RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Serological Surveillance of Wild Boars for *Trichinella spiralis* – **NPB #08-216**

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Industry Summary:

Trichinella spiralis and *Toxoplasma gondii* are important zoonotic parasites, occurring in warm blooded animals and humans worldwide. Among domesticated food animals, domestic pigs and wild carnivores are hosts for *Trichinella*, while pigs, chickens, sheep, and goats are known to be infected with *T. gondii* at varying rates, depending on husbandry. Infections in wildlife with these parasites are generally higher than in domesticated species, however, infection rates in feral swine are unknown. Feral swine are attracted to and have direct contact with non-biosecure domestic swine, which presents opportunity for disease transmission. It is therefore important to determine the prevalence of *Trichinella* and *Toxoplasma* infection in feral swine to understand the risk of transmission of these parasites to domestic swine with which feral swine commingle. A serological survey was conducted to estimate the prevalence of *Trichinella* spp. and *Toxoplasma gondii* in feral swine in the U.S., and risk factors associated with infection. A total of 3262 serum samples were collected from feral swine in 32 states; results are reported from 26 states. Predictive maps based on environmental conditions were created for each parasite to highlight geographical areas with high probability for occurrence of infection. The overall seroprevalence of antibodies to *Trichinella* spp. and *T. gondii*, indicating infection, was 3.0% and 17.5%, respectively. A small proportion of feral swine (0.6 %) was seropositive for both parasites. No

significant difference in infection level between male and female swine was observed for either parasite. The seroprevalence for *T. gondii* infection was significantly higher in adults than in sub-adults or juveniles ($p<0.05$); this trend was not observed in *Trichinella* infected swine. *Toxoplasma* seropositive feral swine were widespread across the South and Midwest, and more restricted in the arid West. *Trichinella* infection was significantly higher in the South than in the Midwest, and higher in the Midwest than in the West region ($p<0.05$). The most probable distribution areas for both parasites are similar, concentrated mostly in the South and the Midwest regions of the U.S. It is concluded that feral swine pose a significant risk for introduction of *Trichinella* and *Toxoplasma* into domestic herds of non-biosecure domestic swine as a result of increasing overlap of the range of feral swine with non-biosecure domestic swine production facilities in the U.S.

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Scientific Abstract:

Trichinella spiralis and *Toxoplasma gondii* are important zoonotic parasites, occurring in warm blooded animals and humans worldwide. Among domesticated food animals, domestic pigs and wild carnivores are hosts for *Trichinella*, while pigs, chickens, sheep, and goats are known to be infected with *T. gondii* at varying rates, depending on husbandry. Infections in wildlife with these parasites are generally higher than in domesticated species, however, infection rates in feral swine are unknown. Feral swine are attracted to and have direct contact with non-biosecure domestic swine, which presents opportunity for disease transmission. It is therefore important to determine the prevalence of *Trichinella* and *Toxoplasma* infection in feral swine to understand the risk of transmission of these parasites to domestic swine with which feral swine commingle. A cross-sectional serological survey was conducted to estimate the prevalence of *Trichinella* spp. and *Toxoplasma gondii* in feral swine in the U.S., and risk factors associated with infection. A total of 3262 serum samples were

collected from feral swine in 32 states; results are reported from 26 states. Predictive maps based on environmental conditions using the maximum entropy (Maxent) approach to species distribution modeling were created for each parasite to highlight geographical areas with high probability for occurrence of infection. The overall seroprevalence of antibodies to *Trichinella* spp. and *T. gondii*, indicating infection, was 3.0% and 17.5%, respectively. A small proportion of feral swine (0.6 %) was seropositive for both parasites. No significant difference in infection level between male and female swine was observed for either parasite. The seroprevalence for *T. gondii* infection was significantly higher in adults than in sub-adults or juveniles ($p<0.05$); this trend was not observed in *Trichinella* infected swine. *Toxoplasma* seropositive feral swine were widespread across the South and Midwest, and more restricted in the arid West. *Trichinella* infection was significantly higher in the South than in the Midwest, and higher in the Midwest than in the West region ($p<0.05$). Species distribution modeling indicated that the most probable distribution areas for both parasites are similar, concentrated mostly in the South and the Midwest regions of the U.S. It is concluded that feral swine pose a significant risk for introduction of *Trichinella* and *Toxoplasma* into domestic herds of non-biosecure domestic swine as a result of increasing overlap of the range of feral swine with non-biosecure domestic swine production facilities in the U.S.

Introduction:

The U.S. feral swine population is estimated at 5 million animals and is growing rapidly (USDA, APHIS pub #799). Feral swine are now found in at least 36 states due to natural range expansion and illegal movement of animals for hunting opportunities. Feral swine are attracted to domestic swine facilities due to the presence of breeding sows, access to food resources, and commingling. As a result, localized populations of feral swine pose an increasing risk to non-biosecure domestic swine facilities by serving as reservoirs for pathogens which might be transmitted to domestic swine. *Trichinella* spp. and *Toxoplasma gondii* are important swine parasites worldwide which have been largely eliminated from domesticated swine in the U.S. Human disease in the case of *Trichinella* spp. results from ingestion of larvae in raw or undercooked meat (Gottstein et al., 2009); *T. gondii* infection results from congenital infection, accidental ingestion of oocysts in the environment, or from ingestion of tissue cysts in raw or undercooked meat (Dubey et al., 2005). Among domesticated food animals,

pigs are most commonly infected with *Trichinella* spp., while chickens, sheep, goats, and pigs are known to be infected with *T. gondii* at varying rates, depending on husbandry. Infection rates in wildlife with these parasites are generally higher than in domesticated species.

In the United States, feral swine are hunted for meat and often used to make cured products which would not involve cooking or freezing to inactivate *Trichinella* spp. muscle larvae and *T. gondii* tissue cysts. Despite the zoonotic potential of trichinellosis and toxoplasmosis, nothing is known of the prevalence of *Trichinella* spp. and *T. gondii* in feral swine in the U.S., and these animals frequently have direct contact with non-biosecure domestic swine, which presents opportunity for disease transmission (Wyckoff et al., 2009).

A cross-sectional, serological survey was conducted to determine seroprevalence of *Trichinella* spp. and *T. gondii* in feral swine in the U.S. In addition, a predictive map based on environmental conditions using the maximum entropy (Maxent) approach to species distribution modeling (Phillips et al., 2006) was created for each parasite to highlight the geographical areas with high probability for occurrence.

Objectives:

The objective of the proposed research is to establish a baseline of *Trichinella* prevalence in wild boar in the U.S. and determine the usefulness of an ongoing surveillance program as an adjunct to the U.S. Trichinae Certification Program. The information gathered will support ongoing equivalency discussions by providing accurate data on the very low prevalence of *Trichinella* in U.S. wildlife populations that pose a risk of *Trichinella* transmission to domestic hogs.

Materials & Methods:

Animals and sampled areas

Whole blood was collected from feral swine trapped or hunted in 32 states during the Comprehensive Feral Swine Disease Surveillance Program of the USDA's Animal and Plant Health Inspection Service, Wildlife Services unit (APHIS-WS) during 2006-2010. The main disease that drives feral swine surveillance is classical swine fever (CSF), and feral swine sampling targeted high risk areas based on potential entry pathways for CSF, such as international borders, and in areas near domestic swine production facilities, landfills, and high risk (backyard) swine producers. The 32 states sampled for feral swine were divided into West, Midwest, South,

or Northeast regions based on designations applied by the U.S. Census Bureau (www.census.gov). In the Program, the number of pigs targeted for collection was determined from the number of samples needed per year for point prevalence estimates for CSF based on the estimated population of feral pigs within each state. The location of collected animals was determined using GPS units standardized to World Geodetic System (WGS-84) datum, collected in decimal degrees. The longitude and latitude coordinates of the collection location, gender, and age class of the animals based on lower jaw tooth eruption criteria (incisor 2 absent=juveniles, less than 2 months old; incisor 2 erupted, deciduous canine=sub-adults, between 2 months and one year old; permanent canine=adults, over 1 year old) were recorded for each collected sample.

Whole blood was collected directly from the heart, clavicle well, or orbital sinus into serum separator tubes. Tubes were labeled with a unique subject ID to link the samples and corresponding results back to the individual pig. Blood was allowed to clot for 5-10 minutes at ambient temperature before being placed in a cooler. Blood was centrifuged within 12 hours of collection, and the serum sample was split and transferred to cryovials. Serum was refrigerated at 4°C and shipped within 3 days post-collection, or frozen at -20^{o}C for shipping within two weeks post-collection. Serum samples were accessioned into the National Wildlife Disease Program Feral Swine Tissue Archive, and stored at -80°C. Samples were periodically batched and shipped frozen to the ARS Animal Parasitic Diseases Laboratory, where they were kept frozen at -20°C until tested. Serum samples were tested in duplicate for the presence of antibodies to *Trichinella* spp. and *T. gondii* using 2 commercial ELISA kits as recommended by the manufacturer (SafePath Laboratories, Carlsbad, California). For the *Toxoplasma* ELISA, sera were tested at a 1:50 dilution; sera were diluted 1:200 for *Trichinella* testing. 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 in the *Toxoplasma* test which exceeded 0.20 after subtraction of the negative control OD were considered positive, while OD values in the *Trichinella* test which exceeded 0.3 after subtraction of the negative control OD were considered positive.

Species' distribution modeling

The maximum entropy method, Maxent 3.2.1 program for model building (Phillips et al., 2006), was used to model the *Trichinella* spp. and *T. gondii* geographic distribution in the U.S. Geographic range prediction maps for both parasites were produced using the same methodology as described by Masuoka and co-workers (2009).

Statistical analysis

The Chi-square test was used to investigate any significant relationship between various characteristics (gender, age, location) and parasitic infections and to assess the difference in prevalence within groups, using Epi Info software (version 3.5.1). Whenever an expected value was less than 5, the exact test probability was computed using the SISA tables program. A p value of <0.05 was considered significant.

Results:

Sera from feral swine in 12 states from the South region (TX, OK, FL, NC, VA, WV, TN, KY, AL, AR, MS, GA); 6 states from the Midwest (NE, IA, KS, MI, MO, OH); 5 states from the West (AZ, NM, CA, HI, CO); and 3 from the Northeast (NJ, PA, NH) were sampled and tested by ELISA (samples from 26 states). Sera from feral swine in 6 additional states were also sampled (Wisconsin, North Dakota, Nevada, Louisiana, Oregon, and New York), however feral swine populations in these states are localized or not well established, and/or too few samples were received; they were not included in the analysis. The overall seroprevalence in 3262 tested feral swine to *Trichinella* spp. and *T. gondii*, indicating infection, was 3.0% and 17.5%, respectively. A small proportion of feral swine (0.6 %) was seropositive for both parasites (Table 1).

Host factors associated with serological status are shown in Table 2. Feral swine sampled from southern states were more likely to be infected with *Trichinella*; the number of *Trichinella* seropositive feral swine was significantly higher in the South than in the Midwest, and higher in the Midwest than in the West region ($p<0.05$). Of the 98 sampled swine that were seropositive for *Trichinella*, 83 were found in the South region, 10 were found in the Midwest (6 in KS and 4 in MO), and 1 each was found in the West and Northeast (1 in NM and 1 in NH). Very high seroprevalence rates were found in several southern states, including Georgia (12.5%) and Virginia (10.3%). Seroprevalence in feral swine from North Carolina, one of the top hog producing states in the U.S., was also quite high (7.7%). Alabama, Florida, Texas, Oklahoma, Tennessee, Mississippi, and West

Virginia had *Trichinella* prevalence rates between 1.4 and 5.5%. Among the 12 southern states surveyed, only Kentucky had no *Trichinella* positive feral pigs, whereas only 4 of the 14 remaining states in the Midwest, West, and Northeast had *Trichinella* seropositive pigs.

Toxoplasma infection in feral swine was more widespread than *Trichinella* infection. Of the 26 states sampled and tested, only 3 (Kentucky, Colorado, and New Hampshire) had no *Toxoplasma* seropositive pigs. Other than Kentucky, all of the states in the South region had *Toxoplasma* seropositive swine (11/12). These 11 states had *Toxoplasma* seroprevalence rates ranging from 6.2% to 34.2%; every state with seropositive pigs other than Tennessee had prevalence rates over 12%. In the Midwest region, feral swine in 6 states were sampled and tested; all but 1 had *Toxoplasma* seroprevalence rates over 20%. In the West region, feral swine sampled and tested in 4 of the 5 states (CA, AZ, NM, CO) had *Toxoplasma* seroprevalence rates below 6%. This may reflect the hot, dry climate in these Western states which could adversely impact survival of *T. gondii* oocysts in the soil, a likely source of infection for feral swine. Only Hawaii had significant numbers of *Toxoplasma* seropositive feral swine (39.7%).

Too few animals were collected in the Northeast (NJ, PA, NH) to make meaningful comparisons with data collected from this region for either parasite.

The age class of the animals tested was found to be a significant factor for the presence of *T. gondii* infection; *Toxoplasma* prevalence was significantly higher in adult feral swine than in sub-adults or juveniles ($p<0.05$). This association was not observed in *Trichinella* infected swine. No significant differences in infection levels between genders were observed for either parasite.

The highest predicted probability of occurrence for *Trichinella* spp. infection in feral swine in the U.S. is concentrated in the Southeastern states, extending west to Texas. The most probable distribution of *T. gondii* includes the Southeastern states and extends further north into the Midwest region than *Trichinella* spp. There is a low probability of occurrence in the arid West and Rocky Mountain states for both parasites (Figures 1 and 2).

Collection sites of *Trichinella* positive feral swine indicate a close proximity with identified locations of pastured pig operations in the southeastern U.S. (Figure 3, adapted from Burke et al., 2008).

Discussion:

The *Trichinella* species and genotypes endemic in wildlife in the U.S. are encapsulated *T. murrelli*, *T. spiralis*, *T. nativa*, and *Trichinella* T6, (Masuoka et al., 2009), and the non-encapsulated *T. pseudospiralis*, which is the only *Trichinella* species documented in feral swine in the U.S. (Gamble et al., 2005). Available serological tests cannot distinguish *Trichinella* infections at the species level; therefore, the seroprevalence documented in the present study reflects infections due to all possible *Trichinella* fauna in feral swine. There is only one species in the genus *Toxoplasma*, therefore, seropositive animals can be presumed to be infected with *Toxoplasma gondii*. Both *Trichinella* and *Toxoplasma* persist in the tissues of infected pigs in an infective state for considerable periods of time (>1 year), and in the case of *Toxoplasma*, for the life of the host.

The seroprevalence of *Trichinella spp.* in feral swine varies worldwide, 0.11% in Slovakia (Hurníková and Dubinský, 2009), 0.2% in Switzerland (Frey et al., 2009), 0.77% in Spain (García-Sánchez et al., 2009), and 19.9% in Vietnam (Vu Thi et al., 2010). The *Trichinella* spp. seroprevalence of 3.0% in feral swine in the U.S. was 4 times higher than in feral swine of Europe. The reason for the higher *Trichinella* spp. seroprevalence in the U.S. compared to Europe may be related to the fact that in contrast to Europe, there has never been a *Trichinella* spp. control program in domestic pigs in the U.S. Most feral swine in the U.S. are descendants of escaped or deliberately released domestic swine, with some interbreeding with imported and released European wild boar.

The seroprevalence of *T. gondii* in feral swine also varies worldwide, 0.33% in Corsica (Richomme et al., 2010), 1.1% in Japan (Shiibashi et al., 2004), 4.5% in Brazil (Fornazari et al., 2009), 8.1% in Slovakia (Antolová et al., 2007), 19% in Austria (Edelhofer and Prossinger, 2009), 26.2% in Czech Republic (Bártová et al., 2006), and 38.4% in Spain (Gauss et al., 2005), as compared to the 17.5% in feral swine found in the U.S. *Toxoplasma* seroprevalence in the present study was higher in adult than in younger animals, indicating postnatal exposure and transmission of *T. gondii* (Dubey, 2009). Transplacental or transcolostral immunity does not play a role in the lower seroprevalence of younger animals since antibodies are not transmitted across the placenta and colostrum-derived antibodies disappear by three months of age (Dubey and Urban, 1990).

Seroprevalence of *Toxoplasma* in confinement raised domestic swine in the U.S. was recently reported to be 2.7% (Hill et al., 2008); seroprevalence in pastured pigs has been reported to be as high as 50-100% (Gamble et al., 2000).

In the prediction maps (Figs. 1 and 2), the most probable distribution areas for *Trichinella spp.* are located in the South, and for *Toxoplasma*, the South and Midwest, while the parasites are virtually absent in the sampled Rocky Mountain and arid Western states. The high seroprevalence of *Trichinella spp.* and *Toxoplasma* in feral swine observed in the South in comparison to the other regions is probably related to a number of factors: 1) the southern states currently host the vast majority of feral swine, and sampling targets were higher in these states, increasing the likelihood that seropositive animals would be sampled; 2) warm temperatures in the South promote prolonged survival of *Trichinella* larvae in tissues of dead animals and *Toxoplasma* oocysts in soil, which serve as sources of infection for feral swine; 3) the freeze resistance limits of both *Toxoplasma* and *Trichinella* (other than *T. nativa* and T6, which are minimally infective to pigs) and the prevailing low temperatures and arid climates of the other regions preclude the survival of the parasites in the tissues of dead animals and shortens oocyst survival in soil (Hershey and McGregor, 1987; Masuoka et al., 2009). Interestingly, 1 of 12 serum samples collected in Sullivan County, New Hampshire was positive for *Trichinella*; this location was the site of a *Trichinella* eradication effort on a game preserve where hunted imported wild boar were found infected with *Trichinella* (Worley et al., 1994).

Since feral swine are frequently the target of sport and game hunters, these groups are at risk when handling and consuming meat from these animals. Food-borne transmission of the parasites is an important route of infection particularly for people eating under-cooked or improperly processed meats. Care should be taken while butchering and handling raw meat to avoid infection with *T. gondii* because of the presence of viable, infectious organisms in the tissues of infected animals. Pregnant women should avoid contact with raw meat due to the risk associated with the presence of *T. gondii* infective stages. The stages of *T. gondii* present in meat are killed by contact with soap and water. To prevent infection of humans by *T. gondii*, thorough washing of hands after handling raw meat is essential. All cutting boards, sink tops, knives, and other materials coming in contact with uncooked meat should be washed with soap and water. *Trichinella* spp. larvae and *T. gondii*

organisms in meat can be killed by exposure to extreme cold or heat; meat from potentially infected animals should be heated throughout to 67°C for at least 4 minutes before consumption, or by cooling to -13°C for 3 days (Kotula et al., 1983, 1990; Gamble et al., 2000; Hill et al., 2009). Tasting meat while cooking or seasoning should be avoided. Adherence to good hygienic measures and safe handling and processing of meats appears to be the most practical and effective methods available to minimize transmission of *Trichinella spp.* and *T. gondii* to humans from game meats such as feral swine.

Feral swine also pose a significant risk for introduction of *Trichinella* and *Toxoplasma* into non-biosecure domestic swine as a result of increasing overlap of the range of feral swine with domestic swine production facilities in the South and Midwestern regions of the U.S. Rearing of pigs outdoors has been identified as a major risk factor for pig infection with both *Trichinella* and *Toxoplasma* due to increased exposure to potentially infected reservoir hosts, as well as exposure to oocyst contaminated soil in the case of *Toxoplasma* (Gamble et al., 2000, 2001; Pyburn et al., 2005; Hill et al., 2010). Consumer demand for 'organically raised', 'humanely raised' and 'free range' pork products has resulted in increasing numbers of hogs being raised in non-confinement systems (Honeyman et al., 2006). Swine producers have been recruited to produce animals for the organic market to fulfill a consumer demand that has increased 20% per year in sales since 1990 (Dimitri and Greene, 2002; http://www.ams.usda.gov/nop/). Though 'humanely raised' and 'free range' products have standards that are less stringently defined, outdoor access is also considered a requirement for labeling. These practices substantially increase the risk of exposure of pigs to *Trichinella* and *Toxoplasma*. Burke et al., (2008) identified pastured pig operations in the eastern U.S.; there is close proximity between some of these operations and the locations of collection spots for *Trichinella* and *Toxoplasma* positive feral swine identified in this study (Figure 3). The practice of field dressing hunted feral pigs and aerial hunting which leaves carcasses in the field should be discouraged, as feral swine carcasses and offals can serve as sources of infection for grazing domestic swine and for sylvatic carnivores that serve as reservoirs of infection for both parasites. Interactions between feral swine and domestic swine should be prevented. Increased surveillance efforts coupled with efforts to reduce or eliminate populations of feral swine should be applied in regions with

significant numbers of pasture raised pigs to prevent introduction of these parasites into domestic animals

destined for human consumption.

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Table 1. Number (n) of feral swine tested for infection with *Trichinella* spp. and/or *Toxoplasma gondii* and seroprevalence (%) of infection within states.

State	State sampling target	N	*Trichinella* spp.		*Toxoplasma gondii*		*Trichinella* spp. and *Toxoplasma gondii*	
			n	%	n	%	n	%
South								
Alabama*	25	79	3	3.8	17	21.5	1	1.2
Florida*	250	288	16	5.5	49	17.0	2	0.7
Kentucky	10	8	0	0.0	0	0.0	0	0.0
North Carolina*	100	182	14	7.7	25	13.7	6	3.3
Oklahoma*	200	425	6	1.4	83	19.5	2	0.4
Tennessee	50	48	2	4.1	3	6.25	0	0.0
Texas*	300	815	27	3.3	101	12.4	4	0.5
Virginia*	20	29	3	10.3	8	27.5	1	3.4
West Virginia	25	19	1	5.2	4	21.0	0	0.0
Arkansas	75	38	1	2.6	13	34.2	1	2.6
Mississippi	75	47	2	4.2	9	19.1	0	0
Georgia	100	88	11	12.5	11	12.5	3	3.4
Midwest								
Iowa	10	2	0	0.0	1	50	0	0.0
Kansas*	75	414	4	0.9	89	21.4	0	0.0
Michigan*	10	34	0	0.0	4	11.7	0	0.0
Missouri*	120	222	6	2.7	45	20.3	1	0.4
Nebraska*	10	20	0	0.0	4	20.0	0	0.0
Ohio	15	7	0	0.0	3	42.8	0	0.0
West								
Arizona	40	17	0	0.0	1	5.8	0	0.0
California	200	177	0	0.0	7	3.9	0	0.0
Hawaii	250	234	0	0.0	93	39.7	0	0.0
New Mexico	50	40	1	2.5	1	2.5	0	0.0
Colorado	10	8	0	0.0	0	0.0	0	0.0
Northeast								
New Jersey	25	7	0	0.0	1	14.3	0	0.0
Pennsylvania	30	2	0	0.0	1	50.0	0	0.0
New Hampshire	NA**	12	1	8.3	0	0	0	0
Total	2075	3262	98	3.0	573	17.5	21	0.6

Table 2. Number (n) of feral swine tested for infection with *Trichinella* spp. and/or *Toxoplasma gondii* and seroprevalence (%) of infection within gender, age, and region. Values significantly different ($p<0.05$) between groups are labeled with the same letter (a, b or c).

Factor	N	*Trichinella* spp.		*Toxoplasma gondii*		*Trichinella* spp. and *Toxoplasma gondii*	
		n	%	n	%	n	%
Gender							
Male	1058	19	1.8	190	18.0	4	0.4
Female	1045	20	1.9	189	18.1	5	0.5
Unknown	303						
Age							
Adult	1319	20	1.5	278	21.1[a,b]	7	0.5
Sub-adult	426	11	2.6	61	14.3[a]	2	0.5
Juvenile	376	8	2.1	45	12.0[b]	0	0.0
Unknown	285						
Region*							
Northeast	5	0	0.0	2	40.0	0	0.0
Midwest	609	6	1.0[b,c]	136	22.3[b]	1	0.2
South	1388	37	2.7[a,b]	213	15.3[a,b]	9	0.6
West	404	0	0.0[a,c]	89	22.0[a]	0	0.0
Total	2406	43	1.8	440	18.3	10	0.4

*Source: http://www.eia.doe.gov/emeu/reps/maps/us_census.html

Figure 1. Predicted probability of occurrence for *Trichinella* spp. infection in feral swine in the US.

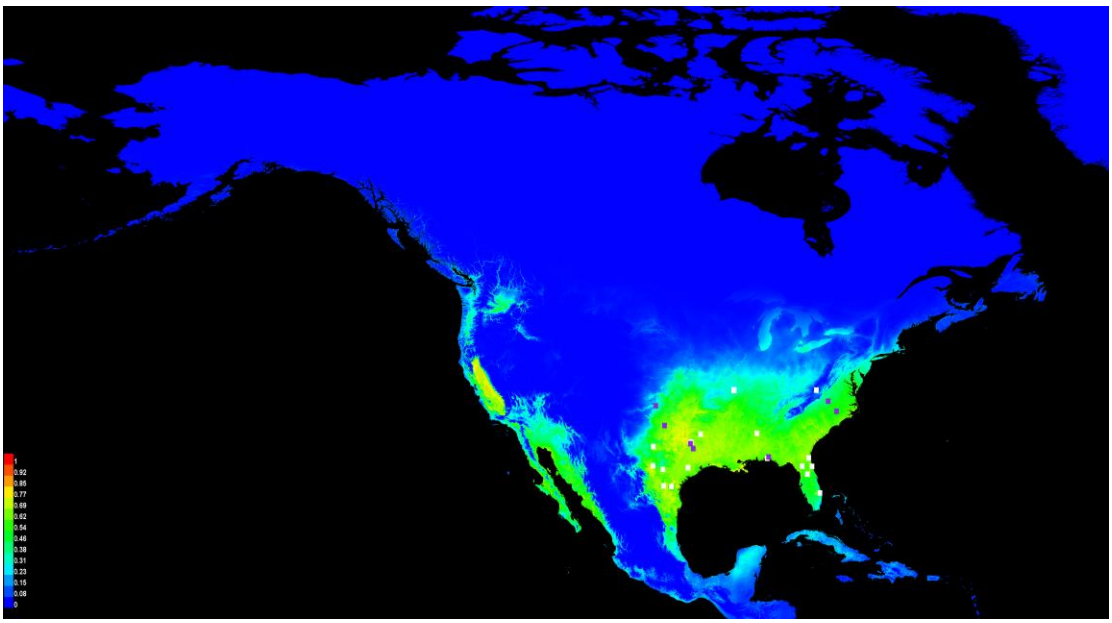


Figure 2. Predicted probability of occurrence for *Toxoplasma gondii* infection in feral swine in the US.

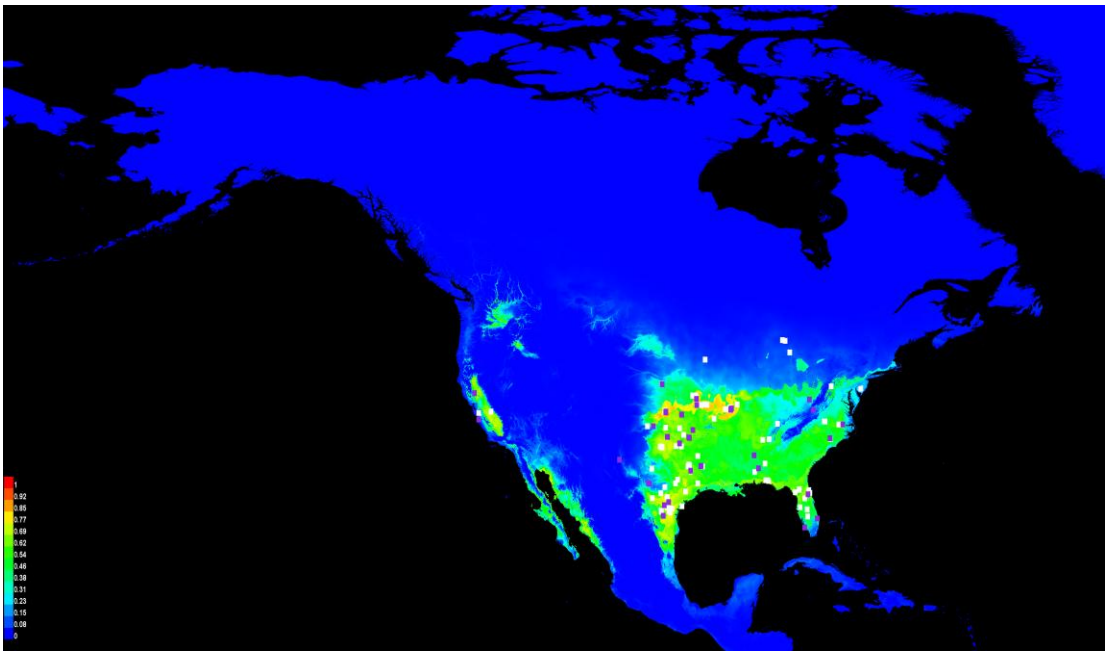
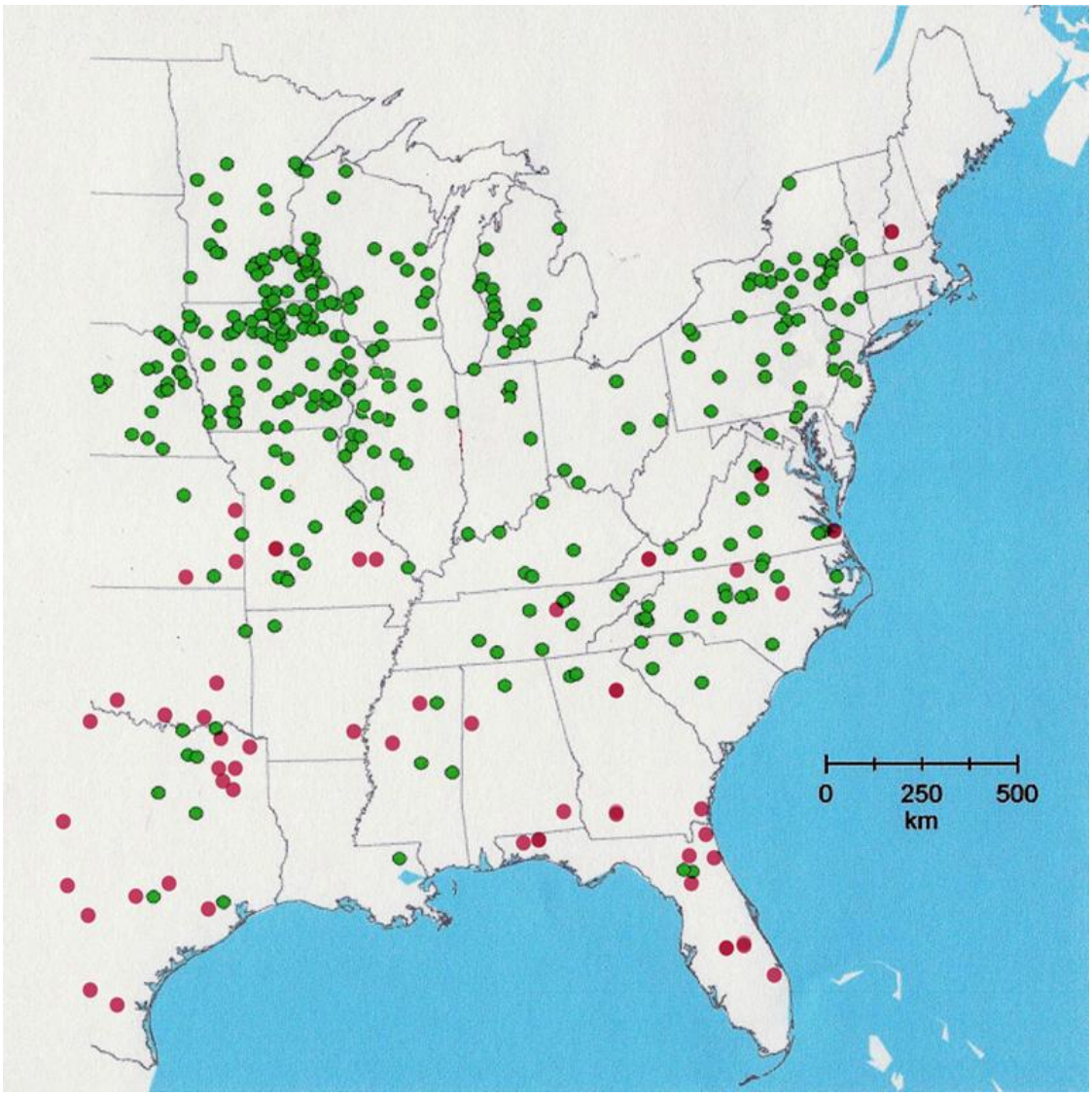


Figure 3. Locations of pastured pig operations (green) and *Trichinella* seropositive feral swine (red) in the southeastern U.S



Adapted from Burke, et al., 2008.