RESEARCH REPORT
PORK SAFETY

Title: Determination of seroprevalence to *Toxoplasma gondii* in the U.S. National swine herd - **NPB # 15-170**

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Industry Summary:

The objective of the study was to complete a national serological survey in market weight slaughter pigs and sows to determine the seroprevalence of *Toxoplasma gondii* and *T. spiralis* in these two populations comprising the national swine herd. We have established seroprevalence of these pathogens as measured in a statistically valid animal level sampling at slaughter in the 50 largest (by volume) plants for market hogs and in the 7 largest sow-only plants in the U.S. The survey encompassed 95% of slaughter production in the U.S., and now provides the most current national dataset for *T. gondii* and *T. spiralis* seroprevalence in market pigs destined for the fresh meat case and sows destined for processed meat products. We used sera collected at the slaughter plants and two commercially available, USDA validated ELISA assays to detect *T. gondii* and *T. spiralis* positive samples; all *T. gondii* ELISA positive samples were retested using the modified agglutination test (MAT). The survey provides a measure of the current national seroprevalence of *T. gondii* and *T. spiralis* infections in market pigs and sows, and provides evidence of the impact of industry-led changes in swine management on reduction of these zoonotic pathogens in the U.S. commercial pork

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supply. In 40,164 market hogs tested for the presence of antibody to *T. gondii*, seroprevalence was found to be 2.3%, while in 13,000 sows, seroprevalence was measured at 4.1%. Testing of the samples for the presence of antibody to *T. spiralis* using a commercial ELISA test kit detected no *Trichinella* positive samples in any of the 53,164 samples tested.

Keywords: *Toxoplasma gondii*, ELISA, seroprevalence, *Trichinella spiralis*, market hogs, sows.

Scientific Abstract:

The objective of the study was to complete a national serological survey in market weight slaughter pigs and sows to determine the seroprevalence of *Toxoplasma gondii* and *T. spiralis* in these two populations comprising the national swine herd. We have established seroprevalence of these pathogens as measured in a statistically valid animal level sampling at slaughter in the 50 largest (by volume) plants for market hogs and in the 7 largest sow-only plants in the U.S.

ELISA results indicated antibody to *T. gondii* was present in 2.3% of 40,164 sera (924 positive sera) from market weight hogs, and in 4.1% of 13,000 sera (533 positive sera) from sows. Sows were almost twice as likely to have antibody to *T. gondii* as market weight hogs. ELISA OD values in *Toxoplasma* positive samples from market weight hogs were above 0.8 OD units in 35% of positive samples, and 397 lots identified with one or more positive samples, resulting in a lot prevalence of 16.7%, and a median within lot prevalence of 11.4%. ELISA OD values in *Toxoplasma* positive samples from sows were above 0.8 OD units in 23% of positive samples, and 202 of 650 lots identified with one or more positive samples, resulting in a lot prevalence of 31% and a median within lot prevalence of 19.2%, significantly higher than that seen in the median within lot prevalence (11.4%) for market weight hogs ($p<0.05$).

No *Trichinella* positive samples were detected in any of the 53,164 samples tested, demonstrating the success of biosecurity measures that have been implemented by industry to eliminate this parasite from the U.S. swine herd.

Introduction: An overview of the researchable question and its importance to producers.

Perhaps the most widespread protozoan parasite affecting humans, *Toxoplasma gondii* infects virtually all warm-blooded animals, including humans, livestock, birds, and marine mammals (Dubey and Beattie, 1988; Hill et al., 2005). Infection in humans occurs worldwide;

however, prevalence varies widely from place to place. Toxoplasmosis is considered one of the Neglected Parasitic Infections, a group of five parasitic diseases that have been targeted by CDC for public health action. In the U.S., the rate of infection with *T. gondii* in humans appears to be declining, though the CDC estimates 1.1 million new infections occur each year. In the most recent serological survey (National Health and Nutrition Examination Survey, 2009-2010; Jones et al., 2014) involving over 7,000 people, 13.2% were positive for *T. gondii* antibodies, indicating infection with the parasite. Prevalence was 6.7% in persons 12-49 years of age and 9.1% in women of child-bearing age. In most adults, infection rarely results in clinical disease; however, there have been reports of focal ocular toxoplasmosis in otherwise healthy adults (Aramini et al., 1998, 1999; Jones et al., 2006; Phan et al., 2008; Wallace and Stanford, 2008). Congenital infection occurs when a woman becomes infected during pregnancy and transmits the parasite to the fetus. Congenital infections acquired during the first trimester are more severe than those acquired later in pregnancy (Desmonts and Couvreur, 1974; Remington et al., 2005). Serious disease can also result in immunosuppressed individuals, such as hose given large doses of immunosuppressive agents in preparation for organ transplants or those with acquired immunodeficiency syndrome.

Humans also become infected by ingesting food or water contaminated with sporulated oocysts from infected cat feces, or through ingestion of tissue cysts in undercooked or uncooked meat (Dubey and Beattie, 1988; Cook et al., 2000; Lopez et al., 2000; Tenter et al., 2000; Jones et al., 2009). Food animals, including pigs, become infected by the same routes, resulting in meat products containing tissue cysts, which can then infect consumers (Smith, 1993; Dubey et al., 1995a, 2005). Some estimates suggest that ingestion of undercooked or improperly cured meats is responsible for approximately one-half of all human infections (Mead et al., 1999). The risk of exposure to *T. gondii* in meats can be determined from prevalence surveys in food animals. In the U.S., various surveys for *T. gondii* infection have been performed in pigs. These have ranged from national surveys, including testing sera collected in the APHIS National Animal Health Monitoring Surveys (NAHMS), to regional

or convenience surveys. In a national survey funded by the USDA, Food Safety and Inspection Service in 1983 and 1984 (Dubey et al., 1991), 23% of market hogs and 42% of sows tested positive. Other sow-only surveys have reported infection rates of 22.2% (Dubey et al., 1995b), 36% (Assadi-Rad et al., 1995), 20.8% (Weigel et al., 1995), and 20% (Patton et al., 1996). More recent testing has focused on market hogs only. Testing of the NAHMS sera for the past four cycles has resulted in prevalence rates of 3.2% (NAHMS 1995, Patton et al., 1998), 0.9% (NAHMS 2000, Patton et al., 2002), 2.6% (NAHMS 2006, Hill et al., 2010), and 3.7% (NAHMS 2012, Hill, unpublished).

Toxoplasma prevalence is linked closely with the type of pork production system. Pigs raised in outdoor systems, such as "organic" production have very high rates of infection (Dubey et al., 2012). For example, a survey of 85 farms in the Northeastern U.S., where pigs were largely raised outdoors, found a prevalence rate of 47.5% (Gamble et al., 1999). On farms where very poor management was practiced, even higher rates were identified - 87.2% - in a 2002 survey in Massachusetts (Dubey et al., 2002) and 68.7% in a 2006 survey in Maryland (Dubey et al., 2008). Alternatively, pigs raised in confinement systems have low rates of infection. A survey of 58 production sites in northwestern Iowa, southwestern Minnesota, southeastern South Dakota, and northeastern Nebraska, in which 8434 pigs were tested, showed an overall prevalence of 0.14%, and the within-herd prevalence for infected herds was 1.2% (Gamble et al., unpublished). Of particular note, 52 of 58 production sites had no positive pigs and on 5 of 6 production sites, infection was eliminated by following some specific bio-security practices, demonstrating that raising pigs free from *T. gondii* infection is an achievable goal.

Toxoplasma ranks second, after *Salmonella*, in annual human disease burden caused by foodborne pathogens, and *T. gondii* in pork ranks second in commodity associated risks for pathogens (Batz et al., 2012). Toxoplasma infection results in 750 deaths each year, making it the second most common cause of death related to food-borne diseases (Scallan et al., 2011). There

are no tests currently available to detect *T. gondii* infection in food animals at slaughter. Therefore, consumers must follow recommended cooking methods to assure safety of meat relative to *T. gondii* just as would be the case for other potential pathogens. While there are no regulations that currently address *T. gondii* as a food safety issue, both FSIS and the European Food Safety Authority have studied this issue, and the Codex Alimentarius and the World Animal Health Organization (OIE) are considering including *T. gondii* in their respective codes.

For decades, pork has been implicated as the major vehicle for transmission of both *T. gondii* to humans in the U.S. Yet, no data currently exists documenting *T. gondii* transmission to humans via pork in the U.S. Industry changes in swine management have resulted in negligible levels of this zoonoses in conventionally raised swine. A recently developed serological assay which can identify oocyst-transmitted *T. gondii* infection in humans has demonstrated that pork, and meat in general, is a minor contributor to *T. gondii* transmission in humans. Development of current *T. gondii* prevalence data in market pigs will provide the industry with information documenting the low risk to humans of acquiring this parasite from U.S. pork and will support future claims of negligible risk for use in the export market. The data will inform policy makers on the effectiveness of industry-led animal management changes in biosecurity that have resulted in reduction of risk to domestic swine of infection with this parasite, and consequently reduction of risk to human health. This study provides data that clearly demonstrates the nationwide seroprevalence of *T. gondii* in market pigs and sows in the U.S. These data will facilitate recognition by consumers and regulators of the impact on public health of industry-driven changes in swine production which have led to the low risk of exposure to this parasite posed by consumption of pork raised under modern management conditions.

Although prevalence of *T. spiralis* in pigs raised in confinement is extremely low in the U.S., extraordinary procedures are required to market U.S. pork domestically and to international trading partners due to the perceived risk from *T. spiralis*. Domestically, pork supplies have never been subject to *T. spiralis* testing. However, pork products which are uncooked, considered ready to eat,

and have not been otherwise tested or treated are subjected to mandatory processes that are known to inactivate *T. spiralis*.

Currently, individual carcass testing for *T. spiralis* is required for shipping to European, Russian, and Asian markets. This testing is labor intensive and costly; more than 50 million pigs have been tested in this mandatory program since 1994, and no positive pigs have been detected. Recent changes in the Codex requirements for assuring freedom from *T. spiralis* in pork have provided alternatives to individual carcass testing based on the establishment of negligible risk compartments. The use of a negligible risk metric for *T. spiralis* infection in pigs was first established by a group of EU subject matter experts (Alban et al., 2011), and is currently used by the European Union in *T. spiralis* control legislation. The Codex standard states that one option for documenting "negligible" risk to consumers should be based on demonstrating, by surveillance testing, a *T. spiralis* prevalence of less than one infection per one million pigs with at least a 95% level of confidence. Modern pork production systems in the U.S. reduce consumer risk for exposure to *T. spiralis* from commercial production to negligible levels, and it is imperative for international competitiveness of the U.S. pork industry to establish a compliant surveillance program to meet Codex standards, increasing marketability of U.S. pork to international trading partners.

Objectives:

The objective of the proposed research was to establish seroprevalence of *T. gondii* and *T. spiralis* in the U.S. swine herd as measured in a statistically valid animal level sampling of market weight pigs and sows at slaughter. This survey covered 95% of slaughter production in the U.S. and provides the most current national dataset for *T. gondii* seroprevalence in market pigs destined for the fresh meat case and sows destined for processed meat products. The resulting data can be used to support science-based decisions on the most effective methods to continue the trend of decreasing seroprevalence of *T. gondii* in market pigs and sows, which are the primary livestock commodity known to be infected with *T. gondii,* and to demonstrate the industry-led elimination of *T. spiralis* from the national swine herd. These data demonstrate the extent to which the level of *T. gondii* and *T. spiralis* have been reduced in commercial pork as compared to previous surveys, and demonstrate the reduction of risk from *T. gondii* and elimination of risk from *T. spiralis* transmission from pork to humans in the U.S. This prevalence data will serve as a baseline against which future surveys can be performed, thus allowing the pork industry to assess progress in reducing prevalence of these pathogens in the U.S. pork supply.

Materials & Methods:

Serum samples were collected by centrifugation of whole blood from market swine and sows at the top 50 (by daily capacity) slaughter plants in the U.S. The serum samples were collected, with consent from plant personnel, from hog slaughter plants in 20 states, representing packers with daily capacity of 36,500 to 100 animals per day; this represented ~95% of the hog slaughter capacity in the U.S., and allowed rapid and economical collection of the over 50,000 serum samples required for a statistically valid study. Whole blood samples were transported to the Beltsville laboratory within 24 hours of collection and maintained frozen at -20^{o} until tested.

For the purposes of this study, commercial market weight hogs were defined as those slaughtered in plants listed in *Pork Quick Facts - Stats* (National Pork Board, 2014); market hogs were sampled since they are the source of most fresh pork consumed in the U.S., and therefore represent the greatest risk for transmission of *T. gondii*. Sows were defined as female breeding stock slaughtered in one of plants listed in *Pork Quick Facts - Stats*; sow only slaughter was verified by contact with the specific plant. To collect samples with as much independence as possible, sampling more than 20 pigs from single lots (i.e. from the same farm/location) was avoided by timing our sampling from each large plant to a morning and an afternoon sampling period, and by sampling smaller plants on non-consecutive days. Previous studies (NAHMS 2006, 2012 results) have shown that pigs from the same lot/farm that is *Toxoplasma* positive for at least one pig have a higher chance of being infected with *T. gondii,* the reason we allowed for correlation when calculating sample size. Smaller plants (<1000 hogs per day) were included in the sampling scheme, since these plants are more likely to accept hogs from small producers, which has been correlated with higher seroprevalence for *T. gondii*. Multiple lots were collected at each plant based on the daily slaughter capacity at the plant. Based on a U.S. annual slaughter capacity of nominally 139,750,040 market hogs, we collected and tested non-pooled samples from 40,164 market weight hogs and 13,000 sows using a non-stratified

sampling plan. Under a worse case scenario of 0.5 correlation of *T. gondii* seroprevalance within a lot, this allowed a precision of 1% in estimation of U.S. seroprevalence. The sampling resulted in collection of 53,164 individual sera and results from 3027 lots/premises.

Each sample was labeled with a unique numerical identifier for the plant, a lot (farm) number, and a within-lot sample number; specific information on the identity of the farm or farm location was not collected. Samples were tested in duplicate by ELISA for the presence of antibodies to *T. gondii* and for *T. spiralis* using two commercial kits as recommended by the manufacturer (SafePath Labs, California). Sera were diluted 1:50 for *T. gondii* testing and 1:200 for *T. spiralis* testing. Specific parasite positive and negative control sera were supplied by the manufacturer and were included on each ELISA plate. ELISA values were reported as the mean optical density (OD) of duplicate wells after subtraction of the OD for the negative control well. Optical densities in the two tests which exceed 0.3 after subtraction of the negative control OD were considered positive. The modified agglutination test (MAT) was used to confirm all *T. gondii* positive results by the method of Dubey, et al. (1995).

Results:

ELISA results indicated antibody to *T. gondii* was present in 2.3% of 40,164 sera (924 positive sera) from market weight hogs, and in 4.1% of 13,000 sera (533 positive sera) from sows. Sows were almost twice as likely to have antibody to *T. gondii* as market weight hogs.

TABLE 1

Toxoplasma gondii seroprevalence from slaughter survey

Sera source	Number Tested	% *T. gondii* positive	% Positive lots/ Median within lot prevalence
Market Weight hogs	40,164	2.3% (n=924)	(n=2377 total lots) 16.7% lots positive (n=397) 11.4% median within lot prevalence
Sows			(n=650 total lots)

	13,000	4.1% (n=533)	31% lots positive (n=202) 19.2% median within lot prevalence

ELISA OD values in *Toxoplasma* positive samples from market weight hogs were above 0.8 OD units in 35% of positive samples, and 397 lots identified with one or more positive samples, resulting in a lot prevalence of 16.7%, and a median within lot prevalence of 11.4% (Table 1). ELISA OD values in *Toxoplasma* positive samples from sows were above 0.8 OD units in 23% of positive samples, and 202 of 650 lots identified with one or more positive samples, resulting in a lot prevalence of 31% and a median within lot prevalence of 19.2%, significantly higher than that seen in the median within lot prevalence (11.4%) for market weight hogs ($p<0.05$).

All sera surveyed for antibody to *T. spiralis* were found to be negative.

Discussion:

The current study represents the most up to date information on seroprevalence of pigs and sows to *Toxoplasma gondii* and *Trichinella spiralis*, 2 parasites of significant food safety concern. Though serological surveillance of the national swine herd for *T. gondii* and *T. spiralis* infection has been conducted at 5-year intervals since 1990 in the National Animal Health Monitoring Survey (NAHMS), recent surveys have not included sows; breeding animals were last included in the survey in 2000.

Early serological surveys completed in the early 1990s documented very high levels of infection with *T. gondii* in both market weight pigs and in sows. Dubey, et al., 1991, documented seroprevalence in sows at 42%, and in market hogs at 23%. Management systems used for pig rearing have had a significant impact on *T. gondii* prevalence rates; numerous recent studies have shown seroprevalence rates have fallen to ~3.0% in market weight pigs raised in confinement, while the most recent sow survey in the NAHMS program in the year 2000 demonstrated a seroprevalence of 6% (Patton et al., 2002). Implementation of confinement rearing, an emphasis on facility biosecurity, and adherence to good production practices known to prevent exposure to *T. gondii* in pigs have greatly reduced the risk of exposure to *T. gondii* in confinement-raised pigs and sows.

The current data show a slowed but continued decrease in *T. gondii* seroprevalence in market weight pigs and sows, down to 2.3 and 4.1% respectively. These data indicate continued industry compliance with biosecurity protocols, but also suggest continued gaps or failures in biosecurity resulting in the low but measurable seroprevalence rates seen here. Previous studies have shown that strict adherence to barn biosecurity, specifically boot hygiene, can successfully eliminate *T.*

gondii from swine barns (Gamble et al., unpublished). The current study also indicates that seroprevalence rates are higher in sows than in market weight swine (4.1 vs 2.3%), and that co-located sows are more likely to be infected (31 vs 16.7% positive lots), and to have multiple animals in a lot infected with *T. gondii* than market weight pigs (19.2 vs 11.4%). These data are similar to findings in previous studies (Patton et al., 2002), and reflect the extended exposure time of sows to environmental contamination due to their longer life span in comparison to market weight pigs. However, since meat from sows is typically used for processed product that is frozen or subjected to other treatments that inactivate *T. gondii*, it is likely that the risk to consumers from sow meat is lower than the low risk posed by fresh meat products derived from market weight pigs. Dubey et al., 2005, demonstrated that the amount of viable *T. gondii* in fresh pork available for consumer retail purchase in the meat case (0.38%) was very low, and could not account for the annual incidence of *T. gondii* infection in humans in the U.S. Subsequent studies have demonstrated that most *T. gondii* infections in humans in the U.S. result from inadvertent consumption of oocysts released into the environment in cat feces (Hill et al., 2011; Boyer et al., 2011). Knowledge on the prevalence of *Toxoplasma* gained from this analysis can be used to develop industry initiatives (pre-harvest risk reduction programs) to reduce the risk of exposure of consumers to infected pork.

The elimination of *T. spiralis* as a food safety and public health risk in the U.S. resulted from coordinated industry and government changes that improved management systems used to house and raise pigs. Biosecure facilities have eliminated access of pigs to wildlife and the sources of *Trichinella* infection. As a result, as demonstrated here and in other nationwide surveys (NAHMS 2006, 20012, Hill, unpublished), *T. spiralis* has been virtually eliminated from the national swine herd. The elimination of this pathogen provides significant opportunity for removal of non-tariff trade barriers such as requirements for individual carcass testing for *T. spiralis* in order to gain access to export markets. The results of this survey provide additional evidence of the absence of this pathogen in the national swine herd, and strengthens the position of the U.S. in discussions to stablish a negligible risk compartment for *T. spiralis* for the U.S. swine herd.

Demands of consumers for pathogen-free meat products have focused the attention of the meat industry on food safety, and the necessity to produce meat that is wholesome, safe and of high quality. This survey provides baseline data for industry to continue development of herd level mitigations to reduce infection with *T. gondii* and to continue the successful elimination of *T. spiralis*.

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