

PORK SAFETY

Title: National Animal Health Monitoring Survey for *Toxoplasma gondii* and *Trichinella spiralis* – NPB #12-121

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Date Submitted: 2/5/2014

Industry Summary:

The United States Department of Agriculture (USDA) initiated the National Animal Health Monitoring System (NAHMS) in 1983 to collect, analyze, and disseminate data on animal health, management, and productivity in U.S. domestic livestock populations. The purpose of this study was to determine the national seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* in grower/finisher pigs using sera collected during the 5th national swine study (NAHMS 2012). Sera was collected during the voluntary survey of 202 grower/finisher swine production sites located in 13 states, accounting for ~90% of U.S. swine production (Iowa, Illinois, Indiana, Kansas, Minnesota, Missouri, Nebraska, North Carolina, Ohio, Oklahoma, Pennsylvania, South Dakota, and Texas). Sera and data on management practices at each surveyed site were collected beginning in July, 2012. Sera were analyzed for antibodies to *T. gondii* and *T. spiralis* using 2 commercially available kits. A total of 5,688 sera were tested. *Toxoplasma* seroprevalence was found to be 3.79%. A single sera was found to be positive for *Trichinella*; further investigation demonstrated that this sera was collected from a poorly managed farm where pigs were kept outdoors with potential access to wildlife. Previous studies have shown that increased risk of infection with *Toxoplasma* is associated with the presence of

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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domestic cats, feral cats, and wildlife, swine access to the outdoors, poor practices for disposal of swine carcasses, and the lack of barn-only boots in infected production sites. Increased risk of infection with *Trichinella* is known to be associated with access to wildlife and poor practices for disposal of swine carcasses. These results suggest that good production practices can be implemented to greatly reduce the risk of exposure to *Toxoplasma* and *Trichinella* in confinement-raised pigs, and that pigs with access to the outdoors are at greater risk for acquiring both *T. gondii* and *T. spiralis*.

Keywords: *Toxoplasma gondii*; Pigs; Good production practices; Food safety; Seroprevalence

Scientific Abstract:

The United States Department of Agriculture (USDA) initiated the National Animal Health Monitoring System (NAHMS) in 1983 to collect, analyze, and disseminate data on animal health, management, and productivity in U.S. domestic livestock populations. The purpose of this study was to determine the national seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* in grower/finisher pigs using sera collected during the 5th National Swine Study (NAHMS Swine 2012). Sera was collected during the voluntary survey of 202 grower/finisher swine production sites located in 13 states accounting for ~90% of U.S. swine production (Iowa, Illinois, Indiana, Kansas, Minnesota, Missouri, Nebraska, North Carolina, Ohio, Oklahoma, Pennsylvania, South Dakota, and Texas). Sera and data on management practices at each surveyed site were collected beginning in July, 2012. Sera were analyzed for antibodies to *T. gondii* and *T. spiralis* by 2 commercial ELISA assays (Hill et al., 2010a; Hill et al., 2010b); all positive sera were retested by ELISA or by Western blot. A total of 5,688 sera were tested. *Toxoplasma* seroprevalence, as determined by ELISA testing, was found to be 3.79%, with a herd prevalence of 29.70%. A single sera was found to be positive for *Trichinella*; further investigation demonstrated that this sera was collected from a poorly managed farm where pigs were kept outdoors with potential access to wildlife. Increased risk of infection with *Toxoplasma* is associated with the presence of domestic cats, feral cats and wildlife, swine access to the outdoors, poor practices for disposal of swine carcasses, and the lack of barn-only boots in infected production sites. Increased

risk of infection with *Trichinella* is known to be associated with access to wildlife and poor practices for disposal of swine carcasses. These results suggest that good production practices can be implemented to greatly reduce the risk of exposure to *Toxoplasma* and *Trichinella* in confinement-raised pigs, and that pigs with access to the outdoors are at greater risk for acquiring both *T. gondii* and *T. spiralis*.

Introduction:

Toxoplasma gondii and *Trichinella spiralis* are zoonotic parasites that can cause serious disease in humans. *Toxoplasma gondii* is a single-celled (protozoan) parasite, whereas *T. spiralis* is a nematode. Both parasites are found in muscle tissue and pose a risk to consumers of raw, undercooked or otherwise improperly prepared meat. A 1990 survey estimated the annual cost of toxoplasmosis in humans in the U.S. to be about 2.6 billion dollars (Roberts and Frenkel, 1990). In 1999, the Centers for Disease Control reported that up to half of human exposures to *Toxoplasma* could be from foodborne sources (Mead et al., 1999), causing consumer advocacy groups to point to meat as a source of infection resulting in birth defects in newborns. It is now estimated that 1,075,242 persons are infected with *T. gondii* each year in the U.S., and approximately 2,839 persons develop symptomatic ocular disease annually (Jones and Holland, 2010). Recent studies (Scallan et al., 2011) estimated that *Toxoplasma* caused 8% of hospitalizations and 24% of deaths resulting from foodborne illnesses. The cost of illness in the United States caused by *Toxoplasma* has been estimated to be nearly 3 billion dollars and an 11,000 quality-adjusted life year (QALY) loss annually (Batz et al., 2012; Hoffmann et al., 2012). Recent publications have linked suicide and schizophrenia to *Toxoplasma* infection (Pedersen et al., 2012; Torrey et al., 2012). Among the major food animals, *T. gondii* has been isolated more frequently from pigs than chickens or cattle. Research has demonstrated a declining, but still significant (2.75% during NAHMS 2006), infection rate for *T. gondii* in market-aged pigs, making pork a target of concern with respect to meat safety.

Consumers have long been aware of the potential presence of worms (*Trichinella spiralis*) in pork. The U.S. requires processing of all ready-to-eat pork products due to this parasite. Trading partners require testing of pork products prior to shipment, and the image of U.S. pork with respect to *Trichinella* infection impacts

accessibility to foreign markets as well as domestic consumption. Although the prevalence *T. spiralis* has declined significantly in the national swine herd and trichinellosis is a rare human disease in the U.S., the lack of any type of testing program, or documentation of the safety of pork is an impediment to both increased domestic consumption of fresh product and to international market access.

The objective of this project was to determine the seroprevalence of the zoonotic parasites *Toxoplasma gondii* and *Trichinella spiralis* in the national swine herd using sera collected during the 5th National Swine Study, NAHMS Swine 2012. Results will be tabulated to determine a national prevalence of *T. gondii* and *T. spiralis* in the U.S. swine herd.

Objectives:

The United States Department of Agriculture (USDA) NAHMS team conducts national studies on the health and health management of America's domestic livestock populations. The purpose of this study was to determine the national prevalence of *Toxoplasma gondii* and *Trichinella spiralis* in pigs using sera collected during the National Swine Study (NAHMS Swine 2012) conducted by the USDA/NAHMS. A statistically representative set of blood samples was collected from randomly selected swine operations in the top hog producing states of the U.S.; participation by producers was voluntary. Samples will be assayed by ELISA for detection of antibodies to *T. gondii* and *T. spiralis* using commercially available test kits. Putative positive samples will be retested to confirm results.

Materials & Methods:

Blood samples (5,688) were collected during the National Swine Study (NAHMS 2012) from randomly selected swine operations in 13 of the top hog producing states in the U.S. In addition, information on management practices was collected by state and federal Veterinary Medical Officers (VMOs) via on-farm

interviews. The 2012 National Swine Survey used a multiple frame sampling technique in 13 states. Producers were randomly selected using multiphasic sampling design in cooperation with the National Agriculture Statistics Service. Selected producers represented 89% of all swine farms with 100 or more pigs in the U.S. A detailed description of the sampling design, including the generation of weights to be used for estimation of the national population and the states surveyed, is described elsewhere (NAHMS, 1992; Bush, 1995; http://www.aphis.usda.gov/animal_health/nahms/swine/). Farrowing rooms were selected using probability proportionate to size methods. Finishers were randomly selected from pens for blood collection. A target number of 30 animals was sampled from each premises. Samples and questionnaire data was coded to protect the identity of the producer. Blood samples collected on-farm were shipped overnight to USDA's National Veterinary Services Laboratory. Serum was stored in 1 ml aliquots frozen at -40°C until used. Aliquots of sera were provided, in turn, to the USDA Animal Parasitic Diseases Laboratory under an inter-agency agreement. Serum samples were tested for antibodies to *T. gondii* and *T. spiralis* using commercially available ELISA kits as developed and validated in our laboratory. These kits employ a freeze-thawed *T. gondii* antigen and a *T. spiralis* excretory-secretory (ES) antigen, respectively. One-hundred microliters (100 μl) of the diluted serum sample (diluted 1:50 for *Toxoplasma* testing, and 1:200 for *Trichinella* testing in dilution buffer supplied by the manufacturer) was added to flat-bottom microtitration plates coated with antigen as described by the manufacturer and incubated at room temperature (25°C) for 10 minutes. Plates were washed 3 times with 200 μl of wash buffer supplied by the manufacturer. After washing, 100 μl of anti-pig enzyme conjugate was added to each well and incubated for 10 min at room temperature, followed by a second washing step as described above. Each well was washed 1 time with 200 μl of distilled water (the *Trichinella* test only); and 50 μl each of substrate solution (A and B) supplied by the manufacturer was added to each well, mixed well, and incubated for 10 minutes. Following the addition of 100 μl of stop solution to each well, plates were read on an automated microplate reader (Molecular Devices, Sunnyvale, CA) at 450/620 to 650 nm. Positive and negative swine serum controls were included on each plate. ELISA values were reported as the mean optical density (OD) values of duplicate wells after subtraction of the OD value for the negative control well. Optical densities which exceeded 0.30 after subtraction of the negative control OD value were considered positive. All sera

which exceed the cutoff value in the initial ELISA were retested by ELISA (*Toxoplasma/Trichinella*) and/or Western blot (*Trichinella*) to confirm results.

Results:

A total of 5,688 samples from 202 farms were tested for antibodies to *Toxoplasma* and *Trichinella*. Antibodies to *Toxoplasma* were detected in 216 of the samples (3.79% animal-level prevalence) collected from 60 farms. The number of positive samples on each farm was between 1 and 24. The mean within-herd prevalence was 12.68%. The 60 farms with one or more *Toxoplasma* positive samples resulted in an apparent farm-level prevalence of 29.7%. One-hundred forty two (142, or 70.29%) sampled farms had no *Toxoplasma* positive samples. A single sera was found to be positive for *Trichinella* (0.017%); further investigation demonstrated that this sera was collected from a poorly managed farm where pigs were kept outdoors with potential access to wildlife.

Discussion:

Toxoplasma gondii is transmitted to pigs by one of two ways – ingestion of meat containing tissue cysts, or by ingestion of infectious oocysts from cat feces or soil contaminated with cat feces. Possible sources of transmission of *T. gondii* to pigs include ingestion of *Toxoplasma*-infected rodents or wildlife, deliberate or accidental feeding of uncooked meat, or ingestion of oocysts contaminating feed, water, or environmental surfaces which may be orally contacted. Pigs raised in confinement barns, on concrete, and under good hygienic conditions would be expected to be at much lower risk of exposure to *T. gondii*, although specific studies of prevalence in confinement production systems are limited. Pigs managed in confinement systems which practice good rodent control, secured feed storage, use barn-only boots, and exclude cats and other wildlife typically are at lower risk of infection with *Toxoplasma*. Pigs reared in outdoor or non-confinement management systems, or where good hygienic practices are not followed, have substantially greater opportunities for exposure to infected rodents and wildlife, as well as to soil and other organic matter containing infectious oocysts.

Nematodes in the genus *Trichinella* are some of the most commonly recognized agents of foodborne parasitic disease. Human trichinellosis has historically been linked to the consumption of raw or undercooked pork or common game meats (e.g., bear, wild boar). *Trichinella* is transmitted from host to host by obligate carnivorousness. Historically, *Trichinella* infection in pigs was associated with the feeding of raw garbage. Exposure of domestic pigs to *Trichinella* is limited to just a few risk factors, especially deliberate or accidental feeding of animal tissues containing parasites, including living or dead rodents, wildlife infected with *Trichinella*, or cannibalism among pigs within an infected herd. When transmission of *Trichinella* to pigs does occur, a simple evaluation of farm management practices can determine the way in which pigs are becoming infected; feeding of any raw or undercooked meat scraps, including table waste, poses a risk of infection. Direct visualization testing of more than 50 million carcasses destined for export (<http://www.ams.usda.gov>) have documented virtual elimination of this parasite in confinement-raised domestic swine, resulting from improvements in animal management and feeding practices by the swine industry.

Animals tested in the NAHMS national surveillance studies (NAHMS 1995, 2000, 2006, 2012) represent a cross-section of the U.S. pork industry, and include all types of pork production systems. Results of these NAHMS surveys have demonstrated that good production practices (GPP) which reduce risk of swine infection with *T. gondii* and *T. spiralis* have been implemented industry-wide, and have resulted in greatly reduced risk of exposure to these parasites in confinement-raised pigs over the last 20 years. The regular surveillance provided by NAHMS using scientifically valid surveillance processes demonstrates the low level of infection in the U.S. swine herd. These data, coupled with industry compliance with GPP, supports acceptance of pork as a safe product by domestic consumers and trading partners. Benefits to U.S. pork producers gained from analysis of the 2012 NAHMS sera include knowledge on the prevalence of *Toxoplasma* and *Trichinella* and associated herd-level risk factors gained from analysis of the NAHMS swine studies. These data can be used as a model to develop additional industry initiatives, such as pre-harvest risk reduction programs, to reduce the risk of exposure of consumers to these and other zoonotic pathogens in pork. Information on the declining prevalence of these parasites can be used for education of consumers regarding the safety of fresh pork.

These data will continue the comparative measure of the prevalence of these two zoonotic parasites for which surveillance has been conducted under the NAHMS program since 1990. These data are critical to the implementation of effective control programs for toxoplasmosis and trichinellosis in domestic swine and for fact-based education of consumers, USDA regulatory agencies, and international trading partners concerning the safety of pork products.

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