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3 **Quantifying the risk of human *Toxoplasma gondii* infection due to consumption of fresh pork in the**
4 **United States**

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Abstract

Toxoplasma gondii is one of the leading foodborne pathogens in the United States. The Centers for Disease Control and Prevention (CDC) reported that *T. gondii* accounts for 24% of deaths due to foodborne illness in the United States. Consumption of undercooked pork products in which *T. gondii* has encysted has been identified as an important route of human exposure. However, little quantitative evaluation of risk due to different pork products as a function of microbial quality at the abattoir, during the production process, and due to consumer handling practices is available to inform risk management actions. The goal of this study was to develop a farm-to-table quantitative microbial risk assessment (QMRA) model to predict the public health risk associated with consumption of fresh pork in the United States. *T. gondii* prevalence in pigs was derived through a meta-analysis of existing data, and the concentration of the infectious life stage (bradyzoites) was calculated for each pork cut from an infected pig. Logistic regression and log-linear regression models were developed to predict the reduction of *T. gondii* during further processing and consumer preparation, respectively. A mouse-derived exponential dose-response model was used to predict infection risk in humans. The estimated mean probability of infection per serving of fresh pork products ranges from 3.2×10^{-7} to 9.5×10^{-6} , corresponding to a predicted approximately 94,600 new infections annually in the U.S. population due to fresh pork ingestion. Approximately 957 new infections per year were estimated to occur in pregnant women, corresponding to 277 cases of congenital toxoplasmosis per year due to fresh pork ingestion. In the context of available data, sensitivity analysis suggested that cooking is the most important parameter impacting human health risk. This study provides a scientific basis for risk management and also could serve as a baseline model to quantify infection risk from *T. gondii* and other parasites associated with meat products.

Key words: *Toxoplasma gondii*, fresh pork, meat, risk assessment

1. Introduction

Toxoplasma gondii is a protozoan parasite which is prevalent in the U.S. population. Based on sample testing from The National Health and Nutrition Examination Survey (NHANES) carried out in 1999-2004, approximately 10.8% of the U.S. population have been infected with *T. gondii* (Jones, Kruszon-Moran, Sanders-Lewis, & Wilson, 2007). In susceptible (not previously infected) populations (approximately 86%), it was estimated that approximately 1.1 million cases of *T. gondii* infection occur annually in the United States (Jones & Holland, 2010), and there were an estimated 4,428 hospitalizations and 327 deaths per year in the early to mid-2000s (Scallan, Hoekstra, Angulo, Tauxe, & Widdowson, 2011).

Most immunocompetent individuals present subclinical symptoms after primary infection; only a small percentage develops severe illnesses such as encephalitis, ocular diseases and pulmonary toxoplasmosis (Montoya & Liesenfeld, 2004). The risk of severe illness is high in specific groups of patients such as congenitally infected fetuses and newborns, and immunologically impaired individuals (UK Food Standard Agency, 2012). *T. gondii* can be transmitted transplacentally from pregnant women to their fetuses when primary infection occurs during gestation. While infected mothers are generally asymptomatic, infected infants may develop chorioretinitis, mental retardation, hydrocephalus, and intracranial calcifications at birth or months to years later (Jones, Ogunmodede, Scheftel, Kirkland, & Lopez et al., 2003). Approximately 190,100 congenital toxoplasmosis cases occur worldwide each year (Torgerson & Mastroiacovo, 2013). In the United States, based on extrapolation of data from regional studies, the Centers for Disease Control and Prevention (CDC) estimated that approximately 400 to 4,000 infants are born with congenital toxoplasmosis each year (Jones et al. 2007). For immunocompromised patients, acquired or reactivation of previously acquired *T. gondii* infection is often severe and sometimes even deadly. Toxoplasmosis was one of the leading causes of death in AIDS patients in the United States before the advent of highly active antiretroviral therapy in the mid-1990s (Luft & Remington, 1992).

Most warm blooded animals can be infected with *T. gondii*. There are three infectious stages of *T. gondii*: tachyzoites, bradyzoites in tissue cysts, and sporozoites contained in oocysts (Hill, Sreekumar, &

Dubey, 2005). Documented human toxoplasmosis outbreaks have been linked to consumption of oocyst-contaminated water and meat contains tissue cysts (Dubey, 2010). Epidemiological studies have demonstrated that meat, particularly pork and lamb, is an important source of infection (Cook et al., 2000; Jones et al., 2009; Boyer et al., 2005). Pork is one of the major consumed meats in the United States, with an individual average consumption of 19.5 kg per capita in 2011, based on boneless retail weight (U.S. Department of Agriculture, 2015a). Pigs are susceptible to *T. gondii* infection and tissue cysts can be harbored in all edible portions of a pig (Guo et al., 2015b). Humans may become infected by ingesting as few as one bradyzoite (i.e., released from tissue cysts in the human gastrointestinal tract) in raw or undercooked meat. Based on expert elicitation, *Toxoplasma*–pork combination was ranked second in terms of predicted foodborne illness burden in the United States, and associated with an average of 35,537 illnesses, 1,815 hospitalizations, 134 deaths, and \$1.2 billion in illness costs annually (Batz, Hoffmann, & Morris, 2012).

Pork is ingested in the United States in two forms: fresh (muscle cuts, usually cooked before consumption) and processed (pork further processed by grinding, curing, smoking, or seasoning prior to wholesale or retail sale) (Davis & Lin, 2005). Processed pork accounts for the majority of pork consumption (13.5 kg per capita in 2011, based on boneless retail weight). However, the infection risk associated with processed pork products is lower than for fresh pork. Meat processing such as salting, freezing, hot smoking, lengthy fermentation, heating, irradiation, and high hydrostatic pressure can reduce or inactivate *T. gondii* tissue cysts in the meat (Mie, Pointon, Hamilton, & Kiermeier, 2008; Guo et al., 2015a). Fresh pork, including pork chops, pork steaks, ribs, fresh ham, other fresh pork, and pork parts, only undergoes a minimal processing, which may not be sufficient to inactivate *T. gondii*. In a U.S. nationwide meat survey conducted in 2005, viable *T. gondii* were detected in 7 of 2,094 fresh pork samples purchased from retail stores (Dubey et al., 2005). A comprehensive and quantitative understanding of infection risk to humans from fresh pork is important in reducing this risk to consumer health.

Quantitative microbial risk assessment (QMRA) is a well-established framework used to understand and assess risks posed by foodstuffs contaminated with pathogenic microorganisms (U.S. Environment Protection Agency & U.S. Department of Agriculture, 2012). While several QMRA studies have been conducted to assess risk associated with foodborne bacteria (U.S. Food and Drug Administration & U.S. Department of Agriculture Food Safety and Inspection Service, 2003; U.S. Department of Agriculture Food Safety and Inspection Service, 2005; U.S. Department of Agriculture Food Safety and Inspection Service, 2001), there are limited risk assessment studies available for *T. gondii*. An Australian qualitative risk-based assessment evaluated the effects of processing on the viability of *T. gondii* tissue cysts in ready-to-eat meats (i.e., a meat or poultry product that is in a form that is edible without additional preparation to achieve food safety). The study concluded that processing steps including freezing, heat treatment, and curing could effectively inactivate *T. gondii* tissue cysts (Mie et al., 2008). A recent QMRA model was developed to quantify *T. gondii* infections associated with meat consumption in the Netherlands (Opsteegh, Prickaerts, Frankena, & Evers, 2011). The model estimated that beef, sheep, pork, and mixed livestock species were responsible for 67.6%, 14%, 11.2%, and 7.1% of meatborne infections, respectively. Approximately 2.8×10^6 new infections per year were estimated in that model in the susceptible Dutch population. To our knowledge, no QMRA study has been carried out for *T. gondii* in meats destined for human consumption in the United States except our recent QMRA study for *T. gondii* due to consumption of domestically produced lamb in the United States (Guo et al., 2016a). Given that there are currently no meat inspection methods that can be used to test for *T. gondii* at slaughter or during processing, it is critical to develop a QMRA model to address the consumer risk of *T. gondii* infection posed by fresh pork. The specific objectives of this study were to (i) systematically collect scientific evidence and information for the QMRA, (ii) develop a farm-to-table risk model to estimate consumer exposure risk due to *T. gondii* from ingestion of fresh pork, (iii) quantify the public health burden associated with ingestion of fresh pork in the U.S. total population and in pregnant women, and (iv) compare the relative risk posed by different fresh pork products.

2. Materials and Methods

2.1 Model overview

In accordance with guidelines by the Codex Alimentarius Commission (CAC) (Codex Alimentarius Commission, 1999), a baseline model was developed to estimate the risk of human *T. gondii* infection due to consumption of fresh pork products in the United States. Since *T. gondii* does not grow outside its host, this model assumed that animal infection only occurs at the farm, and the concentration of *T. gondii* bradyzoites in meat can only be reduced, inactivated, or remain unchanged. Bradyzoite concentration, rather than tissue cyst concentration, was used in this model for three reasons: (i) *T. gondii* tissue cysts are too few to detect in meat (as few as 1 tissue cyst in 50 or 100 g of meat) (Hill & Dubey, 2002), (ii) one tissue cyst could contain a few to several hundred bradyzoites, which increases the uncertainty associated with the model inputs, and (iii) the tissue cyst wall is dissolved in the human gastrointestinal system by enzyme digestion, and the released bradyzoites are responsible for initiating an infection for the intermediate host.

The QMRA model was developed in a Monte Carlo probabilistic framework to account for uncertainty and variability in model parameters and inputs by resampling each distribution using Latin Hypercube sampling over 500,000 iterations. The number of iterations was set to obtain a stable result. Across five independent simulations (each simulation with 500,000 iterations), the arithmetic mean of new infection cases per year varied up to approximately 2.6%, and the probability of infection per serving of arm picnic (i.e., meat comes from the lower part of the pig's shoulder, which is the highest risk cut) varied up to approximately 2.5%. The model was developed in the Microsoft ExcelTM add-in software @Risk (version 6.3, Palisade Inc., Ithaca, NY, USA). For each model iteration, one infected pig was tracked from farm to table (Fig. 1.). The QMRA model divides the exposure assessment into five modules: (1) farm, (2) abattoir, (3) moisture enhancement, (4) cooking, and (5) human consumption. The output of the exposure assessment was used as the input for the dose-response model to estimate the risk of human infection (module 6). The potential exposure to *T. gondii* in a single fresh pork serving was estimated for both the total U.S. population and for the U.S. population of pregnant women.

2.1.1 Module 1: farm

A systematic review and meta-analysis approach was used to select eligible studies, extract data across studies, and integrate results to provide a comprehensive estimate of *T. gondii* prevalence in market pigs (Guo et al., 2016c). The prevalence mean and standard deviation in market pigs was estimated as 0.056 and 0.027, respectively (Guo et al., 2016c). These values were used to define a normal distribution to characterize the variability of *T. gondii* prevalence in market pigs. The normal distribution was truncated at a minimum value of 0.003 and a maximum value of 0.149, the 95% confidence interval (CI) of the computed prevalence (Guo et al., 2016c) (Table 1).

2.1.2 Module 2: abattoir

Market pigs are transported from farm to abattoir for slaughter and further processing. Carcasses are cut into large cuts (four primals: shoulder, loin, side and leg), then further cut to subprimals, portion cuts, and retail cuts (National Pork Board, 2012). Slaughter processes (e.g. scalding, evisceration, and carcass chilling) do not include any steps that could inactivate *T. gondii* tissue cysts residing in the muscle tissue (Guo et al., 2015a). The weight of a live market pig was described as a normal distribution, with the mean and standard deviation set to 122.1 kg and 6.4 kg, respectively. These data were obtained from a pork industry productivity report, which analyzed average weight of conventional finisher pigs from companies representing 35% of the U.S. swine industry production (U.S. Department of Agriculture, 2007). The weight of boneless retail meat obtained from a pig was calculated by multiplying the weight of a market pig by the dressing percentage and retail-cut (boneless) yield rate. Dressing percentage is defined as the yield of dressed carcass from a live animal. Data of average dressing percentage in the United States between 1974 and 2009 (National Pork Board, 2014) were pooled and fit with a triangular distribution. The minimum, most likely (mode), and maximum value of the distribution were set to 70.9%, 70.9%, and 79.0%, respectively (Table 1). The yield rate from carcass to boneless retail cut was set as a fixed value (73.1%) based on USDA data (National Pork Board, 2014). Based on a carcass breakdown diagram (Fig. 2.), the boneless weight of a fresh pork cut can be computed according to the weight percentage of each cut to the total carcass weight (National Pork Board, 2014) (Table 1). Fresh pork cuts included in this QMRA were fresh hams, spare ribs, loins (which includes loin chops and sirloin

roasts), back ribs, tenderloins, arm picnics, blade steaks, and blade roasts. The exposure risk from ground pork was not included with fresh pork and was not estimated in this QMRA because data on cross-contamination during combining and grinding are not available.

No data on bradyzoite concentrations in pork are available at this time. As a result, data were obtained from a real-time PCR study that quantified bradyzoite concentration in muscle samples from 16 experimentally infected goats (Jurankova, Opsteegh, Neumayerova, Kovarcik, & Frencova, 2013). In this experiment, two groups of infected goats (8 goats/group) were killed 30 and 90 days post infection, respectively. For each goat, bradyzoite concentration was quantified in each limb (hind and fore limbs in both left and right sides). A one-way ANOVA analysis and an independent t-test were used to compare the means of bradyzoite concentrations from each limb and at different post infection times, respectively. No statistically significant differences ($p > 0.05$) were found among limbs. Thus, data were pooled together and fit with a lognormal distribution (Table 1). The lognormal distribution was truncated to a minimum value of 0.04 bradyzoite/g (4 bradyzoites/100g) and a maximum value of 41.3 bradyzoites/g (based on the range of the experimental data). The mean of the lognormal distribution was 11.67 bradyzoites/g (Guo et al., 2016a).

T. gondii tissue cysts are unevenly distributed among infected pork cuts. Thus, bradyzoite concentration was calculated individually in fresh pork cuts. Dubey, Murrell, Fayer, & Schad (1986b) reported the uneven distribution of *T. gondii* tissue cysts in commercial pork cuts based on a mouse bioassay. In this experiment, four naturally infected pigs were killed and the carcasses separated into commercial cuts. Ground meat from each pork cut was inoculated subcutaneously in six mice. By combining the data from four naturally infected pigs (total of 24 mice), the probability of *T. gondii* presence in each pork cut was calculated as the ratio of the number of positive mice (mice that became infected) to total mice tested (Table 2). In this QMRA it was assumed that the probability of *T. gondii* presence in loins and back ribs are the same as in tenderloins, because these three pork cuts come from the same primal (loin). A binomial distribution was used to model the probability that a randomly selected pork cut would contain any *T. gondii* (Table 1). Thus, the number of bradyzoites in each pork cut can be

calculated as: bradyzoite concentration (bradyzoites/g) $\times$ boneless weight of a pork cut (g) $\times$ presence of *T. gondii* in one randomly selected pork cut.

2.1.3 Module 3: Moisture enhancement

Fresh pork sold in the United States is often injected with enhancing solutions (a mixed solution of water, salt, and sodium phosphate that can add flavor and moisture to meat products) to extend shelf life, as well as to increase meat quality (U.S. Department of Agriculture, 2013). Pork is passed through a machine with one or more injection needles that inject enhancing solution into the meat (Bohaychuk & Greer, 2003). According to a 2004 retail store survey of enhanced meat, approximately 45% of pork packages were labeled as “enhanced” (American Meat Institute, 2007). Hill et al. (2006) also mentioned that 40-50% of commercially available pork cuts in the United States had been enhanced. A PERT distribution was used to represent the variability of percentage of enhanced pork, with the minimum value, most likely value, and maximum value set to 0.40, 0.45, and 0.50, respectively. A binomial distribution, with parameter p sampled from the above PERT distribution, was used to model the probability that a randomly selected pork cut is injected with enhancing solution (Table 1).

Salt in the enhancing solution can inactivate *T. gondii* bradyzoites in tissue cysts (Dubey, 1997a). A logistic regression model was developed to estimate the effectiveness of inactivation of *T. gondii* due to moisture enhancement, based on data from a published experiment (Dubey, 1997a). In this experiment, brain tissue from *T. gondii*-infected mice and rats were treated under different temperatures (4°C and 10°C), contact time with the enhancing solution (7, 14, 21, 28, 35, 42, 49, and 56 days) and salt concentration (0.85%, 2.0%, 3.3%, and 6% g/ml). Data at higher temperatures (15°C and 20°C) were excluded because the cold chain (<12°C) is generally well maintained through meat processing, transportation, and storage (Richardson, Matthews, Loyd, & Brian, 2009). Treated meats were bioassayed in mice to determine the infectivity, which yielded a dichotomous outcome, mice become infected (assigned a value of 1) or mice do not become infected (assigned a value of 0). Using the JMP pro 11.0 software (SAS Institute, Cary, NC, USA), data were fitted to a nonlinear regression logistic model:

$$P_{\text{pos}} = [1 + \exp (\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3)]^{-1} \quad (1)$$

where P_{pos} (range 0-1) is the probability of a mouse becoming infected by inoculating treated meats under various conditions; X_1 , X_2 and X_3 are three variables in this model, which represent temperature, time, and salt concentration during meat enhancement; $\beta_0 - \beta_3$ are coefficients to be estimated. The Hosmer–Lemeshow goodness-of-fit test statistic was not significant ($\chi^2=69.85$, $p=0.20$), indicating a good fit of the model. The final logistic regression model was:

$$P_{\text{pos}} = [1 + \exp (-0.271 + 0.286 \times \text{temperature } (^{\circ}\text{C}) + 0.022 \times \text{time (days)} - 0.762 \times \text{salt concentration (g/ml)})]^{-1} \quad (2)$$

The three variables in the logistic regression model were described by individual distribution as follows (Table 1). Storage temperature for fresh meat from manufacture processing to home cooking are generally maintained at a cold chain temperature ($<12^{\circ}\text{C}$), except that occasionally (approximately 3% of cases) refrigerated meats could reach 15°C or higher during transportation from retail to home (Ecolab, 2008). No data were found to report transportation temperatures in enhanced pork products. In this model, enhanced pork products were assumed to be kept at retail temperature for most of their shelf life. Thus, the parameters were obtained by fitting display temperature values of the fresh meat counters (Ecolab, 2008) with a normal distribution, and the mean and standard deviation set to 3.39°C and 2.61°C , respectively. The normal distribution was truncated at 0 and 19.44°C . Information on storage time (from moisture enhancement to home cooking) for enhanced pork products were not found, thus, the suggested shelf life of enhanced pork by manufacturers was used. Storage time was described as a Uniform distribution, ranging from 14 to 21 days (Tyson Foods, 2013). Salt (NaCl) concentration in enhanced pork is determined by the equation (Adler et al., 2011):

$$C_{\text{salt}} = \frac{\%ES \times C_{\text{ES}}}{1 + \%ES} \quad (3)$$

where C_{salt} is the salt concentration in an enhanced pork cut, %ES (wt/wt) is the pumping ratio of enhancing solution injected into a fresh pork cut, and initial weight of meat before enhancement, and C_{ES} is the salt concentration in the enhancing solution (Adler et al., 2011; Barbut, 2000). Probability

distributions were used to represent the variability of %ES and C_{ES} (Table 1). Injection normally adds 7 to 15% of enhancing solution by weight to a fresh pork cut (National Pork Board, 1998), and a 12% pumping ratio is commonly used in the meat industry (Miller, 2010). A PERT distribution was used, with the minimum, most likely, and maximum values set to 0.07, 0.12, and 0.15, respectively. For salt concentration in the enhancing solution, a uniform distribution ranging from 0.08 to 0.14 was used, according to the recommended salt concentration in curing brines (Food and Agriculture Organization of the United Nations, 2007). Under each combination of temperature, time, and salt concentration, the probability of infection for a mouse inoculated subcutaneously with an enhanced *T. gondii*-infected meat sample can be calculated by the logistic regression model (equation 2). However, this does not directly reflect the reduction in bradyzoite concentration after moisture enhancement. A binomial distribution (Binomial (1, P_{pos}), which is equivalent to Bernoulli distribution with $p = P_{pos}$, i.e., Bernoulli (P_{pos})) was used to describe the presence of *T. gondii* in one randomly selected meat cut after enhancement, where the value of 1 represents existence of *T. gondii* in meat, and the value of 0 indicates inactivation of *T. gondii* in meat (Table 1).

2.1.4 Module 4: cooking

Cooking is one of the most effective ways to reduce exposure to *T. gondii* in infected fresh pork. However, low cooking temperatures as well as short cooking times may not be sufficient to inactivate *T. gondii* bradyzoites. The U.S. Continuing Survey of Food Intakes by Individuals (CSFII) reported that approximately 82% of fresh pork was purchased at retail stores and prepared at home, while the other 18% of fresh pork was generally purchased from commercial food service establishments, or obtained from places such as school cafeterias and community feeding programs (U.S. Department of Agriculture, 2015b). In this QMRA, it is assumed that 100% of fresh pork is purchased at retail stores and cooked at home by consumers. It is also assumed that cooking is the only factor associated with reduction/inactivation of *T. gondii* in home kitchens. Other factors that could potentially reduce (e.g., consumer marinating fresh pork overnight in the refrigerator before cooking, and consumer freezing fresh pork and thawing it before cooking) or redistribute (e.g. cross-contamination among a cutting knife, a

cutting board and hands) *T. gondii* in fresh pork were not included in this QMRA. The thermal effect of home cooking on bradyzoite concentration was modeled using a time–temperature profile developed by Dubey, Kotula, Sharar, Andrews, & Lindsay (1990). This study associated different time-temperature combinations to a binary outcome (*T. gondii* detected or non-detected, Table 3), thus dividing the time-temperature parameter space into “complete inactivation” and “non-inactivation” zones (Dubey et al., 1990). In this thermal inactivation experiment, *T. gondii*-infected meats were treated for different times (0.01, 3, 6, 9, 12, 24, 48, and 96 minutes) and temperatures (49, 52, 55, 58, 61, 64, and 67 °C), and each treated sample was bioassayed in five mice (Dubey et al., 1990).

Data on home cooking times, or time-temperature combinations, are not available. However, the Ecosure 2007 home cooking data (Ecolab, 2008) contains 275 finished cooking temperatures for pork. Finished cooking temperature is defined as the cooking temperature recorded by consumers upon completion of cooking pork and removal from heat, which varies among consumers according to their cooking preference. Cooking temperature data were fitted with a normal distribution (mean: 69.26°C, standard deviation: 13.42°C), truncated between 27.78°C and 100.56°C (based on the range of the Ecosure 2007 home cooking data for fresh pork).

Based on the finished cooking temperature, resampled from its distribution in each model iteration, three outcomes of the cooking module were considered. First, when the finished cooking temperature is higher than 61°C, pork is assumed to be cooked sufficiently and be completely free of *T. gondii* at the end of cooking. Second, when the finished cooking temperature is lower than 49.9°C, it is assumed no reduction occurred. Third, when the finished cooking temperature is between 49.9°C and 61°C, a log-linear regression was developed to model the percentage of *T. gondii* reduction during cooking. We only selected data for time at 0.01 minute from the thermal inactivation experiment (Dubey et al., 1990) for two reasons: (i) Ecosure 2007 finished cooking temperature is comparable with the measured core temperature in the experiment (Opsteegh et al., 2011), and (ii) data for cooking time for fresh pork are not available at this time, thus, a conservative estimation was conducted. Again, in the experiment by Dubey et al. (1990) *T. gondii* reduction in pork under different cooking conditions was not

measured by bradyzoite counts, but as the percentage of mice becoming infected or not (binary outcome) after being subcutaneously inoculation with a meat sample (Table 3). The regression equation (with the temperature as the independent variable, and the decimal logarithm of the percentage of infected bioassay mice as dependent variable) was calculated in OriginPro 9.0 software (OriginLab Corp., Northampton, MA, USA) and was described as:

$$\text{Log}_{10}(\text{reduction factor}) = 8.583 - 0.172 \times \text{temperature } (^{\circ}\text{C}) \quad (4)$$

where the reduction factor (values between 0-1) is the degree of *T. gondii* inactivation in a pork cut during cooking, the value of 1 indicates no inactivation occurs, and the value of 0 indicates the complete inactivation of *T. gondii* in meat; temperature is the meat internal temperature at the end of cooking. The value of adjusted R^2 for thermal reduction curve is 0.97, indicating an excellent fit (Fig. 3.). The estimated reduction factor is bound to be between 0 (i.e., 100% inactivation) and 1 (i.e., zero inactivation). The fitted regression curve rises above 1 (i.e. crosses the “zero inactivation” line) at a temperature of 49.9°C. Hence, whenever a temperature between 49°C and 49.9°C was selected in the model, the corresponding reduction factor was set to 1.

2.1.5 Module 5: consumption

The purpose of the consumption module was to estimate the number of bradyzoites present in one serving of fresh pork at the time of consumption, and to calculate the numbers of servings per year in both the total U.S. population and the population of pregnant women. Consumption data were extracted from the USDA Food Availability per Capita Data System (FADS) for the total U.S. population. Approximately 20.9 kg of retail pork, corresponding to 19.5 kg boneless pork, were consumed per person in 2011 (U.S. Department of Agriculture, 2015a). By excluding approximately 4.4% of imported meat (U.S. Department of Agriculture, 2015a), the per capita consumption of pork from domestic pork production in 2011 was 18.7 kg.

Pork consumption data for pregnant women or women in the child-bearing age are not available. Thus, consumption data were extracted from the closest group (women in 20-39 years) in the CSFII survey (U.S. Environment Protection Agency, 2003). Per capita consumption was calculated by different

methods in the FADS and the CSFII survey. While the former is based on a “disappearance” model based on the U.S. meat inventory of 2011, the latter was based on 16,000 individuals that recorded their pork consumption data in 24- or 48-hour dietary recalls between 1994 and 1996. An adjustment factor ($19.5 \text{ kg} / 21.9 \text{ kg} = 0.89$) was computed by calculating the difference of per capita consumption between the FADS (19.5 kg) and the CSFII (21.9 kg) in the total U.S. population. Thus, for pregnant women, the per capita consumption of pork in 2011 was computed as 15.5 kg ($17.4 \text{ kg} \times 0.89$ (adjustment factor)). The number of pregnant women per year was described based on the live births in 2011 (3,953,590 of live birth in 2011) (U.S. Centers for Disease Control and Prevention, 2013).

Consumption data for individual fresh pork cuts was estimated by multiplying the total pork consumption by the proportion of consumption of each fresh pork cuts (Davis & Lin, 2005). The proportion of consumption of loin, blade steak, and fresh ham were estimated as 10.8%, 3.7%, and 4.9%, respectively (Davis & Lin, 2005). The consumption data for back rib, spare rib, blade roast, tenderloin, and arm picnic are not available. Thus, the proportion of consumption assigned to these pork cuts was based on the ratio of retail weight of a pork cut to retail weight of a carcass. The proportion of ribs was estimated as 3.7% (Davis & Lin, 2005), and was further allocated to back rib (1.2%) and spare rib (2.5%). Similarly, the share of non-specified fresh pork (13.1%) (Davis & Lin, 2005) was allocated to blade roast (4.9%), tenderloin (0.9%), and arm picnic (7.3%) (Table 1).

The amounts of fresh pork ingested in a single meal (serving size) in the total U.S. population and the pregnant women population were modeled by a normal distribution (mean: 85.0 g, standard deviation: 25.5 g) truncated at 2.8 g and 170.1 g (Miller, Liu, McNamara, & Barber, 2005). The number of servings per year was calculated for each meat cut. For example, the number of servings per year for fresh ham was calculated as follows:

1. Total U.S. pork consumption = 19.5 kg per capita consumption of pork per year $\times$ 312,040,000 of U.S. population in 2011 = 6,084,780,000 kg/year. (in 2011, boneless weight)
2. Total U.S. pork consumption from domestic production = Total U.S. pork consumption $\times$ (1-%import) = 6,084,780,000 kg/year $\times$ (1-4.4%) = 5,817,049,680 kg/year (in 2011, boneless weight)

3. Total fresh ham consumed per year = Total U.S. pork consumption from domestic production $\times$ proportion of fresh ham to total pork consumption = 5,817,049,680 kg/year $\times$ 4.9% = 285,035,434 kg/year (in 2011, boneless weight)

4. Number of servings of fresh ham per year = total fresh ham consumed per year / serving size = (285,035,434 kg/year) / (0.085 kg/serving) = 3,353,358,051 servings/year (in 2011, based on the mean value of the serving size distribution)

2.1.6 Module 6: dose-response model and risk characterization

We developed an exponential dose-response model (Guo et al., 2016b) by fitting experimental data from mice orally infected with Type II bradyzoites (Dubey, 1997b), and validated against dose-response data obtained from rats (Dubey, 1998). The dose-response was described in Fig. 4.

$$P_{DR} = 1 - \exp(-r \times 0.005 \times d) \quad (5)$$

where P_{DR} is the probability of infection, d is the dose per serving (bradyzoites per serving), r is the model parameter that describes the probability of one bradyzoite initiating an infection, and 0.005 is a scaling factor (Guo et al., 2016b). The probability of infection per serving of fresh pork (P_{serv}) was computed by multiplying the probability of infection (P_{DR}) by the prevalence of *T. gondii* in market pigs (Latorre, Pradhan, Van Kessel, Karns, & Boor, 2011):

$$P_{serv} = P_{DR} \times \text{Prevalence} \quad (6)$$

The uncertainty associated with the estimated dose-response parameter r was determined using a bootstrap procedure. Following the procedure recommended by Haas, Rose, & Gerba (2014), 100,000 bootstrap samples were constructed in MATLAB (MathWorks, Inc. Natick, MA, USA) by random sampling with replacement within each dose. The set of 100,000 bootstrapped values of r was fitted with a lognormal distribution, resulting in a mean value of 0.00169 and standard deviation of 0.00067. The lognormal distribution was truncated between 0.00004 and 0.01276, based on the range of 100,000 bootstrapped values of r (Guo et al., 2016a).

In addition to the probability of infection per serving associated with each pork cut, risk was characterized by three other outputs: (i) the number of new infections per year in the total U.S. population,

(ii) the number of new infections per year in the U.S. pregnant women population, and (iii) the number of congenital toxoplasmosis cases per year. The mean number of infection cases per year due to ingestion of an individual pork cut was computed by multiplying the probability of infection per serving by the number of individual pork cut servings per year. The number of infection cases per year associated with consumption of fresh pork was computed by summing all infection cases associated with each fresh pork cut. Individuals already infected with *T. gondii* were excluded from the incidence calculation, because earlier developed immunity prevents them from subsequent infections (Opsteegh et al., 2011). Specifically, *T. gondii* seroprevalence of 10.8% and 11.0% was observed in the U.S. population of age 6-49 years and in women of age 15-44 years, respectively (Jones et al., 2007). The number of infections per year was multiplied by an adjustment factor of 0.892 (1-0.108) and 0.890 (1-0.11) for the total U.S. population and the pregnant women population, respectively, to account for the fact that only a fraction of the population is susceptible. Primary infection during gestation may result in an infant acquiring congenital toxoplasmosis. Dunn et al. (1999) observed 161 congenital infection cases in a cohort study of 557 infants whose mother acquired *T. gondii* infection during pregnancy in France between 1987 and 1995, and estimated the overall maternal-fetal transmission rate to be 29% (161/557). A Beta distribution ($s=161$, $n=557$) was used to model the maternal-fetal transmission rate. The mean number of congenital toxoplasmosis cases per year was estimated by multiplying the mean number of new infections per year in the pregnant women population by the maternal-fetal transmission rate.

2.2 Sensitivity analysis

A sensitivity analysis was performed to provide a quantitative measure of the most important parameters affecting the risk to human health from *T. gondii* via the pathways considered in the model. The Spearman rank correlation test was used to determine the impact of distributed inputs and parameters on the probability of *T. gondii* infection per serving and the number of new infections per year in the total U.S. population.

3. Results

3.1 Estimated number of *T. gondii* infection cases due to consumption of fresh pork

The number of total fresh pork servings consumed per year in the United States was estimated as 24.8 billion. The mean number of new *T. gondii* infection cases per year associated with consumption of fresh pork for the total U.S. population and pregnant women were 94,606 and 957, respectively. Based on the annual number of new infection cases in pregnant women, the mean number of congenital toxoplasmosis cases per year was 277.

3.2 Estimated number of *T. gondii* infection cases due to consumption of individual fresh pork cuts

The probability of infection per serving of fresh pork cut and the number of new infections per year associated with individual cuts are shown in Table 4. The estimated probability of infection per serving for each cut presented a highly skewed distribution. Human infection risk varies by individual fresh pork cut, the mean probability of infection per serving of fresh pork products ranges from 3.2×10^{-7} to 9.5×10^{-6} . Among individual cuts, consumption of arm picnic was associated with the highest probability of infection per serving and number of new infection cases per year. Spare rib, back rib, and tenderloin pose a relatively lower infection risk than pork chop, steak and blade roast. The lowest probabilities of *T. gondii* infection per serving and the number of annual cases were associated with consumption of fresh ham (Table 4). The percentage of *T. gondii*-positive servings of individual pork cuts at the time of consumption ranges from 1.48 to 13.35% (Table 5). For the highest-risk cut, arm picnic, 67,244 out of 500,000 simulated servings (13.35%) contained *T. gondii* bradyzoites, which is consistent with the estimated high number of annual cases. Based on data from these positive servings, the probability of infection per positive serving of each cut was further calculated and presented in Table 5.

3.3 Effect of variability in model parameters on the risk of infection

Sensitivity analyses indicated that cooking temperature and moisture enhancement treatment were the most important factors that affect the probability of infection and number of new infection cases (Fig. 5.). The order of importance of the parameters after moisture enhancement was the presence of

bradyzoites after moisture enhancement, number of bradyzoites in arm picnic, and number of bradyzoites in Boston butt.

4. Discussion

In the present study, we developed a QMRA model to predict the number of human *T. gondii* infection cases associated with fresh pork consumption in the United States, and compared the relative infection risk from different pork cuts. The majority of fresh pork servings were *T. gondii*-negative and free of risk, while a small proportion of servings contained *T. gondii* and could initiate infection. The risk model estimated that approximately 10^5 new infections per year occurred in the total U.S. population due to fresh pork ingestion. Jones et al. estimated that approximately 10^6 persons are infected with *T. gondii* each year, based on the 2009 U.S. Census and NHANES data (Jones & Holland, 2010). The consistency of model estimates with epidemiological studies could be partly attributed to the inclusion of a scaling factor in the dose-response module. Without using the scaling factor, this risk model would overestimate the annual number of new infections in the total U.S. population approximately 60-fold (data not shown). The scaling factor was used to match the number of new infections predicted by this model with epidemiological observations (Guo et al., 2016b). This approach has been previously applied, for example, to develop a risk model for *Listeria monocytogenes* in ready-to-eat foods (U.S. Food and Drug Administration & U.S. Department of Agriculture Food Safety and Inspection, 2003).

For the subpopulation of pregnant women, 957 new infections per year was estimated by the present model, which resulted in 277 congenital toxoplasmosis cases each year in the United States. The CDC estimated that 400–4,000 congenital infections occur per year in the United States (Lopez, Dietz, Wilson, Navin, & Jones, 2000). Based on screening programs in Massachusetts and New Hampshire, Guerina et al. (1994) found that congenital toxoplasmosis occurred in 1 out of 10,000 live births (Guerina et al., 1994). According to this ratio, 3,953,590 U.S. live births in 2011 would result in approximately 395 congenital infections. Our estimate is lower than these epidemiological findings, which is justifiable since fresh pork consumptions is just one of several possible infection sources.

Based on model estimates, among fresh pork cuts included in this QMRA, arm picnic is associated with both the highest number of infections per year and the highest probability of infection per serving. One possible explanation is that *T. gondii* tissue cysts have a high affinity for the muscle tissue used for arm picnic. In the experimental data used to derive the probability of *T. gondii* presence in different cuts, *T. gondii* was detected from the arm picnic of 3 out of 4 naturally infected pigs (Dubey et al., 1986b). All 6 mice inoculated with the arm picnic from these 3 pigs tested positive for *T. gondii*, indicating a higher number of *T. gondii* tissue cysts present in arm picnic than in other cuts. In another study, among all commercial pork cuts *T. gondii* was more frequently detected from arm picnic (3 of 5 infected pigs) than other cuts (Dubey, 1988).

According to the USDA's National Animal Health Monitoring System (NAHMS), *T. gondii* seroprevalence in market pigs has been maintained at a fairly stable low level. *T. gondii* seroprevalence in 1995, 2000 and 2005 NAHMS surveys was 3.2% (Patton et al., 1996), 0.9% (Patton, Faulkner, Anderson, Smedley, & Bush, 2000) and 2.6% (Hill, Haley, Wagner, Gamble, & Dubey, 2010) in grower/finisher pigs, respectively. In this QMRA, prevalence of market pigs was taken from a meta-analysis study that integrated all available prevalence studies in the United States, which includes not only nationwide surveys (e.g., NAHMS), but also small-scale studies. The meta-analysis estimated *T. gondii* prevalence at 5.6% in U.S. market pigs. By doubling *T. gondii* prevalence (11.2%) or decreasing prevalence by 50% (2.8%), the mean number of new infections was changed to 196,175 (207%) and 52,324 (55.3%), respectively. These results suggest that control of *T. gondii* prevalence at the farm level is an important strategy to reduce the human infection burden.

Home cooking temperature was the most important parameter affecting the model outcomes, according to a correlative sensitivity analysis. In 500,000 simulated servings, the number of *T. gondii*-positive arm picnic servings was reduced from 201,475 (40.3%) to 67,244 servings (13.35%) after cooking. In the cooking module, pork was assumed to be free from *T. gondii* when the finished cooking temperature was higher than 61°C. This assumption was based on a thermal reduction curve (proportion of samples with non-detectable *T. gondii* after the treatment, as a function of cooking temperature) for a

0.01-minute cooking time, developed from experimental data (Dubey et al., 1990). In that experiment, *T. gondii* tissue cysts were inactivated when meat samples were treated at 61°C or higher (i.e., 61°C, 64°C, and 67°C) for different treatment times (0.01, 3, 6, 9, 12, 24, 48, and 96 minutes) with an exception of a sample treated at 64°C for 3 minutes (Dubey et al., 1990). Experimental heating conditions (2-mm-thick pouches equilibrated to the experimental temperature in a water bath) are different from how consumers cook pork products at home. Furthermore, experimental samples were a mixture of infected pig heart, tongue and muscle tissues, rat brains, and mouse brains, and the number of *T. gondii* tissue cysts in rodent brains was observed to be 100-fold or higher than in infected pork (Dubey, Brake, Murrell, & Fayer, 1986a). *T. gondii* concentration in experimentally-infected meat samples is also much higher than in naturally-infected pork meat. Therefore, while this study employs all available information on thermal reduction of *T. gondii*, due to the discrepancies between experimental conditions and reality, we still do not possess sufficient data to determine a recommended cooking temperature able to completely inactivate *T. gondii* in fresh pork. The log-linear regression equation reported here was developed based on data at 0.01 minute treatment time from the experimental study (Dubey et al., 1990). The 0.01 minute time count began when experimental samples reached the designated internal temperature, which is comparable to the finished cooking temperatures recorded by consumers at the end of cooking, since these temperatures possibly represent the maximum temperatures reached (Opsteegh et al., 2011). The developed log-linear temperature inactivation relationship for *T. gondii* demonstrates the logarithmic percentage of reduction is inversely proportional to the finished cooking temperature, and provides a good estimation when the finished cooking temperatures are between 49.9 to 61°C (Guo et al., 2016a).

Predicted risk outcomes were also affected by moisture enhancement treatment. *T. gondii* reduction was estimated by a logistic regression model that was developed from experimental data. Mouse brains containing tissue cysts were treated at different combinations of NaCl concentration, temperature, and storage time post-enhancement. Subsequently, the treated samples were inoculated into mice for bioassay. One of the limitations of the animal bioassay is that only dichotomous (infected/not infected) data can be obtained and the actual reduction in *T. gondii* levels cannot be directly estimated.

The reduction was reflected by the probability of mice getting infected, which yielded only two outcomes, *T. gondii* presence in samples (mice become infected, *T. gondii* concentration is unchanged) or *T. gondii* absence in samples (mice do not become infected, *T. gondii* concentration is reduced to 0). Further, in this experiment mouse brains, rather than pork samples, were used. Bradyzoite concentration and survival kinetics in mouse brain tissue could be different from pork meat tissues. In addition, the logistic regression was based on 3 variables considered in experimental trials. It is possible that other factors can also affect the survival of *T. gondii* during moisture enhancement. For example, other ingredients in the enhancing solution (e.g., other salts, phosphates, antioxidants, and flavorings) may also affect the survival kinetics of *T. gondii* in meat. Experiments have shown that sodium lactate, sodium triphosphate, sodium diacetate and potassium lactate can inactivate *T. gondii* in meat (Hill et al., 2006; Hill, Sreekumar, Gamble, & Dubey, 2004), but insufficient data were available to include these factors in the present model. In addition, the distributions of the three variables in the logistic model are mainly based on government guidelines. In reality, most information on the moisture enhancing process is proprietary. Given the potential risk reduction impact of this pre-market processing step, a more systematic investigation of its effectiveness is warranted.

To the best of our knowledge, this is the first QMRA model of *T. gondii* infection associated with consumption of fresh pork in the United States. Previously, a QMRA model was developed to predict meatborne *T. gondii* infection in the Netherlands (Opsteegh et al., 2011), based on data from the Netherlands and with the infection dose calculated indirectly. Another QMRA model was developed to predict *T. gondii* infection through consumption of domestically produced lamb in the United States (Guo et al., 2016a). The *T. gondii* QMRA model presented here reflects the best current knowledge on fresh pork production and processing in the United States. In contrast to the Netherlands QMRA model, *T. gondii* bradyzoite concentration was modeled in pork edible tissues and the dose for the exposure assessment was calculated directly in our QMRA model. In addition, a mouse-derived human dose-response model was developed and was used in our risk model, rather than applying a mouse dose-response model as a human surrogate. Furthermore, the uneven distribution of *T. gondii* tissue cysts was

included in our model, which resulted in bradyzoite concentration that varying among pork cuts. Finally, a method that divides the temperature into three zones (“complete inactivation temperature zone: $>61^{\circ}\text{C}$ ”, “non-inactivation temperature zone: $<49.9^{\circ}\text{C}$ ”, and “reduction temperature zone”: $49.9 - 61^{\circ}\text{C}$ ”) was used in our QMRA model.

Besides the inherent limitations based on assumptions in the current QMRA model, several limitations and knowledge gaps were identified and are described below.

(1) Freezing is acknowledged as an important parameter, but is not incorporated in this QMRA model due to the limited data availability. *T. gondii* in pork meat can be inactivated by freezing either at -10°C for 3 days, or at -20°C for 2 days (Dubey, 2010; Dubey, 1996). Fresh pork can be frozen either by meat producers during processing or by consumers at home. A majority of meat manufacturers claim that their fresh meats have never been frozen. In contrast, a substantial proportion of consumers prefer to store meat in home freezers. In a national mail survey, 66% of 611 American consumers stored meat in home freezers (Li-Cohen & Bruhn, 2002). However, the actual percentage of fresh pork that is frozen, as well as temperature and duration, is largely unknown (based on personal communication with experts from National Pork Board and the USDA); accounting for freezing would lower risk estimates.

(2) Cross-contamination could occur at any point from farm to table, but is not included in this QMRA due to absence of data. The main steps that could contribute to cross-contamination via fluids or meat particles are: bleeding, evisceration, washing, splitting and cutting processes in the abattoir; injecting enhancing solutions during moisture enhancement; and meat preparation in the consumers’ kitchen.

(3) Data to model bradyzoite concentration in pig muscle tissues were obtained from a goat study that used a sequence-specific magnetic capture method for extracting *T. gondii* DNA from samples, and quantified *T. gondii* concentration by real-time PCR. This real-time PCR method has a detection limit of 227 parasites per 100 g meat sample; bradyzoite concentration was quantified by a linear regression model between the number of bradyzoite and the Cp -values

(crossing point-PCR-cycle, i.e., the cycle at which fluorescence achieves a defined threshold) (Opsteegh et al., 2010). In addition, goats were experimentally infected with high doses of oocysts, which is likely different from doses encountered in farm settings. Bradyzoite concentration in pork might be different than in goats. However, bradyzoite concentration in pork is unknown because it is difficult to determine, given tissue cysts are sparse and unevenly distributed (Dubey et al., 1986b). Therefore, bradyzoite concentration data were extracted from a goat study, and although this is a limitation, these are only data available.

(4) For the total U.S. population and pregnant women population, estimates of consumption of spare rib, back rib, tenderloin, arm picnic and blade roast were based on the assumption that the amount consumed is directly proportional to the amount produced. No comprehensive survey of fresh pork consumption is available for the United States. Furthermore, in the total U.S. population, the per capita food availability data were used to model the total annual pork consumption, as a proxy for actual food consumption. The calculation does not account for spoilage and food waste at retail and at home, resulting in an overestimation of actual consumption. The USDA loss-adjusted food availability system estimated fresh pork losses at retail and at home as 4.5% and 29%, respectively (U.S. Department of Agriculture, 2015b). However, these parameters are mainly used to estimate caloric intake, food patterns, and the value of food loss, rather than to predict actual consumption.

(5) The dose-response model used in this QMRA was developed based on the data from a low-virulence strain (Type II). Hence, it may not reflect the dose-infection relationship of strains with higher virulence. Further, risk of infection was used as the endpoint for the total U.S. population in the present QMRA. Except for congenital toxoplasmosis in fetuses, other severe health outcomes (e.g., ocular disease, encephalitis, and death) were not estimated due to the limited data on infection-to-illness probability. The scaling factor developed for the total U.S. population was also used to calculate the number of new infections in pregnant women. Animal experiments have demonstrated an increased susceptibility to *T. gondii* infection in pregnant mice, possibly due to

changes in immune function and hormone levels (Luft & Remington, 1982). Some epidemiological studies in humans have shown that pregnant women have a lower resistance to *T. gondii* (a higher risk of seroconverting) than nonpregnant women (Avelino, Campos, de Parada, & de Castro, 2004; Avelino, Campos, de Parada, & de Castro, 2003). Therefore, the use of an alternative scaling factor would give a more accurate estimate of annual new infection cases in pregnant women.

5. Conclusions

The present QMRA model quantifies the risk of *T. gondii* infection posed by consumption of fresh pork in the United States. Our model estimates that a considerable number of new infections are associated with fresh pork consumption each year, providing evidence for risk managers that more attention is warranted to this route of exposure to *T. gondii*. In addition to further reductions of *T. gondii* prevalence in pigs, our study demonstrated that thorough cooking is another important way to reduce infection risk. Several limitations and data gaps were identified, and more research is needed to reduce the uncertainty associated with the present model. Given the complex life cycle of parasitic pathogens and the bioassays used to detect the pathogen's presence (use animals to detect the presence of *T. gondii*, rather than common enrichment and culture methods), the mathematical tool employed in this QMRA model differs significantly from tools used to model bacterial or viral pathogens. As such, this model could serve as a baseline tool to quantify infection risk from *T. gondii* and other parasites associated with meat products.

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623 Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the
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830 **Table 1**
831 Description and statistical distribution of the QMRA model.

Variable	Description	Value/Distribution	Source
Farm			
Prevalence	Number of positive market/finisher pigs, estimated via a quality-effects meta-analysis.	Normal distribution (μ , σ), $\mu=0.056$, $\sigma=0.027$, truncated to (0.003, 0.149).	Guo et al., 2016c
Concentration	Bradyzoite concentration in pork meat, data obtained from a real-time PCR study.	Lognormal distribution (μ , σ), $\mu=11.67$, $\sigma=97.31$, truncated to (0.04, 41.3) bradyzoites/g.	Jurankova et al., 2013; Guo et al., 2016a
Abattoir			
Weight of a market pig	Weight of a pig at time of slaughter.	Normal distribution (μ , σ), $\mu=122.1$, $\sigma=6.4$ kg.	National Pork board, 2014
Dressing percentage	Yield of dressed carcass from a live pig.	Triangular distribution (0.709, 0.709, 0.790)	National Pork Board, 2012
Boneless retail-cut yield rate	Yield rate from carcass to boneless retail cut.	0.731.	National Pork Board, 2012
Weight of individual fresh pork cut	Weight of each fresh pork cut, includes fresh ham, spare rib, loin, back rib, tenderloin, arm picnic, blade steak, and blade roast.	Calculated individually based on pig carcass breakdown diagram (Fig. 2.).	National Pork Board, 2012
Concentration	Bradyzoite concentration in pork meat (assumed the same in all cuts, if a cut is positive), data obtained from a real-time PCR study.	Lognormal distribution (μ , σ), $\mu=11.67$, $\sigma=97.31$, truncated to (0.04, 41.3) bradyzoites/g.	Jurankova et al., 2013
Probability of <i>T. gondii</i> presence	Probability of presence of <i>T. gondii</i> in a randomly selected commercial pork cut. Yes=1 indicates presence of <i>T. gondii</i> in a pork cut. Probability varies by cut.	Binomial distribution (n, p), where n=1, p varies by pork cut (Table 2).	Dubey et al., 1986b
Number of bradyzoites in pork cut	Bradyzoite load in a considered fresh pork cut.	Bradyzoite concentration $\times$ boneless weight of a pork cut $\times$ probability of <i>T. gondii</i> presence	Calculation
Moisture enhancement			
Percentage of enhanced pork	Percentage of fresh pork that is injected with enhancing solutions in the U.S. market	PERT distribution (0.40, 0.45, 0.50)	American Meat Institute, 2007; Hill et al., 2006
Was one randomly selected pork cut enhanced?	The enhancement status of one randomly selected pork cut. Yes=1 indicates pork cut had been enhanced; No=0 indicates pork cut had not been enhanced.	Binomial distribution (n, p), where n=1, p is the percentage of enhanced pork.	
Storage time	Storage time from enhancement to cooking, estimated based on suggested shelf life of enhanced pork products.	Uniform distribution (14, 21) days	Tyson Foods, 2013
Storage temperature	Storage temperature from enhancement to cooking, estimated based on fitting retail display temperature of fresh meat.	Normal distribution (μ , σ), $\mu=3.39$, $\sigma=2.61$, truncated to (0, 19.44) °C.	Ecolab, 2008
Pumping ratio of enhancing solution	Ratio of enhancing solution added to the meat, and initial weight of meat before enhancement.	PERT distribution (0.07, 0.12, 0.15) wt/wt	National Pork Board, 1998; Miller, 2010
Brine concentration	NaCl concentration in enhancing solution.	Uniform distribution (0.08, 0.14) g/ml	Food and Agriculture Organization, 2007
NaCl concentration in meat	Final NaCl concentration in pork cut	(pumping ratio $\times$ brine concentration) / (1+ pumping ratio), g/ml	Calculation
Probability of infection if enhanced	Probability of a mouse becoming infected by subcutaneously inoculating an enhanced pork cut that	$P_{\text{pos}} = [1 + \exp(-0.271 + 0.286 \times \text{temperature (°C)} + 0.022 \times \text{time (days)} - 0.762 \times \text{NaCl concentration})]$	Dubey, 1997a

	infected with <i>T. gondii</i> , estimated by a logistic regression model.	in pork cut (g/ml)] ⁻¹	
Presence of <i>T. gondii</i> in a specific enhanced pork cut	Probability of <i>T. gondii</i> presence in a randomly selected enhanced pork cut. Yes=1 indicates presence of <i>T. gondii</i> ; No=0, indicates inactivation of <i>T. gondii</i> in an enhanced cut.	Binomial distribution (n, p), where n=1, p is the probability of infection if enhanced (P_{pos})	
Cooking			
Finished cooking temperature	Finished internal cooking temperature recorded by consumers upon completion of cooking pork and removal from heat.	Normal distribution (μ , σ), $\mu=69.26$, $\sigma=13.42$, truncated to (27.78, 100.56) °C.	Ecolab, 2008
Reduction factor (>61°C)	When finished cooking temperature > 61°C, pork is assumed to be free from <i>T. gondii</i> .	Reduction factor=0	Dubey et al., 1990; Guo et al., 2016a
Reduction factor (<49.9°C)	When finished cooking temperature < 49.9°C, it is assumed no reduction occurred.	Reduction factor=1	Dubey et al., 1990; Guo et al., 2016a
Reduction factor (49.9°C-61°C)	When finished cooking temperature is between 49.9°C and 61°C, a log-linear regression was used to predict the percentage of reduction.	$\log_{10}(\text{reduction factor}) = 8.583 - 0.172 \times \text{final cooking temperature (°C)}$	Dubey et al., 1990; Guo et al., 2016a
Reduction factor	Overall <i>T. gondii</i> reduction after home cooking.	$IF(\text{cooking temperature} < 49.9, 1, IF(\text{cooking temperature} < 61, \text{reduction factor}(49.9-61), 0))$	
Number of bradyzoites in a pork cut	Number of bradyzoites in a pork cut at the end of cooking.	Number of bradyzoites in a pork cut before cooking $\times$ reduction factor	Calculation
Consumption			
Pork consumption for the total U.S. population	Pork consumption for the total U.S. population, estimated based on a “disappearance” model.	6,084,780,000 kg/year	U.S. Census Bureau, USDA, 2015a
Pork consumption for pregnant women	Pork consumption of women in the child-bearing age.	61,869,429 kg/year	U.S. Census Bureau, USDA, 2015a; U. S. EPA, 2003
Fresh pork share	Proportion of consumption of each fresh pork cut to total pork consumption.	Loin (10.8%), blade steak (3.7%), fresh ham (4.9%), back rib (1.2%), spare rib (2.5%), blade roast (4.9%), tenderloin (0.9%), and arm picnic (7.3%).	Davis and Lin, 2005
Serving size	Amount of boneless pork ingested in one eating session.	Normal distribution (μ , σ), $\mu=85$, $\sigma=25.5$, truncated to (2.8, 170.1) g.	Miller et al., 2005
Dose-response and risk characterization			
Probability of human infection per positive serving	The probability of human infection per positive serving ingestion, estimated for each pork cut.	Exponential model: $P_{DR} = 1 - \exp(-r \times 0.005 \times d)$, where P_{DR} : probability of infection, d: dose (bradyzoites/serving), r: model parameter, and 0.005 is the scaling factor.	Dubey et al., 1997b; Dubey, 1998; Guo et al., 2016b.
Model parameter r (in the exponential dose-response model)	Probability of one bradyzoite to initiate infection. Data of 100,000 bootstrap r fitted to a lognormal distribution.	Lognormal distribution (μ , σ), $\mu=0.00169$, $\sigma=0.00067$, truncated to (0.00004, 0.01276).	Guo et al., 2016 a, b; Haas et al., 2014
Probability of human infection per serving	The probability of human infection risk in one serving of fresh pork, estimated for each pork cut.	Obtained by multiplying the probability of infection (P_{DR}) by <i>T. gondii</i> prevalence in market pigs: $P_{serv} = P_{DR} \times \text{Prevalence}$	Latorre et al., 2011
Number of servings per year	Number of individual servings of fresh pork cut consumed in a year, estimated for each pork cut.	Total consumption of one specific pork cut divided by serving size.	Calculation
Number of infection	Number of cases caused by fresh pork	Number of servings per year $\times$	Calculation

cases per year	cut consumption in a year, estimated for each pork cut.	probability of human infection per serving.	
New infection cases per year in the U.S. total population	Number of new infections per year due to consumption of fresh pork in the U.S.	Sum of the number of infections per year attributed to each pork cut. Total number of new infections per year = sum of the total cases for the U.S. total population $\times$ 0.892, 0.892 is an adjustment factor (10.8% of the U.S. population of age 6-49 years have been infected with <i>T. gondii</i>).	Jones et al., 2007; Calculation
New infection cases per year in the pregnant women population	Number of new infections per year due to consumption of fresh pork among women in child bearing age.	Number of infections per year attributed to each pork cut was calculated individually and summed together. Total number of new infections per year = sum of the total cases for the pregnant women population $\times$ 0.89, 0.89 is an adjustment factor (11.0% of the women of age 15-44 years have been infected with <i>T. gondii</i>).	Jones et al., 2007; Calculation
Maternal-fetal transmission rate	<i>T. gondii</i> tachyzoite transmission rate from pregnant women to children.	Beta distribution ($s+1, n-s+1$), where $s=161$: number of congenital toxoplasmosis cases observed in the study, and $n=557$: number of mothers who acquired <i>T. gondii</i> infection during pregnancy.	Dunn et al., 1999
Congenital toxoplasmosis cases	Number of congenital toxoplasmosis cases per year associated with consumption of fresh pork.	Number of new infections per year in pregnant women $\times$ maternal-fetal transmission rate	Calculation

832

833

834 **Table 2**
 835 Probability of presence of *T. gondii* tissue cysts in commercial pork cuts, data from Dubey et al., 1986b.

Fresh pork cut	Probability of <i>T. gondii</i> presence in pork cuts (No. of positive mice/total mice tested)
Arm picnic	0.75 (18/24)
Boston butt (blade steak and blade roast)	0.42 (10/24)
Fresh ham	0.29 (7/24)
Loin, back rib and tenderloin	0.21 (5/24)
Spare rib	0.08 (2/24)

836

837 **Table 3**
838 Data used to develop the log-linear relationship between temperature and degree of bradyzoite reduction
839 due to thermal treatment (home cooking module, see also Fig. 3.).

Temperature (°C)	Percentage of <i>T. gondii</i> presence
(No. of positive mice/total mice tested)	
49	100% (45/45)
52	64.4% (29/45)
55	13.3% (6/45)
58	4.4% (2/45)
61	0 (0/45)

840

841 **Table 4**
842 Probability of infection per serving and number of new infections per year in the United States associated
843 with consumption of individual fresh pork cut.

Fresh pork cut	Population	Probability of infections per serving, mean (5 th , 95 th , 99 th percentiles)	Number of new infections/year, mean (5 th , 95 th , 99 th percentiles)
Pork chop	Total U.S. population	2.7×10^{-6} (0, 0, 3.6×10^{-5})	18,064 (0, 0, 256,689)
	Pregnant women	2.7×10^{-6} (0, 0, 3.8×10^{-5})	181 (0, 0, 2,597)
Blade steak	Total U.S. population	5.3×10^{-6} (0, 2.79, 1.2×10^{-4})	12,105 (0, 6,696, 306,058)
	Pregnant women	5.3×10^{-6} (0, 2.76, 1.2×10^{-4})	123 (0, 68, 2,763)
Spare rib	Total U.S. population	9.7×10^{-7} (0, 0, 2.8×10^{-6})	1,492 (0, 0, 4,440)
	Pregnant women	9.6×10^{-7} (0, 0, 2.8×10^{-6})	15 (0, 0, 45)
Back rib	Total U.S. population	2.6×10^{-6} (0, 0, 3.5×10^{-5})	1,935 (0, 0, 27,258)
	Pregnant women	2.6×10^{-6} (0, 0, 3.6×10^{-5})	20 (0, 0, 276)
Fresh ham	Total U.S. population	3.3×10^{-7} (0, 0, 4.8×10^{-6})	982 (0, 0, 15,558)
	Pregnant women	3.2×10^{-7} (0, 0, 5.0×10^{-6})	10 (0, 0, 157)
Tenderloin	Total U.S. population	2.6×10^{-6} (0, 0, 3.9×10^{-5})	1,494 (0, 0, 23,810)
	Pregnant women	2.6×10^{-6} (0, 0, 4.0×10^{-5})	15 (0, 0, 241)
Arm picnic	Total U.S. population	9.5×10^{-6} (0, 1.8×10^{-5} , 2.5×10^{-4})	42,471 (0, 83,691, 1,212,850)
	Pregnant women	9.5×10^{-6} (0, 1.8×10^{-5} , 2.5×10^{-4})	430 (0, 847, 11,420)
Blade roast	Total U.S. population	5.30×10^{-6} (0, 2.8×10^{-6} , 1.2×10^{-4})	16,063 (0, 8,886, 362,283)
	Pregnant women	5.34×10^{-6} (0, 2.8×10^{-6} , 1.2×10^{-4})	163 (0, 90, 3,666)

844

845 **Table 5**
846 Number of *T. gondii*-positive servings at the time of consumption.

Fresh pork cut	No. of servings positive for <i>T. gondii</i>	No. of servings negative for <i>T. gondii</i>	Percentage of servings positive for <i>T. gondii</i>	Mean probability of infection per positive servings in the total U.S. population , mean (5 th , 50 th , 95 th percentiles)
Pork chop	18,826	481,174	3.76%	7.40×10^{-5} (0.013×10^{-5} , 0.70×10^{-5} , 34.19×10^{-5})
Blade steak	18,515	481,485	7.41%	7.09×10^{-5} (0.013×10^{-5} , 0.73×10^{-5} , 34.0×10^{-5})
Spare rib	7,398	492,602	1.48%	7.17×10^{-5} (0.013×10^{-5} , 0.75×10^{-5} , 35.03×10^{-5})
Back rib	18,604	481,396	3.72%	7.34×10^{-5} (0.014×10^{-5} , 0.76×10^{-5} , 34.24×10^{-5})
Fresh ham	26,168	473,832	5.23%	0.94×10^{-5} (0.002×10^{-5} , 0.09×10^{-5} , 4.78×10^{-5})
Tenderloin	18,812	481,188	3.76%	7.44×10^{-5} (0.014×10^{-5} , 0.73×10^{-5} , 38.14×10^{-5})
Arm picnic	66,752	433,248	13.35%	7.23×10^{-5} (0.014×10^{-5} , 0.75×10^{-5} , 34.48×10^{-5})
Blade roast	37,030	462,970	7.41%	7.09×10^{-5} (0.013×10^{-5} , 0.73×10^{-5} , 34.0×10^{-5})

847

848

Fig. 1. Schematic representation of the risk assessment model.

122.1 kg pig

86.6 kg dressed

63.3 kg boneless retail cut

858

859 **Fig. 2.** Diagram of pig carcass breakdown (based on the mean weight, data were obtained from actual

860 cutting tests) (National Pork Board, 2012).

861 Loin cut includes loin chop and sirloin roast.

Fig. 3. Relationship between temperature and reduction in concentration of *T. gondii* tissue cysts in pork (cooking module). Data from Dubey et al., 1990.

865

866 **Fig. 4.** Mouse-derived human exponential dose-response model on a log-log plot, with 95% confidence

867 band deriving from bootstrapping samples.

Fig. 5. Tornado graph of sensitivity analysis for number of new infections in the U.S. total population, carried out via Spearman correlation in @Risk.