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SEROPREVALENCE OF *TOXOPLASMA GONDII* IN MARKET HOGS COLLECTED FROM U.S. SLAUGHTERHOUSES

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KEY WORDS

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Sow
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ABSTRACT

Foodborne pathogens continue to pose a public health risk and can cause serious illness and outbreaks of disease in consumers. The consumption of raw or undercooked infected meat, such as pork containing infectious stages of *Toxoplasma gondii*, may be a major route of transmission to humans. Given the occasional presence of *T. gondii* in pork meat and the frequent use of pork for products not intended to be cooked, such as dry-cured ham, a potential risk exists for *T. gondii* transmission to consumers of these products. The purpose of this study was to determine the seroprevalence of *T. gondii* in U.S. market hogs and sows at slaughter. A total of 20,209 sera samples collected from 22 U.S. slaughterhouses, including 15 of the top 25 largest slaughter plants in the United States, were tested for *T. gondii* antibodies using a commercial ELISA assay. Seroprevalence in this study was 0.74%, with a herd prevalence of 10.86%. We compared seroprevalence of *T. gondii* in market hogs vs. sows from a separate but geographically similar set of slaughterhouse locations, with serum samples screened using the *T. gondii* modified agglutination test. This set of market hogs demonstrated 0% seroprevalence for *T. gondii*, while sows from geographically similar but separate slaughter facilities demonstrated a seroprevalence of 1.03%. Overall, both analyses show low seroprevalence of *T. gondii* in U.S market hogs and sows, respectively, and a marked drop in prevalence in market hogs and sows compared to previous studies.

While the production of safe and healthy products is one of the main objectives of the food industry worldwide, food products continue to be responsible for important outbreaks of disease in consumers. For example, toxoplasmosis has historically been linked to the consumption of raw or undercooked pork. Toxoplasmosis is a zoonotic disease that occurs worldwide and is caused by the protozoan *Toxoplasma gondii*. As reported by the U.S. Centers for Disease Control, toxoplasmosis is a leading cause of illness and death attributed to foodborne pathogens in the United States, with more than 40 million men, women, and children currently infected (https://www.cdc.gov/parasites/toxoplasmosis/index.html). *Toxoplasma gondii* has 3 infectious stages: tachyzoites, bradyzoites (in tissue cysts), and sporozoites (in oocysts) (Dubey et al., 1998). For example, *T. gondii* may be transmitted horizontally, which may involve any of the 3 life-cycle stages, i.e., ingesting infectious oocysts from the environment, ingesting bradyzoites contained in the meat of animals, or by exposure of eyes or broken skin to tachyzoites during butchering and processing of infected meat. Vertical transmission can occur when tachyzoites are transferred from a mother, who has ingested meat contaminated with *T. gondii*, to the fetus via the placenta (Tenter et al., 2000; Munoz-Zanzi et al., 2010). It is not known which route of transmission is more important epidemiologically. Nevertheless, the consumption of raw or undercooked contaminated pork meat may be a significant source of infection of *T. gondii* transmission to humans (Dubey, 2000; Tenter et al., 2000).

Key efforts in many countries for control of *T. gondii* and other foodborne parasitic hazards in pigs have focused on the elimination of the parasites from the human food chain through the implementation of strict biosecurity measures. In the United States, longstanding management practices and strict biosecurity have resulted in the elimination of *Trichinella spiralis* from the national swine herd and a significant reduction in seroprevalence of *T. gondii* in market weight hogs and sows (Weigel et al., 1995; Patton et al., 1996; Gamble, 1997; Jones et al., 2001; Gottstein et

Table I. Geographic location of slaughterhouses from which market hog samples were acquired for ELISA testing for seroprevalence of *Toxoplasma gondii*.

State (no. of plants)	Region	No. of samples tested	Daily slaughter volume
Pennsylvania (2)	Northeast	140	3,350
Virginia (1)	Southeast	880	10,400
North Carolina (2)	Southeast	2,060	37,800
Kentucky (1)	Southeast	920	10,000
Indiana (2)	Midwest	2,580	32,300
Iowa (8)	Midwest	9,200	101,500
Illinois (1)	Midwest	460	4,800
Missouri (2)	Midwest	2,900	10,300
Nebraska (1)	Midwest	960	10,400
Texas (2)	Southwest	109	2,000
Total	—	**20,209**	**221,050**

al., 2009; Hill et al., 2010b; Boyer et al., 2011; Hill and Dubey, 2013; Guo et al., 2015a, 2015b; Rostami et al., 2017; USDA–APHIS 2020). The objective of this study was to determine the current seroprevalence of *T. gondii* in market hogs and sows at slaughter in the United States. The seroprevalence data will serve as a baseline against which future surveys can be measured, thus allowing the pork industry and regulatory agencies to assess progress in reducing the prevalence of this pathogen in the U.S. pork supply.

MATERIALS AND METHODS

Sampled locations and serum collection

Samples were collected from 22 slaughterhouses, including 15 of the top 25 largest slaughter plants in the United States. The locations of slaughter plants from which samples were collected were recorded in GPS units standardized to World Geodetic System (WGS-84) datum, collected in decimal degrees. The longitude and latitude coordinates of the collection location were recorded for each collected sample. The 22 slaughterhouses sampled were divided into geographic regions based on designations applied by the U.S. Census Bureau (www.census.gov; Table I). The number of samples collected from each plant was determined based on the reported daily slaughter volume of the plant for the second quarter, 2014 (National Pork Board Website); the range of percentage of samples collected was between 4.2 and 28%, with an average of 10.9%, of the daily slaughter volume from each plant (Table I).

Within each plant, 20 samples were collected from each lot (defined here as an individual farm) of animals arriving at the plant on a single day. Each farm was sampled only once to prevent oversampling of individual farms. Whole blood (~50 ml) was collected from individual carcasses into sterile plastic 50 ml tubes on the kill floor at the slaughterhouses during exsanguination. Tubes were labeled with a unique subject ID to link the samples and corresponding results to the individual lot and slaughterhouse. Blood was allowed to clot for 5–10 min at ambient temperature before being placed in a cooler on ice and shipped overnight to the USDA Beltsville, Maryland, Laboratory. Blood was centrifuged upon arrival at 2,000 *g* for 10 min, and the serum was collected from the top of the tube. Collected serum was transferred to cryovials and frozen at –20 C until tested. Serum samples were obtained from a total of 20,209 pigs from 22 slaughterhouses in 10 states. Samples were collected from 5–7-mo-old market weight hogs of approximately 250 pounds/113.4 kg.

Ethical approval

No ethical approval was required for this study because no animals were infected or euthanized to carry out this study. These samples were convenience samples collected during humane slaughter operations.

Serological testing

Serum samples were tested for the presence of antibodies to *T. gondii* using a commercial ELISA kit as recommended by the manufacturer (SafePath Laboratories, Carlsbad, California). For the *T. gondii* ELISA, sera were tested at a 1:50 dilution (specificity [Sp] = 98%; sensitivity [Sn] = 88.6%). Specific parasite positive and negative control pig sera supplied by the manufacturer 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 for the *T. gondii* test that exceeded 0.30 after subtraction of the negative control OD were considered positive.

Comparison of *T. gondii* seroprevalence in market hogs vs. sows

Sera from a separate set of market hog and sow-only slaughterhouses were collected from 720 market hogs and 680 sows geographically matched by region as closely as possible in Minnesota, Virginia, Illinois, Iowa, Tennessee, and Wisconsin. Samples were collected from 5–7-mo-old market weight hogs of approximately 250 pounds/113.4 kg or sows >1 yr old. Samples were screened using the *T. gondii* modified agglutination test (MAT) as previously described (Dubey, 1997; Hill et al., 2006). MAT was used to compare titer levels between seropositive animals of both groups. MAT was completed using doubling dilutions of sera beginning at 1:25 and ending at 1:3,200. Samples were considered positive with agglutination at 1:25.

Statistical analysis

Optical density values were transformed using the Box-Cox series of transformations, with $\lambda = -0.3$ (slightly stronger than a log transformation), to produce a dependent variable with homogeneous variances across the farms and locations. A variance decomposition on the transformed values was generated using estimated variances from a linear mixed model with all factors random using the lme4 package in R (Bates, 2015; R Core Team, 2019). This allowed us to estimate the percent variance attributable to state, location (plant), lot (farm), and individual market hogs (residual). Since there was little variation that could be attributed to state and location, we also estimated an overall proportion positive and a 95% confidence interval based on the standard error from binomial distribution theory (sqrt (p [1 − p] / n)), as p ± 2 SE, where p = proportion of market hogs testing positive (OD > 0.3).

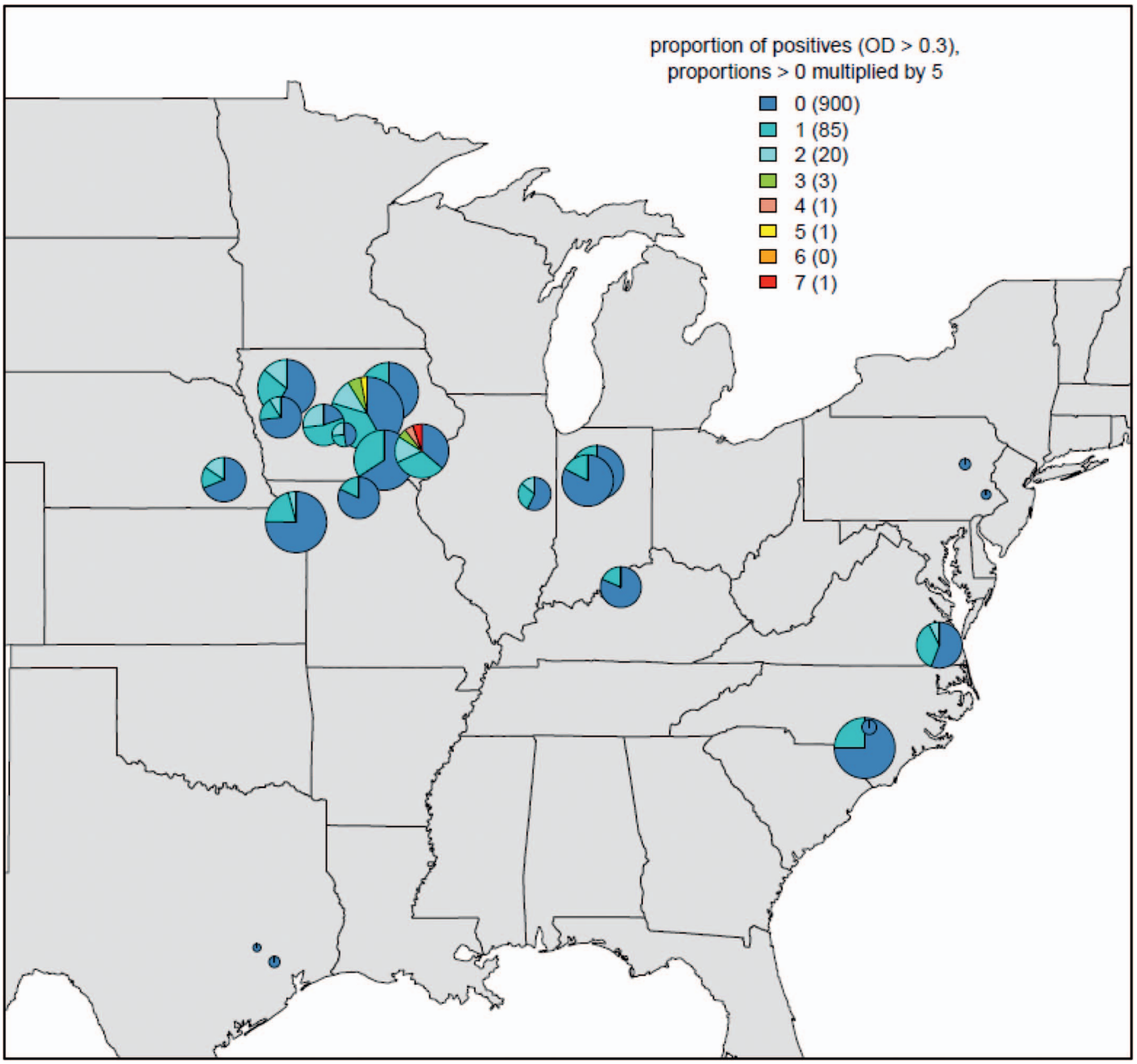

Figure 1. Map showing locations of 22 slaughterhouses from which samples were collected and proportion of positive lots from each slaughterhouse. Circle size indicates number of samples tested. The larger the circle, the more samples collected and tested.

RESULTS

A total of 20,209 samples from 22 slaughter plants were tested for antibodies to *T. gondii* by ELISA. Upon initial testing, antibodies were detected in 150 individual samples (Table II; 0.74% overall seroprevalence for individual animals [CI 95%; 0.621%, 0.863%], and 10.86% of lots had at least 1 positive pig). ELISA results yielded 900 lots with no positive pigs, 85 lots with 1 positive pig, 20 lots with 2 positive pigs, 3 lots with 3 positive pigs, 1 lot with 4 positive pigs, 1 lot with 5 positive pigs, and 1 lot with 7 positive pigs (Fig. 1; results mapped by slaughterhouse; note that some positive pigs were found throughout the area sampled). Of all the market weight pigs sampled, 99.26% were seronegative for *T. gondii*. Location and state accounted for only 18% of variability, while farm (LL) accounted for 48%, and unexplained variation, for 34%. The residual (unexplained) was the average amount of variation within a farm (pig-to-pig variability within a farm); on average, there was more farm-to-farm variation than there was variation within a farm.

MAT results using sera collected from market weight pigs and sows from separate slaughterhouses in Minnesota, Virginia, Illinois, Iowa, Tennessee, and Wisconsin revealed that all market

Table II. Seroprevalence of *Toxoplasma gondii* antibodies detected by ELISA in U.S. market hogs.

State (no. of plants)	No. of samples tested	Positive samples	% of positive samples
Pennsylvania (2)	140	0	0
Virginia (1)	880	7	0.80
North Carolina (2)	2,060	6	0.29
Kentucky (1)	920	2	0.22
Indiana (2)	2,580	13	0.50
Iowa (8)	9,200	103	1.12
Illinois (1)	460	4	0.87
Missouri (2)	2,900	9	0.31
Nebraska (1)	960	6	0.63
Texas (2)	109	0	0
Total	**20,209**	**150**	**0.74**

hog (Table III) samples were seronegative for *T. gondii*. However, 1.03% (CI 95%; 0.2971%, 1.7617%) of serum samples from sows were seropositive (Table IV), and all slaughterhouses where sow samples were collected had at least 1 seropositive sample (Fig. 2).

DISCUSSION

Inspection programs for the testing and detection of *T. gondii* in pigs at slaughter are nonexistent. In addition, there are no regulations mandating that pork undergo additional processing to inactivate the parasite, though recently published guidelines from the U.S. Food Safety and Inspection Service suggest methods for control of parasitic hazards in meat, including *T. gondii*. However, biosecurity and good production practices are crucial in pig farm management and play an integral role in lowering the probability of pigs becoming infected with *T. gondii*.

Serological surveillance of the U.S. national swine herd for *T. gondii* infection began in 1990 with the National Animal Health Monitoring System (NAHMS) survey, conducted by the Animal and Plant Health Inspection Service. The NAHMS surveys evaluate the health and management of the national animal herds and flocks, including swine, and are performed every 5 yr (https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/nahms/nahms swine_studies). The 1990 NAHMS survey sampled sows only; sera collected from 3,479 sows and tested for *T. gondii* infection revealed a seroprevalence of nearly 20% (Patton et al., 1996). In 1995, sow/breeder and grower/finisher populations were surveyed simultaneously, and seroprevalence of *T. gondii* was found to be 15 and 3.2%, respectively (Patton et al., 1996). In 2000, seroprevalence of *T. gondii* in tested sow/breeders was 6% and 0.9% in grower/finisher

Table III. Seroprevalence of *Toxoplasma gondii* antibodies detected by MAT in U.S. market hogs (5–7 mo old).

State (no. of plants)	No. of samples tested	Positive samples	% of positive samples
Iowa	200	0	0
Minnesota	200	0	0
Virginia	320	0	0
Total	**720**	**0**	**0**

Table IV. Seroprevalence of *Toxoplasma gondii* antibodies detected in U.S. sows (1–2 yr old)

State (no. of plants)	No. of samples tested	Positive samples	% of positive samples
Illinois	140	1	0.71
Tennessee	260	1	0.38
Wisconsin	280	5	1.79
Total	**680**	**7**	**1.03**

populations (Patton et al., 2002). The dramatic decline in *T. gondii* seroprevalence from survey years 1990 and 1995 to 2000 in the U.S. sow population likely resulted from large-scale movement of the swine industry towards total confinement rearing (80%) and an emphasis on facility biosecurity (Pyburn et al., 2005).

Due to the larger size of sows vs. market hogs, different equipment, and carcass breakdown methods are required for sows. Therefore, sows are typically slaughtered at separate plants from market hogs. As a result, sampling both populations from the same slaughter plant was not possible. MAT results showed that *T. gondii* antibody was not detectable in sera from sampled market hogs from specific slaughterhouses geographically matched by region to sow-only slaughter plants. However, sera collected from sows at geographically matched slaughter plants demonstrated a seroprevalence of 1.03%. The higher seroprevalence in sow populations, when compared to grower-finisher populations as seen in the current study, has been attributed to the longer lifespan of breeding pigs and consequent enduring risk of exposure to environments contaminated with infectious stages of the parasite (Patton et al., 2002; Dubey, 2009; Klun et al., 2011). There also may be potential biosecurity and management differences at farms housing sows and those housing market weight pigs, which are normally housed indoors from birth to slaughter.

In the current study (20,209 pigs tested), similar results (0.74% seropositive) were seen as in previous NAHMS surveys of grower/finisher swine, which are the source of most fresh pork consumed in the United States (Hill et al., 2010a), and are therefore the population of greatest concern for *T. gondii* transmission to consumers. Seroprevalence of *T. gondii* was 2.6% in the 2006 NAHMS survey of grower/finisher swine (6,238 tested pigs) and 0.8% in the 2012 NAHMS (3,923 tested pigs) in this same population. From 1995 through the 2012 NAHMS survey, and in the current study, seroprevalence ranged consistently between ~1 and 3%, likely resulting from lax enforcement of good production practices known to prevent exposure to *T. gondii* in confinement-reared pigs on a small number of individual swine production sites (Patton et al., 2002; Hill et al., 2010a; USDA–APHIS, 2018).

Data from the current study support this conclusion, since 89% of swine production sites (900) had no seropositive pigs, while of the 111 sites that had seropositive pigs, 105 had 2 or fewer pigs that were seropositive. Macrogeographic location was shown to be of less importance than individual farm identity since slaughter facilities from all sampled geographic locations drew pigs from a small number of farms that had seropositive pigs. In the current study, while most slaughterhouses processed some positive pigs, slaughterhouse location accounted for only 14.4% of the variance in seroprevalence (see the variance decomposition, Fig. 1); 48% of

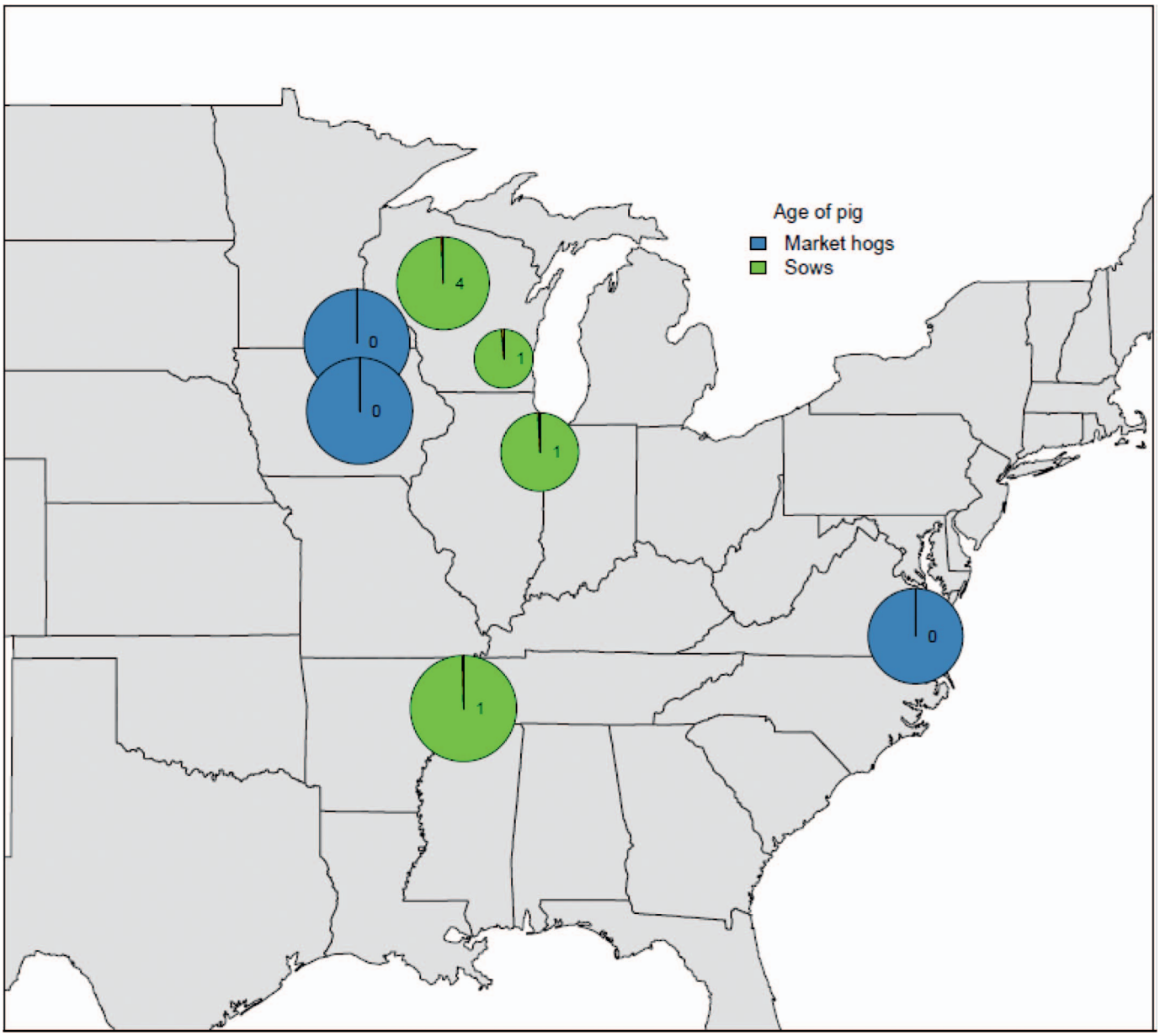

Figure 2. Map showing the 7 slaughterhouses from which market weight (5–7 mo old of age) and sow (female pigs >1 yr old) samples were collected. Circle size indicates number of samples tested. The larger the circle, the more samples collected and tested.

the explainable variance was due to farm-to-farm differences. These data suggest a relationship between farm identity and the presence of positive sera, and microgeographic/management conditions as causative in *T. gondii* infection in the grower/finisher population. The data also demonstrate that adherence to biosecurity measures that exclude *T. gondii* from production facilities is widespread, since 99.26% of tested pigs were seronegative, and that continued adherence to stringent biosecurity and management practices on U.S. swine farms is resulting in a low prevalence of infection with *T. gondii* on a small number of farms.

In the United States, *T. gondii*–infected pigs enter the food chain but are not able to be traced because there is no nationwide system or structure for identifying individual pigs other than the identification system that was established for animals enrolled in the *Trichinella* Certification Program (Pyburn et al., 2005; Hill and Dubey, 2013). This laboratory has shown that *T. gondii* bradyzoites can be inactivated during the processing of pork meat using methods previously approved and required for treatment of hams to inactivate *T. spiralis* in the U.S. Code of Federal Regulations (9 CFR 318.10) and now described in guidance documents from the U.S. Food Safety and Inspection Service (Fredericks et al., 2019, 2020).

In summary, the current study documents seroprevalence from swine entering the food chain from various geographic locations, including 15 of the top 25 largest slaughter plants in the United States. Prior NAHMS-based seroprevalence studies focused attention on seropositivity but more specifically on farm

production factors that influenced seropositivity. Demands of consumers for pathogen-free meat products have focused the attention of government regulators and the meat industry on food safety and the necessity to produce meat that is wholesome, safe, and of high quality (Hill et al., 2014). The current U.S. Farm to Fork Initiative (https://www.farmtoforkinitiative.org/) and European Commission Farm to Fork Strategy (https://ec.europa.eu/food/farm2fork_en) advocate the necessity to ensure and provide healthy, safe food. In this study, we show a substantial decrease in overall seropositivity consistent with these initiatives, which aim to increase food safety.

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