seroprevalence and the conditional probability of animals with viable cysts resulting seropositive were less critical and displaced the mean of PV_{cyst} by 1.7 and 0.7 percentage points respectively.

When the two extreme scenarios were simulated, PV_{cyst_Ti} was 1.3% and 16.0% in best and worst scenarios while the probability of the number of cyst per portion being greater than 1 were 0.4% and 13.7% respectively (Figure 2).

In summary, the results suggest that with the current limited understanding of a number of key parameters such as: (i) the number and size of the cysts in *T. gondii* predilection tissue in infected animals and (ii) the number of cysts that may be detected in non-target organs and (iii) the actual presence of viable cysts in seropositive and seronegative animals; a quantitative risk assessment would not provide any useful information for policymakers with respect to the exposure of *T. gondii* to humans through the consumption of meat products.

4. DISCUSSION

In last decades, QMRAs have been successfully used in a number of food-borne settings worldwide (EFSA, 2010; FDA, 2003; Soboleva, 2013) to model the fate of the hazards along the food chain and generate quantitative estimates in support to the food safety management. However, as any mathematical model, the degree of credibility to risk assessment results is strictly dependent on the quality and quantity of the data, and the assumptions that are made. Inevitably, a model remains a simplification of the real world and this make the structure of the model an assumption itself.

In this study we show that given the current level of understanding and lack of data in key aspects of the parasite-host interaction, it is premature to attempt accurate quantitative estimates of the risk of exposure to *T. gondii* through consumption of meat product.

The main contribution of the model presented in this study is that it explicitly takes into account a number of critical variables that capture the complex interaction of *T. gondii* and the intermediate host, namely: (i) uncertainty in the proportion animals sent to slaughter that are seropositive (i.e. proportion of animals likely to have been exposed to *T. gondii*) as a result of random variation in serological surveys, (ii) the probability that animals found to be seropositive or seronegative have viable cysts (iii) differences in the parasite's tropism that influence the relative amount of cysts in predilected vs. other (edible) tissues, (iv) non-homogeneous distribution of the bradyzoites in edible tissues and (v) the actual number of *T. gondii* cysts in meat of infected animals and the number of infective forms in these cysts.

One of the main improvements of the proposed approach is the use of the 'cyst' rather than the bradyzoite as the first biological unit to which consumers are exposed. While use of the number of bradyzoites/g without proceeding to the conversion into number of cysts/portion is an attractive simplification from a modelling perspective, such an approach overlooks the biological fact that bradyzoites are normally located in discrete tissue cysts and not distributed homogeneously in the muscular tissue. Thus, the consumption of a portion containing at least one viable tissue cyst is the first conditional step to becoming infected. This conceptual difference has proven to be of great impact in the modelling of human exposure in previous *T. gondii* risk assessments. As highlighted by Opsteegh et al (Opsteegh et al., 2011), use of the number of bradyzoites/g as the biological unit of exposure resulted in an overestimation of risk for products consumed in small portions with unrealistic dilution effects when minced meat products were considered. The relevance of this assumption is also evident comparing the results obtained from PV_{cyst} and PV_{cyst_Ti}. In fact, this discrepancy clearly demonstrates that the possibility of not being exposed to *T. gondii* from meat portions obtained from infected animals is taken into account by the stochastic model. Intuitively this event is even more critical when small meat portions are considered or the presence of few cysts are predicted.

In our model, the conditional event leading to human exposure to *T. gondii* was not the presence of bradyzoites themselves, but that of at least one viable cyst; with infective forms normally residing within cysts. This is a closer representation of the nature of the host-parasite interaction and departures from this

conceptualization would be inconsistent with the biology of the parasite with unpredictable effects on model outcomes. Moreover, using the cyst rather than the bradyzoite as the biological unit, the limitations of the previous approach with respect to unrealistic dilution phenomena are overcome making possible the assessment of exposure to the parasite through consumption of products made with meat from different (infected and not infected) animals (i.e. minced meat). This approach also applies to other food matrices where contamination by *T. gondii* has been reported such as vegetables (Lalonde and Gajadhar, 2016) and goat milk (Dubey et al., 2014; Fusco et al., 2007).

Here, our model explicitly identifies key knowledge gaps in the host-parasite interaction dynamics and quantifies the effect that this uncertainty has on the final estimates. The results demonstrate that uncertainty in key parameters continues to undermine our ability to accurately estimate the risk of human exposure to *T. gondii* with a degree of confidence suitable for setting targeted public health policy. This situation can only be addressed by further research, and the parameters identified here should therefore be used to determine new research priorities.

Interestingly, although described by a rather uninformative distribution ranging from (0.01% to 99.9%), the uncertainty in the ratio coefficient (γ) describing the expected number of cysts in the edible tissue *i* given the number of cysts in the predilection tissue was not the most influential input in our model.

Results of sensitivity analysis indicate that the relative impact of the high uncertainty in this input on the calculated mean number of tissue cysts (N_{cyst_Ti}) was sensibly lower than the number of cysts in predilection tissue (N_{cyst}) which is ranked first.

From the results of our model it is evident that without further data on the distribution of the number of cysts in edible tissues and of bradyzoites per cyst, QMRAs for *T. gondii* will continue to be characterized by considerable uncertainty.

Particularly relevant is also the impact of the distribution describing the probability of infected animals resulting seronegative by MAT. In fact, although only 3 of the 70 bioassay-positive animals resulted seronegative by MAT, the sensitivity analysis on PV_{cyst} emphasize the relevance of the expected proportion of false negative animals (point estimate Gamble et al., = 4.3%) in a population characterized by a high prevalence of seronegative (point estimate Lemon et al., = 1- P_{SERA}^{+} = 92.5%).

In addition, it should be considered that the aim of this model was to estimate the exposure to at least one viable cyst in a portion of fresh pig meat; the steps occurring before the actual consumption (e.g. processing, storage conditions, heat treatments or consumer habits) were not taken into account. Those steps are expected to exert a relevant effect on the viability of *T. gondii* (Dubey et al., 1990; Hill et al., 2006; Lundén and Uggla, 1992) and thus, on the PV_{cyst_Ti} at consumption. Furthermore, differences in the host response to different *T. gondii* strains (Saeij et al., 2005) and the lack of accurate strain-specific dose-

response information for humans still represents a limitation to the final quantitative assessment of risk to human health. An animal-derived human dose-response model has recently been proposed by Guo et al.,(Guo et al., 2015b). That model was based on experimental data in mice and validated with experimental data in rats, a scaling factor was then used to match the predicted cases of human infections and adapt the dose-response model to humans. Although the simple quantitative model implemented as a part of that study to estimate the total number of human cases (used to infer the scaling factor) was based on a number of approximations, they filled an important gap and that model represents, to date, the best available information. As our model allows for re-conversion of the number of bradyzoites ingested as a function of the number of cysts (figure 1, dotted line), the dose-response model proposed by Guo et al. to predict the human response after consuming *T. gondii*-infected meats can be easily included. At this respect, it should be remarked that the 'dose' in the dose-response model is inevitably represented by the number of bradyzoites ingested and this fact reinforces the need of quantitative approaches that depart from the assumption of homogeneously distributed bradyzoites in meat. This is also valid for particular processed meat products such as the homogenised meat, where coexistence of homogeneously distributed bradyzoites and entire cysts cannot be excluded.

In conclusion, we have formally identified that the actual number of cysts in *T. gondii* predilection tissue of infected animals, the expected number of cysts in edible tissue and the concordance between serological and bioassay tests are the key data gaps precluding accurate estimation of human exposure to *T. gondii* through consumption of meat products. Most of these data gaps are related to basic aspects of the biology of the parasite and its interaction with the host.

Generating knowledge to fill the identified data gaps might be challenging, but are essential to develop a sound quantitative assessment of *T. gondii* infection through consumption of meat products. In the meantime, available estimates of the number of cases of toxoplasmosis attributed to meat consumption should be interpreted with great caution.

5. AKNOWLEDGMENTS

The study was part of project FS517004 funded by the Food Standard Agency UK.

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Figure captions

Figure 1 Flowchart of the proposed exposure assessment model showing the input and the steps involved in the estimation of the expected number of viable cysts in 100g of pig meat (N_{cyst_Ti}) and the related probability of detecting at least one viable cyst (PV_{cyst_Ti}). The model is structured in four main steps. The ratio coefficient (γ) describes the relationship: $N_{cyst\_Ti}|N_{cyst}$ (i.e. number of cysts in the edible tissue of interest i given the number of cysts in the predilection tissue)

Figure 2 Cumulative probability distribution representing the predicted number of viable cysts in a 100g portion of a hypothetical edible tissue (N_{cyst_Ti}). Grey line = Baseline, bold black line = Best scenario and thin black line = Worst scenario.

Figure 3 Sensitivity analysis. Following simulation, the tornado chart shows the values of the lowest and highest outputs' means of N_{cyst_Ti} that can be obtained as a function of each individual variable when all the others were held at their baseline value. Inputs were then ranked by their effect on N_{cyst_Ti}.

Figure 4 Sensitivity analysis. Following simulation, the tornado chart shows the values of the lowest and highest outputs' means of N_{cyst} that can be obtained as a function of each individual variable when the other was held at its baseline value. Inputs were then ranked by their effect on N_{cyst}.

Figure 5 Sensitivity analysis. Following simulation, the tornado chart shows the values of the lowest and highest outputs' means of PV_{cyst} that can be obtained as a function of each individual variable when the other was held at its baseline value. Inputs were then ranked by their effect on PV_{cyst}.

Figure 1

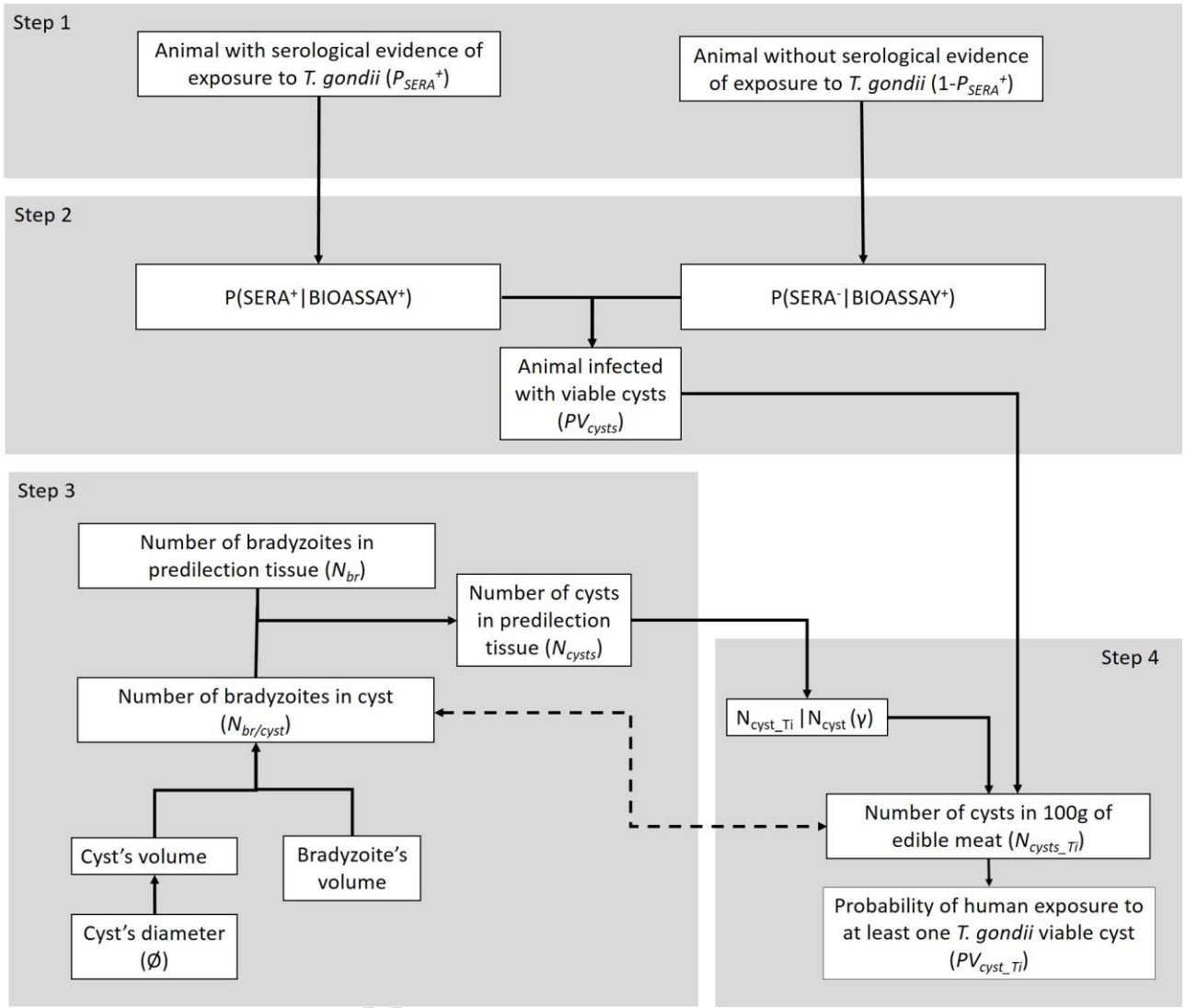


Figure 2

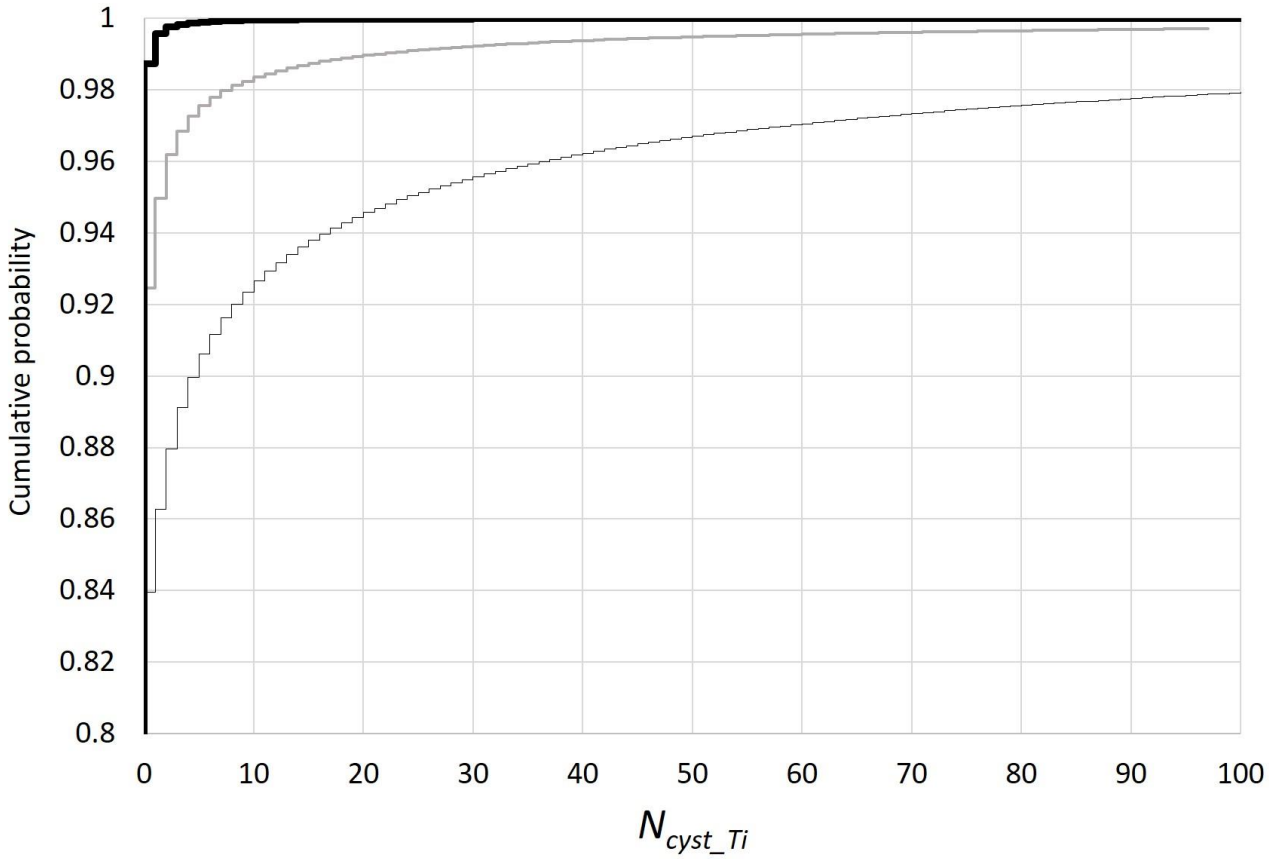


Figure 3

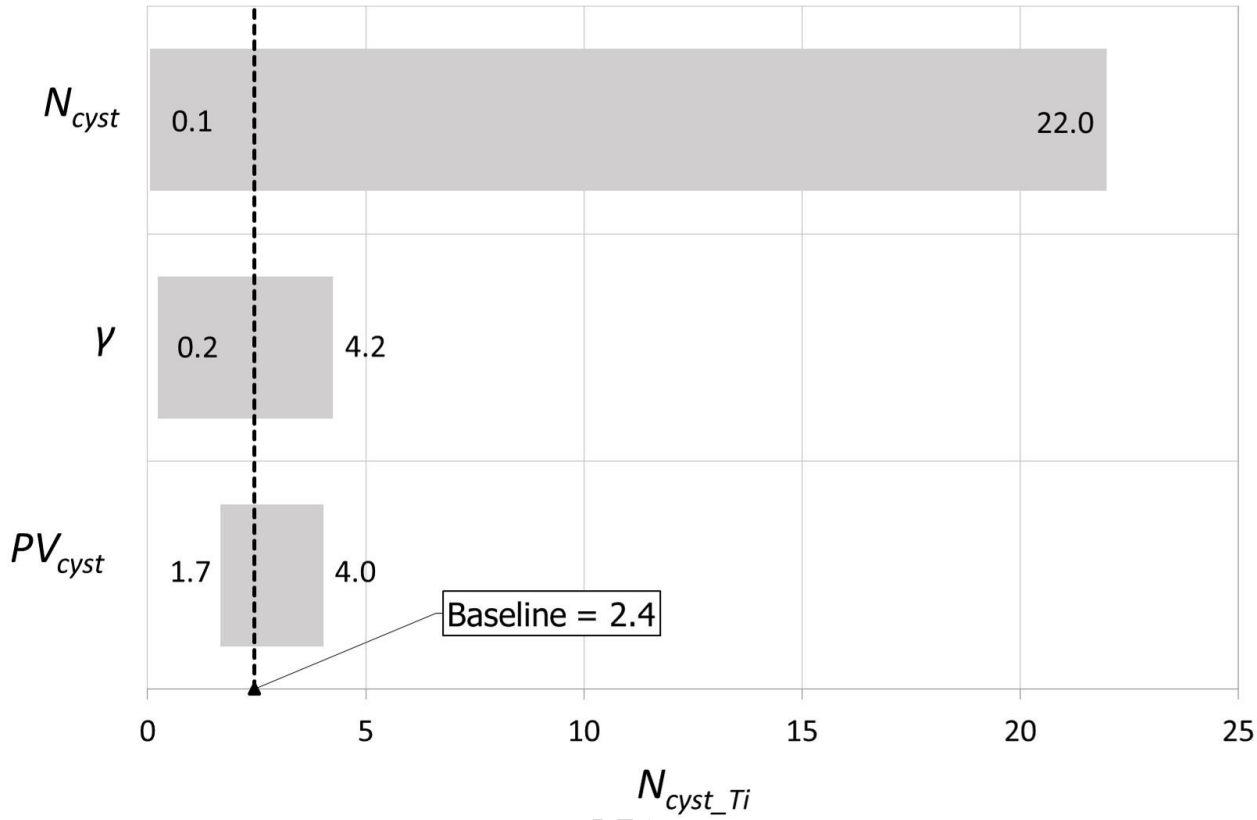


Figure 4

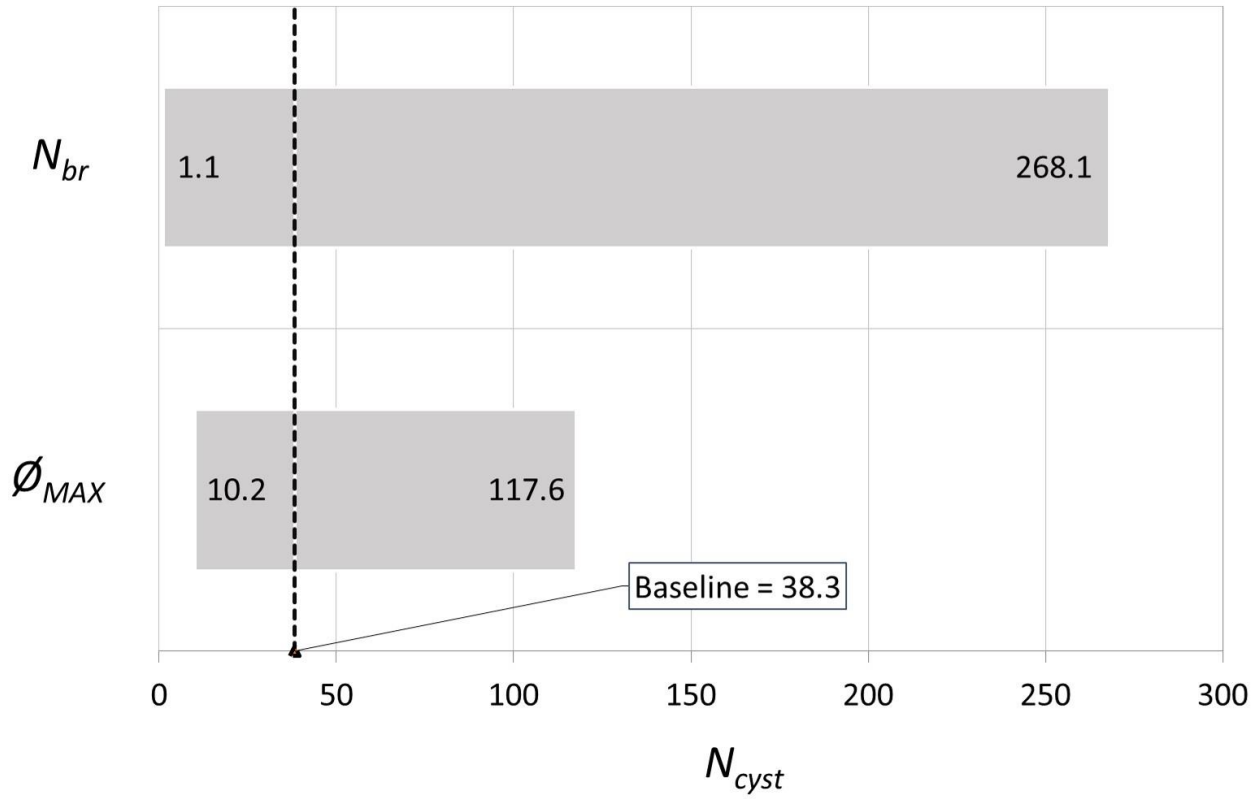
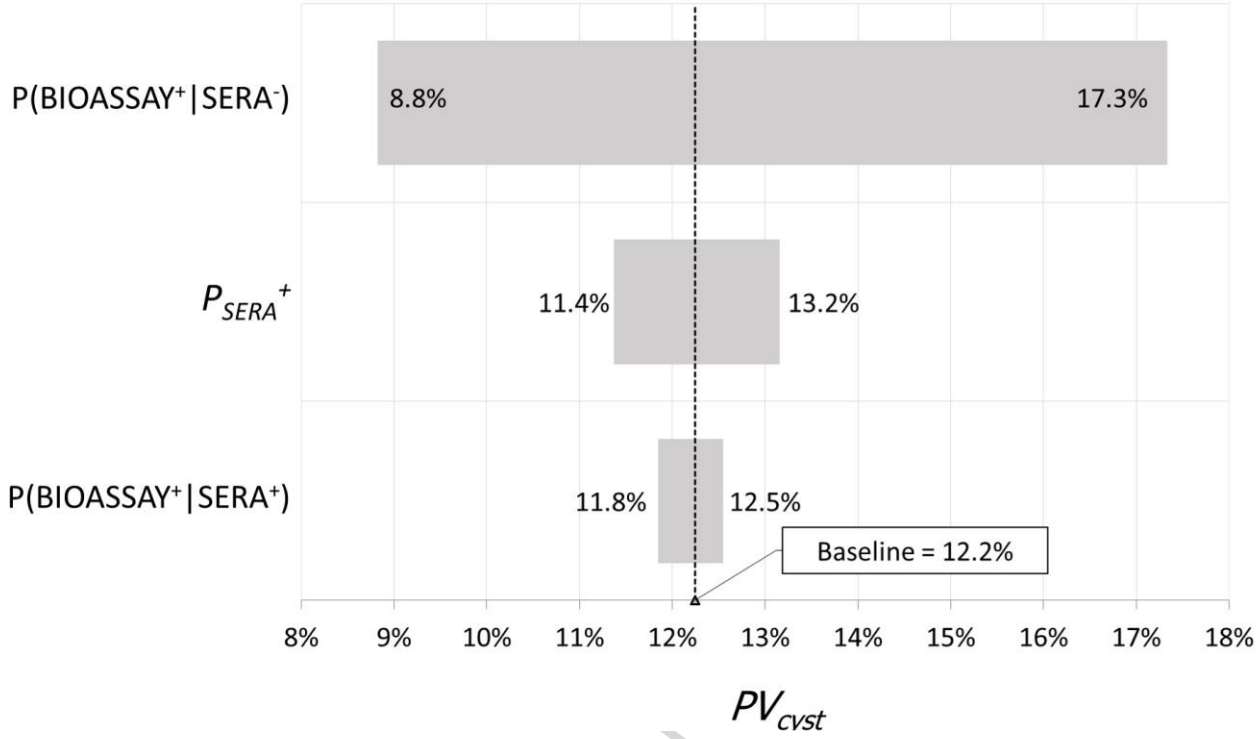


Figure 5



Highlights

Gaps in host-parasite interaction preclude accurate quantitative assessment of exposure to *T.gondii*

The model conceptualized is coherent with current knowledge of the biology of *T.gondii*.

The number of *T.gondii* cysts per portion of infected meat is the variable with high uncertainty