RESEARCH REPORT
PORK SAFETY

Title: Expansion of the *Salmonella* in Pork Risk Assessment: Incorporation of *Toxoplasma* in pork, and assessing the relationship between on-farm factors and risk – **NPB #08-249**

Investigators: Dr. Emma Hartnett, Greg Paoli

Institution: Risk Sciences International, Inc., Ottawa, Canada

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Industry Summary

We extended our previous farm-to-illness model (NPB project number 07-079) to incorporate the risk of human toxoplasmosis as well as salmonellosis, from the consumption of both fresh intact pork cuts, and mixed pork as represented by breakfast sausage. In addition, the effect of two different production methods: continuous flow and all-in/all-out, is explored.

This risk assessment is based on a probabilistic simulation which models the variation that exists in the various factors affecting the addition, growth, partitioning, and inactivation of pathogens along the pork production chain. The prevalence and level of *Salmonella* contamination is simulated by the model, beginning with the arrival of weaned pigs at the production site, and tracked through growing, slaughter, processing and consumer cooking to the point of consumption. *Toxoplasma* level and prevalence in intact meat is represented by estimates from the literature, and the model uses simulated and literature values as input to the mixed meat module, which predicts the level and prevalence of *Salmonella* and *Toxoplasma* contamination in servings of breakfast sausage. The effect of cooking on both pathogens is simulated to yield a dose per cooked serving, which is translated into a risk of illness by dose-response models. In the absence of an accepted dose-response model for *Toxoplasma* cysts in humans, the exponential model is used with two different r-values.

The mean risk of salmonellosis per serving predicted by the model is $2x10^{-6}$ for intact cuts of pork and $6x10^{-6}$ for breakfast sausage. This translates to 50,000 cases of illness annually from fresh intact pork, and 30,000 for breakfast sausage, using available consumption data.

Depending on the r-value applied, the mean risk of toxoplasmosis predicted per serving of intact pork ranged from $8x10^{-7}$ to $8x10^{-6}$, and that per serving of breakfast sausage ranged from $7x10^{-7}$ to $7x10^{-6}$, leading to between 3,000 and 35,000 annual cases from consumption in the home of these two products.

The model supports scenario analysis to explore the impact of:

- proportion of pigs from all-in/all-out sites among pork production facilities
- prevalence of *Toxoplasma* among pigs contributing to mixed meat
- prevalence of *Toxoplasma* among grower pigs
- two levels of *Toxoplasma* cysts in pork
- efficacy of washing of viscera
- proportion of pathogens in protected areas during cooking
- probability of undercooking

This project is of interest to stakeholders at all stages of the pork production chain. The model developed facilitates further understanding of opportunities to manage the risk of both salmonellosis and toxoplasmosis due to pork products. It can also be used to identify important research opportunities.

Contact information: Greg Paoli, Risk Sciences International, Inc.; 200-449 Sussex Drive, Ottawa, ON.; Email: gpaoli@risksciencesint.com

Keywords

Toxoplasma, *Salmonella*, risk, pork, mixed meat

Scientific Abstract

A farm-to-illness probabilistic model previously developed to predict risk of salmonellosis from consumption in the home of fresh, intact pork was extended to consider the additional pathogen *Toxoplasma*, and to simulate the risk from both fresh intact pork and from mixed (ground) pork represented by fresh breakfast sausage. In the extended model, the impact of an all-in/all-out production system is also included. We estimate a mean risk of toxoplasmosis per serving of intact pork or breakfast sausage from $8x10^{-7}$ to $8x10^{-6}$, and from $7x10^{-7}$ to $7x10^{-6}$, respectively, for r-values of 0.01 to 0.1 and assuming an exponential dose-response relationship. Based on available consumption data this is equivalent to 3,000 to 35,000 cases of toxoplasmosis annually from either of these two products. The model predicts a risk per serving due to *Salmonella* of $2x10^{-6}$ for intact fresh pork and $6x10^{-6}$ for breakfast sausage, which will result in 50,000 and 30,000 cases respectively, based on available consumption data. The risk of salmonellosis per serving from breakfast sausage is directly proportional the probability of undercooking, and inversely proportional to the log reduction achieved by antimicrobial washing of viscera. Salmonellosis risk from the intact pork and from breakfast sausage is positively associated with the proportion of pigs raised in sites practicing all-in/all-out management as opposed to continuous flow. A reduction in the risk from *Toxoplasma* in intact pork and breakfast sausage can be achieved by reducing the probability of undercooking, and/or by lowering on-farm prevalence.

Introduction

The protozoan *Toxoplasma gondii* is an obligate, ubiquitous parasite and possibly the only representative of its genus (34). Over 95% of recognized strains belong to one of three clonal lineages exhibiting distinct virulence characteristics in mice (46).

T. gondii is noted for its ability to infect “virtually all warm-blooded vertebrates”, usually by ingestion (46). The cat is the definitive host (16), and ingestion of the parasite by a feline results in the shedding of oocysts in feces. In this form it is capable of remaining viable in the soil for a year or more (20). Within each oocyst,

sporulation gives rise to eight haploid sporozoites (43). Once ingested the sporozoites undergo rapid rounds of asexual reproduction as "tachyzoites" which are dispersed throughout the host via the circulatory system, eventually forming tissue cysts containing "bradyzoites" (13). The tissue cysts, up to 200µm in size (Remington and Desmonts, 1990) likewise may remain infectious for many years within the host (16).

Prevalence and Transmission in Pigs in the United States

Although the earliest indication of the pathogen's veterinary significance was its involvement with abortion storms in sheep, the parasite generally does not cause clinical disease in cattle, poultry or pigs (11). A National Animal Health Monitoring System (NAHMS) survey of 412 swine herds in 17 states, conducted in 1990, measured an average seroprevalence of *T. gondii* antibodies in sows of 20%, with a state-to-state range from 12% to 36% (with the exception of Colorado where no positive hogs were identified) (39). Within the next 5 years samples collected from market-weight pigs in Tennessee and North Carolina indicated a seroprevalence in this age-group ranging from less than 1% to 3%, down from 23% in 1983-84 (39), and consistent with an estimated value of 1.7% in Ontario (40).

Domestic pigs are thought to be capable of becoming infected with *T. gondii* through exposure to cat-derived oocysts in feed, water or soil, or by ingestion of tissue cysts in inadequately-cooked plate waste or infected wild animals (particularly rodents) or through cannibalism (45). In a study of three organic pig farms, a rodent-control initiative progressively reduced *T. gondii* seroprevalence among pigs at each farm during the three-month campaign (22).

For the most part research has implicated oocysts shed into the soil by cats as the most important factor in *Toxoplasma* transmission to pigs. Infectivity in pigs has been demonstrated following inoculation with as few as 1 oocyst (12). Assadi-Rad et al., (1) in a survey of 343 swine farms in Tennessee, found that sows associated with cats were 2.6 times more likely to be seropositive for *T. gondii* antibodies than sows not associated with cats, and that sows that spent any time outdoors were 23 times more likely to be seropositive than sows kept indoors. In nineteen Iowa swine farms, seroprevalence in sows was inversely related to confinement, and appeared to increase with age (where age was indicated by number of pregnancies); the cat population per farm ranged from 2 to 26, and the seroprevalence of *T. gondii* antibodies among cats averaged 41.9% (45). Similarly, seroprevalence in North Carolina finishing pigs kept on pasture, at 19%, was higher than the 0.057% seroprevalence among finishing pigs kept in total confinement (6). Results from the Netherlands also demonstrate a higher seroprevalence of *T. gondii* antibodies in free-range pigs (5.62%) as compared to pigs raised indoors (0.38%) (50).

An exhaustive survey of wildlife and domestic animals at a farm with >95% seropositivity among pigs, revealed a direct relationship between proximity to the pens and seroprevalence in birds and mammals within a 360m radius. This relationship, coupled with the similarity between isolates obtained from the pigs and those obtained from wildlife and farm chickens, is compatible with the existence of a common source of infection on the farm premises. Although no *T. gondii* was isolated from the single barn cat or a road-killed cat found half a mile away (both seronegative), oocysts cannot be ruled out as a source, since they persist for a year or more in the soil. Genetic characterization of 25 *T. gondii* isolates from pigs suggested at least 3 distinct exposure events during their seven months of life (26). Seroprevalence has been known to underestimate true prevalence of exposure to *T. gondii* (9;26).

Survival

Toxoplasma cysts are sensitive to both heat and extreme cold. Using a mouse bioassay, Dubey et al. (27) found that 24 minutes at temperatures of 49°C and above rendered tissue cysts non-infective, and at 58°C inactivation was achieved within 6 minutes. Freezing is also effective: elimination of infectivity of tissue cysts in pork was obtained by 33.6 days at -1°C or -3.9°C, by 16.8 days at -6.7°C, and by 2.8 days at -8°C (24). The authors calculated the theoretical temperature at which *T. gondii* tissues cysts would be inactivated instantaneously as -12.4°C, while acknowledging that one strain of the pathogen originated from the heart of a monkey stored at -20°C for 16 days. Cysts stored at 4°C remained viable for at least 24 days (24).

In addition to temperature, *Toxoplasma* is susceptible to high pressure. Lindsay et al. (28) demonstrated that treatment of tissue cysts in ground pork at pressures of 300 and 400 mPa for 30 to 90 minutes produced only negative results in a mice bioassay, whereas treatment for up to 90 minutes at 200 mPa had no effect on infectivity.

Some commonly-used enhancement solutions reduce the infectivity of *Toxoplasma* tissue cysts. Sodium lactate at 1.5%, potassium lactate at 1.4%, and sodium chloride at 2% removed infectivity from enhanced pork loin (18). The solutions required up to 8 hours to achieve effectiveness (17).

Prevalence and Transmission to Humans in the United States

The 1988-1994 NHANES found age-adjusted seroprevalence of *T. gondii* in the US to be 22.5%, with values of less than 5% in children up to 5 years of age and conversely, values greater than 40% among those 70 years of age or older (20).

Estimation of the infection rate is complicated by the asymptomatic nature of about 85 to 90% of infections (25;29), but an annual rate of at least 0.6% has been suggested based on the seroprevalence among individuals over 60 years of age and assuming equal rates of infection over time (29).

Humans can become infected with *T. gondii* by consuming either tissue cysts in undercooked meat or oocysts in contaminated food or water. The parasite can also be passed to a fetus carried by an infected mother (11). The relative importance of each of these three modes of transmission in the US is unknown, although lower incidence among some vegetarian populations (42), and a sharp increase in prevalence between children and teens, suggests that meat consumption plays an important role (11). Kijlstra and Jongert (22) identify the consumption of under-cooked meat as the single largest risk factor for infection. Mead et al., (29) used 50% as an estimate of the proportion of food-borne transmission of *T. gondii* among humans. While beef and chicken are the two most commonly-consumed meats, neither is considered to play a major role in the transmission of toxoplasmosis (11).

Recently viable *T. gondii* was recovered from a small number of samples in a survey of pork from retail meat stores across the US (10). In a food intake survey conducted in the US, 40% of subjects reported having consumed at least 1 ounce of pork or processed pork products over 2 non-consecutive days (14). Kijlstra et al. (22) noted that an increase in “animal-friendly” production systems, that is, those not using total confinement, may enhance the involvement of pork meat in *Toxoplasma* infections.

Health Effects of Toxoplasmosis in Humans

Toxoplasma is the third leading cause of death attributable to food-borne illness in the US, after *Salmonella* and *Listeria* (5). While infection is largely subclinical in most healthy adults, the parasite poses a significant threat to the immunocompromised, and to unborn children.

Infection in Adults and Children

The clinical course of toxoplasmosis acquired postnatally depends on the immune status of the victim: in immunocompetent subjects, asymptomatic infections are the norm and clinical signs affecting the lymph nodes or retina are seen in only about 10% of those infected (11;33). During an outbreak of toxoplasmosis in British Columbia, Canada, believed to have been caused by water-borne oocysts, half of 100 identified cases developed lymphadenopathy and 19 suffered retinitis (3). Out of an estimated 2894 to 7718 individuals infected, this implies a risk of retinitis of 2.5 to 6.5%, and of lymphadenopathy of 6.5 to 17%. Identification of the 100 cases was by a voluntary pregnancy screening program (37 subjects) or by overt symptoms leading to medical care (60 subjects). Of the 37 women identified by pregnancy screening, 15 remained symptom-free (3).

Lymphadenopathy is the most common clinical symptom in immunocompetent individuals, and in toxoplasmosis describes an enlargement of the lymph nodes that persists for up to 4-6 weeks with no tenderness (33). Occasionally the disorder becomes chronic with fluctuating enlargement over a period of months, and very rarely more serious sequelae occur, such as myocarditis, polymyosotis, pneumonitis, hepatitis, or encephalitis (33).

Toxoplasmic chorioretinitis can cause blurred vision, pain and photophobia (11) and is characterized by clusters of retinal lesions associated with an intense vitreal inflammatory reaction (33). Long-thought to be a late expression or reactivation of prenatal infection, it is now recognized that this disorder can arise as a result of acute postnatal infection, as was seen in the BC outbreak described above. The confusion resulted in part from the fact that the ocular form of toxoplasmosis may not appear immediately; in the 5 years following the BC outbreak, 2 additional cases were diagnosed (19). Silveira et al. (44) reported a 9.5% prevalence of ocular toxoplasmosis among subjects known to have seroconverted within the previous 7 years. In addition, 8.3% of previously seropositive subjects developed ocular toxoplasmosis during the intervening 7 years. The overall prevalence of ocular involvement of toxoplasmosis in the US is estimated at about 2% (19).

In immunocompromised individuals a chronic or latent *Toxoplasma* infection can become re-activated and develop into life-threatening toxoplasmic encephalitis, which, prior to the advent of aggressive therapy affected between 30 and 40% of HIV-AIDS patients (11;47). This disease can present gradually (over weeks) or quickly, with “mental status changes, seizures, focal motor deficits, cranial nerve disturbances, sensory abnormalities, cerebellar signs, movement disorders and neuropsychiatric findings” (33). Disseminated forms of toxoplasmosis may also occur in immune-suppressed patients with cancer and following transfusions or transplants with associated immunosuppressive therapy (11;47). A newly-acquired acute infection in the immunocompromised population can cause severe pulmonary disease or encephalitis (47).

A study done on HIV patients in South Africa suggests that the risk of retinitis and *Toxoplasma* encephalitis among AIDS patients seropositive for *Toxoplasma* are both approximately 4% (15). Kocazeybek et al. (23) reported that individuals with latent toxoplasmosis were over-represented among car accident victims, indicating that the burden of this illness may be greater than formerly recognized.

Congenital Infection

Although reactivation of a latent infection can lead to congenital toxoplasmosis in immune-suppressed pregnant women (11), in most cases the mother's immune system will thwart the vertical transmission to the fetus if the infection of the mother occurs more than 4-6 months prior to the pregnancy (47). The impact of infection of a woman during pregnancy varies with the stage of pregnancy. During the first trimester the likelihood of vertical transmission is lowest (10-15%)(11) but the consequences are most severe, being clinically apparent in nearly 78% of cases (33). The risk of infection of the fetus is 60-90% when maternal infection occurs during the third trimester (11), however in these cases nearly 90% of infections remain subclinical (33). The risk of congenital infection rises gradually throughout the pregnancy, from near 0% if the mother becomes infected at 4 weeks, to nearly 80% at 40 weeks (33).

The more severe consequences of infection range from death of the fetus or spontaneous abortion (10% of cases; (47), to hydro- or micro-cephalus, to retinochoroiditis, CNS disorders, or failure-to-thrive (11). Up to 16% of infected newborns die as a consequence of this disease (47). However most children with congenital toxoplasmosis express no symptoms at birth, instead developing signs of infection with eye or CNS involvement (11).

Toxoplasma in the Pork Production Chain

Since *Toxoplasma* is not an enteric pathogen, control during the on-farm stage of pork production is relatively straight-forward. Pig-to-pig transmission is unlikely barring cannibalism, and the pathogen is unlikely to be introduced into a herd kept away from bare soil or other sources of oocysts (6). Conversely, at one 500-head farm in which sows were kept on dirt lots and finishers in enclosed buildings with poor biosecurity, 51 of 55 growers were positive for *Toxoplasma* (9). In "total containment" type sites there are very few such exposures, and 81% of pigs in the US are reared in this style of housing (49).

Tissue cysts are unaffected by conditions during slaughter, as they are protected within the meat of the pig. The scald step during pork slaughter is roughly 6 minutes at 58.8°C, however the temperature of the meat does not equilibrate; van der Wal et al. (1993) measured a rise of only 1°C (to 40.5° +/- 0.3°C) in the biceps femoris of pigs over a 12 minute scald at 60°C. It is unlikely that this is sufficient to reduce the viability of cysts residing in the muscle; the authors calculated that after 6 minutes the subcutaneous temperature at both 2.5 and 5 cm does not exceed body temperature (51).

Evisceration is not a critical step with respect to *Toxoplasma*, as the pathogen is not enteric, though cross-contamination may occur if cutting knives are not properly de-contaminated between carcasses: infrequent washing of kitchen knives after cutting meat was found to be associated with an increased risk of *Toxoplasma* infection among a group of pregnant women (21).

In a review of control of toxoplasmosis from meat, Kijlstra and Jongert (22) identified pre-harvest monitoring and surveillance programs, and post-harvest decontamination such as freezing as the most promising control practices.

Stated Objectives

The objectives of this project were four-fold:

- to expand the previously-developed farm-to-illness *Salmonella* risk assessment to include *Toxoplasma*, and estimate the number of cases of salmonellosis and toxoplasmosis that occur annually in the US as a result of pork consumption in the home.
- to expand the types of pork product considered in the risk assessment to include fresh mixed pork, and
- to expand the types of swine grower sites to consider All-in/all-out in addition to Continuous production
- to provide an analysis of the impact of on-farm prevalence of *Toxoplasma* on the risk of illness

Like the previous model, this model represents a quantitative risk assessment developed in the software Analytica, and it enables the user to predict the impact of interventions in the farm-to-fork continuum.

Materials and Methods

Model Scope – The model considers the risk from *Salmonella* and *Toxoplasma* associated with fresh cuts of pork (chops, steak, ribs, fresh ham and roasts), and fresh mixed pork (here represented by breakfast sausage), that is consumed at home. The *Salmonella* component of the model simulates the farm-to-illness continuum beginning with pigs entering the grower facility, while the *Toxoplasma* component begins with on-farm prevalence values obtained in the literature. Ready-to-eat products and products prepared outside the home are outside the scope of this assessment.

Mathematical Description of Model Components

In addition to the components presented in the previous report, new components were developed to further explore the risk associated with *Salmonella* and *Toxoplasma* in pork products.

The following components were added to the model:

1. *Toxoplasma* as a second pathogen
2. The farm component has been extended to include, besides continuous production, the all-in/all-out production type.
3. Mixed meat module

Explanation of Model Structure for Toxoplasma infections from Pork

Besides assessing risk of infection by *Salmonella*, we also considering the probability of being infected by *Toxoplasma*. When analyzing the risk of toxoplasmosis, we distinguish between the general public and pregnant women carrying fetuses because the impact of infection is different for those groups.

1) For males, and females not experiencing pregnancy:

A consumer can only become infected with *Toxoplasma* (from consuming contaminated pork for example), if that person has not been infected in the past. Therefore we must first estimate the probability that the consumer has not been infected previously.

First of all a chance distribution is created to model the likelihood of the consumer being in each age group. This is relevant as the probability of previous infection rises steadily with age, assuming constant exposure over time. Then the probability of the consumer being susceptible (i.e. not previously infected) is obtained from the annual infection rate of *Toxoplasma* infection (from all sources) and the age. The probability of being "not previously infected" is distributed according to the following Poisson distribution:

$$\Pr(n) = \frac{\lambda^n e^{-\lambda}}{n!}$$

where λ is the expected number of exposures in a specific period of time. For the case of toxoplasmosis, this value is the product of the annual infection rate, i, and the age at the moment of consumption.

We are calculating the probability of being "not previously infected", i.e., the probability of n=0, so the formula is reduced to a simple form:

$$\Pr(n = 0) = \exp(-iN_{age})$$

The probability of illness is then the probability of a susceptible consumer, multiplied by the probability of infection from intact pork and breakfast sausage (generated by the model), multiplied by the probability of a symptomatic outcome. This latter probability is given the value 15%, since 85% of *Toxoplasma* infections are asymptomatic (29).

This probability is on a per-serving basis, so to obtain an estimate of the annual number of cases expected nation-wide, we multiply the mean probability by the number of servings, calculated as described below.

2) For females experiencing pregnancy

Again, the number of susceptible consumers must first be estimated. Rather than a lifetime age structure, this component of the model uses the age structure only among pregnant females, and calculates the average probability of susceptibility among this population by using pregnancy rate by age combined with the annual infection rate with *Toxoplasma* from all sources.

The probability of infection (from pork) during pregnancy is then calculated by multiplying the probability of being susceptible while pregnant with the probability of infection from pork, based on the value generated by the model.

Since the impact of toxoplasmosis is far greater in fetuses and neonates than in the general population, the possibility that the infection is transmitted to the fetus is calculated based on literature values for this likelihood. The average likelihood of transmission to the fetus is roughly 22%, and the average likelihood of a symptomatic newborn in the case of an infected fetus is 39% (32). Assuming a constant likelihood of infection of the mother over the course of the pregnancy, the number of cases is calculated based on these figures. The number of servings is calculated as below, with the total number of servings being apportioned between pregnant women and the general population strictly on the basis of the size of these populations. The number of symptomatic cases both for pregnant women, and for their unborn children is then calculated.

Toxoplasma dose-response model

The dose-response model permits estimation of the risk of becoming ill after consuming contaminated product. In this module, we estimate a probability of illness caused by a pathogen that is contained in a cooked serving.

Unlike the Beta-Poisson model used to estimate the dose-response relationship for *Salmonella*, we used an exponential model to describe the probability of developing illness from consuming meat products contaminated by *Toxoplasma*:

$$P_{ill} = 1 - e^{-rD},$$

where D is a dose of *Toxoplasma* ingested (predicted by the model) and r is a model parameter specific to a pathogen and host. For *Toxoplasma*, we considered the r-values of 0.01 and 0.1, which represent a range of pathogenicity comparable to that between *Salmonella* and *Shigella*.

To reflect information about the prevalence of a pathogen in a herd, we calculate probability of illness per cooked, contaminated serving, weighted by the probability that the serving will be contaminated prior to cooking. This combination allows us to estimate overall risk of illness per cooked serving.

Farm component: all-in/all-out production type

In addition to the continuous management production model presented in the previous report, a model of all-in/all-out (AI/AO) type production was developed to simulate the prevalence of *Salmonella* in these conditions. Unlike a continuous management type of production, AI/AO management assumes that the entire room must be marketed before a new cycle or turn begins. The farm is assumed to be not contaminated when the new load of pigs arrives, so the dynamics of the disease in a herd is determined by the initial prevalence of *Salmonella* among pigs and the transition rate, i.e., the probability of disease transmission in a contact between a susceptible and an infectious animal. We also developed a modification of this model that considers recovery of the infected pigs through the immune state to again become susceptible. Reversion from the carrier state to susceptible occurs with a constant recovery rate. The probability of transition between excreting and susceptible states, per day, is assumed to be equal to the inverse duration of the disease.

In the model, the herd is considered to consist of 2 classes: excretors and susceptibles. Infection may occur at any moment and is defined by the transition rate, which is the probability of contracting the disease from feces excreted by the infected animals. The output of this module is the estimation of the prevalence of excretors at the end of the period of growth.

The dynamics of the populations of excretors (E) and susceptibles (S) is described by the following equations:

$$\frac{dS}{dt} = -\beta S(N - S) + \alpha E,$$
$$E = N - S$$

where β is the transition rate, or probability of getting the disease in a contact between an excretor and a susceptible, α is the rate of recovery; α=1/D, where D is duration of the disease in days. Under an assumption that there is no recovery, the α parameter in the above equation is set to zero.

According to the model without consideration of recovery, the prevalence of excretors reaches 100% within 10-20 days of the pigs' arrival at the farm, depending on the initial prevalence and infection transition rate,

and remains at this level during the entire stay of the herd at the farm. As pigs stay at the farm for 6 months and the duration of the disease is about 100 days, recovery from excretor to susceptible affects the prevalence of salmonellosis at the end of the growing period: the prevalence of excretors stabilizes at a level of 80% and remains unchanged due to constant recovery of the part of the herd as shown in Figure 1.

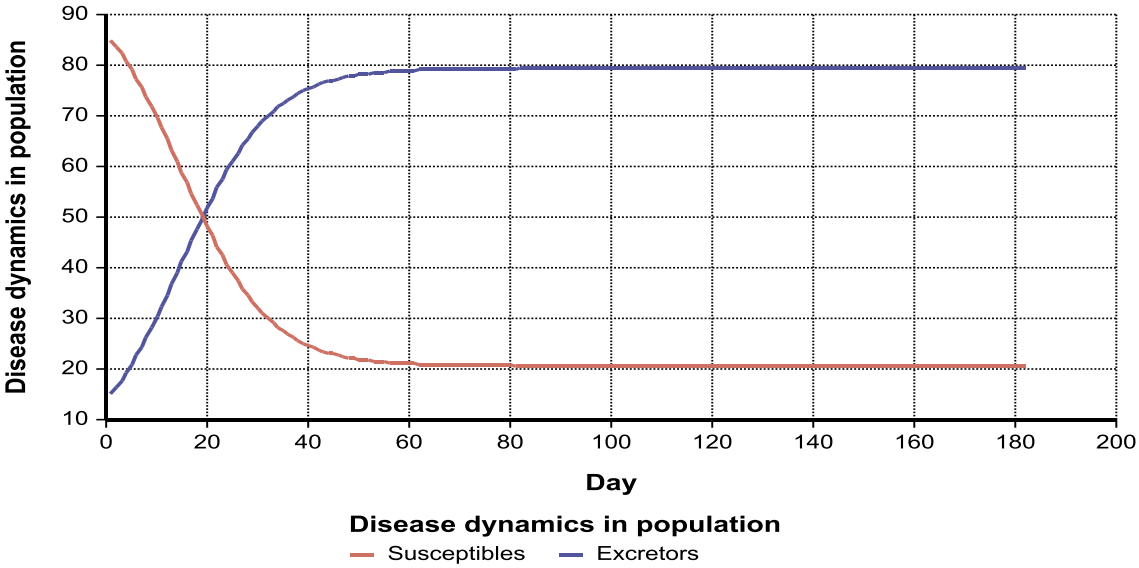

Figure 1. Changes in the proportion of excreting and susceptible pigs in the all-in/all-out type farm.

Since *Toxoplasma* is not transmitted between pigs to an appreciable extent (6), the prevalence of *Toxoplasma* in pigs is determined largely by the age of the pig in concert with the number of opportunities to forage in soil, and access to small animals. The prevalence of toxoplasmosis is therefore assumed to be unaffected by the proportion of pigs that are raised in "all-in/all-out" sites.

Mixed Meat Model Structure

Pork mixed meat is represented by breakfast sausage in this model, and assumed to be composed of trim and by-products. The trim is meat and fat cut from the carcass, and so the enteric pathogen *Salmonella* is only expected to occur on trim if the carcass became contaminated during slaughter. In contrast, *Toxoplasma* occurs in the muscle of an infected animal, so this trim is expected to contain *Toxoplasma* in all pigs infected with *Toxoplasma*. On the other hand, by-products include parts of the digestive tract as well as muscle such as heart, and so by-products may be contaminated with both pathogens in infected pigs. See Table for a list of values and sources. The masses used for trim and for byproduct components were estimated based on the carcass breakdown (38) and individual byproduct values (35). Consideration of cross-contamination is not necessary here as byproducts are mixed in sausage batches.

Trim and by-products from several pigs are combined in a mixer to produce a batch of sausages. We do not assume that the animals contributing trim are the same animals as those contributing byproduct.

In order to estimate the final level of contamination in sausage meat, we simulate the number of contaminated animals contributing trim, and the number of infected animals contributing byproduct, and sum the number of pathogens (cfus or cysts) added from each ingredient (number from trim and number from byproducts). The number for each ingredient is found by consideration of the number of positive carcasses contributing and the level per positive carcass.

The concentration of pathogens in the batch of mixed meat is obtained by dividing the number of pathogens in the mixture (if <>0) by the mass of the batch.

The probability of the carcass (and by implication, trim) being contaminated with *Salmonella* is calculated in the slaughter module. The level of this contamination is estimated based on the fraction of the entire carcass which is removed from it to become trim; the load on the carcass as simulated in the slaughter module is divided between the trim and the remaining carcass according to mass. Thus we obtain the number of *Salmonella*e contributed to the batch by trim from a single pig.

The *Toxoplasma* load of trim is obtained by consideration of the mass of trim used and the concentration of cysts in the muscle tissue of infected pigs (12). This number of cysts is added to the mixer in proportion to the number of *Toxoplasma*-infected pigs that are contributing to the batch.

The predicted level of each pathogen in tissue from the heart, tongue, head meat, ileum, and large intestine is obtained from literature values (4;12), and multiplied by the mass of each tissue used in the batch to calculate the load added. This value is added to the batch depending on the probability of contamination (given infection) of each tissue, a value also obtained from literature (4;16). Table provides a summary of the values used in this expansion of the previous model.

Calculation of Consumption of Pork Products

Appropriate data describing pork product consumption as distributions of amount consumed per eating occasion are lacking, and so the following approach was taken to ensure consistency across the two types of pork considered here.

Davis and Lin (7) reported the total annual per capita amount of pork consumed in the US in several categories, from data gathered in the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-96 and 1998. We have assumed that our intact pork is represented by the sum of “chops”, “steak”, “ribs”, “fresh ham”, and “fresh nonspecified” in Davis and Lin’s report, and that the amount of breakfast sausage consumed is equal to 52.2% of Davis and Lin’s reported consumption of “sausage”. The latter assumption is based on data from the National Pork Board stating that 52.2% of all pork sausage “eatings” occur at breakfast (37). Other data from the NPB indicate that 81% of pork is consumed in the home (36).

These assumptions allow calculation of the annual number of servings per capita of intact pork, and of breakfast sausage eaten in the US, as the total per capita amount of each divided by the serving size. A distribution of serving sizes was generated based on the recommended USDA Pyramid Serving size, as in Miller et al. (31). The annual number of servings per capita was multiplied by population size to arrive at the annual number of servings of intact pork, and the annual number of servings of breakfast sausage, consumed in the US. A further factor of 0.81 (36) was applied to represent pork consumed in the home.

Table 1. Variables and values used in the expansion of the model

Variable	Value	Reference
Proportion of grower/finisher pigs raised in all-in/all-out production sites	0.89	(49) (Swine 2006, Part I)
Toxoplasma prevalence among all pigs	0.08	(52)
Toxoplasma prevalence among growers	Uniform(0.01, 0.03)	(39)
Toxoplasma level in muscle tissue	Uniform(0.01,0.02) cysts/g	Based on (12)
Probability of *Toxoplasma* in heart of infected animal	0.25	(16)
Probability of *Toxoplasma* in tongue of infected animal	0.75	(16)
Log reduction in *Toxoplasma* after inadequate cooking	Normal(0.71,0.59)	Based on D-values in (30)
Transmission of *Toxoplasma* infection to fetus from mother	0.217	(32)
Probability of symptomatic *Toxoplasma* infection in infected newborn	0.385	(32)
Rate of recovery from salmonellosis in pigs	0.0289	Inverse of (duration of disease + 10)
Salmonella level in ileum	10 cfu/g	(4), day 28 pi
Salmonella level in large intestine	10 cfu/g	(4); used cecum as proxy, day 28 pi
Salmonella level in head meat	10 cfu/g	(4); used mandible as proxy, day 28 pi
Salmonella level in heart	10^(Normal(0.67, 0.58))	(4), day 28 pi
Probability of *Salmonella* in heart in infected animal	0.66	(4), day 28 pi
Probability of *Salmonella* in head, ileum and large intestine of infected animal	1	(4), day 28 pi
Mass of mixed meat in batch	450 kg	(2)
Mass of lean trim per pig	(25/2.2)*1000 * 0.42 g	Calculated from (8;38)
Mass of heart per pig	418.2 g	(35)
Mass of tongue per pig	254.5 g	(35)
Mass of head meat per pig	2168.2 g	(35)
Mass of ileum per pig	1550 g	(35)
Mass of large intestine per pig	1495 g	(35)

Results

Objectives 1 and 2: The first two objectives were approached concurrently: to estimate the number of toxoplasmosis and salmonellosis cases in the US due to home consumption of intact and mixed meat pork:

For *Toxoplasma*, the mean risk of illness per serving of fresh intact pork or from breakfast sausage ranged from $8x10^{-7}$ to $7x10^{-6}$, for r-values from 0.01 to 0.1, and assuming the lower of two values used to estimate the number of cysts per gram of tissue (Table).

Table 2. Risk of toxoplasmosis per serving as affected by product type and r-value.

		Product type	
		Intact pork	Breakfast sausage
r-value	0.1	7.5E-06	7.0E-06
	0.01	7.9E-07	7.4E-07

The breakdown of cases by population group is illustrated in Table . From consumption of intact pork, the model predicts between 35 and 331 cases in fetuses, between 62 and 593 cases in pregnant women, and between 3183 and 30,390 cases in the general population, depending on the r-value used.

Though the risk of toxoplasmosis per serving is comparable between the two product types, because consumption data indicated a greater amount of intact pork consumed annually compared to sausage (7), the estimated total number of cases of illness attributed to the two products is lower for breakfast sausage, reaching nearly 5,500 if r=0.1, as compared to over 31,000 from intact pork (Table).

Table 3. Estimated annual cases of toxoplasmosis by population group, as affected by product type and r-value.

			Product type	
			Intact pork	Breakfast sausage
Population group	Fetuses	r=0.01	35	6
		r=0.1	331	57
	Pregnant women	r=0.01	62	11
		r=0.1	593	103
	General population	r=0.01	3183	549
		r=0.1	30390	5250
	Total	r=0.01	3280	566
		r=0.1	31314	5410

The mean risk of salmonellosis predicted by the model per serving of pork consumed in the home was $2x10^{-6}$ from fresh intact pork and $6x10^{-6}$ from breakfast sausage. Using available total annual consumption data (7) and assuming serving sizes consistent with US pyramid recommendations, this risk yields an estimated number of annual cases of salmonellosis from pork consumed in the home in the US of 50,000 for intact pork and 30,000 for breakfast sausage.

Objective 3: The third objective was the expansion of the production methods to include all-in/all-out in addition to continuous flow:

The risk of salmonellosis per serving of intact pork or breakfast sausage rises with the proportion of pigs raised in all-in/all-out sites, as shown in Figure 2.

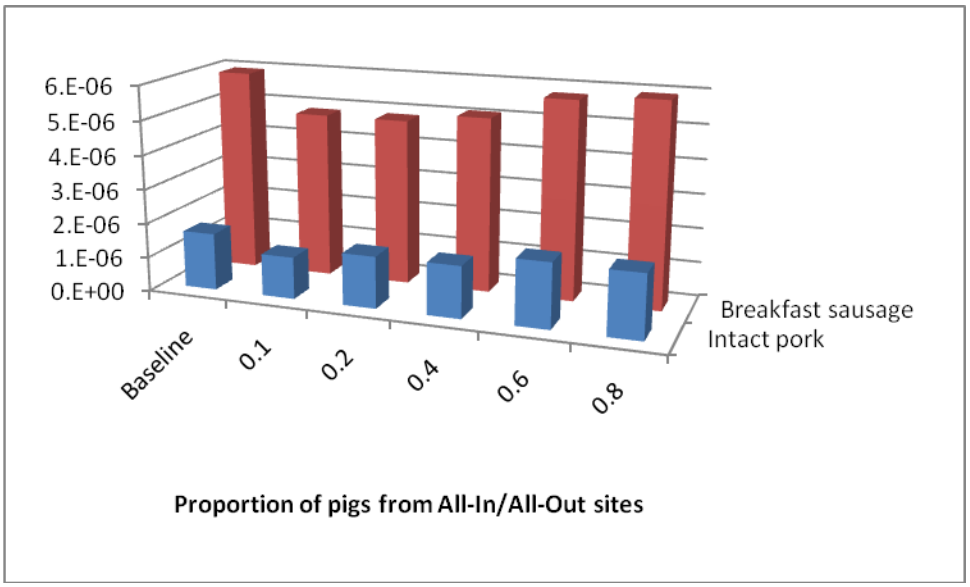

Figure 2. Risk of salmonellosis per serving as affected by the proportion of pigs raised in all-in/all-out sites, as opposed to continuous flow.

Objective 4: The fourth objective was analysis of the impact of on-farm prevalence of *Toxoplasma* on the risk of illness:

Figure 3 illustrates the effect of the prevalence of *Toxoplasma* infection among grower pigs on the risk per serving in intact meat, given two possible r-values for the dose-response. The majority of intact pork is derived from grower pigs, so this prevalence has a direct effect on the risk of illness.

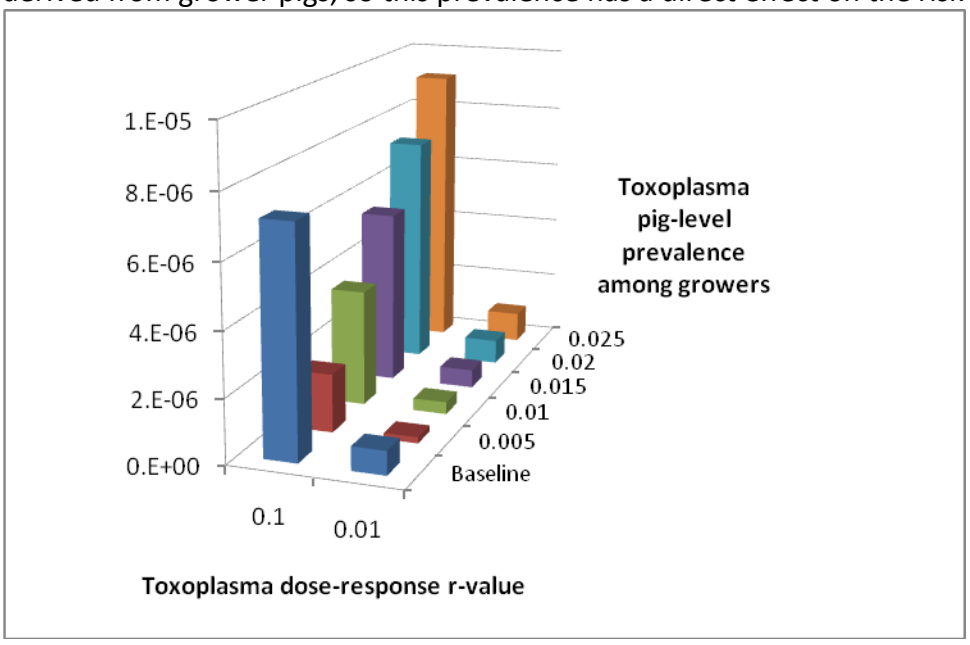

Figure 3. Risk of toxoplasmosis per serving of intact pork as affected by pig-level prevalence in growers and r-value.

Breakfast sausage contains more economical cuts of meat, including those from cull pigs and sows. The prevalence of *Toxoplasma* infection over all pigs influences the risk of illness in this product, as shown in Figure 4.

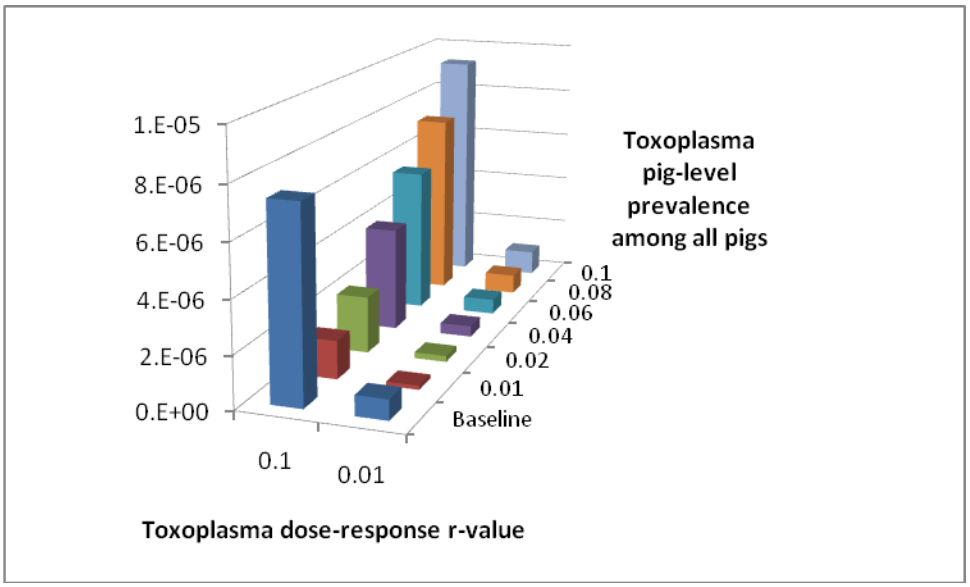

Figure 4. Risk of toxoplasmosis per serving of breakfast sausage as affected by pig-level prevalence among all pigs and r-value.

Discussion

Toxoplasma is not generally transmitted between pigs; instead it is usually contracted through consumption of oocysts in contaminated soil, or tissue cysts in infected small animals such as rodents or birds. This model therefore uses reported prevalence values for *Toxoplasma* among all pigs (52) to generate predictions of *Toxoplasma* prevalence for mixed meat, and the reported prevalence in growers (39) to represent pigs slaughtered for intact pork cuts.

Since the stages of slaughter are assumed to have no effect on the prevalence or level of *Toxoplasma* contamination in pork, the relationship between the on-farm prevalence and the ultimate risk-per-serving is a simple linear one, with prevalence among growers determining the risk per serving for intact pork cuts (Figure 3), and prevalence among all pigs (including sows) determining the risk per serving in sausage (Figure 4).

The dose-response relationship for *Toxoplasma* in humans is not known; we assumed an exponential dose-response and evaluated the risk per serving for two possible r-values. An r-value of 0.1 to 0.01 most closely represents a reported estimated infectious dose of less than 10^4 organisms (41) in (53), assuming 10^2-10^3 organisms per cyst (53). Using these r-values the model predicts between 3000 and 30,000 annual cases of toxoplasmosis from consumption of intact meat (and fewer from breakfast sausage). Published estimates suggest a total of 225,000 annual cases of which half are thought to be food-borne (29).

Although the results presented here are based on a concentration of cysts proposed by Dubey et al. (12) of less than 1 cyst per 50 g of muscle, Warnekulasuriya et al. detected levels of greater than or equal to $5x10^3$ bradyzoites per gram in cured meat. Assuming 10^2-10^3 organisms per cyst (53), that finding would be consistent with a level of at least 1 to 10 cysts per gram. In this case the predicted risk of toxoplasmosis per serving of intact meat would range from $3x10^{-4}$ to $2x10^{-3}$ and the risk per serving of breakfast sausage would range from $3x10^{-4}$ to $3x10^{-3}$, again depending on the r-value (Table).

Table 4. Risk of toxoplasmosis per serving of pork, as affected by estimate of cyst level in meat.

	Estimate for concentration of cysts/g			
	Uniform(0.01,0.02)		Uniform(1,10)	
r-value	Intact Pork	Breakfast Sausage	Intact Pork	Breakfast Sausage
0.1	8 E-06	7 E-06	2 E-03	3 E-03
0.01	8 E-07	7 E-07	3 E-04	3 E-04

The model simulates the *Toxoplasma* level and prevalence in fresh intact pork cuts and fresh breakfast sausage and does not take into account the possibility of cyst inactivation due to freezing, thus producing an estimated risk higher than is likely to obtain in actual products at retail. In addition, any pork treated (by heat or by freezing) to kill Trichinae may also be assumed to be free of infective *Toxoplasma* cysts (13). The addition of salt as in "enhanced" meat may also kill *Toxoplasma* cysts (18). Nearly half of retail pork contains salt (10).

Since *Toxoplasma* is not transmitted between pigs to an appreciable extent, the prevalence of *Toxoplasma* in pigs is determined largely by the age of the pig in concert with the number of opportunities to forage in soil, and access to small animals. In "total containment" type sites there are very few such opportunities, and 81% of pigs in the US are reared in this style of housing (49). The risk of toxoplasmosis from intact pork and from breakfast sausages is therefore presumed to be unaffected by the proportion of pigs that are raised in "all-

in/all-out" sites, but the risk can be reduced to the extent that exposure to soil and wild animals or cats is reduced at the site.

Salmonella does transmit between pigs, and so all-in/all-out sites represent disease transmission and recovery in a closed population. Under these conditions the prevalence soon reaches a steady-state, estimated by the model as 80%. In contrast to "continuous" sites in which each arriving cohort of pigs is separated from previous cohorts, the all-in/all-out model predicts a higher within-herd prevalence at slaughter, and so the risk per serving is directly related to the proportion of pigs raised in all-in/all-out sites, as seen in Figure 2. All-in/all-out sites have become more predominant in the industry of late, increasing from 56.9% of sites to 70.8% of sites between 2000 and 2006 (48;49).

The risk of illness due to sausage meat is influenced by the use of byproducts derived from viscera, the grinding process which distributes superficial contamination into the interior of the final product, and the inclusion of material from several carcasses in each batch of sausage meat. All three characteristics serve to increase the risk of salmonellosis from sausages relative to the risk from intact pork.

When sausage meat is cooked, pathogens distributed throughout the product may survive if the consumer fails to heat the product thoroughly enough to allow all areas of the product to reach the required temperature for inactivation. Parts of the product that are among the last to heat are called "protected areas" and represent potential refuges where pathogens can survive a cooking step. Figure 5 and Figure 6 illustrate the relationship between the probability of undercooking by the consumer and the proportion of pathogen in protected areas, in determining the mean risk per serving of sausage for *Salmonella* and *Toxoplasma*, respectively.

The model assumes a baseline proportion in protected areas of 0.01 to 0.05. A higher proportion might be expected in mixed meat due to the internalization of *Salmonella* organisms during the grinding and blending process, a higher proportion of fat and air, and other factors which may provide thermal protection. As an example of the impact of this assumption, the predicted risk per serving for salmonellosis from breakfast sausage could reach $1x10^{-4}$ when the proportion in protected areas is assumed to be 0.5, given the baseline probability of undercooking of 40% (Figure 5). Similarly, the risk of toxoplasmosis from fresh pork could reach at least $1x10^{-4}$ when the proportion of cysts in protected areas is assumed to be 0.5, and the probability of undercooking is 40% (Figure 6).

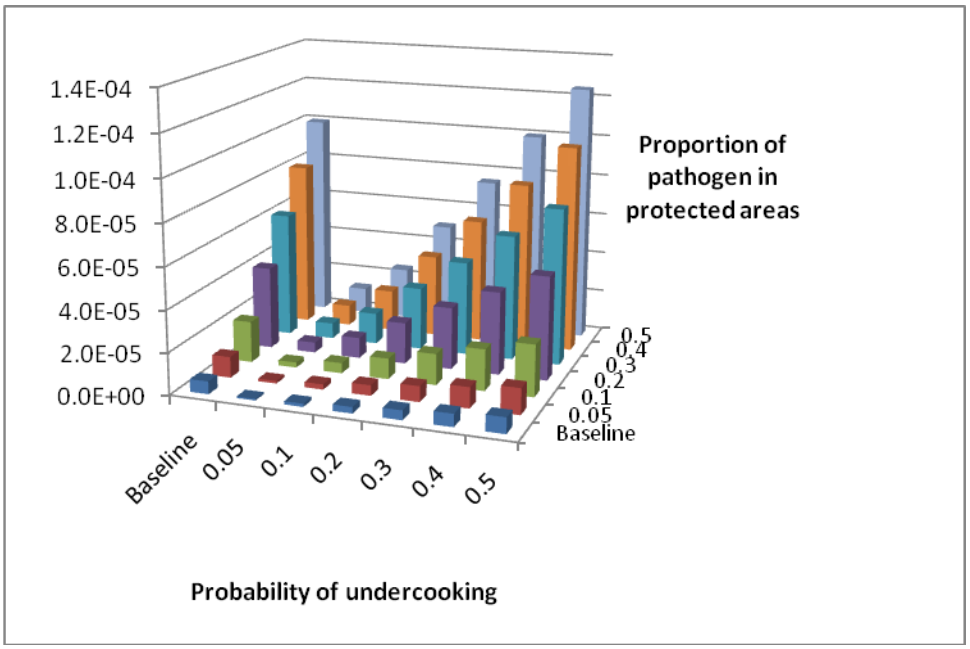

Figure 5. Risk of salmonellosis per serving of breakfast sausage as affected by the proportion of pathogen in protected areas and the probability of undercooking.

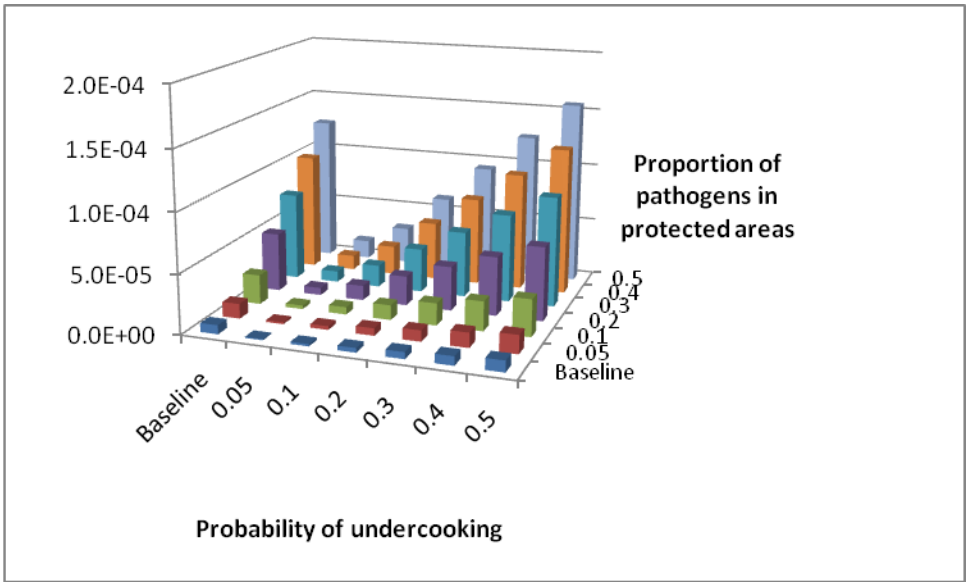

Figure 6. Risk of toxoplasmosis per serving of breakfast sausage as affected by the proportion of pathogen in protected areas and the probability of undercooking (r=0.1).

The carcass and viscera are treated with an antimicrobial wash post-evisceration. This will reduce the level of contamination in a batch of sausage meat in terms of *Salmonella*, but will have no effect on the level of *Toxoplasma* cysts, which reside within the muscle and so are not exposed to the wash solution. The risk of salmonellosis per serving can be reduced by increasing the washing efficacy or by reducing the probability of undercooking, as seen in Figure 7.

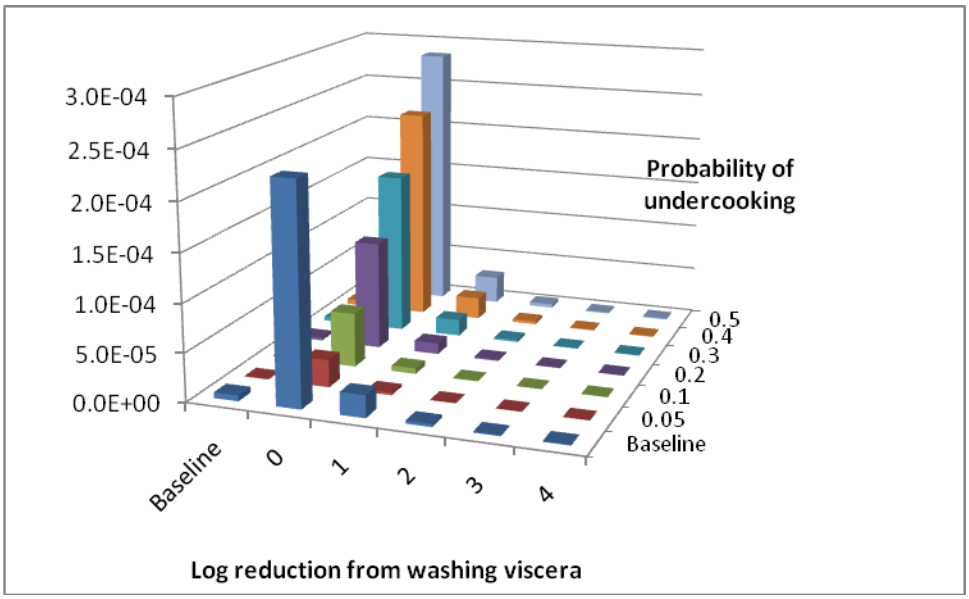

Figure 7. Risk of salmonellosis per serving breakfast sausage, as affected by the efficacy of washing viscera and the probability of undercooking.

For example, a four-fold reduction in risk per serving from breakfast sausage can be achieved either by reducing the probability of undercooking by the consumer to 10%, or by increasing the log reduction from washing of viscera to 3 log (Table).

Table 5. Risk of salmonellosis per serving of breakfast sausage, as affected by the log reduction from washing viscera and the probability of undercooking.

		Log reduction from washing viscera					
		Baseline	0	1	2	3	4
Probability of undercooking	Baseline	5.9E-06	2.2E-04	2.3E-05	3.4E-06	1.5E-06	1.2E-06
	0.05	7.3E-07	2.7E-05	2.9E-06	4.2E-07	1.8E-07	1.5E-07
	0.1	1.5E-06	5.5E-05	5.9E-06	8.4E-07	3.6E-07	2.9E-07
	0.2	2.9E-06	1.1E-04	1.2E-05	1.7E-06	7.3E-07	5.9E-07
	0.3	4.4E-06	1.6E-04	1.8E-05	2.5E-06	1.1E-06	8.8E-07
	0.4	5.9E-06	2.2E-04	2.3E-05	3.4E-06	1.5E-06	1.2E-06
	0.5	7.3E-06	2.7E-04	2.9E-05	4.2E-06	1.8E-06	1.5E-06

Data gaps and limitations of the work

This project addresses two of the deficiencies identified in the previous report, in that in considering all-in/all-out management it represents the pig-flow on the majority of grower/finisher farms in the US, and in that it considers the risk attributable to mixed meat. However, breakfast sausage is only one of many varieties of processed pork product, and the current model does not consider the impact of freezing, curing, enhancement solutions, irradiation, modified atmosphere packaging or other preservation methods. We do not consider cross-contamination from the pork product to other foods.

There is a lack of data in the literature reporting on the level of *Toxoplasma* cysts in the muscle of pigs. We used two divergent estimates (12;53) and simulated the resulting risk for each one. Similarly, no dose-response relationship has been elucidated for *Toxoplasma* infection in humans. We have attempted to address this by assuming an exponential dose-response relationship and including two different values for the

parameter “r”. The level of *Salmonella* in byproducts was based on only two sources. More research into the distribution and level of these two pathogens in pig tissues would be useful.

Managing the risk of toxoplasmosis at the population level is made particularly challenging due to the relative importance that may be placed on congenital toxoplasmosis given the severity and duration of the outcomes, e.g. lifetime institutional care due to severe mental retardation. In some situations reducing the rate of exposure to *Toxoplasma* may increase the rate of congenital toxoplasmosis due to an increased prevalence of seronegative pregnant women. This potential interaction has not been modeled in this work. Some consideration of this potentially elevated risk would ideally be included when contemplating interventions that would reduce the population’s exposure to *Toxoplasma*.

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