ABSTRACT

Title of Document:	EVALUATING FOOD SAFETY RISK OF *TOXOPLASMA GONDII* IN MEAT PRODUCTS CONSUMED IN THE UNITED STATES
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Toxoplasma gondii is one of the leading foodborne pathogens in the United States. The main modes of *T. gondii* transmission are ingestion of food, soil or water contaminated with oocysts, or eating raw or undercooked meat containing tissue cysts. A substantial portion of human *T. gondii* infections is acquired through consumption of meats. The overall goal of this dissertation was to collect and summarize current knowledge and information of *T. gondii* infection, and estimate the risk of human *T. gondii* infection due to consumption of meat products that are potentially infected with *T. gondii* in the United States.

A resource document was developed to collect relevant data of *T. gondii* prevalence in meat animals worldwide, and to identify risk factors associated with *T. gondii* prevalence. Furthermore, a quality-effects systematic meta-analysis was conducted to estimate *T. gondii* prevalence in meat animals raised in the United States.

These results were used to define the risk level of meat category in a farm-to-retail qualitative assessment. Effects of meat processing on the survival of *T. gondii* were assessed, and critical steps for inactivating *T. gondii* were identified. An exponential and a beta-Poisson dose-response models were developed to estimate human infection by using scaling factors. Mouse-derived models were validated against data for the dose-infection relationship in rats.

Two risk models were developed to quantitatively predict the risk of *T. gondii* infection in the United States due to consumption of fresh pork and domestically-produced lamb, respectively. The mean probability of infection per serving of fresh pork ranges from 3.2×10^{-7} to 9.5×10^{-6}, corresponding to 94,606 and 957 new infections annually in the U.S. population and the pregnant women, respectively. The sensitivity analysis suggested that cooking is the most important parameter impacting human health risk. The mean probability of infection of lamb was estimated to be 1.5 cases per 100,000 servings, corresponding to approximately 6,300 new infections per year in the U.S. population. This project systematically evaluated food safety risk of *T. gondii* through meat consumption, and provided scientific evidence for risk managers that attention to *T. gondii* infection through meatborne routes is warranted.

EVALUATING FOOD SAFETY RISK OF *TOXOPLASMA GONDII* IN MEAT
PRODUCTS CONSUMED IN THE UNITED STATES

By

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AIAO   All-In-All-Out

AIC   Akaike Information Criterion

AIDS   Acquired Immune Deficiency Syndrome

AFSSA   Agence Française de Sécurité Sanitaire des Aliments

BIC   Bayesian Information Criterion

CAC   Codex Alimentarius Commission

CCP   Critical Control Point

CDC   Centers for Disease Control and Prevention

CI   Confidence Interval

CSFII   Continuing Survey of Food Intakes by Individuals

DNA   Deoxyribonucleic Acid

ELISA   Enzyme Linked Immunoabsorbent Assay

ERS   Economic Research Service

FAO   Food and Agriculture Organization

IFAT   Indirect Fluorescent Antibody Test

IHAT   Indirect Haemagglutination Antibody Test

LAMP   Loop-mediated Isothermal Amplification

LAT   Latex Agglutination Test

MAT   Modified Agglutination Test

MLE   Maximum Likelihood Estimations

MPN   Most Probable Number

NAHMS   National Animal Health Monitoring System

NASS	National Agriculture Statistics Service
NHANES	National Health and Nutrition Examination Survey
PCR	Polymerase Chain Reaction
PI	Post-infection
PP	Population Prevalence
OIE	World Organization for Animal Health
QALY	Quality-Adjusted Life Year
QMRA	Quantitative Microbial Risk Assessment
RFLP	Restriction Fragment Length Polymorphism
RTE	Ready-to-Eat
SFDT	Sabin-Feldman Dye Test
USDA	U. S. Department of Agriculture
WHO	World Health Organization

Chapter 1: Introduction and literature review

1.1 Life cycle of *T. gondii* and characterization of three infectious stages

Toxoplasma gondii is a protozoan parasite that belongs to the phylum Apicomplexa, subclass Coccidia (1). There are three infectious stages of *T. gondii*: tachyzoites, bradyzoites in tissue cysts, and sporozoites contained in oocysts (2). When a definitive host of *T. gondii*, a member of the Felidae family, ingests tissue cysts, the parasite goes through its entire life cycle and oocysts are shed in feces of the animal 3-10 days post-infection. A single cat may shed more than 100 million unsporulated oocysts into the environment after ingesting as few as one bradyzoite (3). After sporulation in the environment (1-5 days depending on temperature), oocysts become infectious and resistant to environmental conditions. It was reported that oocysts can survive in soil and remain infective for 18 months (4), and in water and seawater for several years at 4°C (5, 6). Oocysts can be inactivated when heated above 55-60°C for 1-2 minutes (7). Constant freezing below -21°C inactivates unsporulated and sporulated oocysts within 1 and 28 days, respectively (8). Oocysts are considered to be less infective to cats, but highly infectious to humans (9).

Most warm-blooded animals can serve as intermediate hosts and allow two stages of parasite multiplication. After a primary infection, tachyzoites rapidly multiply in the intermediate host cells. Tachyzoites are susceptible to proteolytic enzymes and are normally destroyed by the human digestive system (10). After a few multiplication cycles (1-3 weeks), tachyzoites transform into bradyzoites within intracellular cysts. A tissue cyst can contain a few to thousands of bradyzoites depending on the age of the

cyst (11). Although sharing similarities in structure, bradyzoites are more resistant to digestive enzymes than tachyzoites (2). Once intermediate hosts get infected, tissue cysts persist indefinitely for the life of the host, and are unevenly distributed among organs. High affinities occur mainly to the brain, heart, muscle and viscera (12). Tissue cysts can survive at refrigerator temperature and remain infectious in minced meat for up to three weeks (3). The conditions to kill tissue cysts are as follows: 6% NaCl solution at 4-20°C; heating at 67°C or higher; gamma irradiation at a dose of 1.0 kGy or higher; high pressure processing at 300 MPa or higher, and deep freezing (-12°C or lower) (13).

1.2 Virulence and infectivity

Based on the result of restriction fragment length polymorphism (RFLP) assays, the predominant genotypes of *T. gondii* found in North America and Europe can be classified as genotypes I, II, and III (14). These lineages share 98% similarity of their DNA, but they show great differences in virulence. In mouse pathogenicity experiments, genotype I is highly virulent and 100% lethal at all doses ($LD_{100} = 1$ parasite); genotype II, the most widespread strain in the United States, is moderately virulent ($LD_{50} = 10^3$–10^4 parasites); Type III shows low virulence ($LD_{50} > 10^5$ parasites) (15).

The knowledge of strain specific virulence in humans is less well studied, and primarily relies on epidemiological evidence. In Europe and the United States, the majority of human cases have been attributed to genotype II (14). Severe disease in

immunocompetent adults and congenitally-infected newborns have been associated with non-genotype II stains (16, 17).

1.3 Mode of transmission

The main modes of *T. gondii* transmission are ingestion of food, soil or water contaminated with oocysts, or eating raw or undercooked meat containing tissue cysts, or the congenital transmission of tachyzoites from mother to fetus (18). Occasionally transmission occurs during organ transplantation, blood transfusion and drinking unpasteurized milk containing tachyzoites. Oocyst-induced infection is clinically more severe than cyst-acquired infection in humans (19).

1.4 Meat as an important source of *T. gondii* infection in humans

From an epidemiological standpoint, the proportion of human infection through each transmission route is not known for the general population (18). Numerous epidemiological studies have indicated that one of the major sources is meat, especially pork and lamb. Consumers become infected by ingestion of tissue cysts that harbored in the meat animals. In a European multicenter case-control study, it was found that consumption of undercooked or cured meat products and soil contact cause 30-63% and 6-17% of infections in pregnant women, respectively (20). In a recent study in pregnant women, the seropositivity of *T. gondii* has been associated with ingestion of undercooked meat and improperly washed vegetables (21). Muñoz-Zanzi et al. (22) found that 57% of *T. gondii* infections in pregnant women were due to cyst-contained meat consumption, while 43% of infections caused by exposure to the oocysts in the

environment in Chile. In a case-control study involving 148 infected adults in the United States, it was found that consumption of ground beef, eating rare lamb, and eating locally produced cured or smoked meat were all associated with *T. gondii* infection (23). In another retrospective study of 131 mothers who had given birth to children infected with *T. gondii* in the United States, 50% of the mothers recalled having eaten uncooked meat (24).

1.5 Human toxoplasmosis

Immunocompetent individuals can become infected by ingestion of any stage of *T. gondii* and intracellular cysts are formed in the muscles, brain and other organs. Postnatal infections are normally asymptomatic, with only 10-20% of individuals developing glandular, fever-like symptoms (25). Ocular disease is one of the most important clinical manifestations of postnatally-acquired toxoplasmosis.

The risk of severe infection is high in specific groups of patients including congenitally infected fetuses and newborns and immunologically impaired individuals (e.g., AIDS, Hodgkin's disease, and transplant patients) (26). Encephalitis is the most common severe complication in acquired immune deficiency syndrome (AIDS) patients with acquired *T. gondii* infection (27).

Congenital toxoplasmosis occurs when a woman becomes infected with *T. gondii* during pregnancy. While pregnant women are generally asymptomatic after infection, infected infants or babies may develop chorioretinitis, mental retardation, hydrocephalus, convulsions, and intracranial calcifications at birth, after several months or even years (28). Centers for Disease Control and Prevention (CDC) has

estimated that 400-4,000 congenitally infected infants are born each year in the United States (29).

1.6 Human cases: prevalence and outbreak

It was estimated that one third of the world population is seropositive for *T. gondii* infection (30). *T. gondii* prevalence varies among countries (10-80%), where high seroprevalence was found in the population of South American and some European countries (31). Low seroprevalence (10-30%) was observed in the population of North America, East Asia, Northern Europe, and some countries in Africa (32). National Health and Nutrition Examination Survey (NHANES) 1988-1994 estimated that approximately 22.5% of the U.S. population older than 12 years have been infected with *T. gondii* (33). Seroprevalence of *T. gondii* was reduced to 10.8% in NHANES 1999-2004 and further reduction was observed in NHANES 2009-2010 (18, 29). The reduction is possibly attributed to the modern confinement rearing system for meat animals and increased education to both physicians and the public (34).

Outbreaks of toxoplasmosis involving more than a family or small group are rare and infrequently reported. Reported outbreaks have been mainly associated with water or undercooked meat (**Table 1.1**).

1.7 Public health significance of human toxoplasmosis in the United States

Considerable morbidity and mortality in the United States is attributed to *T. gondii* infection. *T. gondii*, along with non-typhoidal *Salmonella enterica*, *Campylobacter* spp., *Listeria monocytogenes*, and *Norovirus* are responsible for 90%

of the quality-adjusted life years (QALYs) lost (35). Among 31 major foodborne pathogens, *T. gondii* was identified as the fourth leading cause of hospitalization and the second leading cause of death, with an estimated 10,964 QALYs, $2,973 million costs due to illness, 86,686 illness, 4,428 hospitalizations, and 327 deaths per year in the United States (36).

Table 1.1 Selected major outbreaks associated with *T. gondii*, 1994-2015

Country	Year	Possible Source	Cases	Reference
Canada	1994	municipal water supply	2,895-7,118	(37)
Australia	1994	kangaroo meat	13	(38)
South Korea	1995	uncooked pork	8	(39)
Brazil	1999	water reservoir	176	(40)
Brazil	2001	water supply	426	(41)
Turkey	2002	exposed to cat litter	171	(42)
France	2003	unknown source	11	(43)
Brazil	2003	non-treated water	8	(44)
Brazil	2003	home-cured sausage contains pork	10	(44)
India	2004	water supply	248	(45)
Brazil	2009	green vegetables	11	(46)

1.8 Detection methods used in meat animals and meat products: an overview

Efficient and robust detection methods are critical to assess the prevalence of infection (7). Methods for the detection of *T. gondii* in meat animals and meat products were developed to study the prevalence of *T. gondii* infection and can be classified into three categories: (i) bioassay in mice or cats, (ii) serological assays, and (iii) detection of *T. gondii* DNA by polymerase chain reaction (PCR)-based techniques.

1.8.1 Bioassay

Bioassay in mice or cats is considered the gold standard to detect viable *T. gondii* (47). A portion of tissues that might contain cysts, typically brain, heart, muscle, tongue and diaphragm, are inoculated into mice parentally or fed to cats orally. For bioassay in mice, tissue samples are homogenized and inoculated subcutaneously into each mouse. The impression smears of lungs from mice that die are stained with Giemsa and examined under the microscope. For each mouse that survives 60 days after inoculation, the brain of the mouse is examined under the microscope for *T. gondii* tissue cysts, and serum is tested by serological assay (48). Finding a *T. gondii* in mouse tissue is dependent upon the concentration of the parasite in the tissue. For bioassay in cats, after feeding cats with meat that might contain tissue cysts, the feces are collected daily for 14 days and are examined for oocysts under the microscope (49).

1.8.2 Serological assays

Although bioassay is an accurate and reliable method to detect the viable *T. gondii* in meat products, it is time-consuming and resource-intensive. Other methods such as serological assays were developed as faster and simpler alternatives to the bioassay method. The indirect haemagglutination antibody test (IHAT), the latex agglutination test (LAT), the indirect fluorescent antibody test (IFAT), the modified agglutination test (MAT), Western Blot and enzyme linked immunoabsorbent assay (ELISA) have all been used to detect anti-*T. gondii* antibody in the serum from meat animals or meat juice. The three most popular serological techniques - MAT, IFAT, and ELISA - have been widely used and validated. These techniques detect anti-*Toxoplasma* IgG and IgM antibodies in serum and tissue fluid. MAT is superior to other

7

agglutination methods but inconvenient for slaughterhouse or field use as it requires large numbers of intact tachyzoites (7). ELISA is simple but requires species specific conjugates and a plate reader. Recently, Al-Adhami and Gajadhar (50) overcame this shortcoming by developing a non-species-specific protein A/G conjugate that could be used for multiple species testing. The advantages of serological assays are that they are rapid and simple to perform. However, serological assays also have certain limitations. The performance of serological assays may differ with regard to sensitivity and specificity and the results of the serological assay are not always consistent with the bioassay results; this is most pronounced in cattle. It was reported that seroprevalence of *T. gondii* in cattle can be as high as 90%, whereas isolation of *T. gondii* from cattle has been generally unsuccessful (51). Viable *T. gondii* has been frequently detected from seropositive swine, sheep and goat. Gamble et al. (47) conducted an experiment to compare MAT and/ELISA results with bioassay in cats of tissue samples from pigs. Among 70 bioassay positive pigs, 60 were positive by MAT (85.7% sensitivity) and 62 were positive by ELISA (88.6% sensitivity).

1.8.3 PCR-based assays

PCR-based techniques have been developed to identify *T. gondii* DNA in meat products and meat animals. By detecting specific and unique genes, *T. gondii* can be quickly characterized and the number of *T. gondii* can even be quantified by real-time PCR. Bezerra et al. (52) detected *T. gondii* from 11 of 20 samples of pork from a butcher shop by PCR. *T. gondii* was found in only 2 of brain samples and none of 20 tongues samples. Lin et al. (53) reported that the real-time PCR assay can quantitatively detect DNA from 1 tachyzoite or less in an assay. Gutierrez et al. (54) reported that

real-time PCR was fast and reliable for detecting infection in aborting ewes. Nevertheless, PCR is not reliable under certain circumstances. PCR detects both viable and dead *T. gondii* in meat samples, which may lead to an overestimate of the infectivity of infected meat. Moreover, PCR based techniques may lack sensitivity because tissue cysts can be unevenly distributed and in low concentration in meat animals (19). In order to resolve low sensitivity problems, new or modified PCR techniques have been developed in recent years. A sequence-specific magnetic capture method for isolation of *T. gondii* DNA was developed by Opsteegh et al. (55). By using this method, DNA could be extracted from 100 g samples and the likelihood of detection of *T. gondii* DNA was increased. Another novel technique, loop-mediated isothermal amplification (LAMP) was also used to detect *T. gondii* infection in meat animals. Zhang et al. (56) reported that LAMP sensitivity (85.7%) was higher than regular PCR (76.9%) when pig lymph nodes samples were tested. Qu et al. (57) reported accurate detection of *T. gondii* from pork samples using the LAMP technique, with a detection limit of 1 tachyzoite in 1 g of pork. Du et al. (58) detected *T. gondii* DNA in 21.1% by PCR and 37.9% by LAMP of 95 soil samples on a pig farm in China. Prevention of contamination while performing these highly sensitive techniques is a major issue.

1.8.4 Comparison of the detection methods

Gamble et al. (47) reported that the ELISA technique is more sensitive for detecting *T. gondii*-specific antibodies in pig samples than MAT. In another study, which compared various methods, Hill et al. (59) further confirmed that ELISA is the most efficient method to detect *T. gondii* infection in pig. In a study that compared

testing of samples from the same infected animals, the sensitivity ranking in descending order was: serum ELISA (100%) > serum MAT (80.64%) > tissue fluid ELISA (76.9%) > real time PCR (20.51%) > semi-nested PCR (12.82%) > direct PCR (0%) (59). In a recent study, Villena et al. (60) compared MAT, ELISA, and bioassay tests on samples from naturally infected sheep and found that ELISA using diaphragm fluids and MAT using heart fluids were in good agreement and both could be used as detection tools for epidemiological studies in sheep. Viable *T. gondii* was isolated from animals with a meat MAT titer of only 1:6. They also found a significant correlation between increased MAT titers and the likelihood of *T. gondii* isolation from heart tissue. New techniques are also emerging. Bokken et al. (61) reported using a bead-based flow cytometric assay to identify *T. gondii*-specific antibodies in pig serum samples. The sensitivity of the bead-based assay was even slightly higher than ELISA.

Serological assays, especially MAT, IFAT and ELISA, are the most popular assays for detecting *T. gondii* infection in meat producing animals and meat products. Serological assays are often used as the first screening method to identify infected animals. Subsequently, tissue samples are further tested to verify the infection via bioassay in mice or cats.

1.9 Microbial risk assessment: an overview

Microbial risk assessment is a well-established method to identify knowledge gaps, characterize the critical risk factors in the food chain, and help identify risk mitigation strategies while providing guidance for determining research priorities in public health and food safety areas (62). According to guidance from the Codex

Alimentarius Commission (CAC) and the World Organization for Animal Health (OIE), risk can be assessed qualitatively or quantitatively, and these two methods are equally valid (63). CAC outlined the framework for risk assessment, which contains four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization (64). In brief, hazard characterization is a description of the microbial hazard that capable of causing adverse health effects and which may be present in a particular food or group of foods. Hazard characterization (dose-response) is the process to collect quantitative data on the adverse health effects upon exposure to different levels of doses of the pathogen. Exposure assessment is an estimate of the dose of a microbial hazard to which the consumer is exposed (65). Risk characterization is the integration of the hazard characterization and exposure assessment to estimate the likelihood and severity of the adverse effects which could occur in a given population.

1.10 Risk assessment studies of *T. gondii* infection

To our knowledge, there are currently no qualitative or quantitative risk assessment studies for *T. gondii* in the United States. A qualitative risk assessment of *T. gondii* infection in ready-to-eat (RTE) small goods processing was published for Australia (66). The assessment rated each ingredient and meat processing step including the addition of spices, nitrates, nitrites and salt, use of fermentation, smoking and heat treatment, and the time and temperature during maturation. Processing was found to effectively inactivate *T. gondii*. A quantitative microbial risk assessment (QMRA) of meatborne *T. gondii* infection was conducted by Opsteegh et al. (67) from

11

the Netherlands in 2011. Their model predicted that beef was responsible for 67.6% of meatborne infections. Mixed meat products, pork and lamb were responsible for 7.1%, 11.2% and 14% of *T. gondii* infection, respectively. The number of new infections in the susceptible Dutch population and pregnant women were 2.8×10^6 and 2.6×10^4 per year, respectively. This number of infected pregnant women would result in approximate 7.6×10^3 congenital infections per year. The QMRA study indicated the importance of heating meat to eliminate viable *T. gondii*. The QMRA identified several knowledge gaps and uncertainties, such as *T. gondii* concentration in meat products and dose-response relationship for humans.

1.11 Project overview

The livestock and poultry industries constitute the largest segment of U.S. agriculture. The main types of meat consumed are poultry, beef, and pork, with lamb, goat, veal and game making up 1% of the total meat consumed (68). Meat animals, both confinement and non-confinement raised, are good reservoirs for *T. gondii*. After infection with *T. gondii*, meat animals can harbor tissue cysts in all edible tissues. Meat animals are raised for human consumption and consumers expect to get *Toxoplasma*-free meat at retail stores. Since *T. gondii* is not visible in the meat with the naked eye, a complete understanding of infection risk to humans from meat animals, development of restrictions, and risk mitigation strategies are important to ensure *Toxoplasma*-free meat products. To our knowledge, there are no nationwide studies currently available on the risk posed to consumers from consumption of meat products in the United States. Epidemiological surveys at the farm level are not sufficient for defining risk at the retail

12

level and at the time of consumption, since meat undergoes a series of processing, which serves to reduce the risk. Therefore, a comprehensive evaluation of food safety risk of *T. gondii* is eagerly needed.

The overall goal of this study was to collect and summarize current knowledge and information of *T. gondii* infection, estimate the risk of human *T. gondii* infection due to consumption of various meat products that are potentially infected with *T. gondii* tissue cysts in the United States. Specific objectives were:

(1) **To develop a resource document that contains globally available data to address *T. gondii* prevalence in meat animals, and identify the risk factors of *T. gondii* infection at farm.** Despite being long recognized as a significant foodborne pathogen, the knowledge of *T. gondii* is typically limited. In particular, information on the wide range of meat animals that can harbor this protozoan pathogen and thus serve as potential reservoirs for human infections is needed.

(2) **To conduct a systematic meta-analysis to provide a quantitative estimate of *T. gondii* prevalence in meat animals reared in the United States.** Prevalence of *T. gondii* in the U.S. domestically-raised meat animals are varied according to geographic location, management system, age of the animal, biological specimen (serum or tissues), year of the study, and detection methods. Thus, a quality-effects meta-analysis is needed to provide a precise estimation of *T. gondii* infection prevalence in meat animals raised in the United States.

(3) **To develop a farm-to-retail framework to qualitatively estimate the relative exposure risk of meat products consumed in the United States.** A qualitative assessment is based on a descriptive model that uses evidence to support a statement

about risk. A qualitative approach is appropriate to initiate the risk assessment because quantitative data and knowledge of *T. gondii* in meat are limited.

(4) To develop dose-response models to predict *T. gondii* infection in humans from ingestion of *T. gondii*-infected meats. A dose-response model establishes the relationship between the magnitude of exposure to a hazard and the probability of occurrence of an adverse health effect. Currently, the dose-response relationship of *T. gondii* infection in humans is poorly understood because of the absence of human volunteer studies. A suitable dose-response model is one of the components required to conduct a quantitative risk assessment (hazard characterization).

(5) To develop a farm-to-table risk model to quantify the public health burden associated with consumption of fresh pork in the total U.S. population and pregnant women. Currently there are no QMRA studies for *T. gondii* in meats destined for human consumption available in the United States. Pigs are susceptible to *T. gondii* infection and tissue cysts can be harbored permanently in all edible portions of an infected pig. Due to the absence of national detection system for *T. gondii* infection in pigs, *T. gondii*-infected pork cannot be identified. Thus, a complete understanding of infection risk to humans from fresh pork is important to ensure *Toxoplasma*-free pork products.

(6) To develop a farm-to-table risk model for predicting number of new infections per year in the U.S. population, through eating U.S. domestically-produced lamb. Sheep are important hosts of *T. gondii* and prevalence of *T. gondii* is high in lambs raised in the United States. To our knowledge, currently there are no risk assessment studies for *T. gondii* in lamb destined for human consumption available.

It is critical to provide science-based information that help in better understanding the risk of *T. gondii* transmission to humans, exposed through eating lamb.

These six objectives influence and complement each other (**Figure 1.1**). Objective 1 serves as a useful resource and information repository for informing risk assessment studies of *T. gondii* infection in humans through meat consumption. Based on information from objective 1, it has been concluded that it is necessary to conduct a risk assessment. A farm-to-retail qualitative assessment (Objective 3) was undertaken as a first evaluation of *T. gondii* infection associated with meat products, to identify critical steps for inactivation of *T. gondii* tissue cysts, and to determine the specific meat products that should warrant more detailed analysis. Prevalence of *T. gondii* in the U.S. produced meat animals (results from Objective 2) were used to define the risk level of each meat category in the qualitative assessment (Objective 3). Based on the qualitative assessment, fresh meat from pork and lamb (both conventional and organic) were identified as important meat products associated with *T. gondii* infection. Except for absence of the dose-response relationship of *T. gondii* infection in humans, current available data could allow us to allocate resources to develop quantitative risk assessment models for conventionally-raised pork and lamb. Thus, human dose-response models (Objective 4) were developed before conducting risk modeling. Resources and information from Objective 1-4 were all used to develop risk assessment models (Objective 5 and 6). A QMRA model associated with consumption of fresh pork was first developed (Objective 5), and was served as the baseline model for developing QMRA models for lamb (Objective 6).
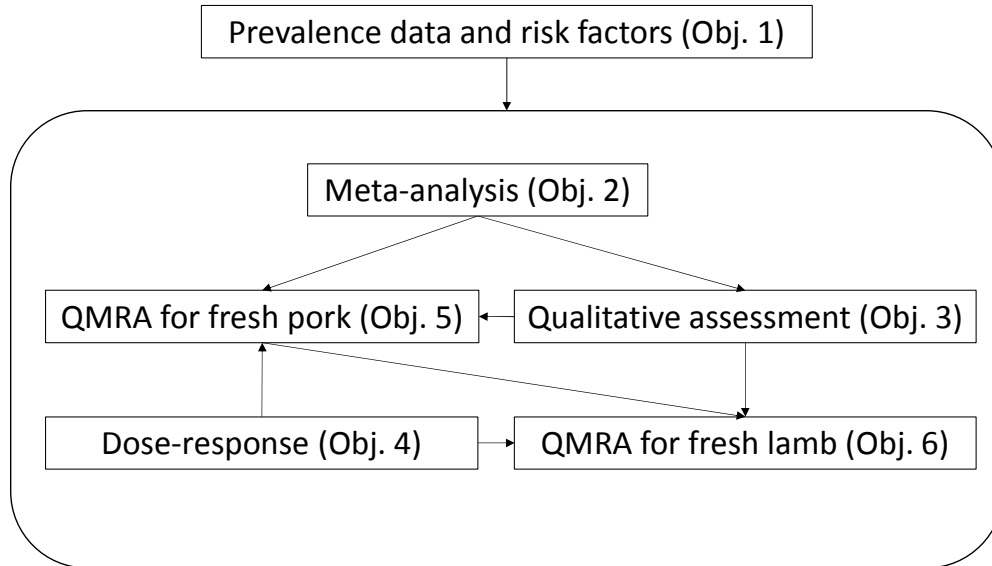
Figure 1.1 Diagram that demonstrate relationships among the six objectives.

Chapter 2: Prevalence and risk factors for *Toxoplasma gondii* infection in meat animals and meat products destined for human consumption

2.1 Abstract

Human *T. gondii* infection results from accidental ingestion of oocysts, from the environment, in water or on insufficiently washed produce or through consumption of raw or undercooked meat products that contain *T. gondii* tissue cysts. The current chapter focused on studies of *T. gondii* infection in meat because substantial proportions of human *T. gondii* infection are acquired through consumption of raw or undercooked meat. Prevalence of *T. gondii* is higher in conventionally reared pigs, sheep and goat as compared with cattle and poultry. Prevalence of *T. gondii* is greater in meat products from organic compared to conventionally reared meat animals because of outdoor access that poses substantially greater opportunities for exposure to infected rodents, wildlife, and oocyst-contaminated feed, water, or environmental surfaces. Risk factors related to *T. gondii* exposure for livestock include farm type, feed source, presence of cats, methods of rodent and bird control, carcasses handling and water quality.

2.2 Introduction

Toxoplasma gondii is a protozoan parasite that was discovered in 1908 (1), and is one of the most important foodborne pathogens in the United States. CDC reported that *T. gondii* is one of three pathogens (along with *Salmonella* and *Listeria*), which together account for >70% of all deaths due to foodborne illness in the United States (36). Epidemiologic data suggest that ingesting raw or undercooked meat containing *T. gondii* is a source of infection for humans in the United States (19, 69, 70). Thus, it is critical to provide science-based information to better understand the risk of *T. gondii* infection linked to consumption of meat products. The goals of this study were to (i) summarize estimates of *T. gondii* prevalence in conventional and organic meat animals and meat products in the past ten years, and (ii) identify risk factors for animal infection at farm level.

2.3 Materials and Methods

Relevant studies in literature were searched in PubMed, Medline, Web of Science and Google Scholar database by key words "*Toxoplasma gondii*" and in combination with "pig", "pork", "sheep", "lamb", "chicken", "poultry", "cattle", "meat" and "organic meat". Government consumption surveys, food risk reports, online statistical tools and personal communication with experts were also used as sources for the study. This study focused on *T. gondii* infection through meat-consumption route. The study excluded transmission through ingestion of oocysts shed by felids.

2.4 Results

Swine, cattle and poultry are the three major meat animals consumed in the United States that can harbor *T. gondii* (71). While lamb and goat meat make up only a small proportion (0.5 kg per capita annually) of total meat consumption per capita (68), they are popular in some ethnic groups. Considering the high *T. gondii* prevalence in sheep and goat, sheep/lamb and goats are included. Current information suggests that *T. gondii* is unlikely to survive in cured products following approved guidelines. From the results of bioassay in mice, it was found that *T. gondii* survived after 7 months of curing process, but not 14 months (72). These results need confirmation because *T. gondii* is highly susceptible to hypertonic salt concentrations (19, 73).

2.4.1 *T. gondii* prevalence in pigs and pork products

Pork is one of the major meat sources associated with human *T. gondii* infections. In a recent risk ranking report, the combination of *Toxoplasma* – pork ranked number 2 among 10 pathogen-food combinations. In the United States, this pathogen/product pair was associated with 4,495 QALYs lost, $1,219 million in illness costs, 35,537 illnesses, 1,815 hospitalizations, and 134 deaths annually (74).

Compared to beef and poultry, pork is more likely to be infected with *T. gondii* because of the high susceptibility of swine to *T. gondii* infection (75). Most studies of *T. gondii* infections in pigs have been focused on epidemiology and seroprevalence data that provide information on the infection rate of swine among farms in different geographic areas. Seroprevalence of *T. gondii* infection in swine has significantly decreased in the last two decades in the United States. Dubey et al. (76) compared seroprevalence in swine from the same area in Illinois at different time periods. In the

first swine survey in that state, which was conducted in 1983–1984, seroprevalence was 23.9% in market pigs and 42% in breeders. In a later survey, conducted in 1992, *T. gondii* seroprevalence dropped to 3.1% in market pigs and 20.8% in breeders (77). According to the National Animal Health Monitoring Survey (NAHMS) that covered more than 90% of U.S. swine production, *T. gondii* seroprevalence in market pigs dropped from 3.2% in 1995 to 2.6% in 2006 using the ELISA method (78, 79). The decline in infection prevalence may be attributed to improvements in farm management including strict confinement housing, restrictive biosecurity regulations, better rodent control and appropriate carcass disposal. A decline in the prevalence of *T. gondii* infection in pigs has also been observed in other countries. For instance, in a study in Austria, Edelhofer (80) reported the seroprevalence of *T. gondii* infection dropped from 13.7% in 1982 to 0.9% in 1992 among slaughtered pigs, and decreased from 43.4% in 1982 to 4.3% in 1992 among breeding sows. The decrease was also attributed to implementation of modern farm management systems.

T. gondii* seroprevalence in swine varies with the age of the animals, farm management practices, and farm location. Seroprevalence increases with age. Available evidence indicates that colostrally derived antibodies decline/disappear by 120 days of age both in experimentally and naturally infected pigs (81). Alvarado-Esquivel et al. (82) found that *T. gondii* seroprevalence was 10.9% in young pigs, and rose to 19.5% in pigs older than 8 months. As already mentioned, seroprevalence is normally higher in breeding sows, which ultimately are used for processed pork products, while younger animals are used for fresh pork products. Based on a study

conducted in the Netherlands in 1995, the seroprevalence of *T. gondii* infection in sows was as high as 30.9%, 17 times more than the prevalence in market pigs (1.9%) (83).

The prevalence of *T. gondii* is dramatically different under various management systems. In modern confinement rearing systems, either all-in-all-out (AIAO, a production system that pigs are moved into and out of facilities in distinct groups) or farrow-to-finish (a production system where pigs are bred and raised to the market weight) operations, *T. gondii* infection is considerably reduced or eliminated with stringent biosecurity measures (84, 85, 86, 87). In contrast, in poorly managed swine farms, the seroprevalence in pigs can still be quite high. Prevalence of *T. gondii* infection was also significantly higher in free-range or backyard raised pigs that were allowed access to the outdoors. Deksne and Kirjusina (85) reported the prevalence of *T. gondii* antibodies in Latvia was significantly greater in free-range domestic pigs (6.2%), compared with those that were intensively reared in confinement (0.4%, $p <$ 0.05). Similarly, Alvarado-Esquivel et al. (84) detected antibodies to *T. gondii* in 58 of 337 (17.2%) backyard pigs by MAT, while only 1 sample (0.5%) was seropositive from 188 confinement raised pigs. *T. gondii* infection prevalence has been reported to be higher in small herds (88). NAHMS 2006 found that small herd pigs had a higher seropositivity rate of 4.1% compared to a 1.9% rate for medium herds and a 0.6% rate for large herds (89). One possible explanation is the risk of *T. gondii* exposure per pig is greater in small herds as compared with medium and large herd sizes (90). Prevalence of *T. gondii* in pigs differs among geographic regions. In the 2006 NAHMS survey, the uneven distribution of *T. gondii* infection was also reported. In the United States, no market pigs from the Southern region were seropositive for *T. gondii* infection, while

seroprevalence was 2.9% in the West region, 3.2% in the East region and 2.9% in the North region (89). The difference in prevalence has been hypothesized to be due to differences in altitude, temperature and humidity, which can impact the survival of *T. gondii* oocysts. Prevalence of *T. gondii* infection in swine over the recent decade is summarized in **Table 2.1** and the demonstration of *T. gondii* from pigs is presented in **Table 2.2**.

T. gondii infection prevalence in swine does not directly relate to prevalence of viable *T. gondii* in pork products. Pork products are often injected with enhancing solutions (a mixed solution of water, salt, and sodium phosphate that can add flavor and moisture to meat products) (91) or undergo processing steps such as cold or heat treatment that can affect the survival of tissue cysts (19). *T. gondii* transmission risk is also associated with consumers' cooking behaviors. Consumers may not properly cook their meat products. According to a 2007 survey, approximately 9% of consumers cooked their pork products to a temperature less than 48°C (92). This temperature may not be sufficient to inactivate *T. gondii* tissue cysts.

Pork consumption in the United States has remained fairly stable over the last two decades. According to the 2011 U.S. Department of Agriculture (USDA) agricultural statistics annual report, pork consumption in the United States is 8,380 million kg/year, 26.8 kg per capita (68). For all pork products, 38% are consumed fresh and 62% are consumed after processing (93). Fresh pork includes pork chops, loins, shoulders, ribs, hams, and other pork parts. Wang et al. (94) reported that 10.2% (42/416) of tissue fluid samples from fresh pork in eastern China were found seropositive for *T. gondii*. Bayarri et al. (95) found two (8%) *T. gondii* positive samples

of 25 fresh pork samples, but not of 25 cured ham samples in Spain by IFAT. Mice inoculated with fresh pork had low IFAT titer and viable *T. gondii* could not be demonstrated in murine tissues by PCR and histology (95). In Mexico, viable *T. gondii* was isolated via bioassay in mice from 1 of 48 pork samples (2.1%) collected from a butcher shop; all samples were negative for *T. gondii* DNA and antibodies (96, 97). In a survey conducted in France, 8.8% of tissue fluid samples from 91 fresh pork samples were positive for *T. gondii* antibody (98). In a nationwide retail meat survey in the United States, Dubey et al. (34) detected viable *T. gondii* in 7 of 2,094 pork samples via bioassay in cats. Infection rates were likely higher in pigs, but tissue cysts can be killed by the injected enhancing solution. Hill et al. (73) found that *T. gondii* tissue cysts in pork loins were killed by injecting 2% sodium chloride or 1.4% potassium or sodium lactate solution, alone or in combination with other components. Hill et al. (99) further confirmed these results and reported that *T. gondii* tissue cysts were killed within 8 hours of injection of enhancing solution.

The average consumption of processed pork products in the United States is approximately 18.6 kg per person annually, which represents the majority of pork consumption (68). Processed pork products are defined as pork treated by grinding, curing, smoking, or seasoning prior to wholesale or retail sale, and products include lunchmeats, hot dogs, bacon, sausage, smoked ham, and other forms of processed pork (100). The infection rate in processed pork products has been reported to be relatively low when compared with fresh products mainly because of the reduction of *T. gondii* viability after meat processing such as salting, freezing, smoking, heating, irradiation, and high pressure (66). *T. gondii* can still be detected from processed pork products

because different processing procedures are used among manufacturers. Dias et al. (101) reported of isolation of viable *T. gondii* via bioassay in mice from 13 of 149 (8.7%) sausage samples. Because some processed pork products are consumed raw (e.g., dry-cured hams, fermented sausages), there is a possibility that such products could serve as a source of human *T. gondii* infections.

2.4.2 *T. gondii* prevalence in sheep and lamb

The USDA classifies, sheep <1 year old and without permanent teeth as lambs (102). Meat from older sheep (over one year of age) is classified as mutton. In the United States, only lamb is slaughtered for human consumption, while mutton is used as an ingredient for pet food or for export to other countries. Sheep and lamb are highly susceptible to *T. gondii* infection. A recent survey indicated that 62.2% of sheep are raised on pasture in the United States (103). Outdoor sheep rearing results in exposure to soil and water, which are both potential sources of *T. gondii* infection.

Reported *T. gondii* prevalence in sheep is summarized in **Table 2.3** and the demonstration of *T. gondii* from sheep is shown in **Table 2.4**. Prevalence varied by animal ages, geographic locations, population densities, grazing areas, and farm management practices. Alvarado-Esquivel et al. (104) found that *T. gondii* seroprevalence in sheep varied among different climate conditions, with a 29.8% in animals from temperate climates and a 7.1% infection rate in semi-arid and warm-humid climates together in Mexico. It appears that temperate climate conditions extend the survival time of oocysts. Differences in *T. gondii* prevalence across different geographic locations were also reported in the Netherlands (105), Finland (106), Nigeria (107), Spain (108) and Mexico (109). The difference in prevalence has been

hypothesized to be due to differences in altitude and climate, which can impact the survival of *T. gondii* oocysts. Sheep can be exposed to *T. gondii* at any stage during their life and prevalence of *T. gondii* infection increases with age (110). Katzer (111) reported that seroprevalence of sheep increased from 37.7% in one year old sheep to 73.8% in ewes that were older than six years.

Based on limited data, any edible lamb tissue can harbor viable *T. gondii* (112). Viable *T. gondii* was detected from 17.7% of 433 lamb diaphragms and hearts from slaughtered lambs in France (113). In another study, *T. gondii* DNA was detected in 6 of 9 commercial lamb cut samples obtained from commercial sources in the United Kingdom (114).

2.4.3 *T. gondii* prevalence in goat and goat meat

According to National Agriculture Statistics Service (NASS) , an increasing demand for goat meat has occurred in recent years due to the growing population of ethnic and faith-based groups who consume goat meat, especially in the southeastern United States (115). Both raw or undercooked goat meat and raw goat milk were identified as potential sources for *T. gondii* infection. Goats are susceptible to *T. gondii* infection, with reported seroprevalence of *T. gondii* infection in goats ranging from 3.7% (116) to 81.8% (117). *T. gondii* seroprevalence is generally lower in goats than sheep based on studies from Iran (118), Nigeria (107), Portugal (119), Brazil (120), and Greece (121). The reasons for the difference in *T. gondii* infection in sheep and goats are not clear; however, it may be related to a difference in susceptibility (121), breed differences (122), and various feeding behaviors (123). Goats tend to consume the tops of grass and small trees, which may have lower *T. gondii* contamination levels

compared to the lower parts of the plants that sheep consume. *T. gondii* seroprevalence was reported to be higher in dairy goats as compared with meat producing goats (124). Possible factors contributing to this difference may be gender and age.

T. gondii prevalence in goat meat is not well defined since there are only a limited number of studies published. *T. gondii* was isolated from 13 out of 24 (54.2%) goat muscle tissue samples using the mouse bioassay in Japan (125). In another study, Silva et al. (126) reported that 13.7% (14/102) of goat tongue, heart or brain samples were positive for *T. gondii* by PCR in Brazil. Dubey et al. (127) detected antibodies to *T. gondii* in 125 (53.4%) of 234 sera extracted from hearts of goats purchased from local U.S. grocery stores; viable *T. gondii* was isolated from 29 of 112 seropositive heart tissues.

2.4.4 *T. gondii* prevalence in cattle and beef

The United States is the world's largest beef producer and beef is one of the major meats consumed nationwide. Variable seroprevalences have been reported in different surveys in cattle (128). However, none of the *T. gondii* serological assays have been critically evaluated in cattle. Garcia Bocanegra et al. (129) detected antibodies to *T. gondii* by ELISA in 420 of 504 cattle (83.3%), which was higher than the prevalence found in sheep (49.3%) and goats (25.1%) in the same study. Berger-Schoch et al. (130) reported that, using a p-30 ELISA, seroprevalence of *T. gondii* infection in cattle was 45.6%, which was higher than in pigs (23.3%), but lower than in sheep (61.6%). However, seroprevalence of *T. gondii* infection in cattle was not consistent with the prevalence of tissue cysts harbored in cattle. In one study, *T. gondii* DNA was detected from two seronegative cattle (131). Cattle are considered a poor hosts for *T. gondii*;

there is no confirmed case of *T. gondii*-induced abortion in cattle (128, 132). In a national retail meat survey, no *T. gondii* was isolated from 2,094 beef samples from retail stores nationwide (34). Thus, beef consumption may not pose a significant risk for human infections in the United States.

2.4.5 *T. gondii* prevalence in poultry and poultry meat

According to the USDA, chicken, broiler chicken in particular, and turkey are the most popular meats in the United States. Chicken consumption (26.3 kg per capita annually) surpassed beef consumption (25.7 kg per capita annually) and became the highest consumed meat product in 2010 (133). Chicken is considered as one of the most important hosts in the epidemiology of *T. gondii* infection (19). The age of chickens and husbandry methods are associated with prevalence of *T. gondii* infection. Older chickens are more likely to be infected with *T. gondii*. The higher prevalence of *T. gondii* infection with age is due to longer exposure to environmental oocysts. Little is known about *T. gondii* prevalence in commercially-raised (indoor) chicken, although it is believed to be low (19). In contrast to indoor chickens, the prevalence of *T. gondii* in free-range chickens is considerably higher (19). "Free-range" refers to animals that are allowed to roam for food, rather than confined in an enclosed system. Infection in free-range chickens has been used as an indicator of environmental oocyst contamination since they roam freely and obtain food directly from the ground (134). The infection rate of free-range chickens can vary widely among chickens from different locales, reflecting differences in extent of environmental oocyst contamination. Backyard raised chickens are different than free-range chickens. While free-range chickens are reared in large numbers in commercial farms, backyard raised

27

chickens are normally raised in the backyard of the household and produced in low numbers and not sold commercially. There are limited data regarding free-range chickens in the United States. Viable *T. gondii* has been isolated from 21 (21.9%) of 96 and 302 (36.3%) of 830 free-range chickens via bioassay in Israel (96) and Austria (135), respectively. In another study, a *T. gondii* infection rate of 34.7% was observed among 308 free-range chicken, while only 2.8% of 210 caged chicken were positive by ELISA in China (136). Although the seroprevalence of *T. gondii* infection in free-range chicken is higher than indoor chicken, free-range chicken might not be an important source for *T. gondii* infection in humans because they are not as an important source of meat and are not available in most retail stores.

T. gondii prevalence in live chickens is not directly proportional to risk of transmission from infected chicken meat. Processing from live chicken to chicken meat may reduce the risk. According to the USDA, chicken sold in the United States is either fresh or frozen. Fresh on a poultry label signifies that a raw poultry product has never been held below 26°F (-3.3°C). Frozen or previously frozen refers to raw poultry held at 0°F (-17.8°C) (91). Freezing at either -10°C for 3 days or -20°C for 2 days efficiently kills *T. gondii* tissue cysts (132, 137). This suggests that frozen chicken meat would have a low risk of being a source for *T. gondii* infections for human. In addition, chicken meat is generally cooked thoroughly, further reducing the risk. Dubey et al. (34) did not isolate *T. gondii* from any of 2,094 chicken breast samples from retail stores in the United States, and all the chicken breast samples were labeled as "not frozen". This result does not indicate that chicken meat is *Toxoplasma*-free. In general, persistence of *T. gondii* in chicken breast is lower than in the heart (19). Many chicken

breasts are also injected with enhancing solutions that can kill some tissue cysts (138). *T. gondii* has been regularly isolated from heart, muscle and brain of chickens, especially from free-range chickens (139, 140, 141, 142). Humans have an increased risk of becoming infected by consuming raw or undercooked chicken heart or free-range chicken meat.

2.4.6 *T. gondii* prevalence in organic meat and poultry

Organic meat and poultry constitutes a small part of the meat industry; however, it is one of the fastest growing sectors in the organic industry. According to data from the USDA Economic Research Service (ERS), organic livestock production increased dramatically between 2000 and 2005, with 20% and 58% average annual increases each year in production of organic beef and milk cows, and organic pigs, respectively (143). National Organic Program Standards require that meat producing livestock and poultry that will be labeled as organic must have "access to outdoors, shade, space for exercise, shelter, clean and dry bedding, fresh air, clean drinking water and direct sunlight". Organic ruminant livestock must have free access to certified organic pasture for the entire grazing season, which ranges from 120 to 365 days per year (144). As discussed above, *T. gondii* prevalence in organic livestock was significantly higher than for conventionally reared animals due to the free access to the outdoors and grazing pasture (19). Access to the outdoors increases the likelihood of meat animals being exposed to infected wildlife, organic material, water and soil containing infectious oocysts. Dubey et al. (145) reported that *T. gondii* infections in confinement-reared market pigs in the United States has decreased significantly over the last 20 years; however, infection levels in organically reared pigs can be quite high. In their study, 30 of 33 (90.9%)

organically-reared pigs were seropositive for *T. gondii* infection by both ELISA and

MAT. *T. gondii* was isolated from heart samples from 17 out of the 33 (51.5%) animals

via bioassay in mice. The differences in *T. gondii* infection rates between conventional

and organic pork meat were also reported in the Netherlands. Van der Giessen et al.

(87) reported 0.38% (1/265), 2.74% (11/402), and 5.62% (10/178) *T. gondii*

seroprevalence in pigs from confinement, organic, and free-range farming systems,

respectively. The likelihood of detecting anti-*T. gondii* antibodies in animals on a free-

range farm was statistically higher ($p < 0.05$) than on an intensive farm, but was not

significantly different when compared with organic farming systems. In another study,

Kijlstra et al. (146) found that 38 of 1,295 (2.9%) pigs from "animal-friendly" systems

tested positive for *T. gondii*, while none of 621 samples from conventional farms were

positive. Meerburg et al. (147) also reported that *T. gondii* prevalence was 3% out of

2,796 pigs from 41 organic farms in the Netherlands. At the farm level, 22 (54%) farms

were positive. To our knowledge, the prevalence of *T. gondii* in organic sheep, lamb

and cattle has not yet been reported.

Demand for organic poultry has experienced a 39% average annual increase

from 2000 through 2005 (143). The demand for organic broilers increased an average

of 53% per annum during that same period. According to the USDA, organic-rearing

of poultry is different from free-range poultry. Organic poultry must be fed only

certified organic feed, grown without artificial fertilizers or pesticides, have not

received hormones or antibiotics and been given reasonable access to outdoors (144).

Based on the USDA regulation, free-range poultry require free, continuous access to

the outdoors for more than half of their lives. *T. gondii* prevalence in organic poultry

has not been well studied. Non-confinement housing is a significant risk factor for *T. gondii* infection in organic chickens.

The organic market is still a niche market targeted to health-conscious consumers. *T. gondii* prevalence in organic meat products and risk to human health has not been well studied. However, based on limited data from swine and free-range animals, it could be speculated that the likelihood of *T. gondii* infection in organic meat and meat animals is higher than in conventional ones.

2.4.7 Risk factors associated with *T. gondii* infection in meat animals

Along with *T. gondii* prevalence studies, several researchers have attempted to establish a correlation between *T. gondii* infection and husbandry risk factors in meat animals, mainly swine and sheep. Risk factors related to increased *T. gondii* infection included farm type, feeding practices, presence of cats, rodent control methods, bird control methods, farm management, carcasses handling and disposal, and water source and quality. The identified risk factors are summarized in **Table 2.5**.

2.5 Conclusions

Despite being long recognized as a significant foodborne pathogen, consumer knowledge of this microorganism is typically limited. In particular, consumers have limited information on the wide range of food animals that can harbor this pathogen protozoa and thus serve as potential reservoirs for human infections. It is clear from the studies reviewed above that prevalence of *T. gondii* was lower in cattle and poultry raised conventionally compared to conventional pigs and sheep. What is not generally appreciated is the increased prevalence of *T. gondii* in organic livestock and poultry, in

large part due to outdoor access. Organic pork and lamb have a higher likelihood of *T. gondii* infection. The limited number of microbial risk assessments performed in a small number of countries is helping identify the critical risk factors influencing the role of husbandry practices on the risk of *T. gondii* infections in meat animals. Knowledge of these risk factors is critical to the identification and evaluation of meaningful intervention strategies. The lack of quantitative microbial risk assessment is hampering the development of effective risk management programs. Thus, it is necessary to develop risk assessment models for human *T. gondii* infection associated with meat consumption. This study serves as a useful resource and information repository for informing risk assessment studies for *T. gondii* infection in humans through meat consumption.

Table 2.1 Seroprevalence of *T. gondii* infection in swine, 2004-2014

Country/Area	Year	Source/type	No. of sampled animals	Seroprevalence (%)	Method	Risk studies*	Reference
Brazil/ Southern Piauí	2014	farm	143	25.5	IgG ELISA	yes	(148)
Brazil/ Rio Grande do Sul	2014	slaughter	100	36.0	IFAT, 1:64	no	(149)
Brazil/ Paraíba	2014	slaughter	190	19.5	IFAT, 1:64	yes	(150)
Mexico/ Veracruz	2014	backyard	402	45.3	MAT, 1:25	yes	(151)
Romania/ Center, West, and Northwest	2013	backyard	2,564	30.5	IFAT, 1:32	no	(152)
		sows	371	12.4			
		finisher	660	0			
China/ Heilongjiang	2013	finisher	1,014	4.6	IHA kit[b], 1:64	limited	(153)
Ireland/ all regions	2013	finisher	317	4.7	LAT, 1:64	limited	(154)
Latvia/ Kurzeme, Latgale, Vidzeme, Zemgale	2013	intensive reared finisher	269	0.4	in-house ELISA	no	(85)
		free-range finisher	543	6.2			
Portugal/ North	2013	farm	254	9.8	MAT, 1:20	limited	(119)
Slovak/ Košice, Prešov, Banská, Bystrica, Nitra, Trnava, Bratislava	2013	slaughter	923	2.16	ELISA kit[a]	no	(155)
		sow	47	4.26			
Brazil/ Pernambuco	2012	slaughter	305	12.5	IFAT, 1:64	no	(156)
Chile/ Araucanía and Los Ríos	2012	slaughter	340	8.8	ELISA kit[c]	no	(157)
China/ Hubei	2012	farm	2,277	29.6	AG-ELISA	limited	(58)
China/ Liaoning	2012	slaughter	1,164	12.0	IHA kit[d], 1:64	no	(158)
China/ Chongqing	2012	slaughter	908	30.6	IHA kit[b], 1:64	no	(159)
Mexico/ Oaxaca	2012	backyard	337	17.2	MAT, 1:25	yes	(84)
		farm	188	0.5			
Brazil/ Rio de Janeiro	2011	slaughter	406	7.64	IFAT, 1:64	no	(160)
Brazil/ Rio de Janeiro	2011	indoor raised	27	11.5	in-house ELISA	no	(161)
		free-range	34	20.6			
Brazil/ São Paulo	2011	farm	200	48.0	IFAT, 1:64	no	(162)
China/ Hubei	2011	large-scale farm finishers	3,558	24.5	AG-ELISA	yes	(163)

China/ Zhejiang	2011	hoggeries	813	53.4	ELISA kit[f]	limited	(164)
Czech/ 8 districts	2011	slaughter	551	36.0	ELISA kit[e]	no	(165)
France/ New Caledonia	2011	slaughter	49	2.0	P-30-ELISA	no	(166)
Italy/ Umbria	2011	indoor raised finisher	960	16.1	IFAT, 1:16	yes	(167)
Mexico/ Durango	2011	slaughter	1,074	12.7	MAT, 1:25	limited	(82)
Serbia/ Belgrade	2011	slaughter	488	9.2	MAT, 1:25	no	(168)
Switzerland/ all areas	2011	finisher	50	14.0	P-30-ELISA	no	(130)
		3-4 years	120	36.0			
		free-range	100	13.0			
Switzerland/ all regions	2011	finisher	50	14.0	P-30-ELISA	no	(169)
		sow	120	36.0			
		free-range	100	13.0			
West Indies/ Grenada and Carriacou	2011	N/S	247	23.1	MAT, 1:25	no	(170)
Brazil/ Mato Grosso	2010	sow	708	12.8	IFAT, 1:64	no	(171)
Brazil/ Patos	2010	slaughter	130	36.2	IFAT, 1:64	no	(172)
Brazil/ Toledo	2010	farm	606	13.4	MAT, 1:25	yes	(173)
China/ Western Fujian	2010	sow	605	14.4	IHA kit[d]	no	(174)
China/ Guangdong	2010	slaughter	1,022	27.0	ELISA kit[g]	no	(175)
Poland/ different regions	2010	N/S	1,754	19.2	in-house ELISA	no	(176)
Spain/ all areas	2010	finisher	1,570	9.7	MAT, 1:25	yes	(177)
		sow	1,400	24.2			
Spain/ Catalonia	2010	finisher	880	20.2	MAT, 1:25	yes	(178)
		sow	322	15.5			
United States NAHMS	2010	indoor raised finisher	6,238	2.6	ELISA kit[c]	yes	(78)
Brazil/ Bahia	2009	farm	465	18.3	ELISA, 1:16	no	(179)
Brazil/ Belém	2009	slaughter	110	50.0	IHT, 1:16	no	(180)
China/ Yunnan	2009	slaughter	831	17.0	IHA kit[b], 1:64	no	(181)
Italy/ Sicily	2009	slaughter	2,160	16.3	ELISA kit[h]	yes	(182)
Brazil/ Paraná	2008	piggeries	408	25.5	IFAT, 1:64	no	(183)
Brazil/ west Paraná	2008	slaughter	304	7.2	MAT, 1:64	no	(184)
Canada/ Ontario	2008	finisher	6,048	0.74	ELISA kit[c]	no	(185)

Country/Region	Year	Type	n	%	Test		Ref
Germany/ 8 Federal States	2008	finisher	4,999	4.1	ELISA	no	(186)
Malaysia/ Perak, Penang, Johor, Selangor	2008	sow	100	0	IFAT, 1:200	no	(187)
Panama/ Cocle´, Chiriquı´, Herrera, Los Santos, Panama´ and Veraguas	2008	confinement raised slaughter	290	32.1	IFAT, 1:20	no	(188)
Poland	2008	slaughter	106	26.4	MAT, 1:40	no	(189)
The Netherlands	2008	organic	406	10.9	ELISA	yes	(190)
Unites States/ Wisconsin, North Carolina, Ohio	2008	outdoor raised finishers	324	6.8	ELISA	no	(86)
		indoor raised finishers	292	1.1			
United States/ Maryland	2008	free-range	48	25.0	ELISA kit[c]	no	(191)
Brazil, Minas Gerais and Sao Paulo	2007	slaughter	262	0	MAT,	no	(192)
Brazil/ Paraná	2007	slaughter	117	8.5	IFAT, 1:64	no	(193)
Brazil/ Registro-SP	2007	small farm	550	20.2	MAT, 1:64	no	(194)
Taiwan/ North	2007	slaughter	395	10.1	LAT, 1:32	no	(195)
The Netherlands	2007	intensive raised	265	0.4	Standard ELISA	no	(87)
		organic raised	402	2.7			
		free-range	178	5.6			
Vietnam/ Ho Chi Min	2007	finisher	325	23.0	MAT, 1:25	no	(196)
		sow	207	32.3			
Brazil/ Rondônia (Amazon)	2006	farm	80	37.5	MAT, 1:25	no	(197)
Brazil/ Rio de Janeiro	2006	slaughter	38	65.8	IFAT, 1:64	yes	(198)
Portugal/ Vinhais	2006	semi-intensive raised	333	15.6	MAT, 1:20	no	(199)
Serbia/ various regions	2006	slaughter	605	28.9	MAT, 1:25	yes	(200)
The Netherlands	2006	organic	2,796	3.0	ELISA	yes	(147)
Brazil/ São Paulo	2005	free-range	286	17.0	MAT, 1:25	no	(201)
Brazil/ São Paulo and Pernambuco	2005	farm	759	1.3	MAT, 1:64	yes	(202)
Brazil/ Paraná	2005	finisher	395	2.6	IFAT, 1:64	no	(203)
		sow	29	20.7			
Germany	2005	finisher	300	5.6	ELISA	no	(204)

Germany/ Munsterland	2005	sow	1,500	9.3	ELISA	no	(205)
Zimbabwe	2005	finisher in good hygiene practice farms	238	19.8	IFAT, 1:100	no	(206)
		backyard scavenging	70	35.7			
Argentina/ Buenos, Cordoba, La Pampa, Santa Fe, San Luis	2004	sow	230	37.8	MAT, 1:25	no	(207)
Germany/ Hesse	2004	sow	2,041	16.5	IFAT, 1:16	yes	(208)
Peru/ Lima	2004	slaughter	137	27.7	Western Blot	limited	(209)
The Netherlands/ Didam, Zevenaar, Apeldoorn, Helmond	2004	conventional raised	621	0	LAT, 1:64	no	(146)
		free-range	635	4.7			
		organic raised	660	1.2			
Taiwan/ Taoyuan County	2004	slaughter	111	28.8	LAT, 1:32	no	(210)
United States/ Georgia	2004	slaughter	152	16.4	Western Blot	limited	(209)

LAT: latex agglutination test; IFAT: indirect fluorescent antibody test; MAT: modified direct agglutination; IHA: indirect haemagglutination antibody; ELISA: Enzyme-linked immunosorbent assay; IAT: indirect antibody tests; Finisher: The phase between the pig's birth and time to go to market; Slaughter pig: including pigs with various age; NAHMS: the National Animal Health Monitoring System; [a]ELISA *Toxoplasma gondii* serum screening, Institut Pourquier, France; [b]Veterinary Research Institute, Jiangsu Academy of Agricultural Sciences, Nanjing, China; [c]SafePath *Toxoplasma gondii*, SafePath Laboratories, USA; [d]IHA commercial kit, Lanzhou Veterinary Research Institute, China; [e]ELISA kit ID Screen Toxoplasmosis Indirect, ID-Vet, France; [f]ELISA kit, Zhuhai S.E.Z. Haitai Biological Pharmaceuticals Co., Ltd., China; [g]ELISA kit, Shenzhen Combined Biotech Co., China; [h]ELISA *T.gondii* serum screening, Institut Pourquier, France; *Risk assessment studies are further discussed in table 4; N/S: not stated.

Table 2.2 Attempts at demonstration of *T. gondii* from pigs

Country	Year	Meat samples	Methods	No. of positive samples /total samples (%)	Reference
Brazil	2014	brain and heart	bioassay in mice	17/36	(149)
Brazil	2014	brain, heart and tongue	bioassay in mice	13/37	(150)
Ireland	2013	diaphragm	nested PCR, 8S-5.8S Rrna ITS region	3/23	(154)
Slovak	2013	brain and muscle	PCR, TGR1E and B1gene	21/21	(155)
Brazil	2012	heart	PCR, B1 gene	21/38	(156)
Jordan	2012	aborted sheep foetal tissue	one-step PCR	43/133	(211)
Serbia	2012	blood clot	bioassay in mice	16/22	(168)
Spain	2012	fresh pork and ham	bioassay in mice	2/50	(95)
Brazil	2011	brain and heart	bioassay in mice	5/35	(212)
Mexico	2010	pork loin and leg	hemi-nested PCR, B1 gene	1/48	(97)
Spain	2010	ham from naturally infected pigs	bioassay in mice	6/6	(72)
United States	2009	heart	PCR-RFLP	29/300	(213)
United States	2008	heart	bioassay in mice and cats	14/38	(191)
Brazil	2007	diaphragm and tongue	PCR, 533bp fragment	diaphragm:17/50,tongue: 33/50	(214)
Brazil	2006	brain	bioassay in mice	6/12	(215)
Portugal	2006	brain and/or heart	bioassay in mice	15/37	(199)
Brazil	2005	pork sausage	bioassay in cats	13/149	(101)
Brazil	2005	heart, brain and tongue	bioassay in mice	7/28	(201)
Brazil	2005	fresh pork sausage	nested PCR	19/70	(216)
Japan	2005	pig lymph node	PCR, SAG2 locus	57/101	(217)
United States	2005	pork loin	bioassay in cats and mice	7/2,094	(34)
Morocco	2004	sheep brain	bioassay in mice	15/50	(218)

Table 2.3 Seroprevalence of *T. gondii* in sheep, 2004-2013

Country/Area	Year	Type	No. of sampled animals	Seroprevalence (%)	Method	Risk studies*	Reference
Brazil/ Bahia	2013	farm	795	30.2	IFAT, 1:64	yes	(219)
China/ Liaoning	2013	sheep	566	4.4	IHA kit[b], 1:64	no	(220)
Italy/ Tuscany	2013	milk sheep	630	33.3	IFAT, 1:64	yes	(221)
Ireland/ all regions	2013	slaughter	292	36.0	LAT, 1:64	limited	(154)
Japan/ Hokkaido, Iwate, and Aomori	2013	sheep	267	28.8	IFAT, 1:40	no	(222)
Spain/ Seville	2013	sheep	503	49.3	ELISA kit[a]	no	(129)
Brazil/ Lages, Santa Catarina	2012	sheep	360	56.9	IFAT,1:64	No	(223)
Greece/ Northern	2012	sheep	1,501	48.6	TgSAG1-ELISA	yes	(121)
Iran/ Kurdistan	2012	sheep	368	21.7	Indirect ELISA kit[c]	no	(224)
Mexico/ Durango	2012	sheep	511	15.1	MAT, 1:25	limited	(225)
Mexico/ Oaxaca	2012	sheep	429	23.1	MAT,1:25	limited	(104)
Portugal/ North	2012	slaughter	119	33.6	MAT, 1:20	no	(119)
Spain/ Southern	2012	sheep	503	49.3	ELISA kit[a]	no	(129)
Bangladesh/ Mymensingh	2011	sheep	25	40.0	Toxotest-MT[f], 1:16	no	(226)
Brazil/ Maranhão	2011	sheep	64	18.8	IFAT,1:40	no	(120)
Brazil/ Pernambuco	2011	sheep	124	48.4	IFAT, 1:16	no	(227)
Brazil/ São Paulo	2011	farm	382	18.6	MAT, 1:16	no	(228)
Brazil/ Minas Gerais	2011	farm	155	46.5	IFAT, 1:64	no	(229)
Brazil/ São Paulo	2011	slaughter	602	11.0	MAT, 1:16	no	(230)
China/ Tibet	2011	sheep	455	5.7	IHA kit[d], 1:64	no	(231)
China/ Heilongjiang	2011	sheep	792	3.0	IHA kit[e], 1:16	no	(232)
Iran/ Urmia	2011	sheep	156	21.1	MAT, 1:20	no	(233)
New Zealand/ various regions	2011	ewes	2,254	85.0	LAT, 1:16	no	(234)
Switzerland	2011	ewes	150	80.7	P-30-ELISA	no	(130)
		lamb	100	33.0			

Location	Year	Type	N	%	Test	Cats	Ref
Turkey/ Afyonkarahisar	2011	sheep	123	99.2	SFD test, 1:16	no	(235)
		lamb	63	98.4			
United Kingdom	2011	sheep	2,685	57.5	IgG-ELISA	yes	(111)
		lamb	204	37.3			
United Kingdom	2011	sheep	3,539	74.0	LAT, 1:64	yes	(236)
West Indies/ Grenada and Carriacou	2011	sheep	204	44.1	MAT,1:25	no	(170)
Austria	2010	sheep	4,079	66.0	IFAT, 1:40	no	(237)
Brazil/ São Paulo	2010	sheep	488	52.0	IFAT, 1:64	yes	(238)
China/ Qinghai	2010	sheep	580	29.8	IHA kit[e], 1:64	no	(239)
Finland/ North-south	2010	sheep	1,940	24.6	DAT, 1:40	no	(106)
France/ 17 districts	2010	lamb	619	17.7	ELISA kit[g]	no	(113)
		sheep	181	89.0			
Mongolia/ 7 provinces	2010	sheep	175	24.0	ELISA	no	(240)
Nigeria/ Borno	2010	sheep	372	6.7	ELISA kit[g]	limited	(107)
Spain/ Galicia	2010	sheep	177	57.0	DAT, 1:40	no	(108)
The Netherlands	2010	slaughter	1,179	27.8	ELISA kit[g,b]	no	(105)
Brazil/ Alagoas	2009	sheep	432	32.9	IFAT, 1:64	yes	(241)
Brazil/ Paraná	2009	farm	167	25.8	ELISA	no	(242)
Brazil/ Central	2009	farm	1,028	38.2	IFAT, 1:64	limited	(243)
Brazil/ Rio Grande do Norte	2009	farm	409	20.7	IFAT, 1:64	limited	(244)
Czech Republic	2009	farm	547	59.0	ELISA	no	(245)
Pakistan/ Punjab	2009	sheep	90	11.2	LAT, 1:8	limited	(122)
Slovakia, Eastern	2009	dairy aborting	382	24.3	indirect ELISA kit[c]	no	(246)
Brazil/ São Paulo	2008	slaughter	495	24.2	MAT, 1:25	no	(247)
Bulgaria/ Zagora	2008	farm	380	48.2	IHA, 1:10	no	(248)
Egypt/ Cairo	2008	slaughter	300	43.7	MAT, 1:25	no	(249)
Iran/ Ahvaz	2008	farm	150	72.5	ELISA	no	(250)
Mexico/ Colima	2008	farm	351	29.1	ELISA	limited	(109)
Mexico/ Puebla	2008	1-4 years, ranch	103	83.7	hmELISA	no	(109)
Poland	2008	farm	41	53.6	IFAT 1:8	no	(251)

Turkey/ Samsun	2008	sheep	95	49.5	SFD test, 1:16	no	(252)
United States/ Maryland, Virginia	2008	lamb	383	27.1	MAT, 1:25	no	(253)
Brazil/ Paraná	2007	ovine	157	7.0	IFAT, 1:64	no	(193)
Brazil/ Lajes	2007	farm	102	29.4	ELISA	limited	(254)
Brazil/ Paraná State	2007	farm	305	51.5	IFAT, 1:64	yes	(255)
France/ Lithuania	2007	farm	354	42.1	ELISA kit[g]	no	(256)
Iran/ Chaharmhal and Bakhtiari	2007	sheep	1,000	29.1	IFAT, N/A	no	(257)
Iran/ Mazandaran	2007	slaughter	588	35.0	IFAT, 1:16	no	(123)
Iran/ Tehran and Mazandaran	2007	slaughter	105	20.9	LAT, 1:8	no	(258)
Italy/ Campania	2007	farm	1,170	28.5	IFAT, 1:100	limited	(259)
Italy/ Sardinia	2007	sheep	1,043	51.3	ELISA	no	(260)
Italy/ Sicily	2007	farm	1,961	49.9	ELISA kit[g]	yes	(261)
Saudi Arabia/ Tabouk	2007	slaughter	397	52.2	IFAT	no	(262)
South Africa/ 5 provinces	2007	slaughter	600	5.6	IFAT, 1:64	limited	(263)
Spain	2007	farm	203	40.4	MAT, 1:40	no	(264)
Turkey/ Kars	2007	farm	460	95.7	in-house ELISA	no	(265)
Brazil/ Rio Grande do Norte	2006	farm	123	39.0	IFAT, 1:20	no	(266)
France/ Haote-Vienne	2006	lamb	164	22.0	MAT, 1:20	no	(267)
		ewes	93	65.6			
Iran/ Ardabil	2006	farm	200	30	ELISA	no	(268)
Italy/ Orobie Alps	2006	sheep	1,056	78.0	LAT, 1:32	no	(269)
Serbia/ various regions	2006	sheep	511	84.5	MAT, 1:25	yes	(200)
Turkey/ Istanbul	2006	farm	181	31.0	ELISA	limited	(270)
France	2005	sheep	100	20.5	MAT, 1:20	no	(271)
		lamb	150	59.1			
Morocco/ Marrakech	2005	slaughter	261	27.6	ELISA	no	(272)
Turkey/ Yalova	2005	sheep	63	65.1	LAT, 1:16	no	(273)
Zimbabwe	2005	sheep	23	47.8	IFAT, 1:50	no	(274)

Brazil, São Paulo	2004	lamb	118	16.1	IFAT, 1:64	no	(275)
		1-4 years	185	24.9			
		>4 years	294	48.3			
Brazil/ Rondônia	2004	farm	141	46.8	IFAT, 1:64	no	(276)
Ethiopia, Nazareth	2004	>6 months	116	56.0	ELISA kit[h]	no	(277)
Poland	2004	sheep	20	55	MAT, 1:40	no	(278)
Turkey, Afyon	2004	>1 years	172	54.6	SFD test, 1:16	no	(279)
Turkey, Niğde	2004	farm	110	50.9	SFD test, 1:16	no	(280)

Toxotest-MT is a commercially provided diagnosis kit; LAT: latex agglutination test; IFAT: indirect fluorescent antibody test; MAT: modified direct agglutination; IHA: indirect haemagglutination antibody; ELISA: Enzyme-linked immunosorbent assay; PCR: Polymerase Chain Reaction; DAT: direct agglutination test. SFD test: Sabin-Feldman dye test; *Risk assessment studies are further discussed in table 4. hmELISA: home-made ELISA; [a]Competitive species-specific ELISA kit, CNEVA-LPPRA, Biot., France; [b]Chekit-Toxotest Enzyme Immunoassay diagnostic kit, Netherland; [c]ELISA kit, ID-VET. Innovative diagnostics, France; [d]IHA commercial kit, Lanzhou Veterinary Research Institute, China; [e]Veterinary Research Institute, Jiangsu Academy of Agricultural Sciences, Nanjing, China; [f]Diagnosis kit, Eiken Kagaku, Japan; [g]ELISA *Toxoplasma gondii* serum screening, Institut Pourquier, France; [h]ELISA kit, Enzygnost Bio Merieux, France.

Table 2.4 Demonstration of *T. gondii* in naturally exposed ovine tissues

Country	Year	Meat samples	Methods	No. of positive samples /total samples (%)	Reference
Ireland	2013	diaphragm	nested PCR, 8S-5.8S Rrna ITS region	3/83	(154)
Jordan	2012	aborted foetal tissue	one-step PCR	43/133	(211)
Brazil	2011	blood, brain, lung, and muscle	PCR	20/66	(230)
Iran	2011	brain, tongue, muscle, liver	nested PCR, GRA6 gene	21/56	(118)
France	2010	heart	bioassay in mice	48/397	(113)
Brazil	2008	brain, diaphragm, heart	bioassay in mice	16/82	(247)
United States	2008	lamb heart	bioassay in mice and cats	53/68	(253)
Iran	2007	brain	bioassay in mice	4/40	(258)
France	2006	heart	bioassay in mice	8/30	(267)

Table 2.5 Risk factors correlated with *T. gondii* prevalence at farm level

Country	Year	Species and numbers	Numbers of farms	Method	Risk factors associated with *T. gondii* prevalence	Reference
Brazil	2014	143 pigs	10 farms	registration	Age, diet, farm management, presence of cats.	(148)
Brazil	2014	190 pigs	N/S	interview	Extensive husbandry (animals are allowed to roam across the farm) and feed pigs with leftovers.	(150)
Mexico	2014	402 pigs	257 homes	questionnaire	Type of feeding, food storage condition, history of animals (deworming, cohabit with other animals).	(151)
Brazil	2013	795 sheep	31 farms	questionnaire	Age, rearing system, and transit of cats.	(219)
Italy	2013	630 milk sheep	33 flocks	questionnaire	Stray cats had access to animals' water.	(221)
Romania	2013	3,595 pigs	11 counties	N/S	The management system (indoor pigs versus backyard pigs)	(152)
Greece	2012	1,501 sheep and 541 goats	69 farms	questionnaire	Farm type, feeding concentration and water supply.	(121)
China	2011	3,558 pigs	37 farms	questionnaire	Exposure to cats, high breeding frequency, presence of mosquitoes and flies, frequency of scavenge, use of sulfonamides.	(163)
Germany	2011	186 poultry	N/S	N/S	Outdoor access.	(281)
Italy	2011	960 pigs	10 farms	questionnaire	Improper carcass handling, cleaning method and non-AIAU housing.	(167)
United Kingdom	2011	3,539 sheep	227 flocks	questionnaire	Number of breeding ewes in the flock, presence of cattle in the same premises and age.	(236)
United Kingdom	2011	3,333 sheep	125 flocks	questionnaire	Lambing location, sheep shared pasture from different farms.	(111)
Brazil	2010	606 pigs	23 farms	questionnaire	Water reservoirs and feed accessed by other animals, uses different employees according to the farm sector, < 10 piglets/sow at weaning.	(173)
Brazil	2010	488 sheep	6 herds	collected from farm	Gender of the sheep, pasturing system, contact with cats, the use of mineral supplements and the type of feed.	(238)
Spain	2010	2,970 pigs	100 farms	interview	Lack of rodent control and presence of cats.	(177)
Spain	2010	1,202 pigs	23 farms	questionnaire and interview	Presence of cats, percentage of mortality in weaning, and outdoor access.	(178)
United States	2010	6,238 pigs	16 states	questionnaire and interview	Carcass Disposal method and lack of rodent control.	(78)
Brazil	2009	432 sheep	27 herds	questionnaire	Sex, age, geographic region, size of property, number of animals, and rearing system.	(241)

Italy	2009	2,160 pigs	274 farms	interview	Farming type, lack of rodent control, manual cleaning of the stables, farm locates < 200 m above sea level, and pig numbers in farm.	(182)
The Netherland	2008	100-400 pigs	3 organic farms	collected from farm	Rodent control.	(190)
Brazil	2007	305 sheep, 24 dogs	9 farms	questionnaire	Age, breed, farm size, semi-intensive rearing, mineral salt supplementation, water source, rodent control, cat presence.	(255)
Italy	2007	1,961 sheep	62 farms	interview	Presence of cats, farm size, using surface water as drinking water	(261)
Serbia	2006	511 ewes and 605 pigs	N/S	questionnaire	For pig: farm type; for sheep: state-owned flocks associated with an increased risk of infection.	(200)
The Netherland	2006	2,796 pigs	41 organic farms	questionnaire	Feeding goat whey to pigs and the presence of a high number of cats.	(147)
Germany	2004	2,041 sows	94 farms	questionnaire	Farm type.	(208)
Norway	1998	1,940 lambs	194 flocks	questionnaire	Cat presence and use of mouse poison.	(282)
United States	1995	3,841 sows	303 farms	questionnaire	Cat presence, outdoor rearing and raised on small farms (< 29 sows).	(283)

N/S: not stated.

Chapter 3: A systematic meta-analysis of *Toxoplasma gondii* prevalence in meat animals produced in the United States

This work has been submitted to *Foodborne Pathogens and Disease* and is currently under review.

3.1 Abstract

Meat animals are reservoirs for *T. gondii* and act as sources for parasite transmission to humans. Based on limited population-based data, the Food and Agriculture Organization (FAO) / World Health Organization (WHO) estimated that approximately 22% of human *T. gondii* infections are meatborne. The objective of the current study was to conduct a systematic meta-analysis to provide a more precise estimation of *T. gondii* infection prevalence in meat animals produced in the United States. Four databases were searched to collect eligible studies. Prevalence was estimated in six animal categories (confinement raised market pigs, confinement raised sows, non-confinement raised pigs, lamb, goats, and non-confinement raised chickens) by a quality-effects model. A wide variation in prevalence was observed in each animal category. Animals raised outdoors or that have outdoor access had a higher prevalence as compared with animals raised indoors. *T. gondii* prevalence in non-confinement raised pigs ranked the highest (31.0%) followed by goats (30.7%), non-confinement raised chickens (24.1%), lambs (22.0%), confinement raised sows (16.7%), and confinement raised market pigs (5.6%). These results indicate that *T. gondii*-infected animals are a food safety concern. The computed prevalence can be used as an

important input in quantitative microbial risk assessment models to further predict public health burden.

3.2 Introduction

Meat animals can be reservoirs for *T. gondii* and act as sources for parasite transmission to humans. Meat animals become infected after ingestion of oocyst-contaminated soil or water or by ingestion of tissue cysts in the muscle of *T. gondii*-infected animals or animal carcasses (e.g., rodents). *T. gondii* infection prevalence has been summarized in certain meat animals worldwide over recent 10 years in Chapter 2 (objective 1). The current chapter focuses on *T. gondii* infection prevalence in these meat animals raised in the United States. Prevalence of *T. gondii* in the U.S. domestically-raised meat animals varies according to geographic location, management system, age of the animal, biological specimen (serum or tissues), year of the study, and detection methods. Thus, it is necessary to summarize these individual studies and provide an overall estimation in a population.

Meta-analysis is a method to synthesize the results of various studies for a given question, and has been applied to address a wide range of food safety questions and to provide scientific-based evidence for decision making and risk management (284). In addition, the quantitative results obtained from meta-analysis have been used as inputs in risk assessment models (285, 286). The advantages of performing a meta-analysis include providing summary statistics based on multiple individual studies, increasing precision in estimating effects, and taking the size of studies into account (287). A quality-effects model was used in the present study, which incorporates a study specific

quality score to redistribute inverse variance weights and obtain more conservative estimates (288, 289). A series of assessment tools have been developed to analyze the quality of observational studies, and only a few of them were targeted to assess incidence/prevalence studies (290). Currently, six critical appraisal tools have been developed, which include the Joanna Briggs Institute's descriptive/case series appraisal tool (291), a Center for Evidence-Based Management critical appraisal of a survey (CEBMa) (292), a STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) checklist (293), a National Collaborating Centre for Environmental Health (NCCEH) critical appraisal of cross-sectional studies (294), a critical appraisal tool for prevalence (295), and a risk of bias tool (296). Among these assessment tools, the risk of bias tool is effective for addressing both external and internal validity, and has been used in several meta-analysis studies (297, 298, 299).

To the best of our knowledge, there has been no systematic meta-analysis conducted regarding *T. gondii* prevalence in meat animals raised in the United States. The objectives of the current study were to collect eligible studies, integrate results from these studies, and provide a quantitative estimate of *T. gondii* prevalence in certain meat animals raised in the United States.

3.3 Materials and Methods

3.3.1 Literature search

A comprehensive literature search was conducted to identify studies of *T. gondii* prevalence published after 1964 in four databases: PubMed, Google Scholar, MEDLINE and Web of Science. The following key words and their combination were

used during the literature search: "*Toxoplasma gondii* or toxoplasmosis" and "United States" and "incidence or prevalence or infection or isolation or epidemiology" and in combination with one of the following animal or meat categories "pig or pork or boar or sow", "chicken or poultry", "sheep or lamb", "goat", and "meat animals or meat".

3.3.2 Eligibility criteria

The search was limited to full-text, peer-reviewed articles, national surveys or government reports that were conducted for meat animals raised in the United States, written in English, and provided information on the study population, sample size, location, detection methods, and number of positive cases.

3.3.3 Data extraction

No studies were found that reported *T. gondii* prevalence in confinement raised chickens in the United States. Thus, all eligible studies were classified into six categories: confinement raised market pigs, confinement raised sows, non-confinement raised pigs, sheep, goats, and non-confinement raised chickens. Quantitative data were extracted from each study, including year of the study, study population, geographic location of the study, type of samples (e.g., serum, tissue, and organ), detection method, number of positive cases, and sample size. For publications that contained multiple studies, data were extracted separately.

3.3.4 Quality assessment of eligible studies

Each eligible study was assessed for quality and bias by using the risk of bias tool, which is a methodological quality assessment checklist for prevalence studies (296). Ten questions are contained in this checklist and each of the ten questions was scored 1 or 0 based on the quality of each eligible study (**Table 3.1**). Seven different

detection methods were used in these eligible studies. Thus, for question 7, which is to determine the reliability and validity of the measurement, MAT, ELISA, and bioassays are considered as reliable diagnostic methods (score 1), and other detection methods such as IHAT, Sabin-Feldman dye test (SFDT), Western blot and PCR-based assays were determined as unreliable methods (score 0). For each eligible study, a quality score was determined by rescaling the sum of scores between 0 and 1 (296). Quality assessment was completed independently by two assessors and a table of quality score computation for each eligible study is provided in **Table 3.1**.

3.3.5 Data analysis

In order for the prevalence values (mean, and 95% confidence interval) to be within 0 and 1, and to stabilize the variance, data were transformed by a double arcsine transformation as described by Barendregt et al. (300). A quality-effects model was used to calculate the population prevalence (PP) across studies as described by Doi et al. (288), which is a more precise method to describe overall prevalence as compared to a random-effects model (301). In the quality-effects model, the inverse variance weights of each study were redistributed based on quality score. The pooled prevalence was back transformed and normalized (300). Heterogeneity among studies was evaluated by Cochrane Q and I^2 statistical methods. A significant value ($p < 0.05$) in the Cochrane Q method suggests a real effect difference in the meta-analysis. A value of I^2 was used to measure the inconsistency across studies. Values of 25%, 50%, and 75% were considered as having a low, moderate and high degree of heterogeneity, respectively (302). Data were constructed in Microsoft Excel, and analyzed by using

MetaXL version 2.0 software (EpiGear Int Pty Ltd, Wilston, Australia), and graphed as forest plots.

3.4 Results

3.4.1 Characteristics of eligible studies

A total of 183 studies were generated by the systematic search and 39 articles met the criteria for inclusion. A total of 58 studies were reported in these 39 articles, and included 1,537 goats, 9,976 sheep/lambs, 43,386 confinement raised market pigs, 35,975 confinement raised sows, and 174 non-confinement raised chickens, 797 non-confinement pigs (**Table 3.2**). High specificity and sensitivity diagnostic methods such as MAT (n=33) and ELISA (n=9) were commonly used in these studies, while low sensitivity detection methods such as IHAT, SFDT and western blot were only used in 3, 3, and 1 studies, respectively. Animal bioassay was used in 8 eligible studies and viable *T. gondii* were detected from these samples.

3.4.2 Quality assessment

Overall, the quality scores in the eligible studies ranged from 5 to 10 (**Table 3.1**). Most studies were considered at moderate risk of bias (quality score ranges from 6 to 8). The study with the lowest quality score (303) had flaws in both internal and external validity. The common quality deficiency in the eligible studies pertained to external validity. A total of 48 of 58 eligible studies were conducted in local farms or regions, which were not representative of the national population. The flaws in internal validity were mainly due to the use of non-optimal detection methods and the application of sampling from a proxy.

Table 3.1 Quality score assessment based on the "risk of bias tool" (296)

Species	Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Summary
Goats	Dubey et al. 1985 SFDT	0	0	1	1	1	1	0	1	1	1	7
	Dubey and Adams 1990	0	0	1	1	0	1	1	1	1	1	7
	Patton et al. 1990 IHAT	0	0	1	1	0	1	0	1	1	1	6
	Patton et al. 1990 MAT	0	0	1	1	0	1	1	1	1	1	7
	Dubey et al. 2011	0	0	1	1	1	1	1	1	1	1	8
Lambs/sheep	Franti et al 1976	0	0	1	1	0	1	0	1	1	1	6
	Riemann et al. 1977	0	0	1	1	1	1	0	1	1	1	7
	Dubey et al. 1985	0	0	1	1	0	1	0	1	1	1	6
	Dubey and Welcome 1988	0	0	0	1	0	1	1	1	1	1	6
	Dubey and Kirkbride 1989	0	0	0	1	0	1	1	1	1	1	6
	Malik et al. 1990 lamb	0	0	1	1	1	1	1	1	1	1	8
	Malik et al. 1990 sheep	0	0	1	1	0	1	1	1	1	1	7
	Underwood and Rook 1992	0	0	0	1	0	1	0	1	1	1	5
	Dubey et al. 2008	0	0	1	1	1	1	1	1	1	1	8
	USDA 2014	1	1	1	1	1	1	1	1	1	1	10
Confinement raised market pigs	Dubey et al. 1985	0	0	1	1	1	1	0	1	1	1	7
	Zimmerman et al. 1990	0	0	1	1	1	1	1	1	1	1	8
	Dubey et al. 1991	1	1	1	1	1	1	1	1	1	1	10
	Dubey et al. 1992	0	0	1	1	1	1	1	1	1	1	8
	Dubey et al. 1995	0	0	1	1	1	1	1	1	1	1	8
	Weigel et al. 1995	0	0	1	1	1	1	1	1	1	1	8
	Patton et al. 1996	1	1	1	1	1	1	1	1	1	1	10
	Davies et al. 1998	0	0	1	1	1	1	1	1	1	1	8
	Gamble et al. 1999	0	0	1	1	0	1	1	1	1	1	7
	Patton et al. 2000	1	1	1	1	1	1	1	1	1	1	10
	Dubey et al. 2002	0	0	0	1	1	1	1	1	1	1	7
	Saavedra and Ortega 2004	0	0	1	1	0	1	0	1	1	1	6
	Dubey et al. 2005 ELISA	1	1	1	1	1	1	1	1	1	1	10
	Dubey et al. 2005 Bioassay	1	1	1	1	1	1	1	1	1	1	10
	Gebreyes et al. 2008	0	0	1	1	1	1	1	1	1	1	8

	Hill et al. 2010	1	1	1	1	1	1	1	1	1	1	10
Confinement raised sows	Zimmerman et al. 1990	0	0	1	1	1	1	1	1	1	1	8
	Dubey et al. 1991	1	1	1	1	1	1	1	1	1	1	10
	Smith et al. 1992	0	0	1	1	1	1	1	1	1	1	8
	Weigel et al. 1995b	0	0	1	1	1	1	1	1	1	1	8
	Dubey et al. 1995	0	0	1	1	1	1	1	1	1	1	8
	Weigel et al. 1995a	0	0	1	1	1	1	1	1	1	1	8
	Assadi-Rad et al. 1995	0	0	1	1	1	1	1	1	1	1	8
	Dubey et al. 1995	0	0	1	1	1	1	1	1	1	1	8
	Patton et al. 1996	1	1	1	1	1	1	1	1	1	1	10
	Hu et al. 1997	1	1	1	1	1	1	1	1	1	1	10
	Patton et al. 1998	1	1	1	1	1	1	1	1	1	1	10
	Patton et al. 2000	1	1	1	1	1	1	1	1	1	1	10
Non-confinement raised chickens	McCulloch et al. 1964	0	0	0	1	1	1	1	1	1	1	7
	Foster et al. 1969	0	0	0	1	1	1	1	1	1	1	7
	Dubey et al. 1981	0	0	0	1	1	1	1	1	1	1	7
	Dubey et al. 2003 Montana MAT	0	0	1	1	1	1	1	1	1	1	8
	Dubey et al. 2003 Montana bioassay	0	0	1	1	1	1	1	1	1	1	8
	Dubey et al. 2003 Massachusetts	0	0	0	1	1	1	1	1	1	1	7
	Dubey et al. 2007	0	0	0	1	1	1	1	1	1	1	7
Non-confinement raised pigs	Dubey et al. 1992	0	0	1	1	1	1	1	1	1	1	8
	Davies et al. 1998	0	0	0	1	1	1	1	1	1	1	7
	Gebreyes et al. 2008	0	0	1	1	1	1	1	1	1	1	8
	Dubey et al. 2008a	0	0	0	1	1	1	1	1	1	1	7
	Dubey et al. 2012	0	0	0	1	1	1	1	1	1	1	7

Questions listed in the checklist (Yes=1, No=0)

Q1: Was the study's target population a close representation of the national population in relation to relevant variables?

Q2: Was the sampling frame a true or close representation of the target population?

Q3: Was some form of random selection used to select the sample, OR, was a census undertaken?

Q4: Was the likelihood of non-response bias minimal?

Q5: Were data collected directly from the subjects (as opposed to a proxy)?

Q6: Was an acceptable case definition used in the study?

Q7: Was the study instrument that measured the parameter of interest shown to have reliability and validity (if necessary)?

Yes (if using MAT, ELISA, and bioassays), No (using other detection methods such as IHAT, Sabin-Feldman dye test, western-blot, and PCR-based assays)

Q8: Was the same mode of data collection used for all subjects?

Q9: Was the length of the shortest prevalence period for the parameter of interest appropriate?

Q10: Were the numerator(s) and denominator(s) for the parameter of interest appropriate?

Table 3.2 Characteristics and quality score of the included studies

Category	State/Area	Study population	Detection methods	Sample size	Prev%	QS	Reference
Goats	Montana	goats	SFDT	123	22.7	7	(313)
	Northwest	dairy goats	MAT	1,000	22.1	7	(116)
	Southeast	dairy goats	IHAT	108	51.9	6	(314)
			MAT	72	65.3	7	
	Northeast	goat hearts from retail	MAT	234	53.4	8	(127)
Lambs	California	domestic sheep	IHAT	1,048	15.0	6	(315)
	West	market lambs	IHAT	1,056	8.0	7	(316)
	Montana	domestic sheep	SFDT	649	13.2	6	(313)
	New York	ewes with high abortion risk	MAT	592	73.8	6	(308)
	North central	ewes with high abortion risk	MAT	1,564	65.5	6	(317)
	Northeastern	market lambs	ELISA	345	55.1	8	(318)
		sheep	ELISA	309	62.5	7	
	Michigan	sheep with high abortion risk	N/A	63	60	5	(303)
	Northeast	market lambs	MAT	383	27.1	8	(253)
	NAHMS	market lambs	MAT	3,967	9.4	10	(319)
Confinement raised pigs	Montana	market pigs	SFDT	413	5.0	7	(313)
	Iowa	market pigs	ELISA	2,029	4.8	8	(88)
	Nationwide	market pigs	MAT	11,229	23.0	10	(76)
	Hawaiian	grain-fed market pigs	MAT	180	33.8	8	(320)
	Illinois	market pigs	MAT	4,252	2.3	8	(321)
	Illinois	market pigs	MAT	1,885	3.1	8	(77)
	NAHMS	market pigs	MAT	4,712	3.2	10	(322)
	North Carolina	market pigs	MAT	2,238	0.6	8	(307)
	New England	pigs of various ages and sex	MAT	1,897	47.4	7	(323)
	NAHMS	market pigs	MAT	5,720	0.9	10	(324)
	Massachusetts	market Pigs from a high risk farm	Bioassay	55	92.7	7	(309)
	Georgia	pigs from slaughter house	Western Blot	152	16.4	6	(209)
	Nationwide	pork from retail stores	ELISA	2,094	0.6	10	(34)
			Bioassay	2,094	0.4	10	

	Midwest	market pigs	ELISA	292	1.1	8	(86)
	NAHMS	market pigs	ELISA	6,238	2.6	10	(78)
Confinement raised sows	Iowa	sows	ELISA	587	10.0	8	(88)
	Nationwide	sows	MAT	613	42.0	10	(76)
	Iowa	sows	MAT	273	14.3	8	(325)
	Illinois	sows	MAT	3,690	19.5	8	(90)
	Illinois	sows	MAT	2,617	15.1	8	(321)
	Illinois	sows	MAT	5,080	20.8	8	(77)
	Tennessee	sows	MAT	3,841	36.0	8	(283)
	Iowa	sows	MAT	1,000	22.2	8	(326)
	NAHMS	sows	MAT	3,479	20.0	10	(322)
	NAHMS	sows	MAT	3,473	19.0	10	(327)
	NAHMS	sows	MAT	3,236	15.0	10	(328)
	NAHMS	sows	MAT	8,086	6.0	10	(324)
Non-confinement raised chickens	Iowa	free-range chickens	Bioassay	12	91.6	7	(329)
	Texas	free-range chickens	Bioassay	11	63.6	7	(330)
	Montana	free-range chickens	Bioassay	11	27.3	7	(331)
	Ohio	free-range chickens	MAT	118	16.9	8	(332)
			Bioassay	118	9.3	8	
	Massachusetts	free-range chickens	Bioassay	11	100	7	(332)
	Illinois	free-range chickens	MAT	11	100	7	(333)
Non-confinement raised pigs	Hawaii	garbage-fed free-range pigs	MAT	199	67.3	8	(320)
	Hawaii	garbage-grain fed free-range pigs	MAT	130	40.0	8	(320)
	North Carolina	market pigs kept on pastures	MAT	63	19.0	7	(307)
	Midwest	free-range pigs	ELISA	324	6.8	8	(86)
	Maryland	free-range market pigs	MAT	48	70.8	7	(191)
			ELISA	48	25.0		
	Michigan	organic pigs	MAT	33	90.9	7	(145)
			Bioassay	33	51.5	7	

QS: quality score; Prev: prevalence; SFDT: Sabin-Feldman dye test; MAT: modified direct agglutination test; IHAT: indirect haemagglutination antibody test; ELISA: Enzyme-linked immunosorbent assay; NAHMS: the National Animal Health Monitoring System.

3.4.3 Population prevalence (PP) in meat animals

A wide variation in prevalence was observed in each animal category (**Table 3.3**), which reflected the heterogeneity of sample sets and real differences in effect size across studies (Large Cochran's Q value with $p < 0.05$, and $I^2 > 95\%$). Among the six animal categories, *T. gondii* population prevalence ranked the highest in non-confinement raised pigs (31.0%, **Figure 3.1**) followed by goats (30.7%, **Figure 3.2**), non-confinement raised chickens (24.1%, **Figure 3.3**), and lambs (22.0%, **Figure 3.4**). Compared to non-confinement raised pigs, indoor-raised pigs showed a lower prevalence. The prevalence was computed as 5.6% and 16.5% in confinement-raised market pigs (**Figure 3.5**) and sows (**Figure 3.6**), respectively.

Table 3.3 Statistics of the quality-effects meta-analysis

Meat animals	No. of studies	PP (%)	95% CI (%)	Cochran's Q	I^2
Confinement raised market pigs	16	5.6	0.4-14.8	7006.0	99.8
Confinement raised sows	12	16.5	10.3-23.7	2098.2	99.5
Lambs	10	22.0	1.3-53.5	3271.4	99.7
Goats	5	30.7	8.7-58.0	148.4	97.3
Non-confinement raised chickens	7	24.1	0.0-71.3	131.2	95.4
Non-confinement raised pigs	8	31.0	3.6-67.2	349.8	98.0
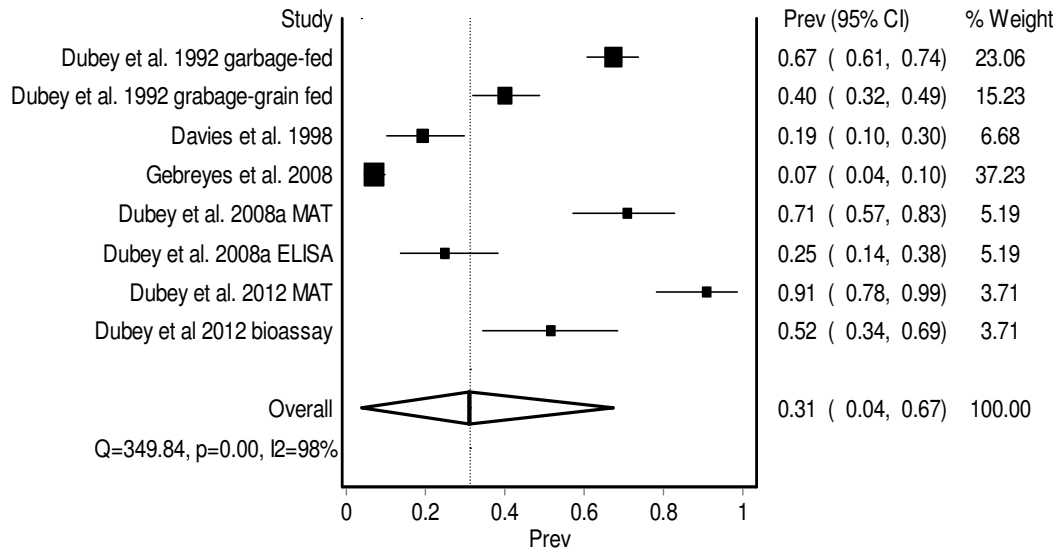
Figure 3.1 Forest plot that showing *T. gondii* infection in non-confinement raised pigs in the United States (quality-effects model).
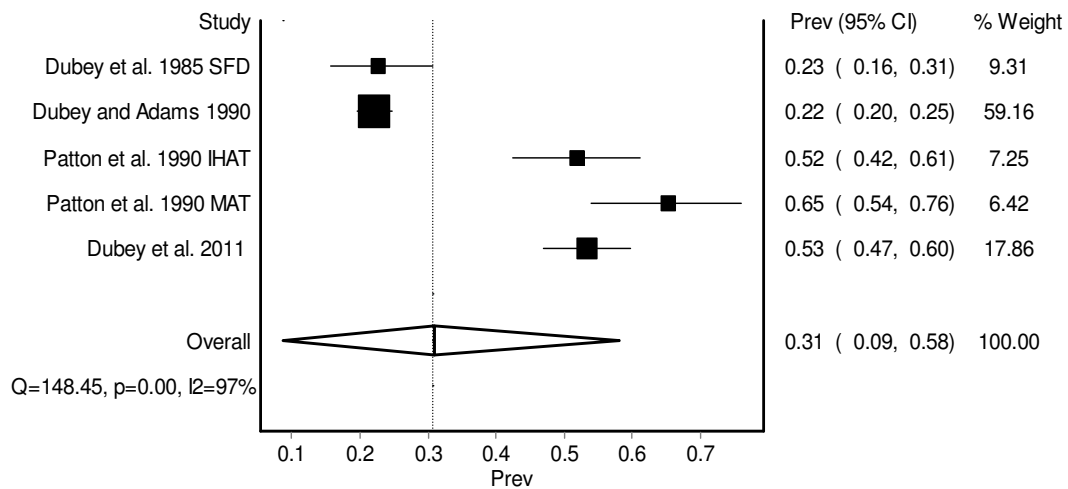
Figure 3.2 Forest plot that showing *T. gondii* infection in goats in the United States (quality-effects model).
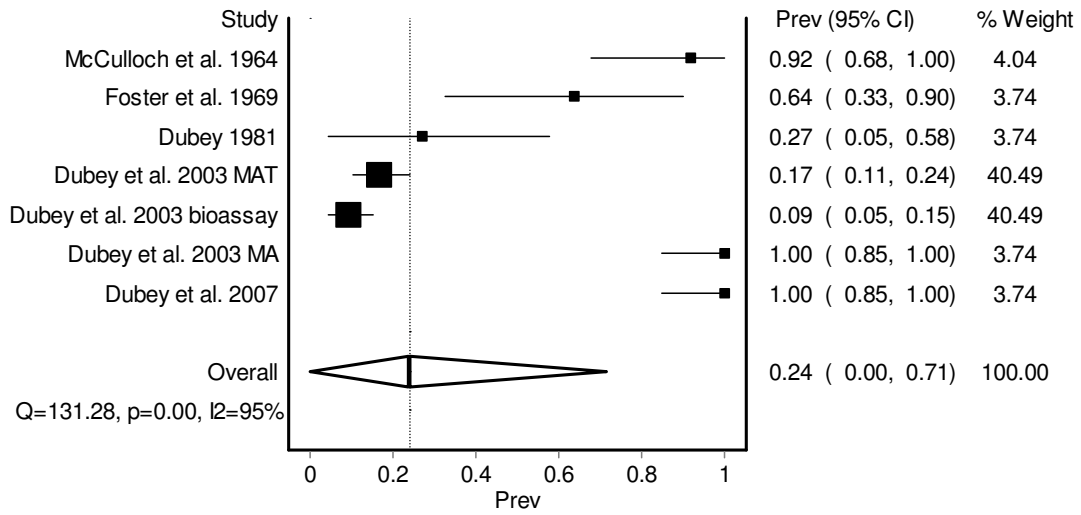
Figure 3.3 Forest plot that showing *T. gondii* infection in non-confinement raised chickens in the United States (quality-effects model).
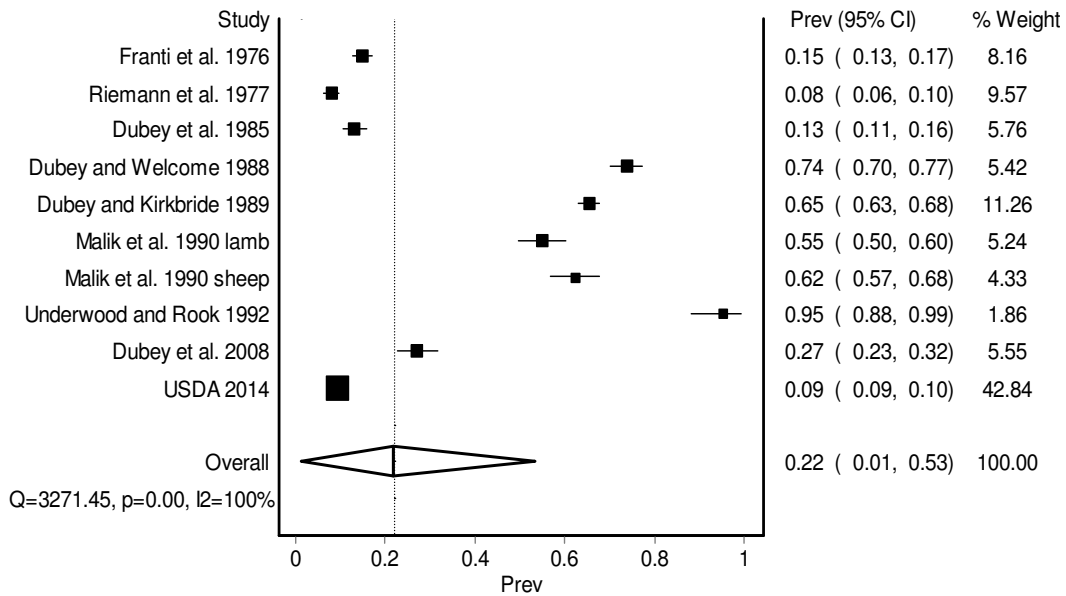
Figure 3.4 Forest plot that showing *T. gondii* infection in lambs in the United States (quality-effects model).
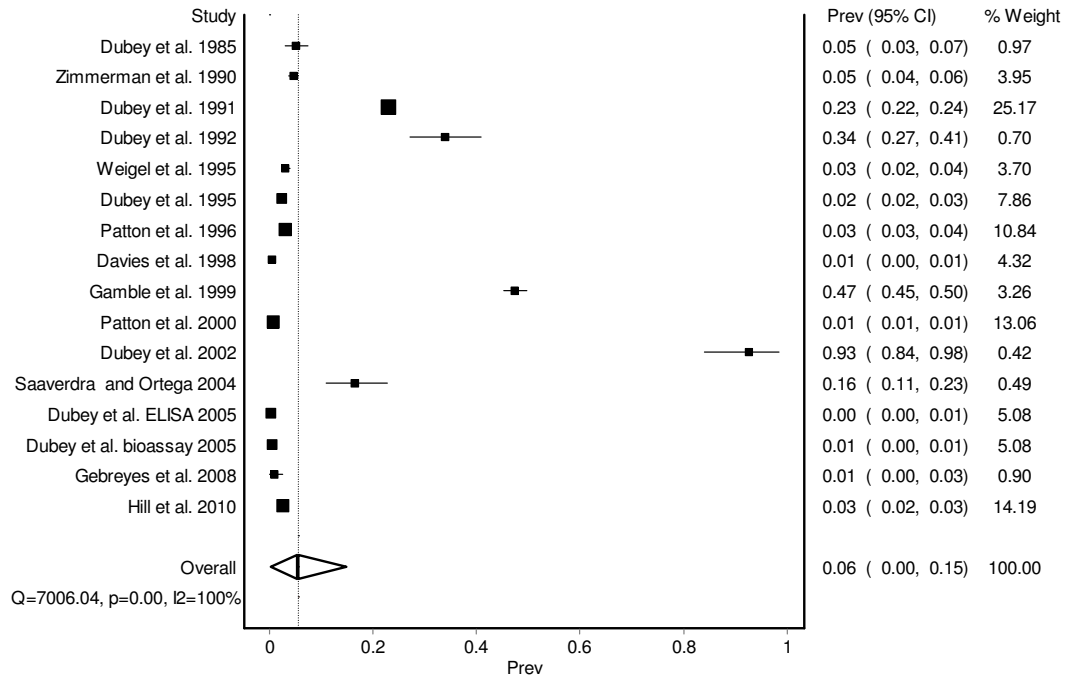
Figure 3.5 Forest plot that showing *T. gondii* infection in confinement-raised market pigs in the United States (quality-effects model).
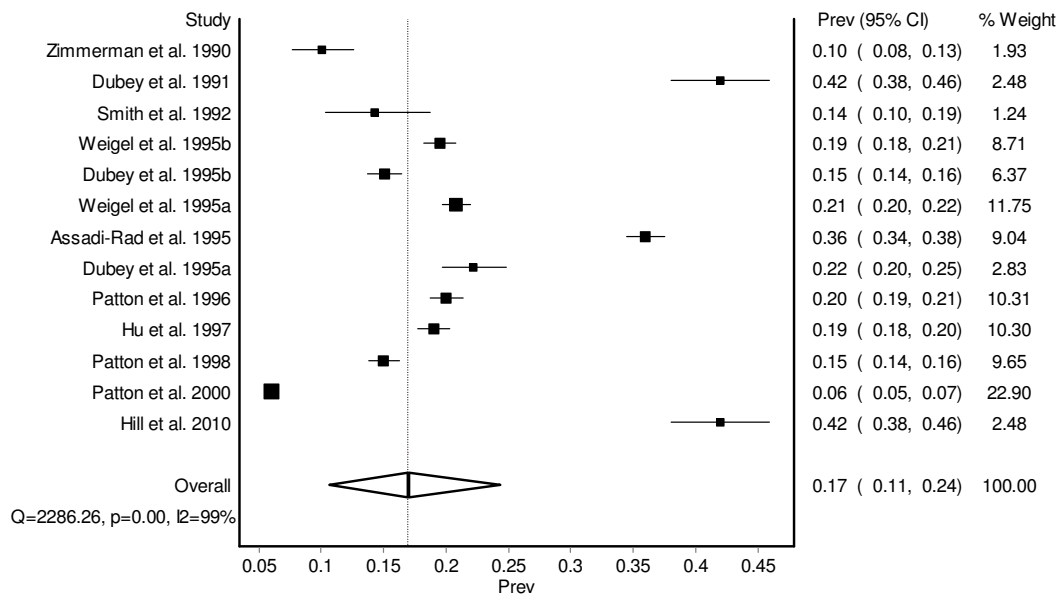
Figure 3.6 Forest plot that showing *T. gondii* infection in confinement-raised sows in the United States (quality-effects model).

3.5 Discussion

The meat and poultry industries are important components of U.S. agriculture. The consumption of meat products that produced from *T. gondii* prevalent meat animals accounts for approximately 25% of the total meat consumption in the United States. Approximately 92.3 billion pounds of red meat and poultry were produced in 2014, among which 22.8 billion pounds (24.7%) and 0.16 billion pounds (0.2%) came from pigs and lamb (304), respectively. An increased interest in goat meat consumption was observed in the United States. In 2006, approximately 1.5 million head of goats were consumed in the United States, and nearly half of the goats were raised domestically (305). This represents an increase of 150% and 320% from 2002 and 1999, respectively. In addition, the U.S. consumer demand for pasture- or organically-raised meats has grown increasingly in recent years. Pork from organically- and free-range-raised pigs is available in retail stores. Health-conscious consumers perceive organic/free-range pork as healthier and higher quality meat as compared to pork from conventionally raised pigs. According to the USDA, 12,373 pigs were certified organic in 2011, which is approximately three times more than the numbers in 2004 (306). From a public health standpoint, it is critical to understand the status of *T. gondii* infections in these high risk meat animals.

According to our results, *T. gondii* infection is widespread in these meat animals, specifically lamb, goats, non-confinement raised chickens and non-confinement raised pigs. Compared with confinement raised market pigs (5.6%), a higher prevalence was found in non-confinement raised pigs (31.0%). In a prevalence study included in the meta-analysis, out of 13 seropositive samples from 2,238 pig

serum samples, 12 (92.3%) were from pigs raised on pasture and only 1 was from pigs kept in confinement (307). Similarly, outdoor access is associated with high prevalence in goats and sheep, since they are raised on pasture and risk for exposure to infective oocysts is continuous. Based on limited studies, *T. gondii* prevalence in non-confinement raised chickens is also considerably higher than chickens raised indoors. These results are consistent with the findings in Chapter 2, which based on *T. gondii* prevalence studies from worldwide.

T. gondii prevalence is higher in breeding sows as compared to market pigs that are less than 6 months old, indicating the increasing exposure with age. Similarly, *T. gondii* prevalence was higher in sheep as compared to lambs (< 1 year). In similar age groups, a wide variation in prevalence was observed in each category of meat animals; this could be attributed to the fact that the studies differ in geographic region, years, and diagnostic methods. The heterogeneity in prevalence could also be related to the presence of specific risk factors identified in Chapter 2, including farm type, feeding practices, presence of cats, rodent control and bird control methods, farm management, carcasses handling and disposal, and water source and quality. Among the various studies of meat animals, the highest prevalence was found in sheep (73.8%) from the farm that experienced *T. gondii*-induced abortion in ewes (308), and in market pigs (92.7%) from poorly managed farms (309). In the study reporting a poorly managed pig farm, viable *T. gondii* were isolated in 51 of 55 market pigs destined for human consumption. The high prevalence might be related to two husbandry risk factors that were observed at this farm, feeding pigs with garbage and presence of feral cats in the farm (309).

Infected meat animals harbor tissue cysts that contain *T. gondii* bradyzoites in virtually all edible portions of the animal (19). Human consumers can be infected by ingestion of one bradyzoite in raw or undercooked meat. However, it is noteworthy to mention that *T. gondii* prevalence in meat animals may not be directly translated into human infection risk through consumption of these meat products. Amount of meat consumption, meat processing, and cooking behavior could change the infection risk at the time of consumption. For example, high *T. gondii* prevalence was observed in non-confinement raised chicken. However, it is not considered as an important source for *T. gondii* infection in humans, due to the fact that consumers tend to cook chicken thoroughly and the amount of non-confinement raised chicken consumption is low in the United States. On the contrary, although *T. gondii* prevalence in confinement-raised pigs is considerably lower than non-confinement raised animals, conventional pork is still an important source for meatborne toxoplasmosis. Based on an expert elicitation, among meat products, pork was associated with 41% of meatborne *T. gondii* infection (310). As one of the major consumed meats in the United States, even low proportion of infected pigs could result in high amount of infected-pork and a considerable number of infections each year.

In this meta-analysis, nationwide studies such as the NAHMS are considered to be good samples that represent the whole animal population. NAHMS represents more than 90% of swine operations with 100 or more pigs, but does not include the animal population from small farms (fewer than 100 pigs) (311). In addition, studies using proxy animals and non-optimal diagnostic methods were also included in the current study to compute the population prevalence. Prevalence studies for sheep were

61

included to compute the population prevalence in lamb (< 1 year old sheep). Quality assessments account for these differences among studies by assigning a quality score to each study. The present meta-analysis was able to integrate the results of each individual study in a way that more weight was assigned to the studies with high quality score (e.g., NAHMS) as compared to the low quality studies. However, caution is warranted to interpret the results of *T. gondii* prevalence in goats, non-confinement raised chickens, and non-confinement raised pigs. Prevalence was analyzed based on limited number of regional studies, and nationwide surveys are not available in these meat animals, which resulted in a wide 95% confidence interval of the estimated prevalence.

Neither the fixed-effects nor the random-effects models for meta-analysis are appropriate for the purpose of the current study. The fixed-effects model assumes prevalence is the same in all the eligible studies, which is not applicable in our analysis. The random-effects model assumes prevalence across the studies follows a normal distribution, but adjusts a study weight without any rationale. Neither model corrects for systematic bias or can provide precise estimates when true heterogeneity exists among the studied populations (312). The quality-effects model discounts a study weight in relation to all other study weights based on its quality score (288). Compared to the random-effects model, the quality-effects model is more appropriate for the purpose of the current study, and provided more conservative confidence intervals (CI) for the mean values. For example, based on the data of confinement sows, 95% CI were estimated as 10.7-24.3%, 17.0-17.8% and 15.0-26.8% for quality-effects, fixed-effects, and random-effects models, respectively.

The main data gaps of the current study were identified: (i) no data were found to observe *T. gondii* prevalence in confinement raised chickens, (ii) no studies were found to evaluate *T. gondii* infection in organically-raised chickens, and (iii) nationwide surveys are needed to understand *T. gondii* prevalence in goats, non-confinement raised chickens, and non-confinement raised pigs.

3.6 Conclusions

Overall, the quality-effects meta-analysis approach in the current chapter provided an estimate of *T. gondii* prevalence in various meat animals raised in the United States with an increased level of precision. The widespread prevalence of *T. gondii* in animals, specifically goats, lamb, confinement raised pigs, and non-confinement raised pigs, indicate a food safety concern in the United States. The results obtained from this meta-analysis can be used as an important input in microbial risk assessment models.

Chapter 4: Qualitative assessment for *Toxoplasma gondii* exposure risk associated with meat products in the United States

This work has been accepted by *Journal of Food Protection* and is currently in press.

4.1 Abstract

The goal of this study was to qualitatively estimate the exposure risk to *T. gondii* from various meat products in the United States. Risk estimates of various meats were analyzed by a farm-to-retail qualitative assessment which included evaluation of farm, abattoir, storage and transportation, meat processing, packaging and retail modules. It was found that exposure risks associated with meats from free-range chickens, non-confinement raised pigs, goats and lamb are higher than those from confinement-raised pigs, cattle, and caged-chickens. For fresh meat products, risk at the retail level was similar to that at the farm level unless meats had been frozen or moisture-enhanced. Our results showed that meat processing such as salting, freezing, commercial hot air drying, lengthy fermentation, hot smoking, and cooking are able to reduce *T. gondii* levels in meat products, whereas nitrite/nitrate, spice, low pH, and cold storage have no effect on the viability of *T. gondii* tissue cysts. Raw-fermented sausage, cured raw meat, non-hot air dried meat, fresh meat, and fresh processed meat were associated with higher exposure risks as compared with cooked meat and frozen meat. This study provides a reference for meat management control programs to determine critical control points, and the foundation for subsequent quantitative risk assessments.

4.2 Introduction

The livestock and poultry industries constitute the largest segment of U.S. agriculture. The main types of meat consumed are poultry, beef, and pork, with lamb, goat, veal and game making up 1% of the total meat consumed (68). Meat animals, especially pigs, chickens, sheep and goats have been frequently reported to harbor *T. gondii* tissue cysts in edible tissues (75). Most published studies have focused on *T. gondii* prevalence at the farm or abattoir, which is not sufficient for defining risk at the retail level, since meat often undergoes a series of processing treatments, which serve to reduce the risk. Thus, a comprehensive evaluation of food safety risk for *T. gondii* is needed.

Microbial risk assessment, which predicts the exposure and adverse human health effects due to a specific pathogen, has increasingly been used as a means of enhancing public health risk management. According to guidance from the CAC and the OIE, risk can be assessed qualitatively or quantitatively, and these two methods are equally valid (63). A qualitative assessment is based on a descriptive model that uses evidence to support a statement about risk, which is an important component of risk assessment. A qualitative approach was applied in the present assessment because quantitative data and knowledge of *T. gondii* in meat are limited. The goals of the current study were to (i) develop a farm-to-retail framework to qualitatively estimate the relative exposure risk of meat products consumed in the United States, and (ii) evaluate the effects of meat processing on the survival of *T. gondii*.

4.3 Materials and methods

A farm-to-retail qualitative assessment was developed by following the guidelines provided by the CAC (63). It includes six modules: (i) farm, (ii) abattoir, (iii) transportation and storage, (iv) meat processing, (v) packaging, and (vi) retail (**Figure 4.1**). The initial step was to estimate the risk status of each meat category based on population prevalence (PP), which is the percentage of animals that have been infected in a population. The PP in each meat category had been evaluated and computed in Chapter 3. Subsequently, changes in risk status were estimated as the meat was handled at the abattoir, during transportation, storage, processing (curing, smoking, drying, fermentation, thermal processing, etc.), packaging, and retail sale, by using the following risk categories: "High", "Medium", "Low", and "Minimal". The number of *T. gondii* tissue cysts can only be inactivated, reduced, or remain unchanged in meat in the current model because *T. gondii* does not grow outside of its host. This qualitative assessment was targeted to analyze exposure risks to healthy adults and may not address subgroups such as pregnant women and immunocompromised patients.

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┌─────────────────────────────────────────────────────────┐
│ **Farm**: *T. gondii* prevalence in meat animals         │
│           Risk estimation of each meat category          │
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                              ▼
┌─────────────────────────────────────────────────────────┐
│ **Abattoir**: Effect of slaughter processing on the viability of *T. gondii* │
└─────────────────────────────────────────────────────────┘
                              │        ┌──────────────────────────────┐
                              │ ──────▶│ **Transportation and storage**│
                              │        └──────────────────────────────┘
                              ▼
┌─────────────────────────────────────────────────────────┐
│ **Meat processing**                                      │
│     ❖ Injection with enhancing solution                  │
│     ❖ Combining meat ingredients                         │
│     ❖ Curing                                             │
│     ❖ Smoking                                            │
│     ❖ Drying                                             │
│     ❖ Fermentation                                       │
│     ❖ Thermal processing                                 │
│     ❖ Other treatments                                   │
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│ **Packaging:** Effect of meat packaging on the viability of *T. gondii* │
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│ **Retail**: Risk estimation for eight subgroups of meat products │
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Figure 4.1 Framework of the farm-to-retail qualitative assessment.

4.3.1 Farm: defining risk of each meat category

Initial risk level of each meat category was based on *T. gondii* prevalence in the corresponding meat animals: fresh pork (market pigs raised in confinement), processed pork (pigs and sows raised in confinement), beef (cattle raised in confinement), organic beef (organically-raised cattle), chicken (chicken raised in confinement), free-range/organic chicken (chicken raised in non-confinement environment), lamb (<1 year

old sheep raised in pasture), goat (goat raised in pasture), organic pork (organically-raised market pig), organic lamb (organic-raised lamb). *T. gondii* prevalence in meat animals was estimated either based on an overall prevalence (estimated by a meta-analysis) or on accepted knowledge/expert opinions when prevalence studies are limited or absent. A systematic meta-analysis approach that was described in Chapter 3 was used to summarize available prevalence studies in farm animals when sufficient studies were found. Risk status of each meat category was defined as a function of *T. gondii* prevalence in meat animals (results were obtained from Chapter 3). The calculated mean value of PP in meat animals was used to define the risk for each meat category. The risk level was defined as Low: PP < 6%, Medium: PP between 6 to 20%, high: PP > 20%.

4.3.2 Abattoir

When meat animals reach market weight, they are transported from farm to abattoir by trucks. After being held in lairage for a period of hours, animals are processed in the abattoir (process flow chart is presented in **Figure 4.2**) (334); the risk from the meat was analyzed at each step (**Table 4.1**) and explained below. We assumed that no new infection occurred during transportation from farm to abattoir and holding in lairage. Similar to meat animals processed in the abattoir, for poultry, birds are processed in the poultry processing plant where they are scalded, eviscerated, washed, and chilled.

Ante-mortem inspection is conducted mainly based on the health-related condition of the animal. Only animals considered to be healthy are allowed to be

slaughtered for human consumption (335). Since *T. gondii* cannot be transmitted from one animal to the other, this step does not affect the viability of *T. gondii* in meat.

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┌─────────────────────────────────────────┐
│   Lairage and ante-mortem inspection     │
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                    ↓
┌─────────────────────────────────────────┐
│         Stunning and bleeding            │
└─────────────────────────────────────────┘
                    ↓
┌─────────────────────────────────────────┐
│      Scalding and dehairing/feather      │
│            removal/skinning              │
└─────────────────────────────────────────┘
                    ↓
┌─────────────────────────────────────────┐
│         Singeing and polishing           │
└─────────────────────────────────────────┘
                    ↓
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│       Head removal and evisceration      │
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│    Splitting, dressing and final washing │
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│            Carcass chilling              │
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│            Meat fabrication              │
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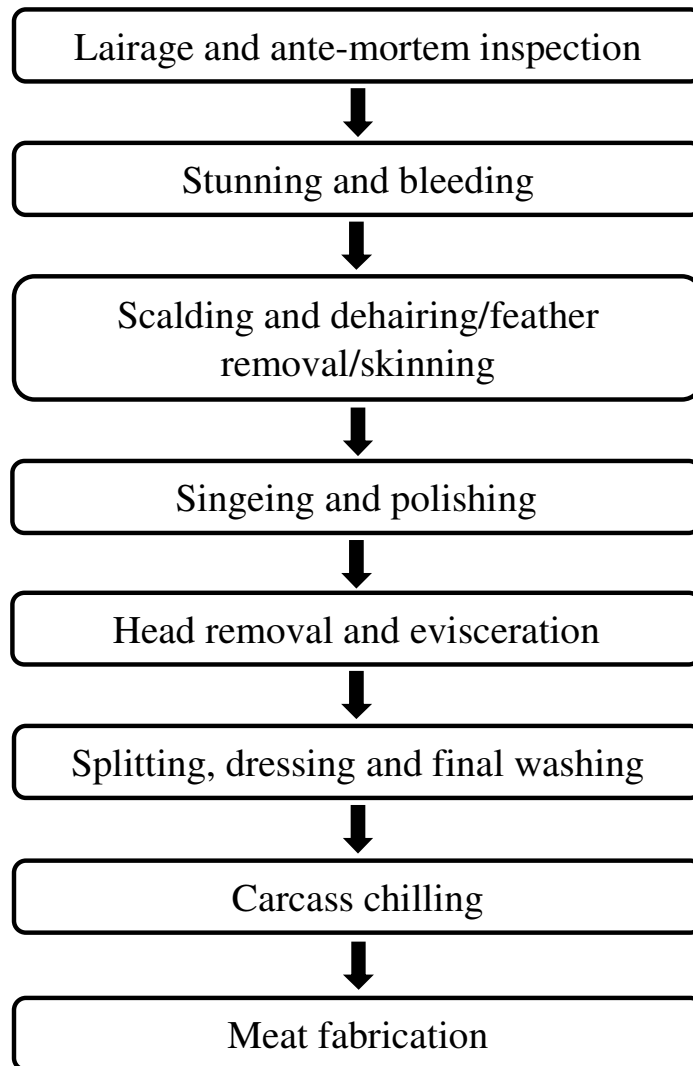
Figure 4.2 Flow diagram of essential abattoir processing steps in the United States.

For slaughter, animals are stunned either by electrical shock or carbon dioxide, after which they are hoisted by one leg for bleeding. The purposes of bleeding are to kill the animals with minimal damage as well as to remove blood. A knife is used severing a major vein (jugular) of the animal, which could internalize microorganisms

on the hide. Since *T. gondii* tissue cysts are not harbored on the skin and the knives are normally sterilized after each operation, we assumed that no cross-contamination occurred during sticking and bleeding.

After slaughter, the hide of ruminants (cattle, sheep, and goats) is removed (either manually or mechanically) through a skinning process. This process does not affect the viability of tissue cysts in carcass. A scalding process is used to remove the hair or feathers of pigs and poultry, respectively, followed by a mechanical dehairing or defeathering process. Pigs are scalded in warm water (57.7–61°C) for about 3-8 minutes to loosen the hair (336), while chickens are scalded in warm water (51-64°C) for shorter time (30-120 seconds) (337). Subsequently the hair/feathers are removed. It was found that bacterial loads (e.g., *Salmonella*) on the carcass surface can be reduced through physical removal and thermal inactivation of the scalding process (338). However, this treatment does not impact *T. gondii* tissue cysts harbored inside. Van der Wal et al. (339) found that the internal temperature of the carcass increased less than 1°C after the scalding process, which is not sufficient to inactivate tissue cysts. Some fecal material may be extruded due to the vigorous action of the dehairing machine (340) and result in microorganism transfer from an infected carcass to a non-infected carcass. However, as *T. gondii* is not shed in the feces of meat animals, this step has no effect on the viability of tissue cysts. Thus scalding has no effect on the viability of tissue cysts.

Following dehairing, the surface of the livestock carcass is exposed to high temperature (800-1000°C) by singeing, which is an effective approach to remove most of the surface contamination (dirt and fecal material) as well as reduce the bacterial

counts on the surface of the carcass. Singeing is followed by polishing in a device used to remove the superficially burnt skin, which may redistribute any remaining bacteria on the surface (341). This step occurs on the surface of the carcass and has no effect on *T. gondii* tissue cysts harbored inside.

The head is removed before evisceration. Evisceration is a critical control point (CCP) in slaughter facilities (342). During this step, the belly is opened and the viscera are removed. Both interior and exterior surfaces of the carcass can be contaminated by intestinal contents even when evisceration is compliant with recommended guidelines (340). During the evisceration process, *T. gondii* tissue cysts may be transferred from one organ to another by transport on cutting knives. Workers normally have two knives, with one in use while the other is held in a sterilizer. One popular sanitization method is to immerse the knife in flowing water higher than 82°C for a few seconds. A few seconds of immersion was found to be sufficient to eliminate bacterial contamination on the blades of the knives (343), which would also be sufficient to inactivate *T. gondii* tissue cysts on the blades (344). Chemical disinfectants such as trisodium-phosphate, polyphosphates, hydrogen peroxide, organic acid, chlorine and isopropyl alcohol are also used on cutting knives (345). Wainwright et al. (346) found that the *T. gondii* oocysts are resistant to high concentrations of chlorine solution, but the effect of chlorine on *T. gondii* tissue cysts is unknown. Tissue cysts are known to be resistant to an acid environment (347), but the effect of other disinfectants on the viability of *T. gondii* tissue cysts is unknown. Thus, there is a small chance that cross-contamination could occur during evisceration if chemical sanitizers are used. However, cross-contamination could not be assessed due to the absence of data.

After evisceration, the carcass is split in half by a mechanical saw. The saw is cleaned between carcasses; thus, we consider cross-contamination is negligible during splitting. Carcass dressing is a procedure where the kidneys are removed and visible contamination is trimmed by knives. Knives are sanitized between each action. If chemical sanitizers are used, cross-contamination could occur, but could not be assessed due a lack of data. The purpose of final washing before chilling is to further remove dirt and blood on the carcass, which could cause a redistribution of microorganisms on the carcass (340). However, there are no studies available to assess redistribution of tissue cysts at this stage of slaughter.

Immediately following slaughter and dressing, the carcass is chilled and stored at low temperature to remove the heat from the carcass and ensure meat quality (348). Three chilling systems may be used in commercial abattoirs: conventional chilling, spray chilling, and blast chilling. Conventional chilling cools the carcass to approximately 1°C for 24 hours. Spray chilling uses cold water (1-5°C) sprayed onto the carcass over a period of approximately10 hours to achieve the target temperature. Blast chilling is highly efficient and fast, and is favored by large slaughter facilities. It involves exposing the carcasses to circulating cold (-20°C to -40°C) air (349). For all three types of chilling systems, the temperature on the carcass is not decreased below the freezing point. *T. gondii* tissue cysts are resistant to the refrigerated temperatures; they are able to survive storage at 4-6°C for up to 2 months (350). Therefore, it is assumed that the chilling process has no effect on the viability of *T. gondii*.

Following chilling, meat is subjected to further cutting. In the United States, most slaughter facilities perform fabrication processing, which involves breaking the

carcass into large cuts (primals), then further into smaller cuts (subprimals), portion cuts, and retail cuts (351). Trimmings, defined as meats trimmed off from large cuts, account for approximately 20% of carcass weight. However, the exposure risk from trimmings was not included in the meat category because of the absence of data for cross-contamination during meat cutting and combining. Cross-contamination during fabrication may occur if knives and equipment are not properly sanitized.

Overall, the risk status of meat after slaughter processing remained the same as risk at the farm level. The scalding process is not sufficient to inactivate *T. gondii* tissue cysts and while cross-contamination may occur during evisceration and fabrication, it was not evaluated in the present model due to the absence of data.

Table 4.1 Effect of various treatments on the viability of *T. gondii* tissue cysts in animal carcasses during abattoir processing

Processing	Risk level	Justification
Lairage and ante-mortem inspection	Unchanged	No effect on the viability of *T. gondii* tissue cysts.
Stunning and bleeding	Unchanged	Assume no cross-contamination occurred.
Scalding and dehairing/ feather removal/skinning	Unchanged	Temperature during scalding is not sufficient to inactivate *T. gondii* tissue cysts.
Singeing and polishing	Unchanged	No effect on the viability of *T. gondii* tissue cysts.
Head removal and evisceration	Unchanged	Cross-contamination may occur during evisceration, but cannot be analyzed because data are not available.
Splitting, dressing and final washing	Unchanged	Cross-contamination may occur during dressing, but cannot be analyzed because data are not available.
Carcass chilling	Unchanged	*T. gondii* tissue cysts survive at refrigerator temperature.
Meat fabrication	Unchanged	Cross-contamination may occur, but cannot be analyzed because data are not available.

"Unchanged" means risk level remains the same as risk at the farm level during each step of the abattoir processing.

4.3.3 Transportation and storage

In the United States, fresh meat from livestock (beef, pork, lamb) is routinely held at refrigerated temperatures from the abattoir to retail display (cold chain), while freezing is usually limited to meat used as stock, for export or for further processing (352). It was reported that *T. gondii* tissue cysts can be inactivated at low temperatures (-20°C) for 4 hours, but common refrigerated temperatures are not able to kill tissue cysts (353). In the United States, many chickens are labeled as frozen, which indicates poultry has been held at 0°F (-17.8°C) or below. This treatment can efficiently kill *T. gondii* tissue cysts and reduce the risk of *T. gondii* infection (137). It was estimated that risk remains unchanged during storage and transportation from the abattoir/processing plant to retail, unless the meat had been commercially frozen during the process. When the frozen conditions met the critical parameters listed in **Table 4.2**, for the purposes of this study, risk was considered to be reduced to a minimal level.

4.3.4 Meat processing: injection of enhancing solution

Enhancing solution is a water-based solution that contains salt, phosphates, antioxidants, and flavorings (354). In the United States, fresh pork and chicken are frequently injected with enhancing solution to extend the shelf life and enhance meat quality. Moisture enhancement treatment may decrease *T. gondii* concentration in meat because the salt solution can inactivate *T. gondii* tissue cysts (355). For meat products labeled as "enhanced meat" which met the critical parameters listed in **Table 4.3**, for the purposes of this study, risk was estimated to be reduced by one level.

Table 4.2 Critical parameters for freezing to inactivate *T. gondii* tissue cysts in meat

Food matrix	Temperature	Inactivation time	Reference
Pork	-12°C	3 days	(356)
Meat	-20°C	1 day	(357)
Minced meat	-12°C	4 days	(51)
	-4°C	22 days	(51)
	-6.7°C	11 days	(51)
Pork and mouse brain	-1°C	33.6 days	(353)
mixture	-3.9°C	33.6 days	(353)
	-6.7°C	16.8 days	(353)
	-8°C	2.8 days	(353)
Mutton leg	-20°C	1 day	(358)
Sheep organs and	-10°C	3 days	(359)
muscles	-20°C	2 days	(359)
Goat meat	-20°C	4 hours	(360)

4.3.5 Meat processing: combining meat ingredients

Mixing is a process in which meat ingredients are combined together, and the ingredients can be from one kind of meat (e.g., ground pork, chicken sausages) or from different kinds of meat (e.g., beef and lamb burgers, pork and chicken sausage). Combining low risk meat ingredients with higher risk meats can result in changing risk status in the final product. For conservative purposes, the risk status of new formulated meat products was estimated based on the ingredient with the higher risk.

Table 4.3 Critical parameters to inactivate *T. gondii* tissue cysts in meat during moisture enhancement and curing process

Temp (°C)	Salt concentration (%)						IT (days)	Reference
	Sodium chloride	Sodium lactate	Sodium triphosphate	Sodium diacetate	Potassium lactate	Nitrite		
< 0	2.0	0	0	0	0	0	2	(361)
< 0	2.5	0	0	0	0	0	2	
4	0	1.5	0.25	0	0	0	7	(73)
4	0	2.0	0	0.2	0	0	7	
4	1.0	0	0	0	0	0	45	
4	0	0	0.25	0.1	1.4	0	7	
4	0	1.4	0.25	0.1	0	0	7	
4	0	0	0.25	0.14	1.96	0	7	
4	0	2.0	0	0	0	0	7	
4	2.0	0	0.5	0	0	0	7	
4	0	1.5	0.25	0	0	0	0.33	(99)
4	0	1.4	0.25	0.1	0	0	0.33	
4	0	2.0	0	0	0	0	0.33	
4	0	2.0	0	0.2	0	0	0.33	
4	0	0	0.25	0.14	1.96	0	0.33	
4	2.0	0	0.5	0	0	0	0.33	
4	0	0	2.5	0.1	1.4	0	0.33	
4	2.0	0	0	0	0	0.5	5	(347)
4	2.5	0	0	0	0	0.5	1	
4	3.0	0	0	0	0	0.5	1	
4	1.9	0	0	0	0	0	14	(360)
4	0.85	0	0	0	0	0	56	(355)
4	2.0	0	0	0	0	0	49	
4	3.3	0	0	0	0	0	21	
4	6.0	0	0	0	0	0	14	
10	2.0	0	0	0	0	0	35	
10	3.3	0	0	0	0	0	28	
10	6.0	0	0	0	0	0	3	
15	0.85	0	0	0	0	0	28	
15	2.0	0	0	0	0	0	21	
15	3.3	0	0	0	0	0	21	
20	0.85	0	0	0	0	0	21	
20	2.0	0	0	0	0	0	14	
20	3.3	0	0	0	0	0	7	

Temp: temperature; IT: Inactivation time.

4.3.6 Meat processing: curing

Curing is a preservation technology that involves adding salt, nitrate/nitrite, spices, and sugar to meat. Numerous studies have found that *T. gondii* tissue cysts can be inactivated under proper combinations of salt, temperature, and treatment duration (critical parameters are summarized in **Table 4.3**). Nitrite and nitrate are both used in the curing process to develop desirable color and extend shelf life. It was reported that the combination of 0.5% nitrite with NaCl may yield a higher degree of inactivation for *T. gondii* tissue cysts than common table salt (347). Condiments such as black pepper and garlic do not affect the viability of *T. gondii* (361), and the effect of sugar has not been reported. We conservatively assumed that the addition of sugar and spices has no effect on the viability of *T. gondii* tissue cysts. The risk status was estimated to be reduced by one level if the processing conditions (salt concentration, time and temperature, adding nitrate/nitrite) met the critical parameters listed in **Table 4.3**.

4.3.7 Meat processing: smoking

Meat is often smoked after curing to obtain a particular flavor and color. A variety of smoking methods and equipment are used by different manufacturers. Smoking can be classified as hot smoking, warm smoking, and cold smoking. Cold smoking is often used to produce raw or fermented sausages, with the meat being smoked between 12-25°C from several hours to 16 days. Warm smoking is carried out at 23-45°C for 4-48 hours. The temperature of both cold and warm smoking does not inactivate *T. gondii* tissue cysts (362). Hot smoking can be divided into different stages, with treatment at 40-50°C for 30 minutes without smoke in the first stage, followed by several stages of smoking that bring the internal temperature of meats to 68-72°C (362).

T. gondii tissue cysts are inactivated at 68-72°C (344). Antimicrobial and aromatic compounds such as organic acids are often added during smoking, which lower the pH of the meat products. However, tissue cysts are not sensitive to an acid environment; they can remain alive after 26 days at pH 5 (347). The effect of antimicrobial and aromatic compounds on the survival of tissue cysts is unknown. Therefore, in this assessment, it was estimated that the smoking process does not change the viability of *T. gondii* tissue cysts unless meats are treated with hot smoking, in which case the risk status was estimated to be reduced to a minimal level.

4.3.8 Meat processing: drying

The water activity of meat decreases during drying. Most microorganisms are not able to grow at low water activities. The survival kinetics of *T. gondii* tissue cysts in dry products has not been reported. The most common dehydration method used in industry is hot air drying. The temperature in drying chambers ranges from 50 to 80°C and meat is held at these temperatures for hours until the desired low moisture is reached. Under these conditions, *T. gondii* tissue cysts are usually inactivated. For the purposes of this study, the risk status for meat treated in this manner was estimated to be reduced by one level during drying.

4.3.9 Meat processing: fermentation

Meat fermentation is a biological acidification method used for preservation and for developing distinctive properties such as color, flavor and texture. Cultured or wild microorganisms are used to ferment meat; the process lowers the pH of the meat due to the production of lactic acid. Addition of salt and drying also lowers the water activity of meat products. It was found that the duration of fermentation is critical to *T.*

gondii survival. Tissue cysts remain viable in fermented sausage after 12 hours of treatment, even with the presence of 2% curing salt containing nitrite. In another study, *T. gondii* tissue cysts were inactivated in dry fermented goat sausages after 3 hours of fermentation, but with a higher concentration (2.5%) of curing salt (360). Under current meat fermentation guidance, fermentation normally takes days to weeks, and *T. gondii* tissue cysts are unlikely to remain viable in the fermented meat *(363)*. Thus, in this assessment, risk level was estimated to be reduced by one level during fermentation.

4.3.10 Meat processing: thermal processing

Thermal processing is often applied to manufacture cured-cooked meat, precooked-cooked meat, and raw-cooked meat. Heating methods vary by temperature and duration time, which is determined by meat variety, equipment, and preference of the processers. Some small processers prefer to use a smokehouse, while larger processors may utilize a continuous cooking oven. Critical parameters required to kill *T. gondii* tissue cysts are listed in **Table 4.4**. Cooking time can also be estimated by a log-linear regression equation ($\log$ (heating time) = 7.918 - 0.146 × (temperature)) (344). In this assessment, risk was estimated to be reduced to a minimal level if thermal processing met the critical parameters described.

4.3.11 Meat processing: other treatments

In order to extend shelf life and eliminate foodborne pathogens, some manufacturers may perform additional treatments such as gamma irradiation and high hydrostatic pressure (HHP) processing before packaging. Dubey and Thayer (364) found tissue cysts can be killed with 0.4KGy dose of Cs-137 gamma irradiation treatment at temperatures between -4 to 16°C. Song et al. (365) also reported that *T.*

gondii tissue cysts in mice and pig tissues can be inactivated with Cobalt-60 irradiation at 0.6 kGy. Infectivity of bradyzoites can be reduced by 10,000-fold after gamma irradiation at 0.45 KGy, and the minimal effective dose to eliminate infectivity of *T. gondii* tissue cysts was 0.55 KGy (366). HHP treatment of 300 MPa also kills tissue cysts in ground pork (367). In this assessment, since the actual conditions of irradiation or HHP treatment applied to a specific meat product could be different from the experimental conditions studied to date, risk was conservatively estimated to be reduced by one level if meat is labeled as irradiated meat or treated by HHP (>300 MPa).

Table 4.4 Critical parameters to inactivate *T. gondii* tissue cysts in meat during thermal processing

Matrix	Temperature	Inactivation Time	Reference
Sheep organs and muscles	60°C	10 mins	(359)
Pork	52°C	24 mins	(344)
	58°C	9.5 mins	
	61°C	3.6 mins	
	64°C	3 mins	
	67°C	1s	
T. gondii tissue cysts	60°C	10 mins	(368)
Brain cysts	50°C	30 mins	(369)
	56°C	10-15 mins	

4.3.12 Meat packaging module

Common meat packaging methods comprise vacuum packs, high oxygen modified atmosphere packs, low oxygen modified atmosphere packs, and controlled

atmosphere packs. All these packaging methods have been proven to effectively inhibit the growth of many foodborne pathogens (370). Neumayerová et al. reported that vacuum packaging process did not affect the viability of *T. gondii* tissue cysts, and viable *T. gondii* were isolated from vacuum packaged meat samples after 42 days (360). Consequently, it was estimated that the risk status during packaging processes remains unchanged.

4.3.13 Retail

Meat could undergo various treatments during the retail phase, such as cutting, curing, repackaging, freezing, and thawing. Analyzing these potential treatments would complicate the present assessment, so this analysis was not conducted. We assumed that risk status remained unchanged at retail and all the risk changes occurred during the prior manufacturing processes. Risk status in both fresh meat and processed meat products were estimated at the retail level. By following the guidelines from the FAO, processed meat products were further categorized into seven subgroups by processing technologies, which include fresh processed meat, cured-raw meat, cured cooked-meat, raw-cooked meat, precooked-cooked meat, raw (dry)-fermented sausage, and dried meat (371).

4.3.14 Risk estimation

The qualitative risk assessment framework was developed in Microsoft Excel, which consisted of modules that affect the viability of *T. gondii* tissue cysts as analyzed above (scheme of risk estimation is demonstrated in **Figure 4.3**). Questions related to critical parameters are listed under each module and risk status of a specific meat can be estimated by answering each of the questions.

4.4 Results

4.4.1 Population prevalence and risk estimation for each meat category

A wide variation in prevalence was observed in each meat category (**Table 4.5**). Meta-analysis was not applied to estimate the risk of caged-chickens and cattle because of limited data availability. Risk was estimated as low in both caged-chickens and cattle because *T. gondii* was not isolated from 2,094 chicken and 2,094 beef samples purchased from retail stores nationwide (34). Since there were no prevalence studies available for organically-raised cattle, the risk for organic beef was also estimated as low based on risk estimation of conventional beef. Based on the mean value of PP (**Table 4.5**), at the farm level, the risk for fresh pork was estimated as low, while the risk for lamb, free-range/organic chicken, goat meat, and organic pork were estimated as high. Ingredients used in processed pork come from both market pigs and sows, thus, risk was estimated as medium based on the PP of sows. No study was found on *T. gondii* prevalence in organically-raised lamb/sheep, and therefore the risk status was estimated as high based on risk estimation of conventional lamb.

4.4.2 Risk estimation of meat products at the retail level

The effect of processing on the viability of *T. gondii* tissue cysts is summarized in **Table 4.6**. The risk status of eight meat subgroups was analyzed and is listed in **Table 4.7**. The risk level was estimated to remain at the initial level in fresh meats and fresh processed meat products unless the moisture enhancement or curing process met the critical parameters listed in **Table 4.3**. Risk was estimated to be reduced by one level (i.e., for organic dry-cured ham, risk level was estimated to be reduced from high to medium) in cured-raw meats because the fermentation process can inactivate *T.*

gondii tissue cysts. Risk was estimated to decrease to a minimal level in cured-cooked meats and precooked-cooked products because thermal processing is sufficient to inactivate tissue cysts. Compared with other processed products, raw-fermented sausage and dried meat originating from lamb, goat, free-range chicken or organically-raised pig have a higher risk because they are normally consumed without cooking; therefore the risk level is only reduced by one level during processing. Overall, risk level is reduced to a minimal level if meat of any origin undergoes a commercial freezing process.

Table 4.5 Risk definition of individual meat category, based on the results of a quality-effects meta-analysis to estimate prevalence in meat animals

| Meat category	Corresponding meat animals	PP (%)	95% CI (%)	Risk status
Fresh pork	Market pigs raised in confinement	5.6	0.4-14.8	Low
Processed pork	Pigs and sows raised in confinement	16.5	10.3-23.7	Medium
Beef (conventional and organic)	Confinement- or organic-raised cattle	N/A	N/A	Low
Conventional chicken	Chicken raised in confinement	N/A	N/A	Low
Free-range/Organic chicken	Chicken raised in non-confinement environment	24.1	0-71.3	High
Lamb	<1 year sheep raised in pasture	22.0	1.3-53.5	High
Goat meat	Goat raised in pasture	30.7	8.7-58.0	High
Organic pork	Organically-raised market pigs	31.0	3.6-67.2	High
Organic lamb	Organically-raised lamb	N/A	N/A	High

N/A is not applicable; PP: population prevalence; CI: confidence interval; definition of risk levels: low < 6%, medium 6-20%, high > 20%.

Table 4.6 Effects of processing on the viability of *T. gondii* tissue cysts in meat

Processing	Effect	Justification	Reference
Abattoir	No effect	Scalding process is not sufficient to kill tissue cysts. Cross-contamination by the cutting knife may occur, but cannot be evaluated because data are not available.	Table 4.1
Transportation and storage	No effect or inactivation if meat has been frozen	Frozen rather than refrigeration temperature is able to kill tissue cysts.	Table 4.2
Moisture enhancement	Inactivation	Salt in the enhancing solution can inactivate *T. gondii* tissue cysts.	Table 4.3
Curing	Inactivation	Salt, rather than sugar and spice, has effect on the viability of *T. gondii* tissue cysts. Salt along with nitrate/nitrite may increase the degree of inactivation.	Table 4.3
Smoking	No effect or inactivation if hot smoked	Hot smoking is sufficient to eliminate *T. gondii* tissue cysts. Antimicrobial and aroma compounds have no effect.	(362)
Drying	Inactivation	Commercial hot air drying can inactivate *T. gondii* tissue cysts.	(344)
Fermentation	Inactivation	*T. gondii* tissue cysts are unlikely to survive during lengthy fermentation process.	(363)
Thermal processing	Inactivation	Thermal processing is able to inactivate *T. gondii* tissue cysts).	Table 4.4
Irradiation	Inactivation	*T. gondii* tissue cysts can be inactivated (irradiation dose > 0.55 KGy).	(366)
High hydrostatic pressure	Inactivation	*T. gondii* tissue cysts can be inactivated (pressure > 300 MPa).	(367)
Packaging	No effect	*T. gondii* tissue cysts are not be inactivated.	(360)

Table 4.7 Qualitative risk estimation of various meat products at retail level, meat subgroups were divided based on the FAO guidelines

No.	Category	Processing	Consumption pattern	Typical examples	Risk estimation at retail level
1	Fresh meat	Minimum processing, maybe moisture enhanced	Cooking is needed.	Pork chop, steak, spare rib	Risk remains the same as risk at the farm level unless the enhancing condition meets the critical parameters listed in Table 4.3.
2	Fresh processed meat	Salted only	Cooking is needed.	Sausage, Kebab, patties	Risk remains the same as risk at the farm level unless the salt condition reaches the critical parameters listed in Table 4.3.
3	Cured-raw meat	Curing, fermentation, ripening	Some consumed without cooking.	Raw cured beef, cured ham, bacon	Risk is reduced by one level during fermentation, and may be further reduced if curing condition reaches the critical parameters listed in Table 4.3.
4	Cured-cooked meat	Curing, heating	Cooking is needed.	Cooked ham	Risk is reduced to minimal level.
5	Raw-cooked product	Mix/batter, heat	Cooking is needed.	Frankfurter, Meat loaf, hotdog	Risk level is reduced to minimal if the product is heated during processing; otherwise risk level remains the same as risk at the farm level.
6	Precooked-cooked	Heat treatment twice	Cooking is needed.	Blood sausage, corned beef	Risk is reduced to minimal level.
7	Raw(dry)-fermented sausage	Fermentation, ripening, drying, no heating	Mostly consumed raw.	Salami, raw fermented sausages	Risk is reduced by one level during fermentation.
8	Dried meat product	Gradual and equal drying	Consumed without cooking.	Beef jerky, meat floss	Risk is reduced by one level during drying.
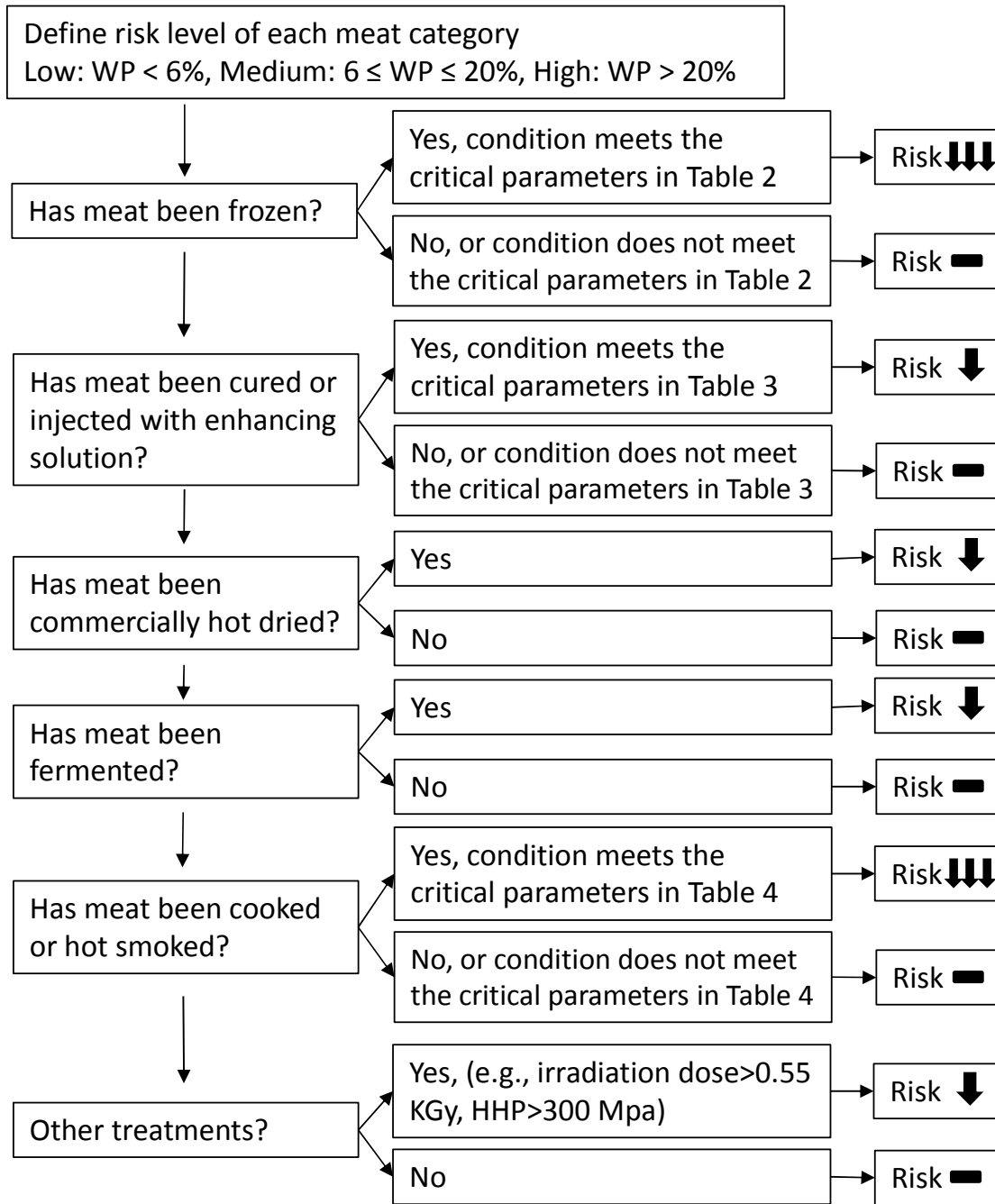
Figure 4.3 Schematic presentation of risk estimation in the qualitative risk assessment.

WP: weighted prevalence; HHP: high hydrostatic pressure

⬇ : Risk is reduced by one level
▬ : Risk is remained unchanged
⬇⬇⬇ : Risk is reduced to minimal level

4.4.3 Demonstration of the application of the qualitative risk assessment tool

Meat products can be analyzed by using the qualitative risk assessment tool (schematic demonstration is shown in **Figure 4.3**) that was constructed in Microsoft Excel (**Figure 4.4**). Two examples of how to use this tool to analyze the risk of meat products are given below.

Dry-cured ham was used as one example. Approximately 40% of processed pork consumed in the United States is ham. Dry-cured ham, also known as country ham, is a traditional processed pork product that involves curing and a light smoking process. Pork used for ham originates from the hind quarters of market pigs. In the farm module, ham belongs to the fresh pork category, and the initial risk is low (Table 4.5). The USDA requires the curing temperature to be in the range of 1.7-7.2°C for an initial 3 days/kg of ham, and higher than 15.6°C for the remaining curing process (372). Some hams are smoked by using a cold smoking method at a temperature of 32.2°C in order to maintain the flavor and aroma. Aging time is normally between 3 to 6 months with the internal temperature less than 35°C (373). The final salt concentration in dry cured hams has been reported to range from 4.0% to 9.7% with an average of 6.5% (374). The critical step during processing is the combination of salt concentration (4.0-9.7%), temperature (1.7-15.6°C) and time (3-6 months). Referring to the critical parameters listed in **Table 4.3**, curing, as described, is sufficient to inactivate *T. gondii* tissue cysts and reduce the risk level from low to minimal at retail.

Organic semi-dried sausage that has never been frozen was used as another example. Dry or semi-dry sausages are comminuted fermented meat widely consumed in the United States, and can be made of beef, pork, poultry, or a combination of various

87

meat products. Here we used dry organic salami made with organic beef and pork as an example. The initial risk level of the combined ingredients was estimated as high based on the PP of organic pigs (**Table 4.5**). Pork and beef are ground and mixed at a cold temperature (-5 to -2°C). Sometimes frozen meat is used in comminution. Since the proportion of frozen meat is not known, combining is not considered as an inactivation step and the risk level remained high after comminution. Subsequently, meat is mixed with 2.5-3% salt, nitrite/nitrate, spices, sugar, and incubated at 2.2-3.3°C for 2-3 days. The curing process is not sufficient to inactivate *T. gondii* tissue cysts when referenced against the critical parameters listed in **Table 4.3**. The meat mixture is stuffed into a casing and incubated at 15-26°C for 2-4 days. Subsequently, the meat is cold smoked at 23.9-42.5°C for more than 18 hours. Finally, the product is dried for 12-21 days with 65-88% relative humidity at 10-20°C (375). The condition in smoking (not hot smoking) and drying (not commercial hot air drying) cannot inactivate *T. gondii* tissue cysts. Fermentation (12-21 days), however, is long enough to inactivate *T. gondii* tissue cysts and therefore reduced the risk level from high to medium. Consequently, the risk level of dry organic salami was estimated as medium at retail.

Name of the product: organic semi-dried sausage					
Farm module: risk estimation of each meat category		Meat category		Risk status	
1. What are the meat ingredients used in the final product?		Fresh pork	No		
		Processed pork	No		
		Beef	No		
		Indoor chicken	No		
		Free-range chicken	No		
		Lamb	No		
		Goat meat	No		
		Organic chicken	No		
		Organic beef	Yes	Low	
		Organic pork	Yes	High	
		Organic lamb	No		
		Risk of total ingredients		High	
Transportation and storage module					
2. Has the meat been frozen?			No		
		Freezing temperature			
		Freezing time			
		Is the condition meet critical parameters?			
		Risk status after freezing		High	
Meat processing Module					
3. Has the meat been injected with enhanced solution?			No		
		Salt concentration in final product			
		Temperature			
		Shelf life			
		Is the condition meet the critical parameters?			
		Risk status after enhancement		High	
4. Has the meat been cured?			Yes		
		Salt concentration in final product	2.5-3.0%		
		Temperature	2.2-3.3°C		
		Duration of curing	2-3 days		
		Is the condition meet the critical parameters?	No		
		Risk status after curing		High	
5. Has the meat been smoked?			Yes		
		Hot smoking	No		
		Warm smoking	No		
		Cold smoking	Yes		
		Risk status after smoking		High	
6. Has the meat been dried?			No		
		Commerical hot air drying			
		Other drying methods (e.g., sun dry, irradiation dry)			
		Risk status after drying		High	
7. Has the meat been fermented?			Yes		
		Fermentation time	12-21 days		
		Is fermentation long enough to inactivate *T. gondii*?	Yes		
		Risk status after fermentation		Medium	
8. Has the meat been cooked?			No		
		Temperature of cooking			
		Time of duration			
		Is the condition meet the critical parameters?			
		Risk status after cooking		Medium	
9. Are other treatments used during processing?		Irradiation	No		
		HHP	No		
		Risk status after other treatments		Medium	
		Risk estimation at retail level		Medium	

Figure 4.4 Spreadsheet developed to conduct the farm-to-retail qualitative assessment, organic semi-dried sausage was used as an example.

4.5 Discussion

The overall exposure risk for *T. gondii* from meat purchased at retail stores was considered as low in the current risk assessment. Risk can be further reduced during meat preparation and cooking at home. Although a high prevalence of *T. gondii* was

observed in organically-raised pigs, free-range chickens, lamb and goats, a low frequency of consumption and effective home cooking are able to reduce the risk. According to the USDA food availability system, lamb and goat consumption in the United States is 0.5 kg per capita (68), and the consumption of organic or free-range meat is also much lower than conventional meat. At the retail level, risk estimation for fresh meat and fresh processed meat that originated from high-risk animals (e.g., goat and lamb) is higher than for other meat products. However, risk can be further reduced by home cooking. According to the EcoSure 2007 survey, a majority of consumers cook pork, beef and chicken to a final temperature higher than 69°C (92), which can immediately inactivate *T. gondii* tissue cysts in meat (344). Anecdotally, lamb is often cooked rare, which is not sufficient to inactivate *T. gondii* tissue cysts. For raw-fermented sausage, the risk posed to consumers at the time of consumption cannot be further reduced because it is normally consumed without cooking. This result is consistent with an evaluation of pork sausage; *T. gondii*-DNA was detected in 4 of 288 (1.4%) raw pork sausage samples (376). This evidence indicates the potential risk of infection by consuming raw-fermented sausages.

In the present risk assessment, exposure risk posed by fresh pork and fresh processed pork was estimated as low at the retail level, and risk can be further reduced by home cooking. However, exposure risk posed by fresh pork and fresh processed pork is not minimal at the time of consumption. As one of the major consumed meat products, pork consumption in the United States was 8,380 million kg/year or 26.8 kg per capita in 2011 (68). Viable *T. gondii* can be detected from retail pork destined for human consumption. For example, Dubey et al. (34) isolated viable *T. gondii* from 7

of 2,094 fresh pork samples in a 2005 national retail survey in the United States. In Mexico, 1 of 48 pork samples purchased from a butcher shop tested positive for *T. gondii* by bioassay in mice (97). Dias et al. (101) detected viable *T. gondii* from 13 of 149 (8.7%) fresh pork sausage samples via bioassay in mice. Depending on how pork products are cooked prior to consumption, these examples suggest that the number of meals prepared with infectious meat products may be quite high considering the large amount of production and consumption of pork each year.

Home cooking may not be effective in further reducing the risk if meat is undercooked, especially for pork and lamb. In recent years, the USDA recommended internal cooking temperature for fresh pork and lamb has been reduced from 160°F (71.1°C) to 145°F (62.8°C) with a minimal three-minute rest time (377). The recommended internal cooking temperature for chicken is 165°F (73.9°C) (377). Experimental study has proven that *T. gondii* tissue cysts can be inactivated from meat when the internal temperature reaches 67°C (344). In this experiment, *T. gondii* tissue cysts were also inactivated instantly at lower temperatures (61°C and 64°C), with an exception of a sample treated at 64°C for 3 minutes (344). Based on the results from the study described here (344), the recommended temperature for chicken is sufficient to inactivate *T. gondii*. However, the recommended temperature for pork and lamb may not be sufficient to inactivate *T. gondii* by home cooking as it does not provide a sufficient margin of error (7). In addition, some consumers tend to undercook their meat products. In a consumer knowledge of home food safety practices survey, only 63% of 1,000 American respondents cooked meat to recommended temperature (378). According to another survey, 47 of 593 (7.9%) people considered rare or medium

cooked pork safe (379). The Ecosure 2007 survey reported that approximately 30% of American consumers cooked pork products at an internal temperature lower than 67°C (92). The chance of the combination of meat with a high load of *T. gondii* and a low cooking temperature is small, but it does occur. In this case, the risk of human infection can be high.

Freezing is another method to inactivate *T. gondii* tissue cysts and thereby reduce the risk posed to consumers. Manufacturers may freeze meat before processing. Chickens sold in the United States are often frozen, the "frozen label" indicating meat held at 0°F (-17.8°C) or below, which is sufficient to eliminate *T. gondii* and reduce the risk to a minimal level (137). For meat that has not been held at a temperature below freezing (e.g., fresh meat), the risk can be reduced through home storage because a substantial proportion of consumers prefer to store meat in the freezer. Maciorowski et al. (380) reported that 38% of consumers prefer to store poultry at freezer at home. In another study, it was found that 66% of 611 respondents preferred to store meat and poultry in the freezer at home (381). Home freezer temperatures range from -16 to 40°F (-26.7 to 4.4°C) with an average value of -15.7°C (92). Studies have demonstrated that *T. gondii* can be inactivated in meat within one day at this temperature (357).

The actual concentration of *T. gondii* tissue cysts in meat is largely unknown, but it has been estimated to be a low concentration (≤ 1 cyst in 50 or 100 g of meat) in naturally infected meat animals (19). A tissue cyst may contain a few to hundreds of bradyzoites depending upon the age of the cyst. After ingestion of *T. gondii*-infected meat, the cyst wall ruptures in the gastrointestinal tract. Liberated bradyzoites penetrate the epithelial cells and initiate replication. Therefore, while the concentration of *T.*

gondii tissue cysts might be low in meat animals, the number of infectious bradyzoites can be high. Quantification of bradyzoites in *T. gondii*-infected meat can be beneficial to estimate the risk. Limited real-time PCR studies have demonstrated that bradyzoite concentrations range from 0.04 to 41.3 bradyzoites/g in muscle samples of experimental-infected goats (382), and less than 2.3 bradyzoites/g in muscle samples of experimentally-infected pigs (55). Based on these numbers, one serving (85 g) of infected-meat may contain a few to thousands of bradyzoites, which is sufficient to initiate an infection in mice (383) and rats (384), and probably humans, although there is no data on humans.

A qualitative assessment of *T. gondii* risk in ready-to-eat (RTE) small goods processing was published to define the risk of each ingredient and analyze the processing steps on the viability of *T. gondii* in Australia (66). The current qualitative assessment adds to the literature by utilizing quantitative data (the result of the meta-analysis) to define the exposure risk by meat categories and to provide a more thorough analysis of processing effects according to the circumstances of the meat industry in the United States. In addition, a packaging module and a retail module were included in the present assessment, and the effects on the viability of *T. gondii* tissue cysts were analyzed to more fully assess risk. Finally, this qualitative assessment identified critical steps and data gaps for conducting a subsequent quantitative risk assessment. Currently, there are no regulations specifically intended for control of *T. gondii* in meat products. To provide a framework for understanding the risk of *T. gondii* to consumers, and to better inform this risk model, the following data gaps should be addressed: (i) the concentration of *T. gondii* in the edible muscle tissues of meat animals, (ii) the number

of bradyzoites contained in 1 tissue cyst, (iii) the relationship of number of bradyzoites and the age of the tissue cysts, (iv) the relationship of seropositivity of meat animals and the concentration of bradyzoites in meat, (v) the probability/magnitude of cross-contamination occurring during slaughter processing (bleeding, evisceration, washing, splitting and cutting), (vi) the survival of *T. gondii* tissue cysts in low water activity environment, (vii) the effects of sugar, spices, and sanitizers on the viability of *T. gondii* tissue cysts, and (viii) the dose-infection relationship of *T. gondii* bradyzoites in humans.

4.6 Conclusions

The qualitative assessment reported here suggests that exposure risk of *T. gondii* posed by meat purchased at retail stores in the United States is low. However, a combination of frequent consumption, high load of parasites in meat, and improper preparation of meat products can lead to human infection. Fresh meat, fresh processed meat, raw-fermented sausages and non-hot air dried meat products pose a higher risk than other meat products. The exposure risk posed by a specific meat can be analyzed by the qualitative assessment framework reported here. Although the risk level for fresh pork from market pigs was estimated as low, pork products are still a food safety concern because pork is one of the major consumed meat products in the United States. A farm-to-table quantitative risk assessment of *T. gondii* infection associated with fresh meat (e.g., pork and lamb) should be conducted, in order to help estimate the public health risk from foodborne transmission, and to assess the effectiveness of different risk mitigation strategies on the predicted risk. This chapter would serve as a reference

for meat management control programs to determine the critical control points, and the

foundation for subsequent quantitative risk assessments.

Chapter 5: Development of dose-response models to predict the relationship for human *Toxoplasma gondii* infection associated with meat consumption

This work has been accepted by *Risk Analysis* and is currently in press (already has been published online in its "Early View" section in October, 2015).

5.1 Abstract

The dose-response relationship for human exposures to *T. gondii*-infected meat is unknown because no human data are available. The goal of this study was to develop and validate dose-response models based on animal studies, and to compute scaling factors so that animal-derived models can predict *T. gondii* infection in humans. Relevant studies in literature were collected and appropriate studies were selected based on animal species, stage, genotype of *T. gondii*, and route of infection. Data were pooled and fitted to four sigmoidal-shaped mathematical models, and model parameters were estimated using maximum likelihood estimation. Data from a mouse study were selected to develop the dose-response relationship. Exponential and beta-Poisson models, which predicted similar responses, were selected as reasonable dose-response models based on their simplicity, biological plausibility and goodness-of-fit. A confidence interval of the parameter was determined by constructing 10,000 bootstrap samples. Scaling factors were computed by matching the predicted infection cases with the epidemiological data. Mouse-derived models were validated against data for the dose-infection relationship in rats. A human dose-response model was

developed as P (d) = 1 - exp (-0.0015 × 0.005 × d) or P (d) = 1 - (1 + d × 0.003 / 582.414)$^{-1.479}$. Both models predict the human response after consuming *T. gondii*-infected meats, and provide an enhanced risk characterization in a quantitative microbial risk assessment model for this pathogen.

5.2 Introduction

Toxoplasma gondii was ranked fourth in importance in the recent multi-criteria based risk ranking of foodborne parasites compiled by the FAO/WHO (385). The versatile biological characteristics make the transmission routes of *T. gondii* complicated (363). Tissue cysts that may contain several hundred bradyzoites are the form present in meat. The tissue cyst wall is dissolved in the human stomach and small intestine by enzymatic digestion, but the released bradyzoites are able to survive and initiate an infection (386).

A suitable dose-response model is one of the components required to conduct a quantitative risk assessment (hazard characterization). It establishes the relationship between the magnitude of microbial exposure and the probability of occurrence of an adverse health effect. In microbial risk assessment, the majority of available dose-response models have been developed to analyze the relationship between exposure to various pathogenic bacteria such as *Escherichia coli* O157:H7 (387, 388, 389) and *Listeria monocytogenes* (390) and frequency of illness. Only two dose-response models have been developed for protozoan parasite infection in humans. These two models for *Giardia* (391) and *Cryptosporidium* (392), were developed based on human feeding trials. Model parameters were computed by fitting experimental data to models by

97

maximum likelihood estimations (MLE) (393). Previously, multiple exponential dose-response curves, which estimated the probability of infection corresponding to different animal studies with various strains, parasite stages, and genotypes of *T. gondii*, were developed by the French Food Safety Agency (AFSSA) (394). These dose-response models are not suitable for predicting human *T. gondii* infections. Currently, the dose-response relationship of *T. gondii* infection in humans is poorly understood because of the absence of human volunteer studies (which would not be ethical). The goals of the current study were to collect available data, and develop dose-response models to predict *T. gondii* infection in humans from ingestion of *T. gondii*-infected meats.

5.3 Materials and Methods

5.3.1 Data collection

Relevant studies were searched in PubMed, Medline, Web of Science and Google Scholar databases by using key words and their combination: "*Toxoplasma gondii*" and "infectivity", "infection", "pathogenicity", "pathogenesis", "exposure", "model", "dose-response", and "inoculation". Eligible studies were those that met two criteria: (i) the study included ≥3 different challenge doses of *T. gondii*, and (ii) at least five animals were challenged at each dose. **Table 5.1** lists studies that met these criteria and provides data potentially applicable for dose-response analysis.

5.3.2 Data selection

Infection, defined as detection of antibody to *T. gondii* and presence of *T. gondii* tissue cysts in brain tissue, was selected as the endpoint in this modeling study. It is justified since minimizing the cases of infection is a public health goal. All data eligible for inclusion in the models were based on animal studies; there were no human studies

available. Animal data were used to develop the shape and position of the dose-response curve (400). Twelve animal studies (**Table 5.1**) met the two criteria of data collection and were included after the first round screening.

Table 5.1 Comparison of animal studies to evaluate the infectivity of *T. gondii*

Animal	Stain	Genotype	Stage	Dose range	Administration	Reference
Mice	M-7741	Type III	oocyst, bradyzoite, tachyzoite	10^0-10^5	oral, s.c. and i.p.	(395)
Pigs	GT-1	Type I	oocyst	10^2-10^4	oral	(396)
Pigs	VEG	Type III	oocyst	0, <1, 1, 10	oral	(397)
Rats	VEG	Type III	oocyst	10^0-10^6	oral	(398)
Mice	VEG	Type III	oocyst	10^0-10^6	oral	(398)
Mice	VEG	Type III	bradyzoite	10^0-10^6	oral and s.c.	(383)
Mice	**ME-49**	**Type II**	**bradyzoite**	$\mathbf{10^0}$-$\mathbf{10^6}$	**oral and s.c.**	**(383)**
Mice	VEG	Type III	oocyst	10^0-10^7	oral	(399)
Mice	VEG	Type III	bradyzoite	0-10^5	oral and s.c.	(384)
Rats	VEG	Type III	bradyzoite	0-10^5	oral and s.c.	(384)
Mice	GT-1	Type I	bradyzoite	0-10^6	oral and s.c.	(384)
Rats	**GT-1**	**Type I**	**bradyzoite**	**0-10^6**	**oral and s.c.**	**(384)**

s.c. = subcutaneous; i.p. = intraperitoneal.

Two selected studies are highlighted in bold. Details are provided in Table 5.2.

The purpose of modeling the dose-response relationship in the current study was to predict the probability of infection from exposures to *T. gondii*-infected meats. Thus, data should come from studies that mimic a *T. gondii* meatborne transmission route. The optimal study was selected based on the following criteria: (i) animal species: a pig is a better candidate for the experiment than a rat or a mouse; (ii) parasite stage: bradyzoites; (iii) genotype of *T. gondii*: Type II; and, (iv) route of administration: oral route. Experiments with genotype Type II bradyzoites orally administrated in pigs

were determined to be the best candidates for developing the dose-response relationship

applicable to humans for the following reasons:

(1) Three animal species (pigs, mice and rats) were used in the eligible studies, and it was demonstrated that infectivity of *T. gondii* to the three species are comparable, irrespective of their body weight (19). In terms of infections obtained through *T. gondii*-infected meat products, a pig is a better candidate than a rat or mouse. Pigs have been used as an appropriate animal model to study microbial infectious diseases due to their anatomic, genetic and physiological similarities to humans (401).

(2) For all eligible studies, various stains of *T. gondii* at different concentrations were administered to test animals in order to determine the infectivity. Based on the results of RFLP assays, the predominant genotypes of *T. gondii* found in North America and Europe can be classified as types I, II, and III (14). These lineages share 98% similarity of their DNA, but they show great differences in virulence. As a predominant genotype in the United States and European countries, the Type II genotype is associated with the majority of human toxoplasmosis cases and is frequently detected from both meat animals and humans in these countries (19). Thus, Type II strains were preferred for the purpose of this study.

(3) The infectivity and pathogenicity of the three stages of *T. gondii* (bradyzoites, tachyzoites, and oocysts) in intermediate hosts can be different, based on the route of inoculation. Because the stage of *T.*

gondii in meat is most likely tissue cyst containing bradyzoites, rather than oocysts or tachyzoites, studies that used bradyzoites were preferred.

(4)   Oral administration mimics the route of meat consumption in humans. Thus, experiments that administered bradyzoites by oral administration were considered superior to studies using intraperitoneal (i.p.) or subcutaneous (s.c.) administration.

5.3.3 Data characterization

None of the twelve eligible studies (**Table 5.1**) met every criterion of data selection. Although pig is the best candidate among three species, two eligible studies in pigs were not selected because Type I and Type III oocysts rather than bradyzoites were used in the experiments. Two studies (**Table 5.2**), conducted in mice and rats, respectively, were selected for model development because they met most of the data selection criteria. The mouse study met three of four criteria for data selection except the non-optimal animal species, while the rat study met two of four criteria except the non-optimal animal species and genotype (GT-1, Type I).

Table 5.2 Dose-response data for *T. gondii* infection

	Mice, ME-49 (383)		Rats, GT-1 (384)	
Dose	Number of mice	Positive (infection)	Number of rats	Positive (infection)
10^6	10	10	5	5
10^5	10	10	5	5
10^4	10	10	5	4
10^3	10	7	5	2
10^2	10	3	5	1
10	10	0	5	0
1	10	0	5	0

These two animal studies originated from the same research group and were conducted in the same manner. Because the number of bradyzoites in tissue cysts can vary several fold, bradyzoites were released from tissue cysts by enzyme digestion (19). Since it is difficult to quantify viable bradyzoites in the inoculum, the Most Probable Number (MPN) was used to estimate the number of viable bradyzoites. Liberated bradyzoites were serially diluted 10-fold to such a degree that none of subcutaneously-inoculated mice in the group were infected (which is the "dilution point"). The subcutaneous route was used to determine the end point because after oral inoculation a variable number of bradyzoites are killed by enzymatic digestion and some may pass in feces. The number of viable bradyzoites was estimated at each dose based on the "dilution point". The inoculated mice were tested both serologically and parasitologically. For this, all inoculated mice were examined for *T. gondii* antibodies at 6 weeks post-inoculation (PI) and brains of all mice were examined microscopically for tissue cysts, irrespective of serological results. Mice were considered *T. gondii*-infected when tissue cysts (in mice that survived) or tachyzoites (in mice that died) were demonstrable. Serological examination or microscopic examination alone is not reliable because some mice do not develop antibodies by 6 weeks PI and tissue cysts maybe too few to be detectable by microscopic examination (19).

The mouse study described toxoplasmosis in female Swiss-Webster mice induced by bradyzoites (383). The mice were orally dosed with an aliquot (0.5 ml) of an ME-49 stain (Type II) bradyzoite suspension for each dilution. None of the mice died because ME-49 is a mildly pathogenic strain in mice. Sera from mice were tested for *T. gondii* antibodies at 48 days PI using a MAT. All mice were killed at 54 days PI

and the brains examined microscopically for tissue cysts. Mice were considered infected when *T. gondii* tissue cysts were identified in brain tissue and antibodies were detected in a 1:25 serum dilution. Tissue cysts were detected in all serologically positive mice. The rat data were extracted from a study that was conducted in a similar fashion to the mouse study (384). In the rat study, five female Sprague-Dawley rats (~100 g) were orally inoculated with serial dilutions of GT-1 bradyzoites. Although GT-1 is highly pathogenic for mice, it is less so for rats and all rats in the experiment survived. Rats were considered infected when *T. gondii* antibodies were detected and *T. gondii* tissue cysts were found in a brain homogenate.

5.3.4 Curve fitting and parameter estimation

The pooled data (bradyzoite concentration as an independent variable, proportion of test animals showing infection as a dependent variable) were fitted into four commonly used dose-response models (**Table 5.3**). Two of these are mechanistic models (exponential and beta-Poisson) and the other two are empirical models (Weibull and Log-logistic). Parameters in the models were computed in MATLAB (MathWorks, Natick, MA) by using MLE, and the mean value of the deviance (-2 log likelihood ratio), Akaike information criterion (AIC) and Bayesian information criterion (BIC) were determined by using the equations described by Haas et al.(393). MATLAB code for curve fitting are provided in Appendix A.

5.3.5 Goodness-of-fit test and model selection

The goodness-of-fit test was conducted as described by Haas et al.(393). Briefly, the value of the optimized deviance is compared with a χ^2 distribution at the upper fifth percentile (95% percentile, i.e. critical value) with k-m degrees of freedom.

Here, k represents the number of doses (in our case, k=7), and m is the number of parameters in the dose-response model (in our case, m=1 or 2). The null hypothesis is the model with parameters estimated from the observed data is a good fit. When deviance from the model is less than the critical value, i.e. $p > 0.05$, the fit (null hypothesis) cannot be rejected, indicating that the model exhibits goodness of fit. When deviance from the model is greater than the critical value, i.e. $p < 0.05$, the null hypothesis is rejected. According to the FAO/WHO guidelines of hazard characterization for pathogens in food and water, the optimal dose-response model was selected based on the result of goodness-of-fit, simplicity, as well as biological plausibility (402).

5.3.6 Determination of the uncertainty of the parameters and confidence interval (CI) of dose-response curve

The uncertainty associated with the estimated dose-response parameters was determined using a bootstrap procedure to estimate the upper and lower limit of the parameters. Following the procedure of Haas et al. (393), 10,000 bootstrap samples were constructed in MATLAB by random sampling with replacement within each dose. Each dose contained three data points (mean dose, total animals tested (n) and number of infected animals (n_{inf}) at each dose). In each bootstrap replicate, the number of infected animals was randomly generated by a binomial distribution with n and p (p= n / n_{inf}) at each dose. Therefore, each bootstrap sample represents an alternative outcome of a dose-infection relationship in mice. Data for each bootstrap sample were fitted into the dose-response model and parameters were estimated by MLE as described in Section 5.3.4. A total of 10,000 sets of parameters and dose-response

curves were constructed. The 95% bootstrap CI of the parameter was determined by the values of 2.5 percentile and 97.5 percentile in the ascending-ordered bootstrap dataset (406, 407). Similarly, the 95% pointwise confidence band of the curve was determined by the values of 2.5 percentile and 97.5 percentile in the bootstrap dataset at each dose (408). The confidence band was plotted by connecting those values in a log scale (from 10^0 to 10^6) with an increment of 0.01. MATLAB code for bootstrapping are provided in Appendix B.

Table 5.3 Mathematical models used for dose-response curve fitting

Model	Equation	Description	Reference
Exponential	$P(d)=1 - \exp(-rd)$	P(d): probability of infection at dose d; d: dose; r: model parameter, interpret as the probability for one cell to successfully initiate a response	Haas et al. (403)
beta-Poisson	$P(d) = 1 - (1 + d / \beta)^{-\alpha}$	P(d): probability of infection at dose d; d: dose; α: model parameter (infectivity); β: model parameter (shape)	Haas et al. (403)
Weibull	$P(d)= 1 - \exp(-q_1 d^{q_2})$	P(d): probability of infection at dose d; d: dose; q_1: model parameter (infectivity); q_2: model parameter (shape)	Krewski et al. (404)
Log-logistic	$$P(d) = \frac{1}{1 + \exp[q_1 - q_2 \ln(d)]}$$	P(d): probability of infection at dose d; d: dose; q_1:model parameter (infectivity); q_2: model parameter (shape)	Prentice et al. (405)

5.3.7 Derivation of mouse-human scaling factor

A quantitative model was developed in Microsoft Excel to predict the annual number of cases attributable to pork based on human epidemiological data (**Table 5.4**). As described by Jones et al., an equal infection rate for each year of age was assumed and an annual population infection rate (Inf) was calculated as 0.35% (409). The proportion of infection cases associated with food (P_{food}) was described as a PERT distribution, which was adopted from a study that analyzed foodborne pathogens associated with major pathogens in the United States (36). The proportion of *T. gondii* infections resulting from pork consumption (P_{pork}) was described as a PERT distribution. In a cohort study, Boyer et al. found that 78% of congenital toxoplasmosis cases were attributable to oocysts (410). Based on this finding, FAO/WHO estimated that approximately 22% of *T. gondii* infection cases are meatborne (385), and therefore this value was used to define the minimum value in the PERT distribution. The most likely and maximum values were based on expert elicitation, which estimated that 41% of foodborne *T. gondii* infections can be attributed to pork (74). Compared to processed pork, fresh pork is more likely to contain *T. gondii* at the time of consumption, since processing such as curing, smoking, and heating can inactivate *T. gondii* tissue cysts (19). In the absence of any data to describe the proportion of infection associated with fresh pork (P_{fpork}), a point value of 50% was assumed. Thus, annual cases of *T. gondii* infection attributed to fresh pork were calculated as: Total Population $\times$ Inf $\times P_{food} \times P_{pork} \times P_{fpork}$. The model was developed in a Monte Carlo probabilistic framework and fifty thousand iterations were conducted by using Latin Hypercube sampling in @Risk 6.0 (Palisade Inc., Ithaca, NY, USA)(67).

106

A dose-response model developed from animal data cannot be applied to humans directly due to the inherent differences between animals and humans. This requires that the relative susceptibility of humans and mice be used to develop a scaling factor for human infection. A scaling factor was computed to adjust the effective dose in the dose-response model to match with the epidemiological data for human infection. Using the mouse dose-response model directly would greatly overestimate the public health burden. The calculation of a scaling factor is based on the difference in the predicted number of annual cases between the quantitative microbial risk assessment (QMRA) of fresh pork (411) and the epidemiological model. The mean number of bradyzoites a consumer might be exposed to is approximately seven per serving of fresh pork (411). Several scenario analyses were conducted to compute the scaling factor. In each scenario, the dose was multiplied with a scaling factor in the animal-derived dose-response model and the number of annual cases was estimated by simulating the QMRA of fresh pork fifty thousand times in @Risk (67). The scaling factor was altered until the mean value of the estimated number of annual cases was close to the mean value of annual cases predicted by the epidemiological model.

Table 5.4. Description and parameters of the epidemiological model

Symbol	Variable	Distribution, Value or Formula	Source
Pop	U.S. population in 2011	312,040,000	http://www.census.gov/
Inf	Infection rate per year	0.0035	Jones et al. (409)
P_{food}	Proportion attribute to foods	PERT (0.4,0.5,0.6)	Scallan et al. (36)
P_{pork}	Proportion attribute to pork	PERT (0.22,0.41,0.41)	FAO/WHO (385), Boyer et al.(410), Batz et al. (74)
P_{fpork}	Proportion attribute to fresh pork	0.5	Assumption

5.3.8 Validation of the mouse-derived human dose-response model

T. gondii rarely leads to identified outbreaks, and the parasite load generally cannot be identified in the outbreaks associated with consumption of meat. Most documented infections are chronic and incident infections are often not clinically apparent or are misdiagnosed. Since there are no good estimates of incidence of human infection, the rat study mentioned earlier (**Table 5.2**) was used to verify the mouse-derived human dose-response model. Similarly, experimental data from rats were fitted to the optimal model that was selected for mice, and the scaling factor was computed. A rat-derived human dose-response model was graphed and compared with a mouse-derived human dose-response model for the purpose of validation.

5.4 Results

5.4.1 Selection of the dose-response model

The best estimates of the parameters of all four models for the mouse data are summarized in **Table 5.5** and plotted for comparison (**Figure 5.1**). Based on goodness-of-fit (**Table 5.5**) and visual comparisons, all four models provided adequate fits to the experimental data with the deviance less than the critical χ^2 value ($p > 0.05$, **Table 5.5**). The exponential model (P (d) = 1 - exp (-0.0015 × d)) and beta-Poisson model (P (d) = 1 - (1 + d / 582.414)$^{-1.479}$) were selected as optimal models to estimate the infectivity of *T. gondii* bradyzoites in the meat products for the following reasons:

(1) **Best estimation of the parameter.** The beta-Poisson and Log-logistic models have lower deviance values as compared with the other two models. Although the deviance value of the exponential model was slightly higher

than the beta-Poisson and Log-logistic models, but it is still far less than the χ^2 critical value, indicating a good fit.

(2) **Simplicity of the model.** Fewer parameters make a model easier to calculate and minimize the errors. Thus, the exponential model (which contains 1 parameter) was considered superior to the other three models (which contain 2 parameters) in terms of simplicity, which is also reflected by the lowest AIC and BIC values.

(3) **Biological plausibility of the model.** The exponential and the beta-Poisson models are both widely used mechanistic models and have been used to describe the dose-response relationship of a number of different biological agents (393). As empirical models, the Weibull and Log-logistic models lack biological plausibility when compared to the mechanistic models (393).

The exponential (**Figure 5.2A**) and beta-Poisson (**Figure 5.2B**) models are both reasonable models to predict the response in mice. Similar results (based on the mean value, which is approximately 1×10^5 infections per annum) were obtained when both models were used in the QMRA model of fresh pork. By plotting both models in a log-log scale graph, the response in mice predicted by these two models are slightly different when extrapolating to low dose (**Figure 5.2C**). Thus, both are presented in this study. The median doses (N_{50}), defined as the dose that 50% of the population is expected to be infected, predicted by the exponential and beta-Poisson models were 462 and 348 bradyzoites, respectively. The mean probabilities of infection by ingestion of a single bradyzoite were 0.0015 and 0.0025 estimated by the exponential and beta-

Poisson models, respectively. **Figure 5.3A** and **Figure 5.3B** demonstrate the

distribution of the bootstrap samples for the exponential and beta-Poisson models,

respectively. Both graphs demonstrate that bootstrap samples surround the best

estimation from the original data. The 95% bootstrap CI for model parameters is

summarized in **Table 5.6** and the 95% confidence band of the dose-response curve is

shown in **Figure 5.2A** and **Figure 5.2B.**
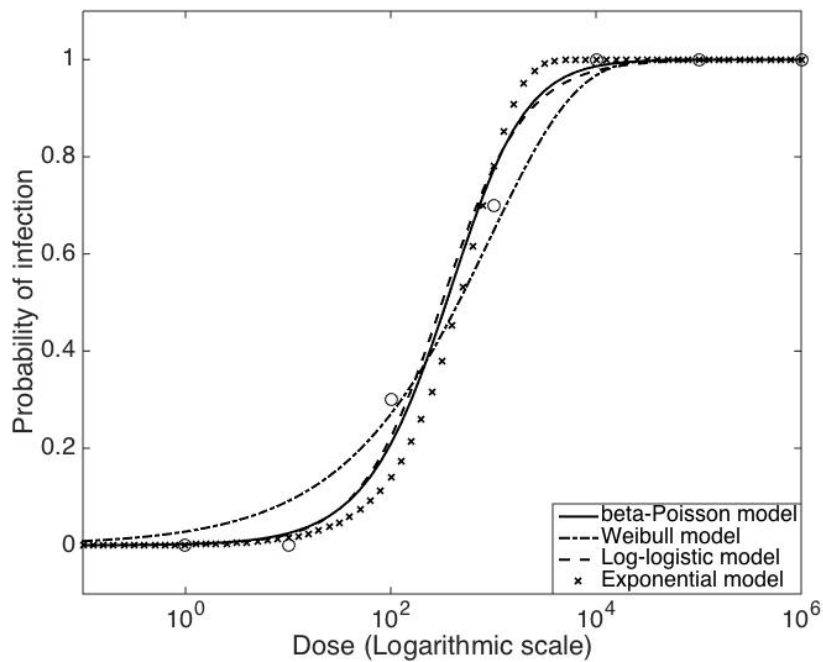
Figure 5.1 Comparison of the curve fitting results of four dose-response models

based on mouse data. Open circles (o) correspond to observed data.

Table 5.5 Comparison of dose-response analysis and goodness-of-fit test based on mouse data

| Model	Parameters	Deviance	AIC	BIC	df	Critical χ^2	p value
Exponential	r=0.0015	2.726	5.526	4.672	6	12.591	0.84
beta-Poisson	α=1.479	1.572	8.571	5.464	5	11.070	0.90
	β=582.414						
Weibull	q_1=0.039	3.726	10.355	8.648	5	11.070	0.59
	q_2=0.496						
Log-logistic	q_1=6.270	1.648	7.247	5.540	5	11.070	0.89
	q_2=1.089						

df: degree of freedom

Table 5.6 95% bootstrap CI of model parameters based on mouse data

| Model	Parameters	N_{50}	2.5% percentile	97.5% percentile
Exponential	r=0.0015	462	0.00070	0.00332
beta-Poisson	α=1.479	348	0.4544	6.6296
	β=582.414		54.0010	3544.7

5.4.2 Computation of the scaling factor between mice and humans

After 50,000 iterations, the mean values of the two models were close to each other, both near 1.0×10^5 cases per year. Based on this value, the scaling factors were estimated as 0.005 and 0.003, respectively, for the exponential and beta-Poisson models, which resulted in shifting the mouse dose-response curve to the right by 2.3 and 2.5 logs, respectively (**Figure 5.4A** and **Figure 5.4B**).
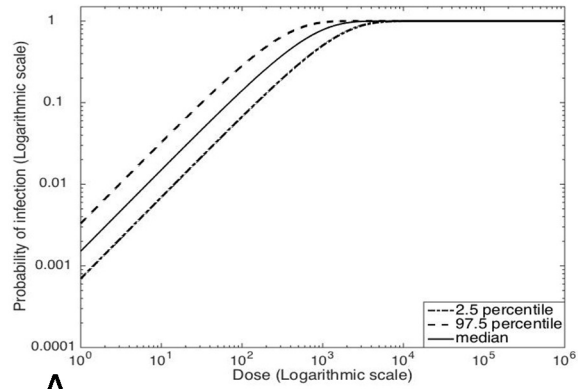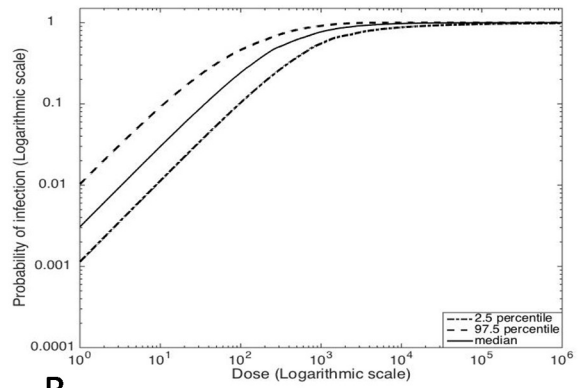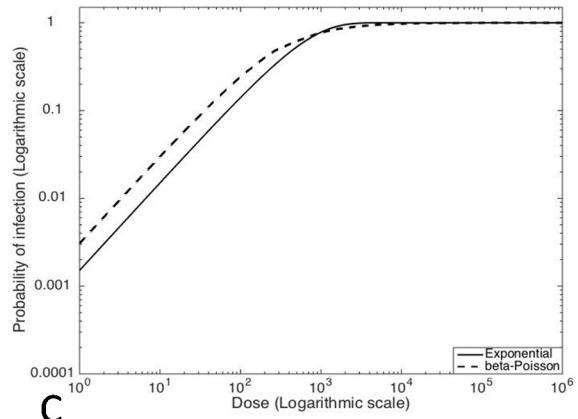
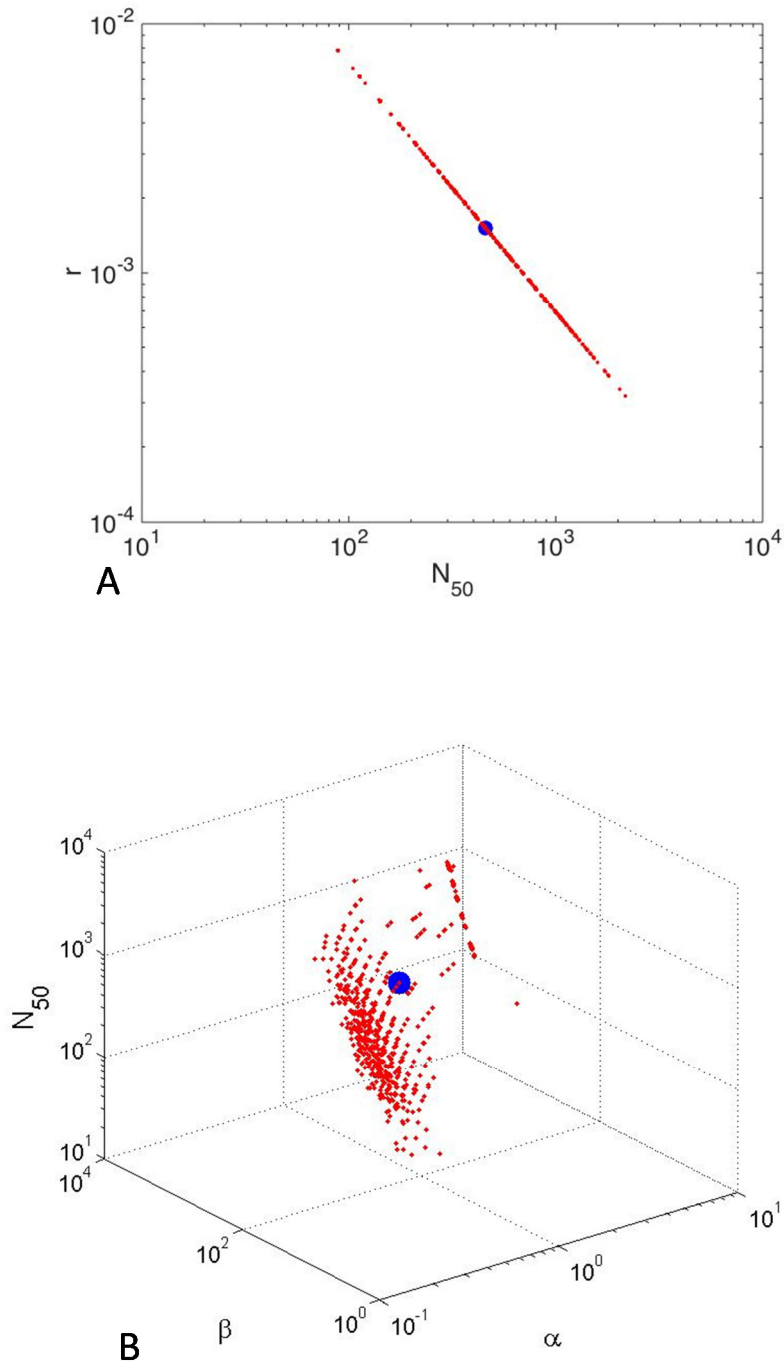**Figure 5.2** Mouse dose-response model on a log-log plot for **A**: exponential model with 95% confidence band, **B**: beta-Poisson model with 95% confidence band, **C**: low dose extrapolation (based on the median value).

Figure 5.3 Bootstrap distribution of parameter estimates for **A**: exponential model ($N_{50}=\ln(0.5)/-r$), **B**: beta-Poisson model ($N_{50}=\beta(2^{1/\alpha} - 1)$), based on mice data. Blue dots correspond to observed data, and red dots correspond to bootstrap data.

5.4.3 Model validation

Experimental data from rats were fitted to the exponential and beta-Poisson models, and the data fit both models well (**Table 5.7**, $p > 0.05$). The exponential and beta-Poisson models were developed as P (d) = 1 - exp (-0.00028 × d), and (P (d) = 1 - $(1 + d / 613.72)^{-0.681}$), respectively (**Table 5.7**, **Figure 5.5A** and **Figure 5.5B**). The N_{50} was approximately 10^3 bradyzoites, which is greater than the median dose estimated by the mouse dose-response model. The difference may result from the error of the MPN method and the virulent difference between ME-49 and GT-1 strains. The scaling factors were calculated as 0.027 and 0.007 for the rat exponential and beta-Poisson models extrapolated to humans, respectively (**Figure 5.6A** and **Figure 5.6B**). The adjusted dose-response curve for rats overlapped with the mouse-derived human dose-response curve (**Figure 5.7A** and **Figure 5.7B**), indicating a consistent response between species, as well as validating the accuracy of the mouse-derived human dose-response model.

**Table 5.7** Results of parameter estimation and goodness-of-fit tests based on rat data

| Model	Parameters	Deviance	AIC	BIC	df	Critical χ^2	p value
Exponential	r=0.00028	4.125	6.925	6.071	6	12.591	0.66
beta-Poisson	α=0.681	1.224	8.225	5.117	5	11.070	0.94
	β=613.72						

df: degree of freedom

5.4.4 Dose-response model and assumptions

The human dose-response models were described as an exponential ($P(d) = 1 - \exp(-0.0015 \times 0.005 \times d)$) or beta-Poisson ($P(d) = 1 - (1 + d \times 0.003 / 582.414)^{-1.479}$) model. Both models were developed based on the following assumptions: (i) Swiss-Webster mice and Sprague-Dawley rats are considered to be representative of their species; (ii) a single bradyzoite is sufficient to cause an infection; (iii) bradyzoites are randomly distributed in a serving of meat (the probability of ingesting a bradyzoite in a serving of meat is described as a Poisson distribution); and (iv) given a certain probability of infection at each dose, the numbers of infected people at that dose are binomially distributed. The difference between the exponential model and the beta-Poisson model is that the former assumes a constant host-pathogen interaction (described by parameter r), while the latter assumes the interaction as a beta distribution (described by parameter α and β in the beta-Poisson model).
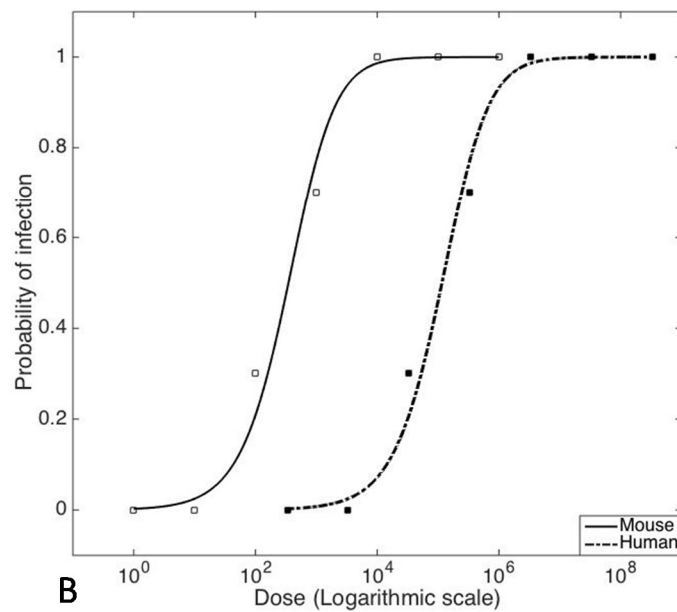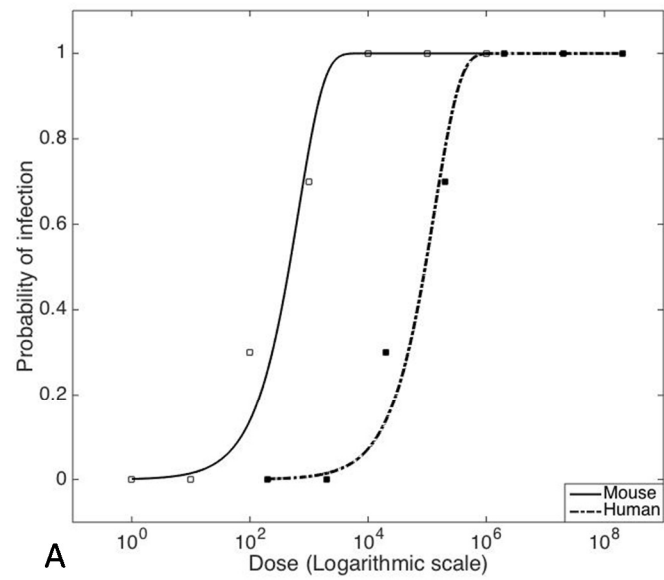
Figure 5.4 Mouse and mouse-derived human models. **A**: exponential model (scaling factor=0.005), **B**: beta-Poisson model (scaling factor=0.003). Open squares correspond to observed data, and closed squares correspond to data adjusted with the scaling factors.
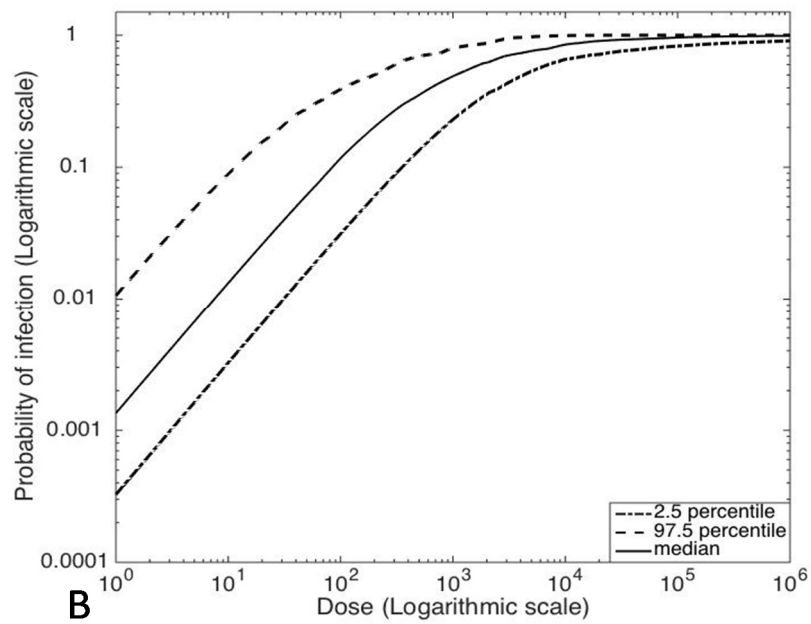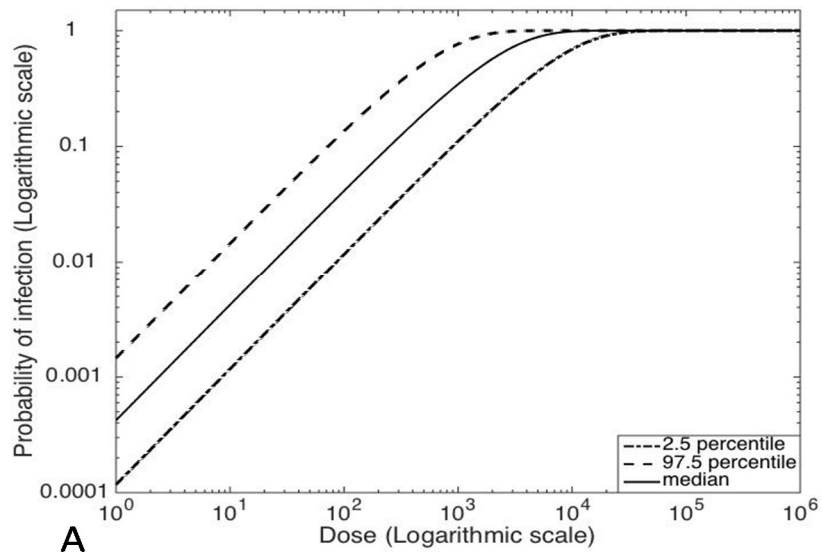
Figure 5.5 Rat model on a log-log plot with 95% confidence band for **A**: exponential model, **B**: beta-Poisson model.

117
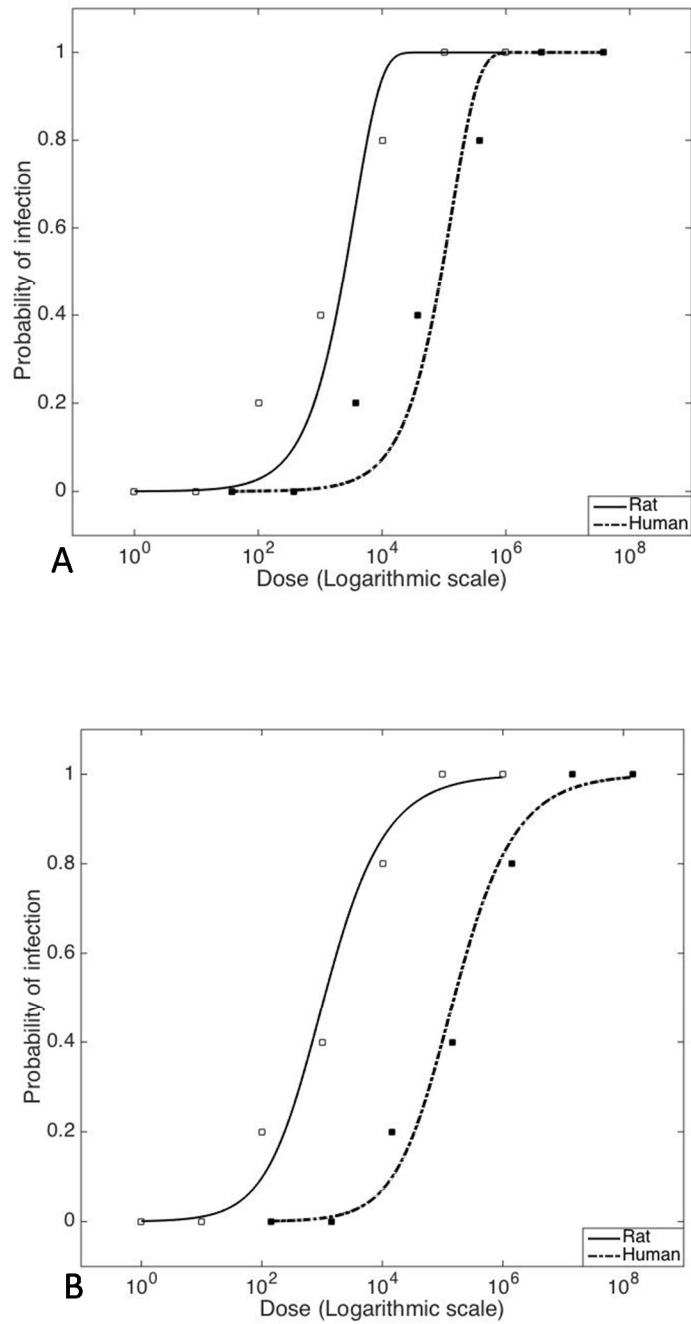
Figure 5.6 Rat and rat-derived human models. **A**: exponential model (scaling factor=0.027), **B**: beta-Poisson model (scaling factor=0.007). Open squares correspond to observed data, and closed squares correspond to data adjusted with the scaling factors.
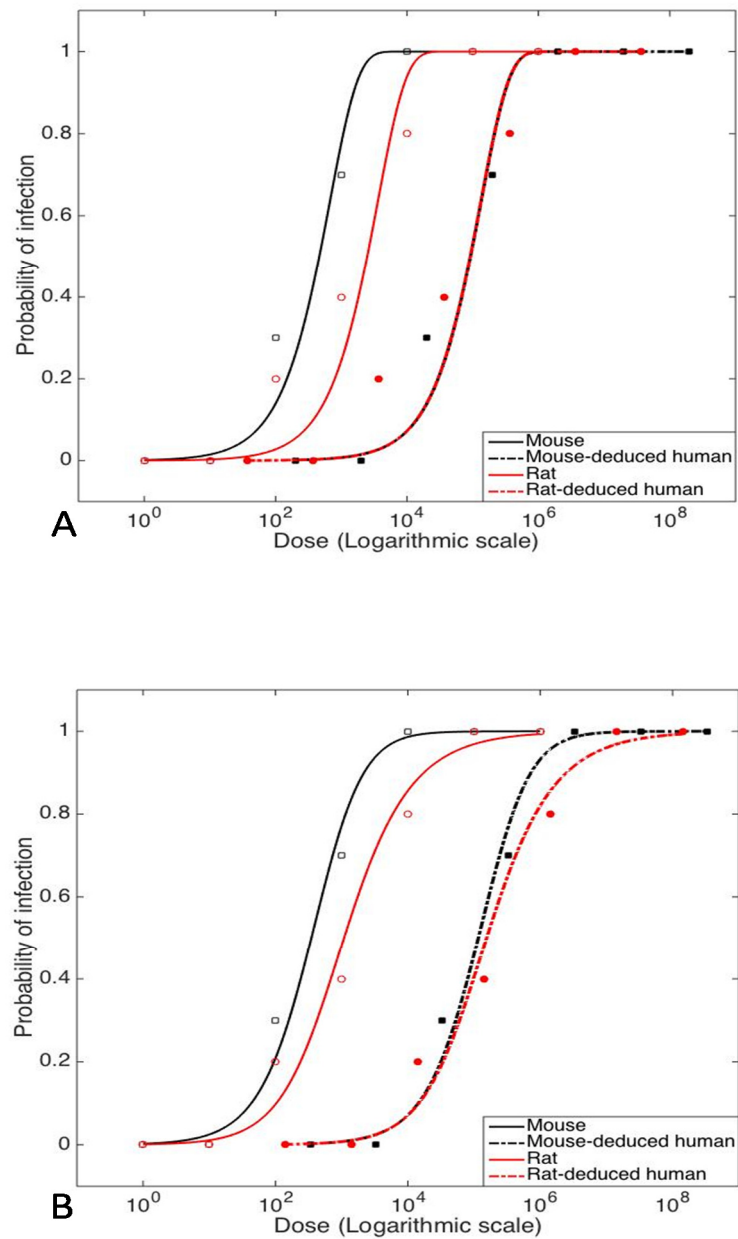
Figure 5.7 Comparison of mouse-derived and rat-derived human models. **A**: exponential model, **B**: beta-Poisson model. Open squares and open circles correspond to observed data from mice and rats, respectively. Closed squares and closed circles correspond to observed data adjusted with the scaling factors from mice and rats, respectively.

5.5 Discussion

The goal of this study was to develop a dose-response model to predict the probability of human infection by *T. gondii* due to consumption of infected meat products. The dose-response model, can then be used in a quantitative risk assessment of meatborne *T. gondii* infections to further describe the public health burden. Previously, multiple exponential dose-response curves were developed to compare infectivity between bradyzoites and oocysts, not to predict the human response (394). The exponential model is simple, based on biological assumptions, and is commonly used for risk estimation. Dose-response models for two other protozoan parasites, *Giardia* and *Cryptosporidium* were also described using exponential models (393). Opsteegh et al. (67) adopted the exponential model developed by AFSSA and used it as one of the inputs in a quantitative microbial risk assessment for meatborne *T. gondii* infection in the Netherlands. This model overestimated the annual infection cases attributed to meats; one of the possible reasons for overestimation was the application of an animal-derived dose-response model directly to humans without adjustment. Inbred experimental animals are different than humans in various aspects, such as body weight, metabolic rate and immunity to pathogen exposure. In the present chapter, data were fitted to four common dose-response models and both an exponential and a beta-Poisson model were selected based on an evaluation of goodness-of-fit, simplicity and biological plausibility. The parameter r estimated here in the current exponential model differs from the result computed by AFSSA, which shifted the dose-response curve to the right by more than 2 logs. Both the exponential and the beta-Poisson models are reasonable and provide a similar estimation based on the mean value. In order to apply

the animal-derived dose-response model to humans, a scaling factor was computed and added to the dose-response model so that the prediction is in agreement with the epidemiological data.

Human volunteer studies are potentially the best candidates for developing a dose-response relationship, but this is not possible because even the lowest mouse virulent *T. gondii* strains (Type II or III) may lead to severe health outcomes in humans (19). Further, such studies are based on the response in healthy adults who might provide poor estimates of susceptibility associated with high risk subpopulations (i.e., immunocompromised individuals). Thus, animal studies were used as surrogates to predict the human response, which required dose adjustment and data validation to correlate animal models to humans. Although the principles of extrapolating animal studies to human risk assessment have not been well defined, an animal-derived dose-response model is generally validated by outbreak or surveillance data (393). Only a few outbreaks of human *T. gondii* infection that linked to meats have been reported over the most recent two decades (19). The sources of infection in these outbreaks were identified based on serological examination of patients and epidemiological description, without isolation and quantification of the parasite in the meats. Thus, in the present chapter, a human dose-response model was developed based on experimental data in mice, and was validated with experimental data in rats. A scaling factor was determined for each species by matching the predicted annual cases derived using the animal dose-response model with an estimate of human incidence per year derived from epidemiological data. This approach has been previously used to develop dose-response models for *Listeria monocytogenes* in RTE foods (390). The purpose of

121

the scaling factor is to bridge the complex relationship between response in humans and animals, so that animal-derived dose-response models can estimate the right number of human cases per annum. The scaling factor was used to account for several uncertainties in the dose-response model, including intra- and interspecies variability between surrogate animals and humans, strain/genotype bias, and food matrix effects (390). For example, in the present study, the ME-49 strain (Type II) and GT-1 strain (Type I) were used in mice and rats, respectively. The virulence of these strains differ among mice, rats and humans; application of the scaling factor enabled us to account for these differences. In the absence of reported incidence of human infections, the quantitative models were developed in order to estimate the number of infections per annum from an epidemiologic standpoint.

It was estimated that 20% of the U.S. population belongs to high-risk groups for clinical manifestations of *T. gondii* infection (412). Compared to immunocompetent people, severe outcomes from infection with any of the three stages of *T. gondii* are often observed in immunocompromised adults (413). However, whether the dose-infection relationship for *T. gondii* changes with age and immunity status of a person is largely unknown. Preliminary animal studies indicated that the infection rate might be higher in high-risk than low-risk populations. Dubey et al. reported that the percentage of infected animals is higher in IFN-γ knockout mice (68.6%, 35 of 51) than healthy mice (51.6%, 48 of 93), when mice were orally given the same inoculum of *T. gondii* contaminated goat heart (127). The alteration of immune function and hormone levels during pregnancy also leads to increased susceptibility to *T. gondii* infection (414). Luft and Remington observed a remarkably decreased resistance to *T. gondii*

infection in pregnant Swiss-Webster mice during the third trimester. Compared to their virgin counterparts (40% mortality rate), all (100%) pregnant mice died day 5 PI with *T. gondii* strain C37 (415). These findings suggest that individual susceptibility to *T. gondii* infection and disease may also vary in humans. Mouse-derived human dose-response models reported here are an effort to describe the dose-infection relationship in the human general population. By using alternative scaling factors, it can also be used to predict the dose-infection relationship in high risk or susceptible subpopulations such as pregnant women, the fetus, or immunocompromised individuals, when relevant data (e.g., epidemiological, meat consumption) are available.

The results of this chapter include (i) establishment of a good fit of biologically plausible models from observed data sets; (ii) identification of scaling factors to account for the intra- and interspecies variability; (iii) validation of mouse-derived human dose-response models using the dose-infection data in rats; and, (iv) correlation of animal-derived dose-response models with human response using a scaling factor, which can be directly used in risk assessment for human infections.

In addition to the inherent limitations based on assumptions in the models, the results of this chapter have several limitations, including: (i) current dose-response models were developed based on the data from a specific strain (less-virulent Type II ME-49 strain); the pathogenicity and virulence of strains of other genotypes were not integrated in the model; and, (ii) the present dose-response models were developed for estimating the health risk in the general population, and the development of dose-

123

response curves for several high-risk subpopulations were not conducted because data were unavailable.

To address these gaps, the following studies are recommended for future research: (i) more doses should be added near the median dose to improve the curve fitting and reduce the uncertainty of the current models; (ii) additional data should be collected from the Type II strains in animal species, such as rats and pigs, in order to better evaluate interspecies variability; (iii) more animal studies should be conducted using virulent *T. gondii* strains (Type I) to evaluate other disease endpoints (illnesses and deaths); (iv) animal studies need to be focused on the infectivity of *T. gondii* for sensitive populations; pregnant animals and gene knockout animals are both excellent candidates to challenge with *T. gondii* infection, and a dose-response model can be developed for each of the subgroups with the acquired data; and, (v) efforts should be devoted to study the pathogenicity and infectivity of broader genotypes and strains, especially strains isolated from human patients.

5.6 Conclusions

This chapter has demonstrated that exponential and beta-Poisson models provide an effective means of describing animal dose-response relations for *T. gondii*. By computing a scaling factor, the annual numbers of human infections could be predicted using an animal-derived human dose-response model, which is concordant with epidemiological findings. The developed dose-response models can be directly used in risk assessment studies for *T. gondii* to predict public health outcomes.

Chapter 6: Quantifying the risk of foodborne human *Toxoplasma gondii* infection due to consumption of fresh pork in the United States

This work has been submitted to *Applied and Environmental Microbiology* and is currently under review.

6.1 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 abattoir, production process, and 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 burden in the United States associated with consumption of fresh pork. *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 in each pork cut of an infected pig. A logistic regression and a log-linear regression models were developed to predict the reduction of *T. gondii* during moisture enhancement and home cooking, respectively. An exponential mouse-derived dose-response model was used to predict infection burden

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 94,606 new infections annually in the U.S. population. Approximately 957 new infections per year were estimated to occur in pregnant women, corresponding to 277 congenital toxoplasmosis cases per year. In the context of available data, the sensitivity analysis suggested that cooking is the most important parameter impacting human health risk. This study provides the scientific basis for risk management and also could serve as the baseline model for developing risk models of *T. gondii* infection associated with other meat products.

6.2 Introduction

Pork is one of the major consumed meats in the United States, with an individual average consumption of 26.8 kg per capita in 2011 (68). Pork is consumed in the United States in two forms: fresh (muscle cuts, usually cooked before consumption) and processed (pork transformed by grinding, curing, smoking, or seasoning prior to wholesale or retail sale) (416). Processed pork accounts for the majority of pork consumption (18.6 kg per capita in 2011). However, the infection risk associated with processed pork products is lower than for fresh pork (8.2 kg per capita in 2011). 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 (66, 417). 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,

viable *T. gondii* was detected from 7 of 2,094 fresh pork samples purchased from retail stores (34).

Quantitative microbial risk assessment (QMRA) is a well-established framework to understand and assess risks posed by foodstuffs contaminated with pathogenic microorganisms (418). While several QMRA studies have been conducted to assess risk associated with foodborne bacteria, there are limited risk assessment studies available for *T. gondii*. To our knowledge, no QMRA study has been carried out for *T. gondii* in meats destined for human consumption in the United States. 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 to *T. gondii* due to consumption of fresh pork, (iii) quantify the public health burden associated with consumption of fresh pork in the total U.S. population and in pregnant women, and (iv) compare the relative risk posed by different fresh pork products.

6.3 Materials and Methods

6.3.1 Model overview

In accordance with guidelines by the CAC (64), a baseline model was developed to estimate the risk of human *T. gondii* infection due to consumption of different fresh pork products in the United States. Since *T. gondii* does not grow outside its suitable hosts, this model assumed that animal infection only occur at farm, and *T.*

127

gondii bradyzoites concentration can only be reduced, inactivated or remain unchanged in fresh pork in this QMRA. 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) (419), (ii) one tissue cyst could contain a few to several hundred bradyzoites, which increases the uncertainty associated with the model inputs, and (iii) tissue cysts 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 method over 5×10^5 iterations. The number of iterations was set to obtain a stable result. Across five independent simulations (each simulation with 5×10^5 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 (highest risk cut) varied up to approximately 2.5%. The model was developed in the Microsoft Excel add-in software @Risk. For each model iteration, one infected pig was tracked from farm to table (**Figure 6.1**). 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 in both the total U.S. population and the pregnant women population.

6.3.2 Module 1: farm

Prevalence of *T. gondii* in market pigs was obtained from the systematic meta-analysis conducted in Chapter 3. As a result of the meta-analysis, mean and standard deviation of *T. gondii* prevalence in market pigs was estimated as 0.056 and 0.027, respectively. 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% CI of the computed prevalence (**Table 6.1**).

No data on bradyzoite concentration 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 (382). In this experiment, two groups of infected goats (8 goats/group) were killed after 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). One-way ANOVA analysis (**Table 6.2**) and independent t-test (**Table 6.3**) were used to compare the means of bradyzoite concentration from each limb and at different post infection time, respectively. No statistically significant differences ($p > 0.05$) were found among limbs. Thus, data were pooled together and fit with a lognormal distribution (**Table 6.1**). 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 is 4.36 bradyzoites/g.
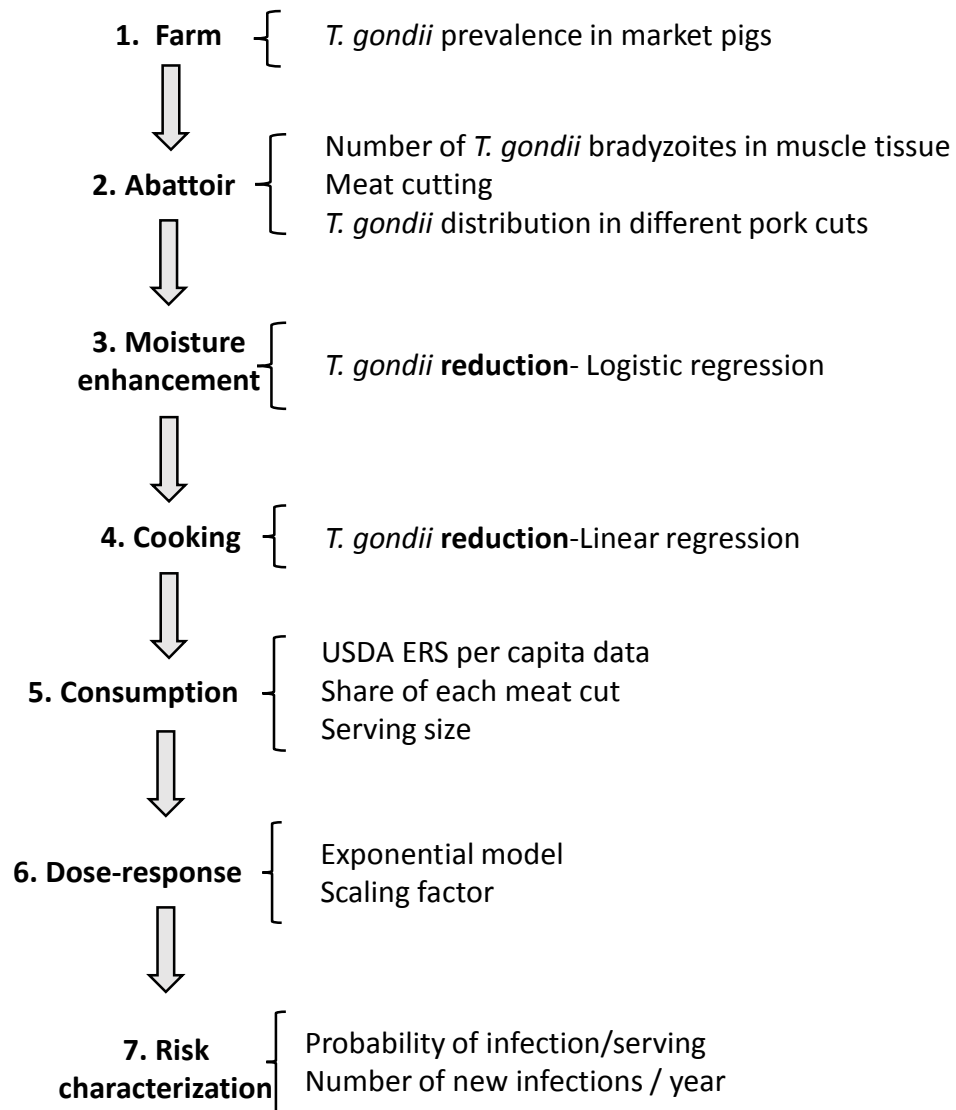
Figure 6.1 Schematic representation of the risk assessment model.

130

Table 6.1 Description and statistical distribution of the QMRA model

Variable	Description	Distribution	Source
Farm			
Prevalence	Number of positive market/finisher pigs, estimated via a quality-effects meta-analysis.	Normal distribution (μ, σ), μ=0.056, σ=0.027, truncated to (0.003, 0.149).	Guo et al, 2015a
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), μ=11.67, σ=97.31, truncated to (0.04, 41.3) bradyzoites/g.	Jurankova et al., 2013
Abattoir			
Weight of a market pig	Weight of a pig at time of slaughter.	Normal distribution (μ, σ), μ=122.1, σ=6.4 kg.	Stalder, 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.	Constant, 0.73.	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 (Figure 6.2).	National Pork Board, 2012
Probability of *T. gondii* presence	Probability of presence of *T. gondii* in a randomly selected commercial pork cut. Yes=1 indicates presence of *T. gondii* in a pork cut. Probability varies by cut.	Binomial distribution (n, p), where n=1, p varies by pork cut (Table 6.2).	Dubey et al., 1986
Number of bradyzoites in pork cut	Bradyzoite load in a considered fresh pork cut.	Bradyzoite concentration × Boneless weight of a pork cut × Probability of *T. gondii* 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 (0.4, 0.45, 0.45)	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), μ=3.39, σ=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	FAO, 2007

NaCl concentration in meat	Final NaCl concentration in pork cut	(pumping ratio × brine concentration) / (1+ pumping ratio)	Calculation
Probability of infection if enhanced	Probability of a mouse becoming infected by subcutaneously inoculating an enhanced pork cut that infected with *T. gondii*, estimated by a logistic regression model.	$P_{pos}= [1 + \exp (-0.271 + 0.286 \times$ temperature (°C) + 0.022 × time (days) - 0.762 × NaCl concentration in pork cut $(g/ml))]^{-1}$	Dubey, 1997
Presence of *T. gondii* in a specific enhanced pork cut	Probability of *T. gondii* presence in a randomly selected enhanced pork cut. Yes=1 indicates presence of *T. gondii*; No=0, indicates inactivation of *T. gondii* 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), μ=69.26, σ=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 *T. gondii*.	Reduction factor=0	Dubey et al., 1990
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
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 (reduction factor) = 8.583-0.172× final cooking temperature (°C)	Dubey et al., 1990
Reduction factor	Overall *T. gondii* reduction after home cooking.	IF(cooking temperature <49.9, 1, IF(cooking temperature <61,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 × 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, 2014
Pork consumption for pregnant women	Pork consumption of women in the child-bearing age.	61,869,429 kg/year	U.S. Census Bureau, USDA, 2014; 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), μ=85, σ=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., 1997; Dubey, 1998; Guo et al., 2015b.
Model parameter r (in the exponential dose-response model)	Probability of one bradyzoite to initiate human infection. Data of 100,000 bootstrap r fitted to a lognormal distribution.	Lognormal distribution (μ, σ), μ=0.00169, σ=0.00067, truncated to (0.00004, 0.01276).	Guo et al., 2015b., 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 *T. gondii* prevalence in market pigs: $P_{serv}= P_{DR} \times$ 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 cases per year	Number of cases caused by fresh pork cut consumption in a year, estimated for each pork cut.	Number of servings per year × Probability of human infection per serving. Assumes all servings consumed in a year are the same.	Calculation
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 × 0.892, 0.892 is an adjustment factor (10.8% of the U.S. population have been infected with *T. gondii*).	Jones et al., 2007
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 × 0.89, 0.89 is an adjustment factor (11.0% of the women of age 15-44 years have been infected with *T. gondii*).	Jones et al., 2007
Maternal-fetal transmission rate	*T. gondii* 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 *T. gondii* 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 × Maternal-fetal transmission rate	Calculation

Table 6.2 Comparison of bradyzoite concentration among four limbs

Group		Sum of squares	df	Mean square	F	p
30 dpi	Between groups	181.9	3	60.6	0.51	0.68
	Within groups	3332.3	28	119.0		
	Total	3514.2	31			
90 dpi	Between groups	286.4	3	95.5	1.16	0.34
	Within groups	2304.0	28	82.3		
	Total	2590.5	31			

df: degree of freedom; dpi: days post infection.

Table 6.3 Comparison of bradyzoite concentration at different post infection time

Group	Levene's test for equality of variance		t test for Equality of means				
Equal variance assumed	F	p	t	df	Sig. (2-tailed)	Mean difference	Std. error difference
	2.179	0.145	1.420	62	0.160	3.524	2.481

df: degree of freedom.

6.3.3 Module 2: abattoir

Market weight pigs are transported from farm to abattoir for meat processing. In the abattoir, pigs are slaughtered and carcasses are cut into large cuts (four primals: shoulder, loin, side and leg), then further cut to subprimals, portion cuts, and retail cuts (351). Abattoir processing (e.g. scalding, evisceration, and carcass chilling) does not include any step that could inactivate *T. gondii* tissue cysts residing in the muscle tissue (417). The weight of a live market weight pig was described as a normal distribution, with 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 (420). 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 to 2009 (421) 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 6.1**). The yield rate from carcass to boneless retail cut was set as a fixed value (73.1%) based on USDA data (421). Based on carcass breakdown diagram (**Figure 6.2**), boneless weight of a fresh pork cut can be computed according to the weight percentage of each cut to the total carcass weight (421) (**Table 6.1**). Fresh pork cuts included in this QMRA are fresh ham, spare rib, loin (which includes loin chop and sirloin roast), back rib, tenderloin, arm picnic, blade steak, and blade roast. The exposure risk from ground pork was not listed as fresh pork and estimated in this QMRA because data of cross-contamination during combining and grinding are not available.

T. gondii tissue cysts are unevenly distributed among infected pork cuts. Thus, bradyzoite concentration was calculated individually in fresh pork cut. Dubey et al. (422) reported the unevenly 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 6.4**). In this QMRA it was assumed that the probability of *T. gondii* presence in loin and back rib are the same as in tenderloin, 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 6.1**). Thus, number of bradyzoites in each pork cut can be calculated as: bradyzoite concentration (bradyzoites/g) × boneless weight of a pork cut (g) × presence of *T. gondii* in one random selected pork cut.

122.1 kg pig　　**86.6 kg dressed**　　**63.3 kg boneless retail cut**

Fresh ham

Loin →　　→ Side (bacon)
　　　　　　→ Spare rib
Boston butt →　→ Arm picnic

- Fresh ham (1.1%): 696.3 g
- Spare rib (3.8%): 2,405.4 g
- Loin (11.1%): 7,026.3 g
- Back rib (1.8%): 1,139.4 g
- Tenderloin (1%): 633.3g
- Arm picnic (7.8%): 4,937.4 g
- Blade steak (2.9%): 1,835.7 g
- Blade roast (5.2%): 3291.6 g
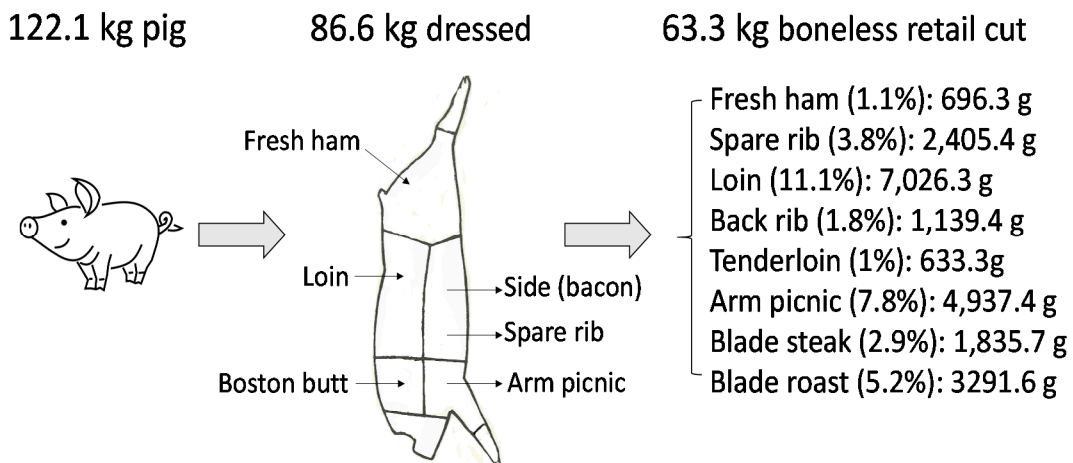
Figure 6.2 Diagram of pig carcass breakdown (based on the mean weight, data were obtained from actual cutting tests) (421).

Loin cut includes loin chop and sirloin roast.

Table 6.4 Probability of presence of *T. gondii* tissue cysts in commercial pork cuts (data from Dubey et al., 1990)

Fresh pork cut	Probability of *T. gondii* 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)

6.3.4 Module 3: moisture enhancement

Fresh pork sold in the United States is often injected with enhancing solutions to extend shelf life, as well as to increase meat quality (91). Pork is passed through a machine with one or more injection needles that inject enhancing solution into the meat (423). According to a 2004 retail store survey of enhanced meat, approximately 45% of pork packages was labeled as "enhanced" (354). Hill et al. (99) 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 minimal 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 6.1**).

Salt in the enhancing solution can inactivate *T. gondii* tissue cysts that containing bradyzoites (355). 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 (355). In this experiment, brain tissue from *T. gondii*-

137

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 as cold chain (<12°C) is generally well maintained through meat processing, transportation, and storage (424). 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 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:

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

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; β_0 - β_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 the good fit of the model. The final logistic regression model was:

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

The three variables in the logistic regression model were described by individual distribution as follows (**Table 6.1**). Storage temperature for fresh meat from manufacture processing to home cooking are generally maintained at a cold chain temperature (<12°C), except that occasionally (approximately 3%) refrigerated meats could reach at 15°C or higher during transportation from retail to home (92). No data

138

were found to report transportation temperatures in enhanced pork products. In this model, enhanced pork products were assumed to be kept at retail for most of the shelf life time. Thus, the parameters were obtained by fitting display temperature values of fresh meat counter (92) 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 of 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 (425). Salt (NaCl) concentration in enhanced pork is determined by the equation (426):

$$C_{salt} = \frac{\%ES \times C_{ES}}{1 + \%ES}$$

(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 (426, 427). Probability distributions were used to represent the variability of %ES and C_{ES} (**Table 6.1**). Injection normally adds 7 to 15% of enhancing solution by weight to a fresh pork cut (428), and a 12% pumping ratio is commonly used in the meat industry (429). Thus, a PERT distribution was used, with the minimum, most likely and maximum value 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 (371). Under each combination of temperature, time, and salt concentration, the probability of infection for a bioassayed mouse subcutaneously inoculated with an enhanced *T. gondii*-infected

meat can be calculated by the logistic regression model (equation 2). However, this is not directly reflect the reduction in bradyzoite concentration after moisture enhancement. A binomial distribution (Bionomial (1, 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 6.1**).

6.3.5 Module 4: cooking

Cooking is one of the most effective barriers to reduce exposure to *T. gondii* via ingestion of infected pork. However, low cooking temperatures as well as short cooking times may not sufficiently 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 (416). 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 as follows by using a time–temperature profile developed by Dubey et al.

(344). This study associated different time-temperature combinations to a binary outcome (*T. gondii* detected or non-detected, **Table 6.5**), thus dividing the time-temperature parameter space into a "complete inactivation" and a "non-inactivation" zones (344). 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 bioassasyed in five mice (344).

Data on home cooking times, or time-temperature combinations, are not available. However, the Ecosure 2007 home cooking data (92) contains 275 finished cooking temperatures measured for pork in the United States. The finished cooking temperature is defined as the internal temperature measured 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 at 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 described earlier (344) for two reasons: (i) Ecosure 2007 finished cooking temperature is comparable with the measured core temperature in the experiment (67), and (ii) data of cooking time for fresh pork are not available at this time, thus, a conservative estimation was conducted. It was also assumed that the temperature throughout the entire piece of pork is the same as the temperature at the coldest spot (i.e. the finished cooking temperature). Again, in the experiment by Dubey et al. (344), *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 subcutaneously inoculated with a meat sample (**Table 6.5**). 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:

$$\text{Log (reduction factor)} = 8.583 - 0.172 \times \text{temperature (°C)} \tag{4}$$

where 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 completed 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 a good fit (**Figure 6.3**). The estimated reduction factor is bound to be between 0 (i.e., 100% inactivation) and 1 (i.e., zero inactivation). The fitted regression curve raises above 1 (i.e. crosses the "zero inactivation" line) at a

142

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.

Table 6.5 Data used to develop the log-linear relationship between temperature and degree of bradyzoite reduction due to thermal treatment

Temperature (°C)	Percentage of *T. gondii* 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)
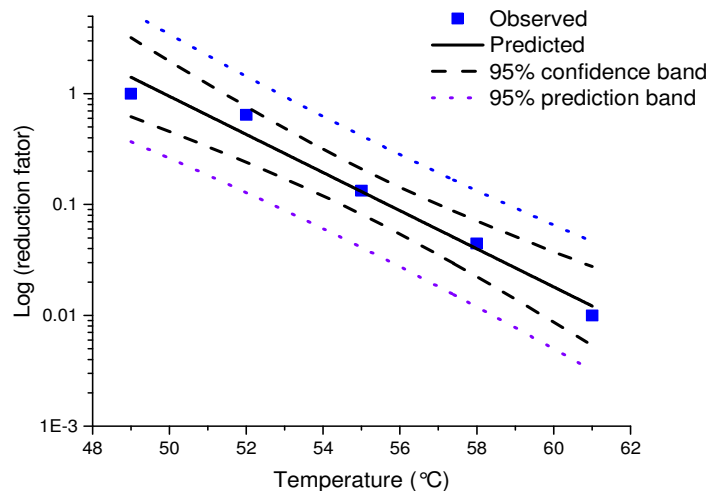
Figure 6.3 Relationship between temperature and decimal logarithm of the reduction

factor during cooking. Data from Dubey et al., 1990.

6.3.6 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

143

calculate the numbers of servings per year in both the total U.S. population and the pregnant women population. Consumption data were extracted from the USDA Food availability per capita data system 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 (68). By excluding approximately 4.4% of imported meat (68), 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 (430). Per capita consumption was calculated by different methods in the USDA Food availability per capita data system 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 kg / 21.9 kg = 0.89) was computed by calculating difference of per capita consumption between the food availability per capita data (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 kg × 0.89 (adjustment factor)). The number of pregnant women per year was described based on the data of live birth in 2011 (3,953,590 of live birth in 2011) (431).

Consumption data for individual fresh pork cut was estimated by multiplying the total pork consumption by the proportion of consumption of each fresh pork cut (416). The proportion of consumption of loin, blade steak, and fresh ham were estimated as 10.8%, 3.7%, and 4.9%, respectively (416). The consumption data for

144

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% (416), and was further allocated to back rib (1.2%) and spare rib (2.5%). Similarly, the share of non-specified fresh pork (13.1%) (416) was allocated to blade roast (4.9%), tenderloin (0.9%), and arm picnic (7.3%) (**Table 6.1**).

The amount 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 (432). 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 × 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 × (1 - %import) =6,084,780,000 kg/year × (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 × Proportion of fresh ham to total pork consumption = 5,817,049,680 kg/year × 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)

6.3.7 Module 6: dose-response model and risk characterization

The dose-response model estimated the relationship between the magnitude of *T. gondii* exposure and the probability of infection in humans. In Chapter 5, two dose-response models have been developed, by fitting experimental data from mice orally infected with Type II bradyzoites (383), and validated against dose-response data obtained from rats (384). The exponential model was selected in the current QMRA model and was described as (**Figure 6.4**):

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

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 to initiate an infection, and 0.005 is a scaling factor (433). 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 (434):

$$P_{serv} = P_{DR} \times \text{Prevalence} \tag{6}$$

The uncertainty associated with the estimated dose-response parameter r was determined using a bootstrap procedure that was described in Chapter 5. 100,000 bootstrap samples were constructed in MATLAB and fitted into the exponential model to estimate parameter r. The set of 100,000 bootstrapped values of r was fitted with a lognormal distribution, resulting in a mean value 0.00169 and standard deviation

0.00067. The lognormal distribution was truncated between 0.00004 and 0.01276, based on the range of 100,000 bootstrapped values of r (**Figure 6.5**).

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. This calculation of the mean number of new infections per year was based on the assumption that for each cut, all the servings consumed in one year are identical, i.e. contain the same number of bradyzoites. And the risk from exposure of each serving of pork cut was independent from other exposures. 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 (67). 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 (29). 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 baby developing congenital toxoplasmosis. Dunn et al. (435) observed 161

147

congenital infection cases in a cohort study of 557 babies 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 infection per year in the pregnant women population by the maternal-fetal transmission rate.

6.3.8 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.
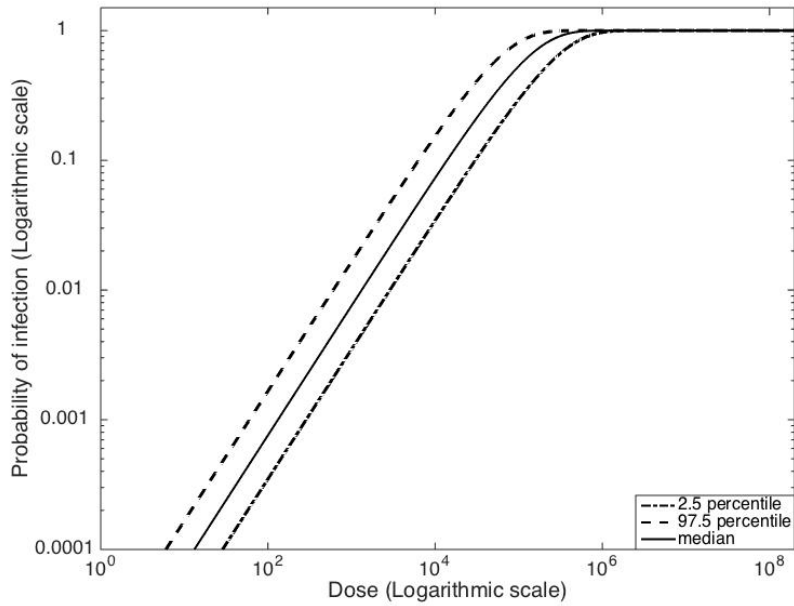
Figure 6.4 Mouse-derived human exponential dose-response model on a log-log plot,

with 95% confidence band deriving from bootstrapping samples.
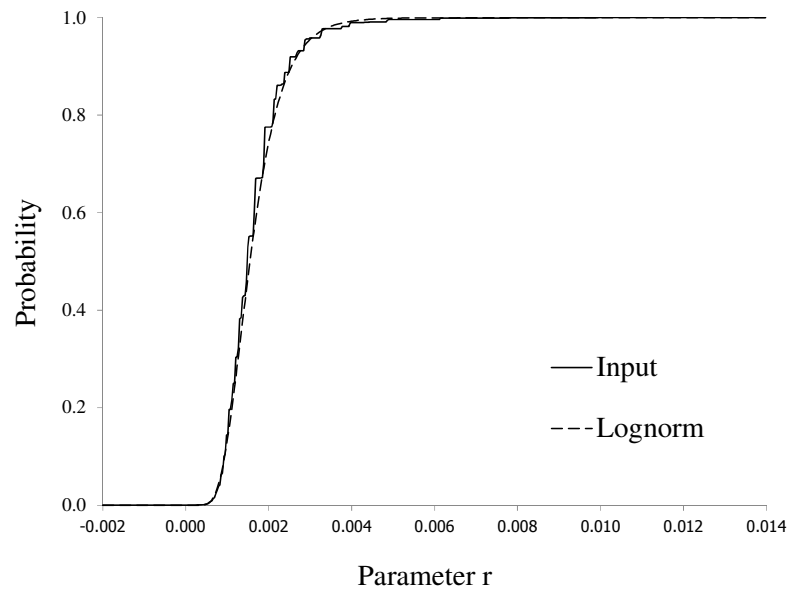
Figure 6.5 Cumulative frequency of 100,000 values of parameter r, fit with a lognormal

distribution.

149

6.4 Results

6.4.1 Estimated number of *T. gondii* new infections 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* infections 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 infections in pregnant women, the mean number of congenital toxoplasmosis cases per year was 277.

6.4.2 Estimated number of *T. gondii* infections 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 cut is shown in **Table 6.6**. 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 as 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 6.6**). The percentage of *T. gondii*-positive servings of individual pork cuts at the time of consumption ranges from 1.48 to 13.35% (**Table 6.7**). 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 of these positive servings, the probability of infection per positive serving of each cut was further calculated and presented in **Table 6.7**.

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

Sensitivity analysis indicted that cooking temperature and moisture enhancement treatment were the two most important factors to affect the probability of infection and number of new infection cases (**Figure 6.6**). The order of importance of the parameters after moisture enhancement was the presence of bradyzoites after moisture enhancement, presence of bradyzoites in arm picnic, and presence of bradyzoites in Boston butt.

6.5 Discussion

In the present chapter described here, 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 to compare the relative infection risk from different pork cuts. The majority of fresh pork servings are *T. gondii*-negative and free of risk, while a small proportion of servings contained *T. gondii* and could initiate infection. This risk model estimated that approximately 10^5 new infections per year occurred in the total U.S. population. 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 (409). 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 (433). This approach has been previously applied, for example, to develop a risk model for *Listeria monocytogenes* in ready-to-eat foods (390).

Table 6.6 Probability of infection per serving and number of new infections per year in the United States associated with consumption of individual fresh pork cut

Fresh pork cut	Population	Probability of infections per serving, mean (5th, 95th, 99th percentiles)	Number of new infections/year, mean (5th, 95th, 99th 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)

Table 6.7 Number of *T. gondii*-positive servings at the time of consumption

Fresh pork cut	No. of servings positive for *T. gondii*	No. of servings negative for *T. gondii*	Percentage of servings positive for *T. gondii*	Probability of infection per positive servings in the total U.S. population, mean (5th, 50th, 95th 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})
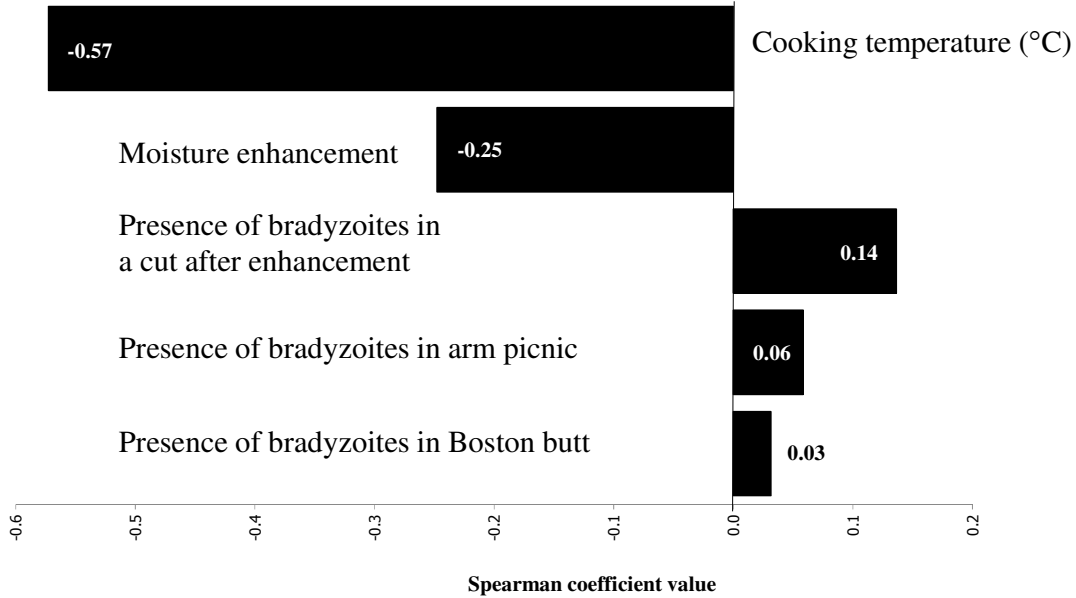
Figure 6.6 Tornado graph of sensitivity analysis for number of new infections in the U.S. total population, carried out via Spearman correlation in @Risk.

For the subpopulation of pregnant women, approximately 10^3 new infections occurred annually, which resulted in 296 congenital toxoplasmosis cases each year in the United States. CDC estimated that 400–4,000 congenital infections occur per year in the United States (69). Based on screening programs in Massachusetts and New Hampshire, Guerina et al. found that congenital toxoplasmosis occurred in 1 out of 10,000 live births (436). 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 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 probability of infection per serving. One possible explanation is *T. gondii* tissue cysts have a high affinity 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 (422). 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 (356).

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% (322), 0.9% (324) and 2.6% (78) in grower/finisher pigs, respectively. In this

QMRA, a meta-analysis approach was used to integrate all available prevalence studies in the United States, which is not only including 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. In the sensitivity analysis, *T. gondii* prevalence was not identified as an important factor affecting model outcomes, likely due to the narrow distribution of this variable. However, by increasing *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 farm level is an important strategy to reduce the human infection burden. An analysis of the risk factors associated with *T. gondii* prevalence at farm level are quite complicated (363) and are beyond the scope of the present study.

Home cooking temperature was the most important parameters affecting the model outcomes, according to a correlative sensitivity analysis. In 500,000 simulated servings, number of *T. gondii*-positive arm picnic servings were 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 the thermal death curve for a 0.01-minute cooking time, developed from a trial with artificially infected mice (344). 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 (344). Experimental settings (2-mm-thick pouches equilibrated to the experimental temperature in a water bath) were 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 (437). *T. gondii* concentration in experimental 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. In the cooking module, it was assumed that temperature throughout the pork is the same as the finished cooking temperature. In reality, a temperature gradient from the meat surface to the coldest spot is created during cooking. Tissue cysts on the meat surface are quickly killed, while the tissue cysts located in interior portions die progressively. By not taking thermal diffusion within the pork into consideration, the present risk model would underestimate the effect of cooking and overestimate the number of new infections per year. The log-linear regression equation reported here was developed based on data at 0.01 minute from this experimental study (344). The 0.01 minute started to count 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 (67). The developed log-linear 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.

156

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 contained 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 get 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 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 (73, 99), but no sufficient data were available to include these factors in the present model. More data are needed in order to include these variables in the logistic model. In addition, the distribution 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 Dutch population (67), based on data from the Netherlands and with dose calculated indirectly. The *T. gondii* QMRA model presented here reflects the best current knowledge on fresh pork production and processing in the United States. Compared with the Netherland QMRA model, *T. gondii* bradyzoite concentration was modeled in pork edible tissues and the dose for the exposure assessment was calculated directly in our model. In addition, a mouse-derived human dose-response model was developed and was used in our QMRA model, rather than applied a mouse dose-response model as a human surrogate. Furthermore, the uneven distribution of *T. gondii* tissue cysts was included in our risk model, which resulted in bradyzoite concentration varied among pork cuts. Finally, a method that divides the temperature into three zones ("complete inactivation temperature zone: > 61°C", "non-inactivation temperature zone: < 49.9°C", and "reduction temperature zone": 49.9 - 61°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 model due to the lack of data. *T. gondii* in pork meat can be inactivated by

freezing either at -10°C for 3 days, or at -20°C for 2 days (132, 137). Fresh pork can be frozen either by meat producers during processing or by consumers at home. A majority of meat manufacturers claimed 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 (381). 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) In the present model, infection risk associated with consumption of ground pork was not calculated individually. Although ground pork is a combination of lean meat and fat from virtually any part of the carcass (438), ground pork is mainly produced from shoulder meat (Boston butt) in the United States. Boston butt could be used as a tentative proxy for ground pork.

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

(4) 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, and bradyzoite concentration was quantified by a linear regression model between the number of bradyzoites and the Cp-values (crossing point-PCR-cycle, is the cycle at which fluorescence achieves a defined threshold) (55). Bradyzoite concentration in pork might be different than in goats. In addition, goats were experimentally infected with high dose of oocysts, which is likely different from doses encountered in farm settings.

(5) For both populations (total U.S. population and pregnant women population), consumption data of spare rib, back rib, tenderloin, arm picnic and blade roast are based on the assumption that 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 are 4.5% and 29%, respectively (439). However, these parameters are mainly used to estimate caloric intake, food patterns, and the value of food loss, rather than to predict the actual consumption. Fresh pork consumption data in pregnant women was estimated based on a food intake survey conducted between 1994 and 1996. Consumption data of women in 20-39 years groups were extracted from this survey, to be the proxy for pregnant women.

(6) 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 stains with higher virulence. Further, risk of infection was used as the endpoint for the total U.S. population in the present QMRA. Except congenital toxoplasmosis in fetuses, other severe health outcomes (e.g., ocular disease, encephalitis, and death) were not estimated due to the absence of the 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 (415). 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 (440, 441). Therefore, the use of a specific scaling factor would give a more accurate estimate of annual new infection cases in pregnant women.

6.6 Conclusions

Overall, the present QMRA model quantified the risk of *T. gondii* infection posed by consumption of fresh pork in the United States. Our model estimated that a considerable number of new infection cases is associated with fresh pork consumption each year, providing evidence for risk managers that more attentions is warranted to this route of exposure to *T. gondii*. Our study also demonstrated that thorough cooking is the most effective 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, the mathematical tool employed in this QMRA model differ 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.

Chapter 7: Quantifying the risk of foodborne human *Toxoplasma gondii* infection due to consumption of domestically-produced lamb in the United States

This work has been prepared to be submitted to *Journal of Food Protection*.

7.1 Abstract

The aim of this study was to develop a farm-to-table quantitative microbial risk assessment (QMRA) model to predict the public health burden in the United States associated with consumption of U.S. domestically-produced lamb. Following the baseline model for fresh pork that we have developed in Chapter 6, the present risk model was divided into five modules. *T. gondii* prevalence in market lambs was pooled from the 2011 NAHMS survey, and the concentration of the infectious life stage (bradyzoites) was calculated in the current model. Same as the baseline model for fresh pork, a log-linear regression and an exponential dose-response models were used to model the reduction of *T. gondii* during home cooking and to predict the probability of infection, respectively. The mean probability of infection per serving of lamb was estimated to be 1.5 cases per 100,000 servings, corresponding to approximately 6,300 new infections per year in the U.S. population. Based on a sensitivity analysis, cooking was identified as the most effective method to influence human health risk. This study provides a QMRA framework for *T. gondii* infection through consumption of lamb, and quantified the infection risk and public health burden associated with lamb consumption.

7.2 Introduction

Lamb, defined as a sheep less than 1 year old and without permanent teeth, is one of the meat animals that is frequently infected with *T. gondii*. Infected lambs harbor *T. gondii* tissue cysts in edible tissues *(75)*, and humans become infected by ingestion of tissue cysts containing bradyzoites in raw or undercooked lamb. A case-control study of 148 infected adults and 413 non-infected controls in the United States found that consumption of rare lamb is one of the risk factors associated with *T. gondii* infection (23). In a European multicenter case-control study, eating uncooked lamb was also identified as a risk factor for *T. gondii* infection in pregnant women (20).

Lamb is a less frequently consumed meat in the United States, compared to pork, beef and chicken. According to the USDA food availability data, lamb consumption has remained fairly stable over the recent years, at approximately 0.5 kg per capita per year (68). Approximately half of lamb consumed in the United States is imported, mainly from Australia and New Zealand. U.S. domestically-produced lamb accounts for the other half of lamb consumption (68), and 60% of these lamb is sold at retail outlets (442). Lamb is typically sold as fresh cuts (muscle cuts that undergo minimal processing and is cooked before consumption).

Sheep are important hosts of *T. gondii* and seroprevalence of *T. gondii* is high in lamb raised in the United States (110, 417). According to the 2011 NAHMS survey that was conducted in 22 major sheep producing states, nearly half of the U.S. sheep operations had animals which were seropositive for *T. gondii* and overall 9.4% of lambs tested positive for *T. gondii* antibodies (443). Approximately 62 % of these operations raise sheep on pasture (443); outdoor rearing results in sheep exposed to oocyst-

contaminated environment. Domestic lamb is often sold fresh or chilled at retail stores, whereas imported meat is typically sold frozen (444). Freezing can inactivate *T. gondii* tissue cysts and reduce the risk of *T. gondii* infection to a minimal level (137). However, risk associated with fresh lamb is not reduced during processing since the conditions do not inactivate *T. gondii* tissue cysts (417). Likewise, consumer preparation which may include rare or medium rare cooking methods for lamb may not inactivate *T. gondii* and therefore not affect the risk for transmission at the time of consumption. Considering the relative high prevalence of *T. gondii* in domestically produced lamb, it is important that scientific information related to the potential for lamb consumption to be a vector for human *T. gondii* infections be integrated to assess the magnitude of the risk and evaluate potential for risk mitigation strategies.

To our knowledge there have been no risk assessment studies for *T. gondii* in lamb destined for human consumption available. The QMRA model for fresh pork that developed in Chapter 6 was served as the baseline model for the current risk model for lamb. The objectives of the current study were to (i) collect available science-based information, and (ii) develop a farm-to-table risk model for quantitatively estimating lamb-consuming consumer exposure to *T. gondii,* and predicting the number of infection cases per year in the U.S. population associated with eating domestically-produced lamb.

7.3 Materials and Methods

7.3.1 Model overview

Following the baseline model for fresh pork, a farm-to-table QMRA was developed in the Microsoft Excel add-in software @Risk. To estimate the risk of human *T. gondii* infection through consumption of lamb in the United States, 5×10^5 iterations were conducted using Latin Hypercube sampling to obtain a stable result. Across five independent simulations (each simulation with 5×10^5 iterations), the arithmetic mean of new infection cases per year varied up to approximately 1.0%, and the probability of infection per serving varied up to approximately 1.3%. Similar to the baseline model, the present model also modeled bradyzoite concentration in infected lamb, and was divided into five modules: (1) farm, (2) abattoir, (3) home cooking, (4) human consumption, and (5) dose-response and risk characterization. The main assumptions of this model were: (i) all domestically-produced lamb meats are sold as fresh or chilled and have never been frozen, (ii) all lamb is sold at retail stores and cooked at home by consumers, and (iii) meat products produced from a live lamb are all in fresh form (sold as fresh cuts), and only undergo a minimal processing.

7.3.2 Model 1: farm

T. gondii prevalence data were pooled from the 2011 NAHMS survey (443) and fitted with a Beta distribution (**Table 7.1**). No data on bradyzoite concentration in lamb were available. As the baseline model (chapter 6), bradyzoite concentration data were pooled from a real-time PCR study (382) and fitted using Distribution Fitting option in @Risk software. Lognormal distribution (**Table 7.1**) with the best fit (χ^2 test) was used to define the bradyzoite concentration.

Table 7.1 Description and statistical distribution of the QMRA model

Variable	Description	Distribution	Source
Farm			
Prevalence	Number of positive market lambs.	Beta distribution (s+1, n−s+1), where s=373: number of positive lambs observed in the study, and n=3,967: total number of lambs sampled.	USDA APHIS, 2014
Concentration	Bradyzoite concentration in lamb muscle tissues, data pooled from a real-time PCR study.	Lognormal distribution (μ, σ), μ=11.67, σ=97.31, truncated to (0.04, 41.3) bradyzoites/g.	Jurankova et al., 2013;
Abattoir			
Weight of a market lamb	Weight of a lamb at time of slaughter.	PERT distribution (29.0, 63.5,70.3) kg	USDA NASS, 2015
Dressing percentage	Yield of dressed carcass from a live lamb.	PERT distribution (0.44, 0.51, 0.56)	USDA NASS, 2015; South Dakota State University, 2015
Boneless retail-cut yield rate	Yield rate from carcass to boneless retail cut.	Constant, 0.658.	USDA per capita data system, 2015
Yielded retail weight (boneless)	Weight of boneless retail cuts obtained from a lamb.	Weight of a market lamb × Dressing percentage × Boneless retail-cut yield rate	Calculation
Probability of *T. gondii* presence	Probability of presence of *T. gondii* in a randomly selected lamb cut. Yes=1 indicates presence of *T. gondii* in a lamb cut.	Binomial distribution (n, p), where n=1, p = 38/64.	Dubey et al., 1989
Number of bradyzoites in lamb cut	Bradyzoite load in total lamb cuts.	Bradyzoite concentration × Boneless weight of total lamb cuts × Probability of *T. gondii* presence	Calculation
Cooking			
Finished cooking temperature	Finished internal cooking temperature recorded by consumers upon completion of cooking meat and removal from heat.	Normal distribution (μ, σ), μ=68.2, σ=11.6, truncated to (37. 8, 104.4) °C.	Audits International and FDA, 2000
Reduction factor (>61°C)	When finished cooking temperature > 61°C, lamb is assumed to be free from *T. gondii*.	Reduction factor=0	Dubey et al., 1990
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
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 (reduction factor) = 8.58 - 0.17 × final cooking temperature (°C)	Dubey et al., 1990
Number of bradyzoites in lamb	Number of bradyzoites in lamb at the end of cooking.	Number of bradyzoites in lamb cut before cooking (abattoir) × Reduction factor	Calculation
Consumption			
Consumption for the U.S. population	Domestically-produced lamb consumption for the total U.S. population, estimated based on a	Per capita × U.S. total population × Proportion of domestically-produced lamb (45%)	U.S. Census Bureau; USDA per capita data system, 2015

	"disappearance" model in 2011.		
Serving size	Amount of boneless lamb ingested in one eating session.	Normal distribution (μ, σ), μ=85, σ=25.5, truncated to (2.8, 170.1) g.	Miller et al., 2005
Dose-response and risk characterization			
Probability of human infection per serving	The probability of human infection per serving.	Exponential model: P = (1- exp (-r × 0.005 × d)) × Prev, where P is probability of infection per serving, d is the dose (bradyzoites/serving), r is the model parameter, 0.005 is the scaling factor, Prev is *T. gondii* prevalence in market lamb.	Guo et al., 2015
Parameter r	The probability of one bradyzoite to initiate an infection. Data of 100,000 bootstrap r were fitted to a lognormal distribution.	Lognormal distribution (μ, σ), μ = 0.00169, σ = 0.00067, truncated to (0.00004, 0.01276).	Guo et al., 2015; Haas et al., 2014.
Number of servings per year	Number of individual lamb servings consumed in a year.	Total lamb consumption divided by serving size.	Calculation
Number of new infection cases per year	Number of new infections/year due to consumption of infected lamb.	The probability of infection per serving × The number of lamb servings per year × Adjustment factor (0.892). 0.892 is an adjustment factor (10.8% of the U.S. population have been infected with *T. gondii*).	Calculation

APHIS: Animal and Plant Health Inspection Service; NASS: National Agricultural Statistics Service.

7.3.3 Model 2: abattoir

Market weight lambs (generally 6-8 months old) are slaughtered and processed to meat products at the abattoir. In chapter 4, it has been concluded that none of the abattoir processing steps for small ruminants would be expected to inactivate *T. gondii* tissue cysts in the muscle tissue (417). The weight of a live market weight lamb was described with a PERT distribution, with the minimum, most likely and maximum values of the distribution were set to 29.0, 63.5, and 70.3 kg, respectively (**Table 7.1**). The minimum and maximum values were obtained from the 2014 lamb slaughter data from 35 states, and the most likely value is the average live weight of federally-inspected lambs in 2014 (445). The weight of boneless retail meat obtained from a lamb was calculated by multiplying the weight of a market lamb by dressing percentage (ratio of carcass weight to live weight of a lamb) and boneless retail-cut yield rate

(ration of boneless retail weight to carcass weight). Dressing percentage is normally between 44-56% (446), with an average value of 51% (445). These values were used as parameters of a PERT distribution for modeling dressing percentage (**Table 7.1**). Based on the USDA data (68), a fixed value (0.658) was used to convert carcass weight to boneless retail weight.

T. gondii tissue cysts have a higher affinity for lamb muscle tissues (i.e., the meat products for human consumption) as compared with heart and tongue tissue (112). In a mice bioassay experiment, samples of limb muscle and intercostal muscle from infected lambs (total 8 lambs) were digested and inoculated subcutaneously in mice. It was observed that 18 of 32 mice and 20 of 32 mice tested positive for *T. gondii* when inoculated with limb muscle and intercostal muscle samples, respectively (112). No significant differences ($p > 0.05$) were found between the numbers of positive mice inoculated with limb muscle samples versus inoculating intercostal muscle samples by Fisher's Exact Test. Thus, data were combined to estimate the probability of *T. gondii* presence in lamb cuts, which is the ratio (38/64) of the number of positive mice (mice that became infected) to total mice tested. A binomial distribution was used to model the probability that a randomly selected infected lamb commercial cut would contain any *T. gondii* from and infected lamb (**Table 7.1**). Since no distribution differences of tissue cysts were observed in muscle tissues, it was assumed that *T. gondii* tissue cysts are randomly distributed in fresh cuts (e.g., shoulder, rack, shank/breast, loin, and leg) of an infected lamb, and the bradyzoite concentration is the same in all fresh lamb cuts. Unlike the baseline model for fresh pork, the number of bradyzoites was calculated for all lamb cuts together, rather than individual calculations for each cut. The number of

bradyzoites in all lamb cuts was calculated as: bradyzoite concentration in muscle tissue (bradyzoites/g) × boneless weight of a lamb (g) × presence of *T. gondii* in one randomly selected lamb.

7.3.4 Model 3: cooking

Cooking was assumed to be the only factor related to the viability of *T. gondii* during consumer preparation, and other factors that could reduce or redistribute *T. gondii* in lamb were not included in this model. For cooking lamb, data on home cooking times, or time-temperature profiles are not available. However, the 1999 U.S. food temperature evaluation contains 584 finished cooking temperatures (i.e., internal temperatures) measured for meat (pork/beef /lamb) by consumers upon completion of cooking and removal from heat (447). Finished cooking temperature data were fitted with a normal distribution (mean: 68.2°C, standard deviation: 11.6°C), truncated between 37.8°C and 104.4°C (based on the range of the 1999 U.S. food temperature dataset for cooking meat). As with the baseline model, a thermal effect on the viability of *T. gondii* was modeled by using a reduction factor (**Table 7.1**), and the details of the calculation and assumptions were described in Chapter 6. In brief here, the calculation of the reduction factor was based on the finished cooking temperature. When the finished cooking temperature was lower than 49.9°C, a value of 1 was assigned to the reduction factor, indicating no inactivation occurs. When the finished cooking temperature was higher than 61°C, a value of 0 was assigned to the reduction factor, indicating the completed inactivation of *T. gondii* in meat. For finished temperatures between 49.9-61°C, a log-linear regression model was used to calculate the reduction factor (value is between 0-1). It was assumed that the temperature throughout the entire

piece of lamb is the same as the temperature at the coldest spot (i.e., the finished cooking temperature).

7.3.5 Model 4: consumption

According to the USDA Food Availability Per Capita Data System, approximately 280 g of boneless retail lamb was consumed per person in 2011 (68). The consumption of domestically-produced lamb in 2011 was 130 g per capita, which accounts for approximately 45% of total consumption (68). It was assumed that consumers eat at least one serving of lamb each year. The amount of lamb consumed per serving was assumed with a normal distribution, with a mean value of 85.0 g and a standard deviation of 25.5 g, and truncated to a minimal of 2.8 g and to a maximum of 170.1 g (432).

7.3.6 Model 5: dose-response and risk characterization

The exponential dose-response model developed in Chapter 5, was used to link the dose of bradyzoites ingested to the probability of infection per serving (433). A scaling factor of 0.005 was used, and the uncertainty of parameter r was described by a lognormal distribution (**Table 7.1**).

The primary outcome of the risk assessment was the probability of infection per serving, calculated by multiplying the probability of infection obtained from the dose-response model by the prevalence of *T. gondii* in market lambs (**Table 7.1**). The mean number of infections per year associated with lamb consumption in the United States was computed by multiplying the probability of infection per serving by the number of lamb servings per year. It was assumed that for each cut, all the servings consumed in one year were identical (contain the same number of bradyzoites), and the risk from

171

exposure of each serving of lamb was independent from other exposures. The number of new infections per year was further calculated by multiplying an adjustment factor of 0.892 by the number of infections per year, since approximately 10.8% of the U.S. population of age 6-49 years old has been infected with *T. gondii* (29).

7.4 Results and Discussion

The mean number of U.S. reared lamb servings consumed per year in the United States was estimated as 4.6×10^8. The mean probability of infection was estimated to be 1.5 cases per 100,000 servings, corresponding to approximately 6,300 new infections per year (**Table 7.2**). Based on the epidemiological estimation, approximately 10^6 persons were estimated to be infected with *T. gondii* each year in the United States (409). Our estimation of new infections per year through ingestion of domestically-produced lamb accounts for less than 1% of the total number of new infections each year. However, compared with the baseline model, the number of new infections associated with consumption of fresh pork is only 16-fold more than the number of infections due to consumption of lamb, given that the number of fresh pork servings (24.8 billion servings per year) is approximately 54 times more than the number of lamb servings per year. The probability of infection per serving of lamb is higher than the pork arm picnic (highest risk pork cut, 0.95 case per 100,000 servings), indicating that lamb poses a higher per serving risk to human consumers than fresh pork. The high probability of infection through ingesting infected lamb is associated with a high prevalence of *T. gondii* in live lambs, a high affinity of *T. gondii* for lamb

172

muscle tissues, and no critical steps that inactivate *T. gondii* in lamb during slaughter or processing.

Table 7.2 Probability of infection and number of new infections per year in the United States associated with consumption of U.S. reared lamb

|	**Mean**	**5th percentile**	**Median**	**95th percentile**
Probability of infection per serving	1.5×10^{-5}	0	0	3.2×10^{-5}
Number of new infections per year	6,300	0	0	13,726
Probability of infection per positive serving [a]	9.5×10^{-5}	2.35×10^{-7}	1.02×10^{-5}	4.63×10^{-4}

[a]Probability was calculated based on 78,396 positive servings of 500,000 simulated servings.

Sensitivity analysis indicated that cooking temperature is the most important factor affecting the probability of infection and number of new infection cases resulting from lamb (**Figure 7.1**). In 500,000 iterations of the current model, for infected lamb, cooking reduced the number of *T. gondii* positive servings from 296,875 (59.4%) to 78,396 (15.7%). Based on data from these 78,396 positive servings, the mean probability of infection per serving of lamb was 9.5×10^{-5} (**Table 7.2**).

T. gondii prevalence in the current QMRA was described with a Beta distribution, and the data were extracted from the 2011 NAHMS survey (443). This national lamb survey represents 87.4% of the U.S. sheep inventory and 70.1% of U.S. farms with ewes; blood samples of 3,967 lambs from 353 operations that were tested for *T. gondii* (443). However, as described in Chapter 3, *T. gondii* prevalence in domestically-grown lambs tested in regional studies was higher than the prevalence observed in the national survey (417). For example, in an observational study conducted in Maryland, Virginia and West Virginia, 104 (27.1%) of 383 market lambs

destined for human consumption tested positive for *T. gondii* antibodies (253). In another study, of 345 lamb blood samples collected from slaughterhouses in northeastern United States, prevalence of *T. gondii* was 55.1% (318). In Chapter 3, *T. gondii* prevalence in market lambs raised in the United States was estimated with a mean value of 22.0%, based on a meta-analysis approach. Therefore, the present model could underestimate the number of new infections per year by using the data from the national survey. In a sensitivity analysis, *T. gondii* prevalence was not identified as an important factor affecting model outcomes, likely due to the narrow distribution of this variable. However, model outcomes are heavily influenced by *T. gondii* prevalence. By setting prevalence to 22.0% (the result obtained from the meta-analysis), the predicted number of new infections of this QMRA was 14,571 cases per year, which is 2.3-fold more than the prediction with prevalence set to 9.4% (mean prevalence of the 2011 NAHMS survey). This result suggests that reducing *T. gondii* prevalence in lambs at the farm level is an important strategy for reducing human infection burden.

In addition to limitations of the current cooking module that have been described in Chapter 6, another limitation is that the finished cooking temperatures used in this model are not specific for lamb, but rather a combination of data for beef, pork and lamb. Anecdotally, lamb is often cooked rare to medium rare, which could result in the finished cooking temperature for lamb being lower than the temperature used in this model. In this case, this model would underestimate the probability of infection and number of new infections attributed to ingestion of lamb per year.
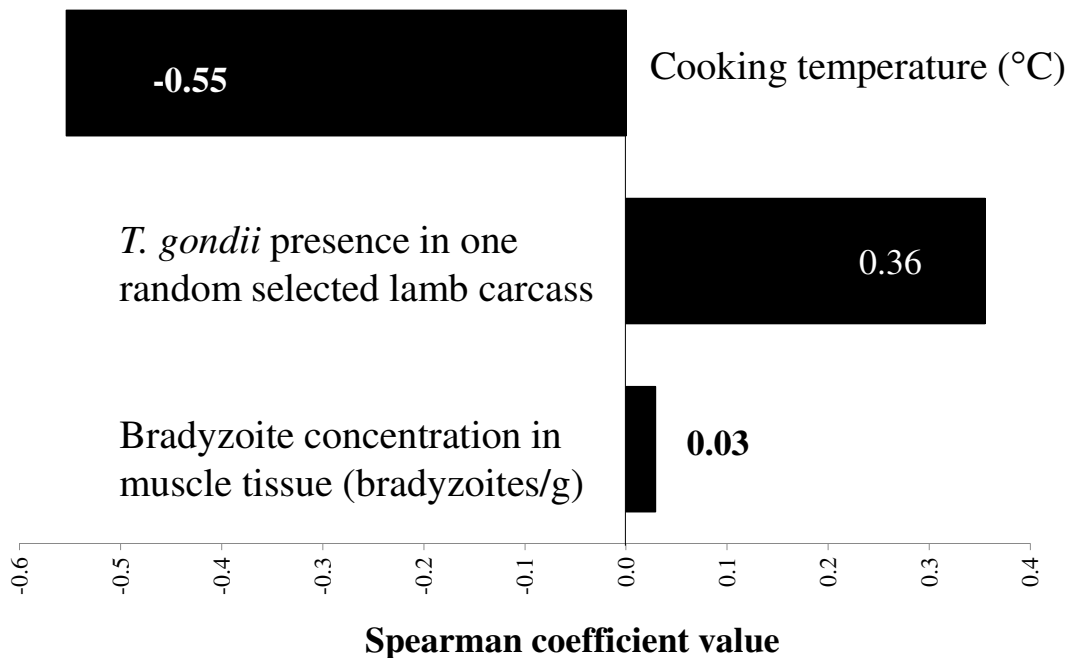
Figure 7.1 Tornado graph of sensitivity analysis for number of new infections in the U.S. total population, carried out via Spearman correlation in @Risk.

The current QMRA only included domestically-produced lambs, and did not consider imported lamb due to the absence of data. As discussed in the introduction, imported lamb meat, mainly from Australia (60-70%) and New Zealand (30-32%), accounts for approximately 55% of the total lamb consumption in the United States (68). The trend in chilled rather than frozen lamb imports has been increasing in recent years due to improvements in distribution systems and consumers demand for fresh lamb (448). Percentage of fresh or chilled lamb imports increased from 20% twenty years ago (444) to approximately 45% in recent years (448). *T. gondii* tissue cysts are inactivated during commercial freezing, and therefore do not pose any risk to consumers. Limited information constrains the assessment of infection risk associated

175

with imported lamb in the United States. No studies were found to report *T. gondii* prevalence in lamb raised in Australia and New Zealand over the past 30 years. However, a toxoplasmosis outbreak associated with eating raw lamb was reported in Australia in 1984, involving 5 family members (449). In another study, 1,917 of 2,254 (85%) blood samples from ewes (sheep older than 1 year) raised in New Zealand were seropositive for *T. gondii (234)*. Given lambs in Australia and New Zealand are mostly raised on open pasture (444) and exposure to potentially oocyst-contaminated environment is continuous, *T. gondii* prevalence in sheep/lambs raised in these two countries could be higher than in U.S. domestically-raised lambs. Thus, the human infection risk associated with consumption of imported chilled lamb needs to be assessed and included in this model when data are available.

The current risk model was developed based on the framework of the baseline model for fresh pork, and reflected the best current knowledge on lamb production and processing in the United States. The modules and inputs simplified for the process steps from lamb production to consumption, due to the fact that only limited information is available. Except the limitations have been discussed for the baseline model in Chapter 6, several limitations and knowledge gaps were identified as follows.

(1) All Meat from a live lamb was assumed to be converted to fresh meats in this QMRA. In reality, a small proportion of lamb meat was manufactured as processed products, such as smoked lamb leg and summer sausage. Processed lamb meats were not included in the model because information for human consumption is limited.

(2) Lamb was assumed to be kept at refrigerated temperatures from farm to table. Freezing, which could inactivate *T. gondii* in meat, was not included in this QMRA because no data were found related to the proportion of U.S. lamb that had been frozen either by manufacturers or consumers.

(3) Lamb was assumed to be all cooked at home by consumers. In reality, lamb is also eaten in commercial food service establishments, or obtained from places such as school cafeterias and community feeding programs, and medium-rare or rare lamb is sometimes served. Consumption of lamb at these locations were not included in this model due to the limited data availability.

(4) *T. gondii* bradyzoite concentration in lamb muscle tissue was described based on a real-time PCR study of limb muscles from experimentally-infected goats. Bradyzoite concentration in muscle tissue from a naturally infected lamb might not be the same as experimentally-infected goats. In addition, PCR-based detection methods are not able to distinguish the viable and dead bradyzoites, resulting in an overestimation of actual bradyzoite concentration in muscle tissue.

(5) The probability of *T. gondii* presence in lamb muscle tissue was modeled based on a study of congenitally-infected lambs, rather than naturally-infected lambs. Congenitally infected lambs acquire infection from ewes through the placenta, not through exposure to an oocyst-contaminated environments.

(6) Differing from the baseline model, a moisture enhancement module was not included, since no data were found regarding moisture enhancement for lamb, the proportion of lamb that are enhanced, or the conditions of enhancement.

(7) Risk outcomes were only assessed for the U.S. population, but not for subpopulations such as pregnant women, due to the fact that lamb consumption data were not available for women of childbearing age. Likewise, consideration of increased lamb consumption by various ethnic groups in the United States was not considered.

7.5 Conclusions

In summary, the current QMRA model quantified *T. gondii* infection risk associated with consumption of domestically-produced lamb in the United States. Our model estimated that less than 1% of new infections each year are attributable to ingestion of domestically-produced lamb. However, the probability of infection per serving of lamb is higher than the highest risk pork cut. This model identified that thorough cooking is the most effective method to prevent exposure risk in *T. gondii*-infected lamb, but may not be the optimal risk management approach due to the propensity for lamb to be eaten by a significant portion of consumers after rare or medium-rare cooking temperatures. The current risk model for lamb not only provides scientific evidence for risk management, but also identifies several limitations and data gaps associated with *T. gondii* infection through ingestion of lamb that could be addressed by future research.

Chapter 8: Summary and future studies

8.1 Summary

Toxoplasma gondii has been long recognized as a significant foodborne pathogen in terms of public health impact, however, knowledge of risk posed by this pathogen is typically limited in the United States. This project systematically evaluated food safety risk posed by *T. gondii* through meat consumption, since meat has been identified as an importance source of *T. gondii* infection in humans.

Chapter 2 focused on evaluating *T. gondii* prevalence in different meat animals, and on identifying risk factors and available risk studies, to serve as an information repository for informing risk assessment studies for *T. gondii* infection in humans. Based on observational studies of prevalence in meat animals worldwide, it is clear that prevalence was higher in sheep, goat, and pigs compared with cattle and poultry. An increased prevalence was observed in organically-raised meat animals compared with conventionally-raised counterparts, mainly due to outdoor access. Risk studies were limited to identify risk factors at the farm level, which based on interviews and questionnaires to owners or workers. No systematic risk assessment studies were found in the United States, which hampered the development of effective risk management programs for *T. gondii*. It has been concluded that it is necessary to conduct risk assessment studies of *T. gondii* infection associated with meat consumption in the United States.

Furthermore, in chapter 3, a meta-analysis approach was used to collect available studies, and pool data to increase precision of estimates for *T. gondii*

prevalence in meat animals raised in the United States. The trend of *T. gondii* prevalence in meat animals raised in the United States is consistent with prevalence in animals raised from other countries. The quantitative data of prevalence obtained from the meta-analysis could be used in risk assessment studies to model *T. gondii* prevalence in meat animals. Specifically, these data were used to define risk level of meat category in a qualitative assessment for analyzing *T. gondii* exposure risk (Chapter 4), and to model prevalence in market pigs in a quantitative risk assessment for fresh pork (Chapter 5).

Chapter 4 focused on developing a qualitative assessment tool to estimate risk level of various meat products at the retail level, which served as the first round evaluation of infection risk associated with meat consumption. Changes of risk level of meat products can be assessed from farm production, through abattoir processing, storage and transportation, meat processing, packaging, until arrival at retail. It was found that salting, freezing, commercial hot air drying, lengthy fermentation, hot smoking, and cooking are able to reduce/inactivate *T. gondii* levels in meat products, whereas abattoir processing, cold storage, nitrite/nitrate, spice, low pH, and packaging have no effect on the viability of *T. gondii* tissue cysts. Therefore, among various meat products consumed in the United States, fresh meat posed a higher risk compared with processed meat, since it only undergoes a minimal processing that is not sufficient to inactivate *T. gondii*. Critical parameters were summarized in chapter 4, which provide references for meat management control programs to determine critical control points. Data gaps associated with risk assessment were also identified. Based on this qualitative assessment, it was concluded that it is necessary to develop a quantitative

risk assessment of *T. gondii* infection associated with meat products, to further quantify the public health burden, especially for fresh pork and lamb.

A suitable dose-response model is an important component required to construct a quantitative risk assessment. Currently, the dose-response relationship for human exposures to *T. gondii*-infected meat is unknown because no human data are available. Thus, chapter 5 focused on collecting relevant data for dose-response in animals, developing dose-response models to predict human infection, and validating these models. Among eligible studies, a mouse study was selected to develop the dose-response models, while a rat study was used to validate the mouse-derived models. Following the guidelines of hazard characterization for pathogens in food and water from FAO/WHO, exponential and beta-Poisson models, were selected as reasonable dose-response models based on their simplicity, biological plausibility and goodness-of-fit. These two animal-derived models were derived to predict human infection by using two scaling factors. Scaling factors were computed by matching the predicted infection cases in the QMRA for fresh pork with the epidemiological data. Mouse-derived models were further validated against data for the dose-infection relationship in rats. These two dose-response models provide an enhanced risk characterization in a QMRA model for *T. gondii*.

Information and results from the previous chapters established the foundation for conducting QMRA studies. Based on data availability and risk estimation of meat animals, two QMRA models were developed to predict public health burden through consumption of fresh pork and fresh lamb, respectively. In Chapter 6, a farm-to-table QMRA model was developed based on the best of our knowledge. This model

estimated that approximately 10% of human *T. gondii* infections are associated with consumption of fresh pork in the United States. Among all fresh pork cuts, arm picnic cut poses the highest risk, and accounts for 44.9% of infection cases attributed to fresh pork. Cooking was identified as the most effective way to reduce infection risk. The mathematical tool employed in this model differ significantly from tools used to model bacterial or viral pathogens, given the complex life cycle of *T. gondii* and the detection methods used to detect this pathogen.

In Chapter 7, by using the QMRA for fresh pork as the baseline model, another QMRA model was developed to predict public health burden by ingestion of domestically-produced lamb in the United States. The mean probability of infection per serving of lamb was estimated to be 1.5 cases per 100,000 servings, which is higher than arm picnic (0.95 case per 100,000 servings), indicating that fresh lamb poses a higher risk to human consumers than fresh pork. Approximately 6,300 new infections occurred each year in the U.S. population. Cooking was also identified as the most effective method to influence model outcomes.

To our knowledge, this is the first systematic study to assess risk for *T. gondii* in meat products in the United States. The current project collected all relevant data on *T. gondii* in meat animals and meat products. For a specific meat product, risk can be assessed by the qualitative assessment tool, which is an easy and straightforward method to predict risk of meat products purchased from the retail stores. When data of a specific meat product are sufficient, following the framework of the baseline model, a QMRA model for this meat could be developed to further predict the public health burden, if necessary.

The outcomes of the current project provide scientific evidences for risk managers, policy makers, and researchers as well as direct future research efforts. More attention is warranted to better understand *T. gondii* infection through meatborne route so that risk mitigation strategies could be developed and implemented. Potential intervention strategies to reduce meatborne transmission are discussed as follows.

(1) Based on the results of risk models, control of *T. gondii* prevalence in food animals could reduce the human infection risk considerably. Measures to prevent infection of food animals include restricting cats' access to feed, water, animal housing areas, and storage; implementing strict rodent control and bird control programs; prohibiting feeding food animals with non-pasteurized goat whey, offal and garbage; and establishing proper carcass handling procedure. These preventive strategies for animals raised indoor (confinement) are unlikely to reduce *T. gondii* infection in outdoor reared food animals.

(2) Reduction of environmental contamination with oocysts is beneficial to outdoor-reared food animals, given that a vaccine targeted to prevent the formation of tissue cysts in food animals is currently not available. Methods to reduce the oocyst contamination in pasture or farm land include preventing feral cats' entrance and using the litter box for farm cats.

(3) Decontamination of meat by manufacturers is another effective strategy to reduce the infection risk. The effects of processing on the viability of *T. gondii* is listed in Chapter 4. High risk meats, which included raw-fermented sausage, cured raw meat, non-hot air dried meat, fresh meat, and fresh processed meat, have been identified. For high-risk meats, infection risk can be reduced by

freezing the meat, injecting enhancing solutions to the meats, irradiating the meat, and implementing high hydrostatic pressure treatment to the meat. However, consumers' acceptance and cost of the treatment could be potential problems for implementing these methods.

(4) Strengthen the role of education to consumers, physicians, and pregnant women. Given that a proportion of consumers may not be aware of the potential risk of infection through meat consumption, the knowledge of preventing *T. gondii* transmission to humans should disseminated through possible risk communication channels such as media, websites and physicians' offices.

8.2 Future studies

The current study represents our current best knowledge of *T. gondii* infection through ingestion of meat products. Several data gaps were identified and were elaborated in each chapter. Some possible areas of research pertaining to meatborne *T. gondii* infection are proposed as follows.

(1) Studies of *T. gondii* infection prevalence in certain meat animals raised in the United States is needed. These meat animals include indoor-raised chicken, goat, non-confinement raised pig, and non-confinement raised chicken.

(2) Besides the risk factors identified at the farm level, a risk model describing transmission dynamics of *T. gondii* among intermediate hosts, definitive hosts, and the environment would provide science-based information to prevent infection in meat animals at the farm level. A few mathematical models have been developed to mimic the transmission of *T. gondii* in a farm system, and to

184

identify the important risk factors (450, 451, 452). However, these models were not integrated to a QMRA model to further assess human infection risk through consumption of meat. Including the transmission dynamics in the farm module of the current QMRA model would allow risk assessors to investigate the influence of farm management, and to determine the preventive strategies to mitigate the risk during production.

(3) A complete understanding of *T. gondii* bradyzoite concentration in muscle tissue of a naturally-infected meat animal, and concentration changes along with post infection time is needed. The number of *T. gondii* tissue cysts in a naturally-infected animal is quite low, approximately 1 tissue cyst in every 50 g or 100 g of tissues. Thus, an effective detection method is needed to quantify the low number of *T. gondii* in a meat cut. In addition, the relationship of the number of bradyzoites and the age of a tissue cyst should be investigated, since one tissue cyst contains a few to several hundred bradyzoites depend upon the age of the tissue cyst.

(4) The effects of low water activity environment, sugar, spices, sanitizers, and any new meat processing technology on the viability of *T. gondii* tissue cysts need to be investigated.

(5) Development of the dose-infection relationship in the high-risk population. Two possible approaches are suggested. One is to compute alternative scaling factors by matching number of new infections in the high-risk population predicted by a QMRA model with the epidemiological data accordingly. The other method is to conduct dose-response experiments in high-risk

experimental animals (e.g., pregnant mice and gene knockout mice), and further extrapolate these findings to predict human infection.

(6) Freezing module should be included in the current QMRA models when data are available. To add a freezing module, following data are needed: the percentage of fresh meat that has been frozen, the freezing temperature, and storage time during freezing.

(7) Several surveys could fill some of the data gaps in the current project, which include: comprehensive consumption surveys for pork and lamb, and a survey to evaluate consumers' cooking temperatures for lamb.

(8) More efforts should be devoted to experimental studies that evaluate the effect of cooking temperature on the viability of *T. gondii* tissue cysts. Current available data are not sufficient to suggest a safe cooking temperature for meat products. The USDA minimal internal cooking temperature for fresh pork and lamb has been reduced from 160°F (71.1°C) to 145°F (62.8°C) with a minimal three-minute rest time since 2011, due to elimination of the risk of infection with *Trichinella* in commercial pork (377). The current recommended minimal internal cooking temperature is sufficient to inactivate *Salmonella* spp., another concerned pathogenic microgram in red meat. Based on the USDA guidelines for *Salmonella* lethality, a 6.5 Log CFU/g inactivation can be achieved when cook red meat at an internal temperature of 145°F for 4 minutes (453). In a risk assessment study for thermal inactivation of *Salmonella* in fresh pork, it was showed that for all fresh pork cuts (enhanced and unenhanced pork chops and roasts), surviving CFU per serving and associated risk per serving were only

predicted at the 99th percentiles and higher, when a cooking end-point temperature was set to 145°F for 1 minute and 15 seconds (454). However, more research efforts should be allocated to investigate the effect of the reduced minimal cooking temperature to the survival of *T. gondii* in pork and lamb. The experimental data have demonstrated that *T. gondii* tissue cysts can be inactivated immediately at internal temperature at 61°C and 64°C, respectively, with an exception of a sample treated at 64°C for 3 minutes (18). More experiments should be undertaken in order to verify this result, since it is critical in terms of determining whether the current recommended cooking temperature would be sufficient to inactivate *T. gondii* tissue cysts in pork and lamb.

(9) Imported lamb should be included in the QMRA for lamb when data are available. The combination of an increased amount of chilled lamb imports and a high prevalence of *T. gondii* in imported lamb may result in an increased number of new infections associated with ingestion of imported lamb.

(10) Model functional validation is needed to demonstrate the accuracy of the model. The ideal validation is to evaluate the model by data from real world and to perform a statistical comparison of simulated outcomes and observations (455). However, it is very difficult to obtain such data. For the present risk models, a possible way is to collect data (e.g., consumption data, cooking temperatures and *T. gondii* infection prevalence in this group) from a specific group. Predicted outcomes can be obtained by simulating the risk models with model inputs that replaced with the observed data. If the predicated outcome (number of new

infections per year) match the annual number of new infections in this group, the

present risk models can be validated.

Appendices

Appendix A

MATLAB code for model fitting in Chapter 5

------ Four models comparison ---------

```matlab
clear all
clc
close all

display('Input a valid method: expo_mle, bp_mle, weibull_mle, log_logistic_mle');


global n t x y;
n=5;
%n=[7 7 7 11 9 8 7 3];
t=10.^[6 5 4 3 2 1 0];
%t=9.*[0.001 0.01 0.1 1 10 100 1000 10000];
%x=[0 0 1 8 8 7 5 3];
%x=[10 10 10 7 3 0 0];
%x = [5 5 4 2 1 0 0];
y=[0.99999999999 0.99999999999 0.8 0.4 0.2 0.0000000001 0.0000000001];
%y=[0.00000000000001 0.00000000000001 0.14 0.73 0.89 0.88 0.71
0.999999999999];
x=n.*y;


deviance = [];
para = [];

X0 = [-1:0.1:6];
X = 10.^X0;

for trial = 1:4

   if trial == 1
      method = 'bp_mle';
      [deviance_cur w2] = predRecoveryErr(method);
      deviance = [deviance; deviance_cur];
      para = [para; w2'];
      N50(1,1) = w2(2,1)*(2^(1/w2(1,1))-1);
      AIC(1,1) = deviance_cur + 2*length(w2)*(length(t)/(length(t)-length(w2)-1));
      BIC(1,1) = deviance_cur + length(w2)*log(length(t));
```
189

```matlab
            prd2=1-(1+t/(w2(2,1))).^(-w2(1,1));
            r2(1,1)=1-sum((prd2-y).^2)/sum((y-mean(y)).^2);
            GOF(1,1) = sum(((prd2-y).^2)./y);
            y_est=1-(1+X/(w2(2,1))).^(-w2(1,1));
            semilogx(X,y_est,'k','LineWidth',2);
            hold on
    elseif  trial == 2
            method = 'weibull_mle';
            [deviance_cur w2] = predRecoveryErr(method);
            deviance = [deviance; deviance_cur];
            para = [para; w2'];
            N50(2,1) = w2(2,1)*(2^(1/w2(1,1))-1);
            AIC(2,1) = deviance_cur + 2*length(w2)*(length(t)/(length(t)-length(w2)-1));
            BIC(2,1) = deviance_cur + length(w2)*log(length(t));
            prd2=1-exp(-w2(1,1)*(t.^w2(2,1)));
            r2(2,1)=1-sum((prd2-y).^2)/sum((y-mean(y)).^2);
            y_est=1-exp(-w2(1,1)*(X.^w2(2,1)));
            semilogx(X,y_est,'k-.','LineWidth',2);
            hold on
    elseif trial == 3
            method = 'log_logistic_mle';
            [deviance_cur w2] = predRecoveryErr(method);
            deviance = [deviance; deviance_cur];
            para = [para; w2'];
            N50(13,1) = w2(2,1)*(2^(1/w2(1,1))-1);
            AIC(3,1) = deviance_cur + 2*length(w2)*(length(t)/(length(t)-length(w2)-1));
            BIC(3,1) = deviance_cur + length(w2)*log(length(t));
            prd2=1./(1 + exp(w2(1,1) - w2(2,1)*log(t)));
            r2(3,1)=1-sum((prd2-y).^2)/sum((y-mean(y)).^2);
            y_est=1./(1 + exp(w2(1,1) - w2(2,1)*log(X)));
            semilogx(X,y_est,'k--','LineWidth',2);
            hold on
    elseif trial == 4
            method = 'expo_mle';
            [deviance_cur w2] = predRecoveryErr(method);
            deviance = [deviance; deviance_cur];
            para = [para; [w2' 999]];
            AIC(4,1) = deviance_cur + 2*length(w2)*(length(t)/(length(t)-length(w2)-1));
            BIC(4,1) = deviance_cur + length(w2)*log(length(t));
            y_est = 1-exp(-w2(1,1)*X);
            semilogx(X,y_est,'kx','LineWidth',2);
            hold on
    else
            display('error')
    end
```

```matlab
end

semilogx(t,y,'ko','markersize', 10);
hold on
axis([10^-1, 10^6, -0.1, 1.1])
xlabel('Dose');
ylabel('Probability of infection');
gac=legend('beta-Poisson model', 'Weibull model','Log-logistic model', 'Exponential
model')
set(gca,'FontSize',14);
```

------ Exponential function -------

```matlab
function loglik = expo_mle(w)
% EXPO_MLE The log-likelihood function of the exponential model
 global n t x y;


 p=1-exp(-w(1,1)*t);% exponential model prediction
 p=(p < ones(length(t),1)').*p+(p >= ones(length(t),1)')*1;% ensure that p lies
between 0 and 1

 p_all = p;
yu = find(p_all==1);
for i = 1:length(yu)
   p_all(yu(i)) = p_all(yu(i))-0.0000000001;
end
yl = find(p_all==0);
for i = 1:length(yl)
   p_all(yl(i)) = p_all(yl(i))+0.0000000001;
end
p=p_all;

 loglik = (-2)*(x.*log(p./y)+(n-x).*log((1-p)./(1-y)));
% minus log-likelihood for individual observations
 loglik = sum(loglik);% overall minus log-likelihood being minimized


------ Beta-poisson function -------

function loglik = bp_mle(w)

global n t x y;

y_all = y;
```

```matlab
yu = find(y_all==1);
for i = 1:length(yu)
    y_all(yu(i)) = y_all(yu(i))-0.00001;
end
yl = find(y_all==0);
for i = 1:length(yl)
    y_all(yl(i)) = y_all(yl(i))+0.00001;
end

 p=1-(1+t./(w(2,1))).^(-w(1,1));% Beta-Poisson model prediction
 p=(p <= ones(length(t),1)').*p+(p >= ones(length(t),1)')*1;% ensure that p lies
between 0 and 1
 loglik = (-2)*(x.*log(p./y_all)+(n-x).*log((1-p)./(1-y_all)));
% minus log-likelihood for individual observations
 loglik = sum(loglik);% overall minus log-likelihood being minimized



------ Weibull function -------

function loglik = weibull_mle(w)
 global n t x y;

 p=1-exp(-w(1,1).*(t.^w(2,1)));% Weibull model prediction
 p=(p <= ones(length(t),1)').*p+(p >= ones(length(t),1)')*1;% ensure that p lies
between 0 and 1
 loglik = (-2)*(x.*log(p./y)+(n-x).*log((1-p)./(1-y)));
% minus log-likelihood for individual observations
 loglik = sum(loglik);% overall minus log-likelihood being minimized




------ Log-logistic function -------

function loglik = log_logistic_mle(w)
 global n t x y;

y_all = y;
yu = find(y_all==1);
for i = 1:length(yu)
    y_all(yu(i)) = y_all(yu(i))-0.00001;
end
yl = find(y_all==0);
for i = 1:length(yl)
    y_all(yl(i)) = y_all(yl(i))+0.00001;
end
```

```matlab
 p=1./(1 + exp(w(1,1) - w(2,1)*log(t)));% log-logistic model prediction
 p=(p <= ones(length(t),1)').*p+(p >= ones(length(t),1)')*1;% ensure that p lies
between 0 and 1
 loglik = (-2)*(x.*log(p./y_all)+(n-x).*log((1-p)./(1-y_all)));
% minus log-likelihood for individual observations
 loglik = sum(loglik);% overall minus log-likelihood being minimized
```

Appendix B

MATLAB code for bootstrapping in Chapter 5

------ Exponential bootstrapping -------

```matlab
clear all
clc
close all

global n t x y;

display('Input a valid method: expo_mle, bp_mle, weibull_mle, log_logistic_mle');

%n=10;% number of independent binomial trials (i.e., sample size)
n=5;
%n=[7 7 7 11 9 8 7 3];
t=10.^[6 5 4 3 2 1 0];% time intervals as a column vector
%t=9.*[0.001 0.01 0.1 1 10 100 1000 10000];
y=[0.99999999999 0.99999999999 0.8 0.4 0.2 0.0000000001 0.0000000001];
%y=[0.99999999999 0.99999999999 0.99999999999 0.7 0.3 0.0000000001
0.0000000001];
%y=[0.00000000000001 0.00000000000001 0.14 0.73 0.89 0.88 0.71
0.999999999999];
%x = [10 10 10 7 3 0 0];
%x = [5 5 4 2 1 0 0];
%y = x./n;
x=n.*y;

method = 'expo_mle';
[deviance_ori w2_ori] = predRecoveryErr(method);
N50_ori = log(0.5)/(-w2_ori);
pit = 1-exp(-w2_ori.*t);
pit(find(pit==1))=pit(find(pit==1))-0.000000001;
pi0 = y;
```

```matlab
sigma = (pi0-pit)./(sqrt(pit.*(1-pit)./n));


nbootstrap = 10000;

x_all = [];
y_all = [];
for i = 1:nbootstrap
    icur = randi([1 length(pi0)], [1 length(pi0)]);
    pim = pit + sigma(icur).*sqrt(pit.*(1-pit)./n);
    pim_norm = (pim-min(pim))./(max(pim) - min(pim));
    pim_norm(pim_norm==1) = 1-0.000001;
    pim_norm(pim_norm==0) = 0.000001;
    x_cur = binornd(n, x./n);
    %x_cur = binornd(n, pim_norm);
    x_all = [x_all; x_cur];
    y_cur = x_cur./n;
    y_cur(find(y_cur==1))=y_cur(find(y_cur==1))-0.000000001;
    y_cur(find(y_cur==0))=y_cur(find(y_cur==0))+0.000000001;
    y_all = [y_all; y_cur];
end



deviance = [];
para0 = [];
N500 = [];
for ifold = 1:nbootstrap
if mod(ifold, 10)==0
    ifold
end
x = x_all(ifold, :);
y = y_all(ifold, :);

method = 'expo_mle';
[deviance_cur w2] = predRecoveryErr(method);
%N50(ifold,1) = w2(2,1)*(2^(1/w2(1,1))-1);
deviance = [deviance; deviance_cur];
para0 = [para0; w2];
N500 = [N500; log(1/2)/(-w2)];
end


para = para0(para0<=inf&para0>=0);
N50 = N500(para0<=inf&para0>=0);
N50_new = [];
```

```matlab
N50_loc = [];
para_new = [];
for i = 1:length(N50)
    if N50(i)<=inf && N50(i)>=0 && para(i)<=inf && para(i)>=0
        N50_new = [N50_new; N50(i)];
        para_new = [para_new; para(i)];
        N50_loc = [N50_loc; i];
    end
end

loglog(N50_ori,w2_ori,'b.','MarkerSize',50);
hold on
for i = 1:length(N50_new)

    %plot(N50(i),para(i,1),'r.','MarkerSize',10);

    loglog(N50_new(i),para_new(i),'r.','MarkerSize',10);
    hold on
end
xlabel('N_(150)','FontSize',20);
ylabel('r','FontSize',30);
axis([10 10^4 10^-4 10^-2]);
%axis([10^-1 100 min(para(:,1)) max(para(:,1))]);

%X0 = [1:1000:10^6];
X0 = [0:0.01:6];
X = 10.^(X0);
Y = [];
y_est = [];
for i = 1:length(para_new)
    %y_est=[y_est; 1-(1+X./(para_new(i,2))).^(-para_new(i,1))];
    y_est=[y_est; 1-exp(-para_new(i).*X)];
end
y_sort = sort(y_est);
lb = ceil(length(para_new)*0.025);
ub = floor(length(para_new)*0.975);

for i = 1:length(X)
    Y(1,i) = y_sort(lb,i);
    Y(2,i) = y_sort(ub,i);
    Y(3,i) = y_sort(ceil(length(para_new)/2),i);
end

loglog(X,Y(1,:),'r-.','LineWidth',2);
hold on
loglog(X,Y(2,:),'r--','LineWidth',2);
```

```
hold on
loglog(X,Y(3,:),'r','LineWidth',2);
hold on
axis([1 10^6 0.0001 1.5])
 yTickOld = get(gca,'YTick');
yTickNew = num2str(yTickOld);
set(gca,'YTickLabel',yTickNew)
set(gca,'YTickLabel',yTickOld)

xlabel('Dose', 'Fontsize', 15);
ylabel('Probability of infection', 'Fontsize', 15);
h_leg = legend('2.5 percentile', '97.5 percentile', 'median');
set(h_leg,'FontSize',15)
```

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202

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214

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219

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248

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