Modeling the risk of human toxoplasmosis in the Danish population from ingestion of tissue cysts present in pork products

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Abstract
In this study we present a quantitative risk assessment model to estimate the risk of human toxoplasmosis from consuming pork in Denmark. The model conceptualized in this study estimates the risk of human toxoplasmosis from ingestion of tissue cysts present in pork. The model predicted that approximately 8-13% of the human population would be infected with the *T. gondii* parasite after 50 years of consuming certain pork products. The total probability of infection is higher for infected pork portions coming from conventional pigs, as compared to organic pigs, due to a much higher consumption rate (despite a higher risk per portion for organic meat). Our model suggests that utilization of tissue cysts as unit of exposure in the model, instead of bradyzoites, is more suitable as a means to quantify risk of exposure to *T. gondii* infection in humans.

Background
Toxoplasma gondii is an important foodborne zoonotic pathogen worldwide. One possible route of infection is via tissue cysts in meat. Meat from infected pigs can infect warm-blooded animals, as experimentally shown for e.g., mice and cats[1,2]. Therefore, eating raw, or undercooked pork may transmit *T. gondii* to humans. *Toxoplasma gondii* infections in humans are usually mild and asymptomatic but can lead to severe complications in immune-compromised individuals. Moreover, in pregnant women, toxoplasmosis can result in congenital toxoplasmosis that can cause abortions or risk to infants of developing postinfectious sequelae over their lifetime. Quantitative microbial risk assessment (QMRA) has been increasingly used to assess risk of acquiring toxoplasmosis from eating meat and meat products, as well as to evaluate the effect of potential mitigation[3-4]. We undertook a QMRA for *T. gondii* in pork. Previous modeling efforts within QMRA for *T. gondii* have been built on the assumption that all infectious bradyzoites are homogenously distributed in a meat portion. However, in reality bradyzoites are clustered within tissue cysts, and it is rare to find more than one cyst per 100g portion of pork. Many portions from the same infected pig may be free of tissue cysts, while a few portions may harbor many bradyzoites concentrated in one or few tissue cysts. Therefore, use of bradyzoites as a unit of exposure may result in overestimation of the risk of exposure per consumed portion. For this reason, researchers have recently advocated using tissue cysts instead[5]. We therefore decided to develop a tissue-cyst-based QMRA model to quantify risk of *T. gondii* from ingestion of several types of pork products, differing in processing steps and food preparation style.

Materials & Methods
The QMRA model was built in the R statistical software[6] to evaluate the risk of human toxoplasmosis from consuming conventional and organic pork products in Denmark. An overview of the modeling steps implemented are shown in Figure 1. Data for the model input

parameters originated from published experiments (mice bioassay data for effect of heat/salting/freezing treatments on cyst survival probability), field studies (true seroprevalence data), simulation models (effect of salting on cyst inactivation and risk of human toxoplasmosis from ingestion of an oocyst) and survey data (consumption)[1-5,7-8]. The QMRA model was fitted with suitable distributions for each parameter. However, sensitivity analysis was applied to evaluate the robustness of the model outcome to the choice of prior distribution for the salting inactivation probability (Beta parameter, U-shaped versus uniform) and the probability of human infection after exposure to a single tissue cyst in an infected portion, also known as the single hit probability.

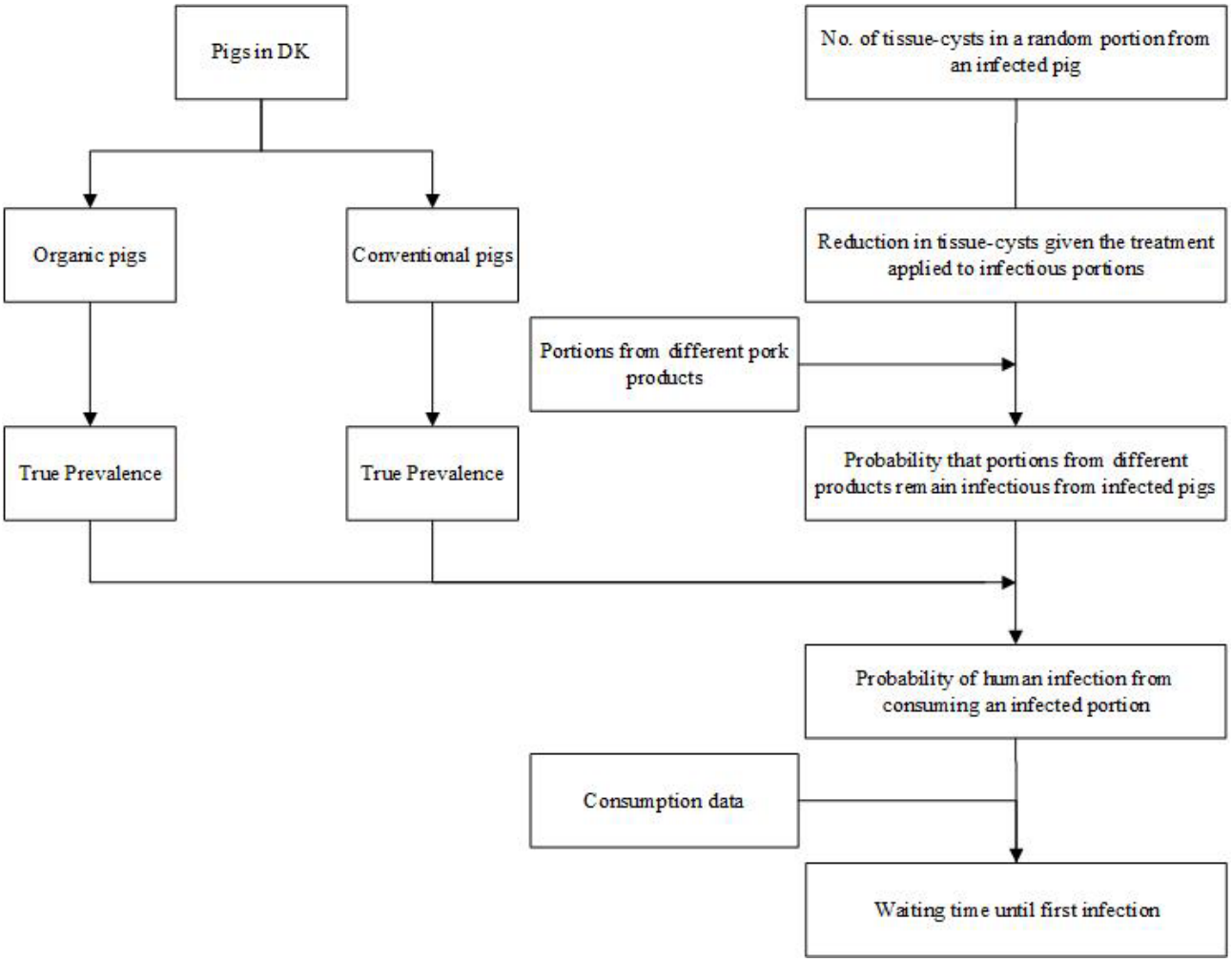

Figure 1: Flow diagram illustrating the model steps in the quantitative microbial risk assessment model developed for predicting risk of human toxoplasmosis.

The QMRA model identified higher-risk pork products originating from conventional and organic finishers by quantifying the number of tissue cysts that remained viable during consumption i.e., after freezing/cooking/salting. By factoring in both the animal-level prevalence and the human risk of infection, where the latter was quantified using a dose-response model considering the single hit probability of an oocyst, the overall probability of an individual becoming infected was quantified. Thereafter the model utilized the consumption data to quantify *T. gondii* infection prevalence as a function of age. For the salting component of the baseline model, the effect of salting on cyst survival was

investigated by taking data from an experimental study conducted under different experimental settings. In the scenario analysis, two additional scenarios were explored for salting component. In the first scenario, the model utilized a published logistic mixed-effects regression model. In the second scenario, the original salting baseline model was extended by using some recent data on the effect of salting. These were not included in the baseline scenario in order to be able to compare the baseline to other QMRA studies that did not yet include the most recent studies.

Results

At the time of consumption, the model estimated viable cysts in about 0.6% of the portions, with the majority (92%) of the infectious portions containing only one cyst. The baseline model predicted approximately 12.5% of the population to have eaten at least one infectious portion after 50 years of consuming portions originating from conventional finishers. Even though the portions from organic pigs constituted only 3% of all the pork consumed, approximately 8% of the population at the same age was estimated to have eaten one or more infectious portion from organic finishers. Moreover, consumption of undercooked portions of pork and dry-cured sausages contributed equally and twice as much as the smoked products to the *T. gondii* prevalence in humans. In the alternative scenario (no.2) for salting, only undercooked portions and dry-cured sausages were identified as high-risk products, whereas smoked products were free of tissue cysts. Hence, in this scenario, the *T. gondii* prevalence in humans at the age of 50 was lower than the prevalence predicted by the baseline model
(approx. 6.6% from conventional pigs; approx. and 4.5% from organic pigs).

Discussion

We developed a tissue-cyst based QMRA model to evaluate risk of *T. gondii* from consuming pork products in Denmark. Use of a tissue cyst as an exposure unit instead of bradyzoites has been previously proposed. In the previous study, a mathematical model was built to estimate the probability of 100g portion containing tissue cysts in a fresh uncooked portion of pork[5]. Our model is conceptually different from the previously published model in that it predicts the number of infectious portions individuals ingest from a prepared product, followed by a dose response model to obtain the proportion of individuals becoming infected with *T. gondii*. The results of this are in line with the previous studies that have identified specific pork products such as undercooked pork, smoked and dry-cured sausages/salamis, as high-risk products[3-4]. We found organic portions have higher hazard, but lower risk due to lower exposure. Therefore, consumption of conventional pork is associated with a higher overall probability of infection than organic pork, because a higher proportion of infectious portions originated from conventional production. The results from the sensitivity analyses suggest the model to be robust. The model outputs were not highly sensitive to the prior choice between Beta (0.5, 0.5) and Beta (1, 1), even though the predicted prevalence was slightly lower with Beta (0.5, 0.5). Additionally, as expected, *T. gondii* prevalence was almost directly proportional with the choice for the hit probability (low = 0.3, average=0.46 high=0.6). In this study, we have considered salting data from recent studies that have shown that dry-cured sausages to be non-infectious in bioassay experiments, even at low salt concentrations (1.3%) within 6hrs. The model output shows that the mean age of first infection with dry-cured sausages is 40 with a 95%confidence interval of [4, 69], which suggests a significant level of uncertainty. This large uncertainty is due to limited amount of data on salting. Hence, it is possible for the attribution to deviate significantly from the mean, and an attribution of a

lower value is still plausible within the given range of uncertainty, which is being explored in the final paper. Hence, the contribution of dry-cured sausages needs further investigation. In this paper, only selected results have been presented and discussed. Additional analysis and insights are contained within an in-preparation peer-reviewed article.

Conclusion

Our QMRA model is based on the use of tissue cysts for estimating risk of becoming infected with *T. gondii* parasite from pork consumption in Denmark. The model allows for flexibility in implementation in a new setting such as in other countries, products and in the future source attribution models for predicting the risk of human toxoplasmosis.

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