2014

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Veterinary Parasitology 205 (2014) 653–665

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Surveillance of feral swine for *Trichinella* spp. and *Toxoplasma gondii* in the USA and host-related factors associated with infection

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ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 23 July 2014
Accepted 25 July 2014

Keywords:
Feral swine
Geographic distribution
Risk factors
Spatial scan statistic
Toxoplasma gondii
Trichinella

ABSTRACT

Trichinella spp. and *Toxoplasma gondii* are important zoonotic parasites that infect warm blooded animals and humans worldwide. Among domesticated food animals, pigs are the main host for *Trichinella spiralis*. 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. Feral swine act as reservoirs of infection in the sylvatic ecosystem for *Trichinella* spp. and *T. gondii*, acting as sources of infection for peridomestic carnivores whose home ranges overlap with domestic pigs. Feral swine can have direct contact with non-biosecure domestic pigs, presenting opportunity for direct disease transmission through cannibalistic behavior. Determination of the prevalence of *Trichinella* spp. and *T. gondii* infection in feral swine is needed to understand the risk of transmission of these parasites to domestic pigs. A cross-sectional serological survey was conducted between 2006 and 2010 to estimate the antibody prevalence of *Trichinella* spp. and *T. gondii* and risk factors associated with infection in feral swine in the USA. Serum samples were tested from 3247 feral pigs from 32 states; results are reported from 26 states. Maximum entropy ecological niche modeling and spatial scan statistic were utilized to predict the geographic range and to examine clusters of infection of *Trichinella* spp. and *T. gondii* in feral pigs. The seroprevalence of antibodies to *Trichinella* spp. and *T. gondii* was 3.0% and 17.7%, respectively. Species distribution modeling indicated that the most probable distribution areas for both parasites was similar, concentrated primarily in the South and the Midwest regions of the USA. A follow up survey conducted during 2012–2013 revealed that 2.9% of 984 sampled feral swine were seropositive for

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http://dx.doi.org/10.1016/j.vetpar.2014.07.026
0304-4017/Published by Elsevier B.V.

Trichinella spp., and 28.4% were seropositive for *T. gondii*. Three hundred and thirty (330) tongues were collected from the 984 sampled animals during 2012–2013; 1.81% were tissue positive for *T. spiralis* muscle larvae; no other genotypes were found. The potential exists for introduction of these pathogens into domestic herds of non-biosecure domestic pigs as a result of increasing overlap of the range of feral pigs with non-biosecure domestic pigs production facilities in the USA.

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1. Introduction

The USA feral swine population is estimated at five million and is growing rapidly. Feral swine are now found in at least 39 states due to natural range expansion, accidental or intentional release, and illegal movement of animals for hunting opportunities (USDA, 2011, APHIS Bulletin # 2086). Feral swine approach domestic pig facilities that overlap their home range 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 pig facilities by serving as reservoirs for pathogens which might be transmitted to domestic pigs. *Trichinella* spp. and *Toxoplasma gondii* are important parasites worldwide which have been largely eliminated from domestic pigs in the USA (Pyburn et al., 2005; Hill et al., 2010). Human trichinellosis 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 (Gamble et al., 1999; Dubey et al., 2002; Hill et al., 2010). Infection rates in wildlife with these parasites are generally higher than in domestic species (Zarnke et al., 2000; Dubey et al., 2009; Aubert et al., 2010).

Despite the zoonotic potential of trichinellosis and toxoplasmosis, very little is known of the prevalence of *Trichinella* spp. and *T. gondii* in feral swine in the USA. Also of concern is the potential for direct contact of feral swine with non-biosecure domestic pigs, which has been documented, and which presents opportunity for disease transmission (Wychoff et al., 2009). Transmission of *Trichinella* and *T. gondii* has been observed to occur due to cannibalism in free-ranging pigs (Dubey et al., 1986a; Hanbury et al., 1986; Hill et al., 2010), and commingling of feral swine and non-biosecure domestic swine provides an ideal circumstance for this behavior to occur. Hunters commonly field dress feral swine, resulting in partial carcasses being left in the field and available for scavenging by free-ranging hogs (Giurgiutiu et al., 2009). In addition, some states allow aerial hunting of feral swine, leaving entire carcasses in the field for scavenging (Moarthland, 2011).

A cross-sectional, serological survey was conducted from 2006 to 2010 to determine the seroprevalence of *Trichinella* spp. and *T. gondii* in feral swine in the USA. 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 both parasites to highlight the geographical areas with high probability for occurrence. The spatial scan statistic (SaTScan) was utilized to investigate clusters of infections. A follow up study was conducted in 2012 through 2013 to assess continuing prevalence of *T. gondii* and *Trichinella spiralis*, and to attempt isolation and genotyping of muscle larvae from tongues collected from feral hogs to determine the species of *Trichinella* spp. circulating in these animals.

2. Material and methods

2.1. Animals and sampled areas

Samples were collected from feral swine trapped or hunted in 32 states according to guidelines developed by USDA's Animal and Plant Health Inspection Service, Wildlife Services (Wildlife Services, 2008, USDA-APHIS-WS, NWDP, Comprehensive Feral Swine Disease Surveillance Procedure Manual) during 2006–2010. The main disease that drives feral pig surveillance is classical swine fever (CSF), and feral pig sampling targeted high risk areas based on potential entry pathways for CSF, such as international borders, and in areas near domestic pig production facilities, landfills, and high risk pig producers (small producers of non-contract animals for personal use or limited distribution). The 32 states sampled for feral pigs were divided into West, Midwest, South, or Northeast regions based on designations applied by the USA 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 (Fig. 1). 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 two months old; incisor #2 erupted, deciduous canine = sub-adults, between two months and one year old; permanent canine = adults, over one year old; Matschke, 1967) 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 min at ambient temperature before being placed in a cooler. Blood was centrifuged

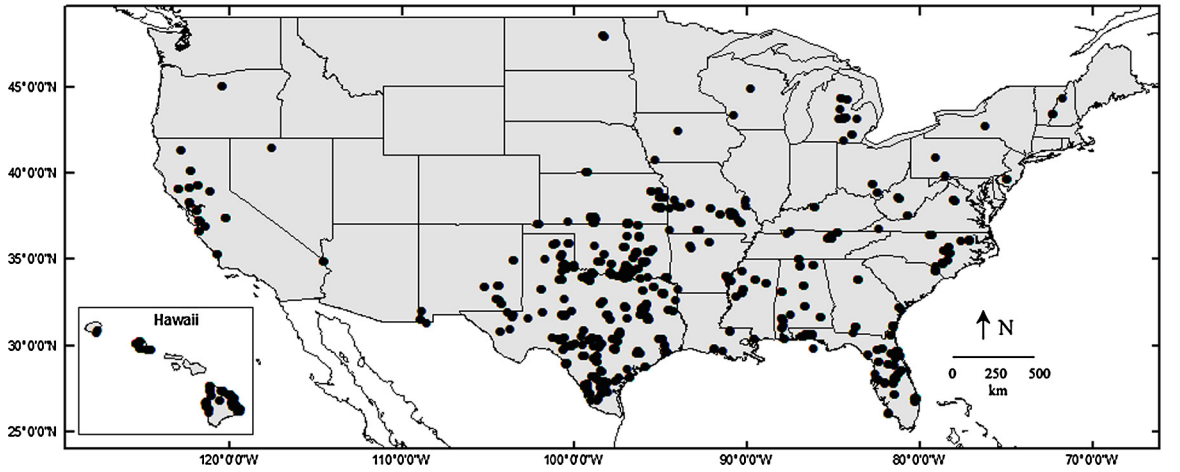

Fig. 1. Sampled areas for feral swine in 32 states in the USA.

within 12 h of collection, and the serum sample was split and transferred to cryovials. Serum was refrigerated at 4 °C and shipped within three days post-collection (p.c.), or frozen at −20 °C for shipping within two weeks p.c. Serum samples were accessioned into the National Wildlife Disease Program Feral Swine Tissue Archive, and stored at −80 °C. Samples were periodically batched shipped frozen to the Agricultural Research Survey (ARS) Animal Parasitic Diseases Laboratory, where they were stored frozen at −20 °C until tested.

2.2. Testing

Serum samples were tested in duplicate for the presence of antibodies to *Trichinella* spp. and *T. gondii* using two commercial ELISA kits as recommended by the manufacturer (SafePath Laboratories, Carlsbad, CA, USA). For the *Trichinella* ELISA, sera were diluted 1:200 (specificity (Sp), 99.6%; sensitivity (Sn), 98.4%); for the *T. gondii* ELISA, sera were tested at a 1:50 dilution (Sp = 98%; 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 *Trichinella* test which exceeded 0.30 after subtraction of the negative control OD were considered positive, while optical densities for the *T. gondii* test which exceeded 0.20 after subtraction of the negative control OD were considered positive.

2.3. 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 USA. Geographic range prediction maps for both parasites were produced using the same types of environmental data and ecological niche modeling as previously described (Masuoka et al., 2009; Koum et al., 2010).

WorldClim version 1.4 climate data (Hijmans et al., 2005) were obtained from the WorldClim website (http://www.worldclim.org). WorldClim provides global gridded climate data with a high spatial resolution of about 1 km^2 in a form appropriate for mapping and spatial ecological modeling in a GIS or with other computer programs. Input data were gathered from a variety of sources primarily between 1950 and 2000 period to obtain the best possible spatial representation since this significantly increased the number of records for some areas. The WorldClim database comprises means for each month for precipitation, and minimum and maximum temperatures. The data are further processed into a series of bioclimatic variables which have an approximate spatial resolution of 1 km and were used for this project.

Elevation data were also downloaded from the WorldClim website. WorldClim processed this data set from NASA Shuttle Radar Topography Mission (SRTM) data to have the same projection and resolution as the other WorldClim layers.

Land cover data were downloaded from the United States Geological Survey's (USGS) Global Land Cover Characteristics Database, version 2 Global (http://edc2.usgs.gov/glcc/glcc.php). The land cover data were developed from 1-km Advanced Very High Resolution Radiometer (AVHRR) satellite data from April 1992 to March 1993. This high resolution dataset is still widely used in studies of global change, in climate, weather, and ecological modeling, biodiversity assessment, fire hazard and other issues, and is used in many current ecological modeling studies as a reference land cover dataset. Land cover data are available in multiple classification schemes; the Global Ecosystems land cover classification, which contains 100 classes, was used for the modeling work.

Presence locations for both parasites were obtained by matching the positive ELISA tests from the serological survey with the coordinates of the location of the corresponding animal.

2.4. Spatial cluster analysis

The presence of clusters was investigated using the spatial scan statistic as described by Kulldorff (1997) and

implemented in SaTScan software, version 8.0 (www.satscan.org). The analysis was performed under the Bernoulli probability model, using test-positive animals as cases and test-negative animals as controls. The maximum cluster size was set as 5% and 20% of the population at risk for *Trichinella* and *T. gondii* infection respectively, based on the prevalence observed in the present study. The significance of cluster was assessed using Monte Carlo hypothesis testing (999 simulations). The most likely cluster was considered significant at 5% and the secondary cluster at 10%, as the secondary clusters have conservative *p*-values (Kulldorff and Nagarwalla, 1995; Odoi et al., 2004).

2.5. 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.

2.6. Follow up survey

From 2012 through 2013, a follow up cross-sectional survey was conducted by testing serum and tissue samples collected from an additional 984 feral swine trapped or hunted in 26 states during the Feral Swine Disease Surveillance Program (APHIS-WS) described above to continue monitoring of seroprevalence to the two parasites and to determine the species of *Trichinella* circulating in feral swine. In the follow up survey, sera were tested for antibodies to *T. gondii* by the modified agglutination test (MAT; (Sp = 90.29%; Sn = 82.9%; Dubey et al., 1995)) as described by Dubey and Desmonts (1987). Initially sera were screened at 25, 50, 100, and 200 dilution; seropositive samples were titrated further to 3200. Sera were also tested for antibodies to *T. spiralis* using the commercially available kit described above. In addition, tongues were collected from 330 of the feral swine to attempt isolation of *Trichinella* spp. from muscle larvae for genotyping to determine the species of *Trichinella* circulating in feral swine. Tongue collections were concentrated in counties of states where feral swine were identified in 2006–2010 as having high seroprevalence to *Trichinella* to maximize the chances of successful larval isolation. For isolation of muscle larvae, the mucosal surface of the entire tongue was removed using a sharp knife, and the remaining muscle was coarsely chopped before being ground using a table top grinder (Magic Bullet, Homeland Housewares, Los Angeles, CA, USA). The ground meat was placed in a 500-1 L beaker (depending on the size of the tongue). Digest solution was prepared by adding granular pepsin (1:10,000 U/mg; American Laboratories Inc., Omaha, NE, USA) to 45 °C acidified water (1% HCl)) to a final pepsin concentration of 1%. Digest solution was added to the beaker containing the ground tongue sample at 10 times the volume of the ground meat along with a teflon-coated stir bar, and the beaker was covered with Parafilm to prevent evaporation. The beaker was then placed on a magnetic stirrer with stirring to create a vortex, and digested for 45 min at 45 °C in a floor model incubator (model RI40, Sheldon Mfg., Cornelius, OR, USA).

Following digestion, the samples were each poured through a #45 copper sieve (355 μm mesh) into a 300 ml Pilsner-style (conical bottom) glass. Samples were individually washed and settled in the Pilsner glasses two times in warm (37 °C) tap water to remove debris and clarify the suspension. Each washing and settling step was carried out for 30 min. After the last settling step, the Pilsner glasses were gently poured off, leaving ~15–20 ml of sediment in the bottom of the glass. This remaining sediment was poured into a square, gridded Petri dish, and allowed to settle for 1 min, then examined using a dissecting microscope for the presence of muscle larvae. All muscle larvae observed in each sample were collected into a 1 ml microfuge tube using a 20 μl pipette (Eppendorf) and frozen at −80 °C until processed for genotyping. All muscle larvae from each sample were genotyped by PCR for determination of the species composition by the method of Zarlenga et al. (1999). Amplification of the internal transcribed spacer (ITS) region of the ribosomal DNA using a set of multiplex primers produces a characteristic amplicon (173 bp) in *T. spiralis* that differentiates it from all sylvatic species extant in North America, and produces amplicons from *T. murrelli* of 127 and 316 bp, *Trichinella pseudospiralis* of 310 and 330 bp, *Trichinella nativa* and T-6 of 127 bp, differentiating them from each other. Amplicons were visualized using an eGene HDA-GT 12 Genetic Analyzer; molecular weight was calculated using the installed Biocalculator software (eGene, Inc., Irvine, CA, USA).

2.7. Ethics

All experiments were performed in accordance to protocols approved by the United States Department of Agriculture.

3. Results

3.1. General consideration

From 2006 to 2010, sera from feral swine in 12 states from the South (Alabama (AL), Arkansas (AR), Florida (FL), Georgia (GA), Kentucky (KY), Mississippi (MS), North Carolina (NC), Oklahoma (OK), Tennessee (TN), Texas (TX), Virginia (VA), West Virginia (WV)); six states from the Midwest (Iowa (IA), Kansas (KS), Michigan (MI), Missouri (MO), Nebraska (NE), Ohio (OH)); five states from the West (Arizona (AZ), California (CA), Colorado (CO), Hawaii (HI), New Mexico (NM)); and three from the Northeast (New Hampshire (NH), New Jersey (NJ), Pennsylvania (PA)) were sampled and tested by ELISA (totalling 26 states). Sera from feral swine in six additional states were also sampled (Louisiana (LA), North Dakota (ND), New York (NY), Nevada (NV), Oregon (OR), and Wisconsin (WI)); however feral swine populations in these states are localized or not well established, and too few samples were received; they were not included in the analysis. The overall seroprevalence in 3247 feral swine to *Trichinella* spp. and *T. gondii*,

Table 1
Seroprevalence of *Trichinella* and *T. gondii* in feral swine in the USA by state.

State	State sampling target	*N*	Trichinella spp.		*Toxoplasma gondii*		Trichinella spp. and *Toxoplasma gondii*	
			n	%	*n*	%	*n*	%
South								
Alabama[a]	25	79	3	3.8	17	21.5	1	1.3
Florida[a]	250	288	16	5.6	49	17.0	2	0.7
Kentucky	10	8	0	0.0	0	0.0	0	0.0
North Carolina[a]	100	180	15	8.3	25	13.9	6	3.3
Oklahoma[a]	200	425	6	1.4	83	19.5	2	0.5
Tennessee	50	47	2	4.3	5	10.6	1	2.1
Texas[a]	300	814	27	3.3	101	12.4	4	0.5
Virginia[a]	20	29	3	10.3	8	27.6	1	3.4
West Virginia	25	18	1	5.6	4	22.2	0	0.0
Arkansas	75	38	1	2.6	13	34.2	1	2.6
Mississippi	75	47	2	4.3	9	19.1	0	0.0
Georgia	100	82	11	13.4	11	13.4	3	3.7
Regional total	1230	2055	87	4.2	325	15.8	21	1.0
Midwest								
Iowa	10	2	0	0.0	2	100	0	0.0
Kansas[a]	75	413	4	1.0	89	21.5	0	0.0
Michigan[a]	10	34	0	0.0	4	11.8	0	0.0
Missouri[a]	120	223	7	3.1	45	20.2	1	0.4
Nebraska[a]	10	20	0	0.0	4	20.0	0	0.0
Ohio	15	7	0	0.0	3	42.9	0	0.0
Regional total	240	699	11	1.6	147	21.0	1	0.14
West								
Arizona	40	17	0	0.0	1	5.9	0	0.0
California	200	176	0	0.0	7	4.0	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	7	0	0.0	0	0.0	0	0.0
Regional total	550	474	1	0.0	102	21.5	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[b]	12	1	8.3	0	0.0	0	0.0
Regional total	55	21	1	4.8	2	9.5	0	0.0
USA total	2075	3247	98	3.0	576	17.7	22	0.7

Number (*n*) of feral swine tested for infection with *Trichinella* spp. and/or *Toxoplasma gondii* and seroprevalence (%) of infection within states.
[a] State sampling target was reached.
[b] State sampling target not set due to low numbers of feral swine in state.

indicating infection, was 3.0% and 17.7%, respectively. A small proportion of feral swine (0.7%) were seropositive for both parasites (Table 1).

There was a significant correlation between the collection location of feral swine and infection with *Trichinella* and *T. gondii*. Feral swine sampled from southern states were more likely to be infected with *Trichinella* (Fig. 2); the number of *Trichinella* seropositive feral pigs was significantly higher in the South than in the Midwest, and higher in the Midwest than in the West region ($p < 0.05$ in both cases). Of 98 feral swine that were seropositive for *Trichinella*, 85 were collected in the South, 11 were identified in the Midwest (four in KS and seven in MO), and one each was detected in the West and Northeast (one in NM and one in NH). Very high seroprevalence rates were found in several southern states, including GA (13.4%) and VA (10.3%). Seroprevalence in feral swine collected in NC, was also quite high (8.3%). *Trichinella* seroprevalence rates were between 1.4% and 5.6% in AL, FL, MS, OK, TN, TX, and WV. Among the 12 southern states surveyed, KY was the only state with no *Trichinella* positive feral swine, whereas only four of the 14 remaining states in the Midwest, West, and Northeast had *Trichinella* seropositive pigs.

T. gondii infection in feral pigs was more widespread than *Trichinella* infection (Fig. 3). Of the 26 states included in the analysis, only three (CO, KY, and NH) had no *T. gondii* seropositive pigs. Other than KY, all of the states in the South region had *T. gondii* seropositive pigs (11/12). These 11 states had *T. gondii* seroprevalence rates ranging from 10.6% to 34.2%; every state with seropositive pigs other than TN had prevalence rates over 12%. In the Midwest region, feral swine in six states were tested; all but one had *T. gondii* seroprevalence rates over 20%. In the West region, feral swine tested from four of the five states (AZ, CA, CO, NM,) had *T. gondii* seroprevalence rates below 6%. Of the Western states, Hawaii was the only state with significant numbers of *T. gondii* seropositive feral pigs (39.7%).

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

Host factors associated with serological status are shown in Table 2. Gender was associated with *Trichinella*

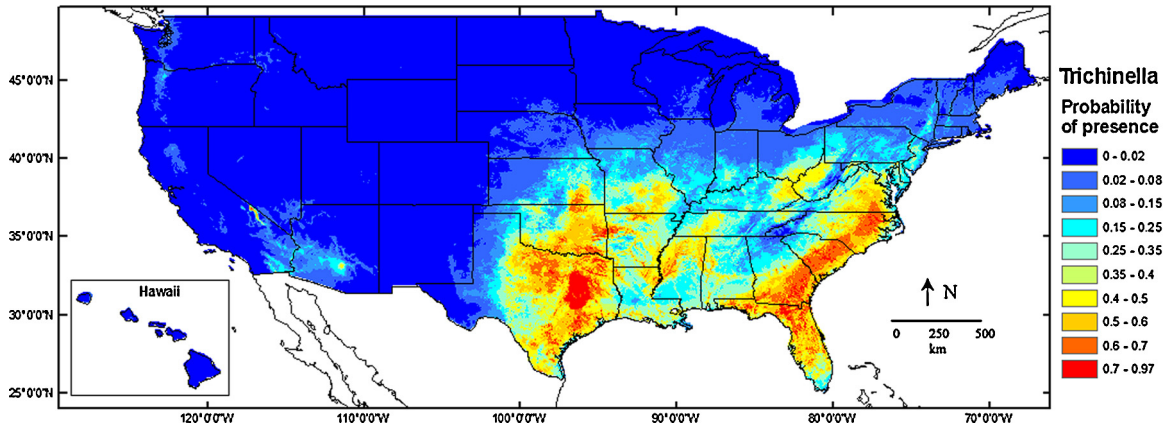

Fig. 2. Predicted probability of occurrence for *Trichinella* spp. infection in feral swine in the USA.

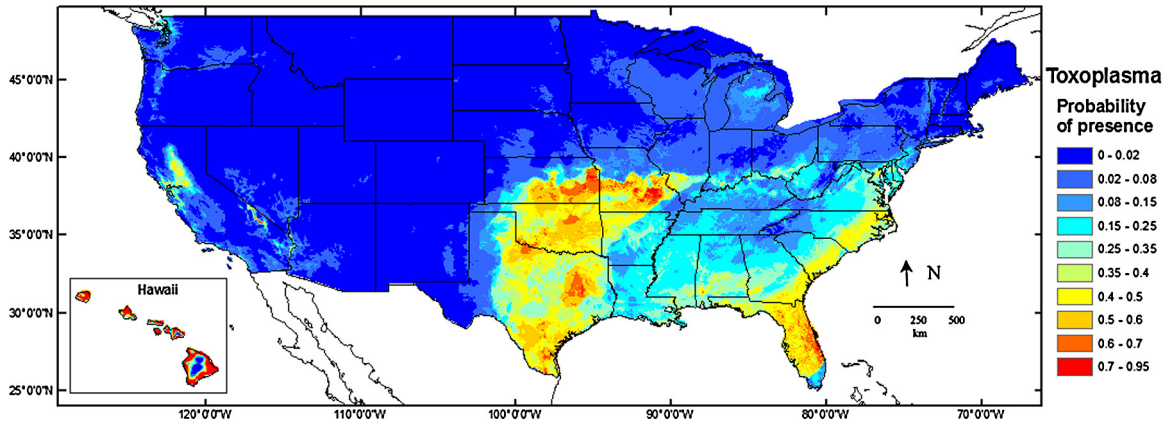

Fig. 3. Predicted probability of occurrence for *T. gondii* infection in feral swine in the USA.

Table 2
Risk factors and geographic variables associated with seroprevalence of *Trichinella* and *T. gondii* in feral swine in the USA.

Factor	*N*	Trichinella spp.		*Toxoplasma gondii*		Trichinella spp. and *Toxoplasma gondii*	
		n	%	*n*	%	*n*	%
Gender							
Male	1427	36	2.5[b]	252	17.7	7	0.5
Female	1516	58	3.8[b]	263	17.3	14	0.9
Unknown	304						
Age							
Adult	1947	54	2.8[b]	388	19.9[b,c]	17	0.9
Sub-adult	583	30	5.1[b,c]	80	13.7[b]	2	0.3
Juvenile	432	10	2.3[c]	52	12.0[c]	2	0.5
Unknown	285						
Region[a]							
Northeast	21	1	4.8	2	9.5	0	0.0
Midwest	699	11	1.6[c]	147	21.0[c]	1	0.1
South	2053	85	4.1[b,c]	325	15.8[b,c]	21	1.0[b]
West	474	1	0.2[b]	102	21.5[b]	0	0.0[b]
Total	3247	98	3.0	576	17.7	22	0.7

Number (*n*) of feral swine tested positive 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 (b or c).
[a] Source: ⟨http://www.eia.doe.gov/emeu/reps/maps/us_census.html⟩.

Table 3
Significant clusters of *Trichinella* and *Toxoplasma* infections in feral swine in the USA.

Species	Cluster	State/county	Number of cases	Expected cases	Relative risk	*p*-Value
Trichinella	Most likely	North Carolina/Johnston	9	1.00	9.91	0.001
	Secondary	Georgia/Thomas	12	2.39	5.61	0.003
	Secondary	Texas/Navarro	6	0.65	9.76	0.026
T. gondii	Most likely	Hawaii/Honolulu	93	40.12	2.61	0.001
	Secondary	Oklahoma/Pittsburg	30	12.09	2.57	0.002

infection only; seroprevalence was significantly higher in female than in male feral swine ($p < 0.05$). There was a significant association between the age of animals and both *Trichinella* and *T. gondii* infection ($p < 0.01$ and $p < 0.001$ respectively). *Trichinella* seroprevalence was significantly higher in sub-adults than in adults and juveniles ($p < 0.01$ and $p < 0.05$ respectively), whereas *T. gondii* seroprevalence was significantly higher in adults than in sub-adults or juveniles ($p < 0.001$ in both cases).

The highest predicted probability of occurrence for *Trichinella* spp. infection in feral pigs in the USA 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 (Figs. 2 and 3). The results of the spatial scan statistic analyses showed one most likely cluster ($p = 0.001$) and two secondary clusters ($p = 0.003$ and $p = 0.026$ respectively) for *Trichinella*, and one most likely cluster ($p = 0.001$) and one secondary cluster ($p = 0.002$) for *T. gondii* (Table 3).

Collection sites of *Trichinella* positive feral swine from 2006 through 2010 indicate a close proximity with locations of pastured pig operations in the southeastern U.S. (Fig. 4, adapted from Burke et al., 2008).

3.2. Model validation

AUC (area under the curve) values are 0.866 and 0.951 for the *Trichinella* and *T. gondii* models respectively, indicating that the models are good to very good (Table 4). The *p*-values using minimum training presence as the threshold are very low for both models indicating very good predictions.

3.3. Effect of environmental variables on the model

The mean temperature of the warmest quarter (BIO10) was the variable that achieved the highest training when

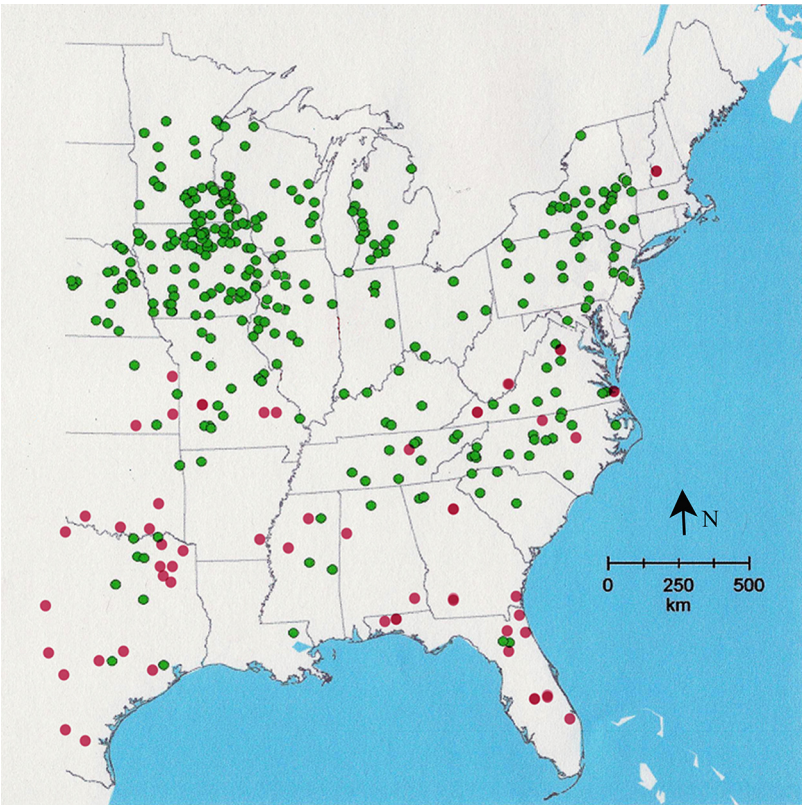

Fig. 4. Locations of pastured pig operations (green) and *Trichinella* seropositive feral swine (red) in the southeastern U.S.

Table 4
Statistical evaluation of the Maxent model.

Species	Number of presence records (training/testing)	AUC for training data	AUC for test data	*p*-Value using minimum training presence as threshold
Trichinella	46/15	0.949	0.866	0.0001
T. gondii	205/68	0.951	0.951	0.0001

used to build a model for *Trichinella* with no other variables (Fig. 5; Table 5; Hijmans et al., 2005). Land cover was the variable that achieved the highest training when used to build a model for *T. gondii* with no other variables. Other variables that show high training gain when modeling only a single variable included elevation, annual mean temperature (BIO1), and land cover for *Trichinella* and annual mean temperature (BIO1), mean temperature of coldest quarter (BIO11), and min temperature of coldest month (BIO6) for *T. gondii.* Land cover types that occurred at the species sampling areas are shown in Table 6. The primary land covers for *Trichinella* are forest and crops while for *T. gondii* they are woody savanna, crops, grasses, and forest.

During the follow up survey conducted in 2012–2013, antibodies to *T. gondii* were found in 280 (28.4%) of 984 feral swine with titers of 25–3200 (Table 7).

Most samples were from the South region where 209 (28.3%) of 736 pigs were seropositive. Host factors associated with serological status are shown in Table 8. Age was the main risk assessment factor; seroprevalence in older pigs was about twice that in juvenile pigs. With respect to geography, prevalences had a similar trend although the number of animals sampled from the West and the Northeast was relatively small.

Antibodies to *Trichinella* were detected in 29 (2.9%) of 984 tested animals in the follow up survey. All seropositive animals were collected in the South, and West to MO and TX. Of 330 tongues collected from a subset of these same animals, six (1.81%) were tissue positive for *Trichinella* muscle larvae. All six tongue samples from which larvae were isolated were seropositive; three additional tongue samples was serologically positive but parasitologically negative. Tissue positive samples were collected from MO (Reynolds County; $n = 2$), NC (Swain County; $n = 2$), and TX (Baylor and Hemphill Counties; $n = 2$). All larvae collected and genotyped from tongue tissue were identified as *T. spiralis* (data not shown). No other genotypes and no mixed infections were detected.

Variable	Trichinella	Toxoplasma
BIO1	0,6581	0,7662
BIO2	0,3533	0,4536
BIO3	0,3962	0,3841
BIO4	0,3119	0,5408
BIO5	0,4898	0,2535
BIO6	0,5686	0,5921
BIO7	0,3195	0,6167
BIO8	0,2921	0,3095
BIO9	0,456	0,4824
BIO10	0,7956	0,5416
BIO11	0,5811	0,6374
BIO12	0,4845	0,5032
BIO13	0,5106	0,4911
BIO14	0,5022	0,4696
BIO15	0,3151	0,2843
BIO16	0,3561	0,3614
BIO17	0,5627	0,5398
BIO18	0,2712	0,318
BIO19	0,4137	0,3398
Elevation	0,6846	0,4598
Land cover	0,5866	0,8847

Fig. 5. Training gain achieved by models using single variables. Length of bar represents the training gain value. A longer bar represents a higher training gain.

Table 5
List of WorldClim bioclimatic variables used in the model (Hijmans et al., 2005).

Bioclimatic variable	Description
BIO1	Annual mean temperature
BIO2	Mean diurnal range (mean of monthly (max temp–min temp))
BIO3	Isothermality (P2/P7) (×100)
BIO4	Temperature seasonality (standard deviation × 100)
BIO5	Max temperature of warmest month
BIO6	Min temperature of coldest month
BIO7	Temperature annual range (P5–P6)
BIO8	Mean temperature of wettest quarter
BIO9	Mean temperature of driest quarter
BIO10	Mean temperature of warmest quarter
BIO11	Mean temperature of coldest quarter
BIO12	Annual precipitation
BIO13	Precipitation of wettest month
BIO14	Precipitation of driest month
BIO15	Precipitation seasonality (coefficient of variation)
BIO16	Precipitation of wettest quarter
BIO17	Precipitation of driest quarter
BIO18	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter

Table 6
Number of sample localities within different land cover categories of the USGS Global Ecosystems land cover classification.

Land cover (class number)	*Trichinella*	*T. gondii*
Tall grasses and shrubs (7)	3	7
Wooded wet swamp (13)	–	1
Inland water (14)	–	2
Sea water (15)	–	4
Cool rain forest (20)	–	2
Cool conifer forest (22)	–	3
Cool mixed forest (23)	–	13
Mixed forest (24)	3	7
Cool broadleaf forest (25)	3	43
Deciduous broadleaf forest (26)	14	37
Conifer forest (27)	7	35
Cool crops and towns (30)	5	18
Crops and Town (31)	13	33
Corn and beans cropland (35)	3	5
Hot irrigated cropland (37)	–	4
Cool irrigated cropland (38)	–	2
Cool grasses and shrubs (40)	1	3
Hot and mild grasses and shrubs (41)	8	47
Savanna (woods) (43)	–	2
Dry woody scrub (47)	–	2
Semi desert shrubs (51)	–	1
Cool fields and woods (55)	–	8
Forest and field (56)	3	18
Cool forest and field (57)	10	29
Woody savanna (91)	5	53
Crops, grass, shrubs (94)	14	48

4. Discussion

Seroprevalence of *T. gondii* in confinement raised domestic pigs in the USA was recently reported to be 2.7% (Hill et al., 2010); seroprevalence in pastured/free-range pigs has been reported to be as high as 50–100% (Gamble et al., 1999). Results of this study indicate high prevalence of *T. gondii* in feral swine in the USA. *T. gondii* persists in the tissues of infected pigs in an infective state for considerable periods of time (>1 year), and potentially for the life of the host. To our knowledge there were three previous reports of *T. gondii* infection in feral swine in the USA (Diderrich et al., 1996; Dubey et al., 1997; Sandfoss et al.,

Table 7
Seroprevalence of *T. gondii* in feral pigs in the USA (2012–2013).

State	No. of pigs	No. of pigs positive with titers of:								No. positives	% positives
		25	50	100	200	400	800	1600	≥3200		
Alabama	64	6	7	6	3	4	2	0	0	28	43.7
Arizona	136	3	6	3	13	6	1	0	0	32	23.5
Arkansas	17	0	0	0	1	0	0	0	0	1	5.8
California	20	0	1	0	0	0	0	0	0	1	50.0
Florida	93	9	3	2	2	1	1	0	1	19	20.4
Georgia	49	3	2	3	0	1	0	0	0	9	18.3
Hawaii	34	5	3	11	3	3	0	0	0	25	73.5
Illinois	13	0	0	0	1	0	0	0	0	1	7.6
Indiana	23	0	1	3	0	0	0	0	0	4	17.3
Kansas	43	3	6	4	10	1	1	0	0	25	58.3
Kentucky	5	3	0	1	0	0	0	0	0	4	80.0
Louisiana	98	14	5	3	5	2	1	1	1	32	32.0
Michigan	6	0	0	2	0	0	0	0	0	2	33.0
Missouri	22	1	1	5	0	0	0	0	0	7	31.8
Mississippi	90	8	7	8	7	3	2	0	0	35	38.8
North Carolina	39	1	1	3	3	1	1	1	0	11	28.2
New Jersey	3	0	0	0	0	0	1	0	0	1	33.0
New Mexico	22	1	0	0	0	0	0	0	0	1	4.5
Nevada	5	0	0	0	0	0	0	0	0	0	0
Ohio	5	1	1	0	1	0	0	0	0	3	60.0
Oklahoma	64	3	3	1	0	1	1	0	0	9	14.0
South Carolina	20	3	2	0	1	2	1	0	0	9	45.0
Tennessee	17	0	2	0	1	2	1	0	0	6	35.2
Texas	90	5	1	2	1	4	1	0	0	14	15.5
Virginia	5	0	0	0	0	0	0	0	0	0	0
West Virginia	1	0	1	0	0	0	0	0	0	1	100
Total	**984**	**69**	**53**	**57**	**52**	**31**	**14**	**2**	**2**	**280**	**28.4**

Table 8
Risk factors and geographic variables associated with seroprevalence of *a gondii* in feral swine according with 2 surveys in the USA.

Factor	(2006–2010)		(2012–2013)	
Year	ELISA		MATMAT	
Test	***n***	**%**	***n***	**%**
Gender				
Male	252	17.7	460	28.4
Female	263	17.3	372	28.4
Unknown	–	–	4	25.0
Age				
Adult	388	19.9[b,c]	728	33.3
Sub-adult	80	13.7[b]	200	14.0
Juvenile	52	12.0[c]	54	14.8
Unknown	–	–	2	50.0
Region[a]				
Northeast	2	9.5	3	33.3
Midwest	147	21.0	112	37.5
South	325	15.8	736	28.3
West	102	21.5	98	28.5
Total	576	17.7	984	28.4

2011). Seroprevalence in these studies varied from 0.9-to-50.0%, depending on the geography and the serologic test used. Diderrich et al. (1996) detected *T. gondii* antibodies in 34.2% of 257 feral swine from the Great Smoky Mountains National Park, SC. Sandfoss et al. (2011) detected *T. gondii* antibodies in 27.7% of 83 feral swine from the Howell Woods Environmental Learning Center in eastern NC. In both Carolina surveys, the MAT test was considered positive at a titer of 32 or higher; the MAT was the same test used here during the 2012–2013 survey, and thus results are comparable. Dubey et al. (1997) reported that a cat-free environment controlled seroprevalence in feral swine from GA. Anti-*T. gondii* antibodies were detected in 18.2% of 170 feral swine from the mainland and 0.9% of 1264 feral swine from a remote island (Ossabaw Island) by MAT with a titer of 25 considered positive. The low seroprevalence on Ossabaw Island was attributed to the absence of cats on this island; seroprevalence in a few of the feral swine (11 of 1264) was attributed to cat fecal contamination of the grain that was imported from the mainland and fed to feral swine.

Worldwide seroprevalences of *T. gondii* in feral swine up to 2009 were recently summarized (Dubey, 2010). Since then, seroprevalences of 17.6–55% were reported in feral swine from Europe, Brazil, and Malayasia (Richomme et al., 2009; Berger-Schoch et al., 2011; Opsteegh et al., 2011; Antolová et al., 2007, Bártová et al., 2006; Deksne and Kirjušina, 2013). The successful isolation of viable *T. gondii* from pigs from France (Richomme et al., 2009) and from Malaysia (Puvanesuaran et al., 2013) from tissues of pigs seropositive by MAT support the validity and usefulness of MAT for the diagnosis of toxoplasmosis in pigs. Richomme et al. (2009) isolated viable *T. gondii* from the tissues of 21 pigs with MAT titers of 1:6 out of a total of 60 pigs and Puvanesuaran et al. (2013) isolated viable *T. gondii* from tissues of six of 30 wild boars with MAT titer of 1:24 or higher.

The *Trichinella* species and genotypes endemic in carnivorous wildlife in the USA are the encapsulated species *T. murrelli*, *T. spiralis*, *T. nativa*; the genotype *Trichinella* T6 (Masuoka et al., 2009); and the non-encapsulated species *T. pseudospiralis*, which is the only *Trichinella* species previously documented in feral swine in the USA (Gamble et al., 2005). The sylvatic genotypes are minimally infective to domestic pigs as opposed to the domestic genotype (*T. spiralis*), and show poor persistence in pig tissues (Kapel and Gamble, 2000). On the other hand, even though *T. spiralis* reproduces well in most carnivore hosts, it is found in the USA almost exclusively in pigs and peridomestic carnivores. Most feral swine in the USA are descendants of escaped or deliberately released domestic pigs, with interbreeding with imported and released European wild boar. *T. spiralis* is rarely detected in wildlife species that do not associate with pigs; *T. murrelli* is the predominant genotype identified in sylvatic carnivores in North America (Zarlenga et al., 1991; Pozio and La Rosa, 2000; Hill et al., 2008). The seroprevalence of *Trichinella* spp. in feral pigs varies worldwide, 0.11% in Slovakia (Hurníková and Dubinský, 2009), 0.2% in Switzerland (Frey et al., 2009a), 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 USA seen in the current 2006–2010 survey was four times higher than in feral swine of Europe. The reason for the higher *Trichinella* spp. seroprevalence in the USA 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 USA.

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* species in feral swine. Isolation of larvae from the tongue of infected animals and genotyping by multiplex PCR is the typical method used to identify larvae to species. Naturally infected animals typically have very low worm burdens (<10 larvae per gram of tissue); the detection limit of the digestion assay used here is 1–3 larvae per gram. As a result, larvae are frequently missed even in seropositive animals, which explains the difference in the number of seropositive animals seen in 2012–2013 (9/330) as compared to the number of tongues from which larvae were successfully recovered (6/330). Our results here confirm that *T. spiralis* is circulating in feral swine, while other sylvatic genotypes were not detected.

The effect of gender and age on the seroprevalence of *Trichinella* across host species is inconsistent. *Trichinella* seroprevalence in the present study was higher in female than male feral swine. In humans, a higher seroprevalence has been observed in females in Mexico (de-la-Rosa et al., 1998) but higher in males in Papua New Guinea (Owen et al., 2005). No gender based differences were found in red foxes and Eurasian lynxes (Frey et al., 2009b) and wolves (Bagrade et al., 2009).

Trichinella seroprevalence in the present study was higher in sub-adult feral swine (5.1%) than adults (2.8%) and juveniles (2.3%). Previous studies in our lab (Hill et al., 2010) demonstrated that naturally infected, juvenile feral swine are capable of seroconversion and of harboring infectious muscle larvae of *T. spiralis*, so the finding of 10 seropositive juveniles in this study was not suprising. Seroprevalence for *Trichinella* infection was not statistically correlated with

age in foxes (Frey et al., 2009b) and wolves (Bagrade et al., 2009), while seroprevalence was significantly higher in adult lynx compared to juveniles (Frey et al., 2009b).

In contrast, *T. gondii* seroprevalence in both the 2006–2010 and 2012–13 surveys was higher in adults than juveniles, suggesting postnatal exposure and transmission of *T. gondii* (Dubey, 2009). Transplacental or transcolostral immunity does not play a role in the lower seroprevalence for *T. gondii* observed in younger animals since antibodies are not transmitted via the placenta, and colostrum-derived antibodies disappear by three months of age (Dubey and Urban, 1990).

In the prediction maps (Figs. 2 and 3), the most probable distribution areas for *Trichinella* spp. are located in the South, and for *T. gondii* the South and Midwest, while the parasites are virtually absent in the sampled Rocky Mountain and arid Western states. The low *T. gondii* seroprevalence rates (<6%) in feral swine in the West regions (CA, AZ, NM, CO) 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. The high seroprevalence of *Trichinella* spp. and *T. gondii* in feral pigs observed in the South in comparison to the other regions is probably related to a number of factors: (1) feral swine populations are concentrated in southern states, and consequently more samples were collected 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 *T. gondii* oocysts in soil, which serve as sources of infection for feral swine; (3) the freeze resistance limits of both *T. gondii* and *Trichinella* (other than *Trichinella nativa* and *Trichinella*-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*; the site where the samples were collected was part of a *Trichinella* eradication effort on a private game preserve where hunted imported wild boar were found to be infected with *Trichinella* (Worley et al., 1994).

The significant clusters of infection for *Trichinella* and *T. gondii* detected in North Carolina and Hawaii respectively during 2006–2010 fall in areas with high probability ($p > 0.7$) of presence predicted by the Maxent program. The underlying causes of increased risk of infection in the identified areas are not known but they may be linked to both abiotic (climate, vegetation, and landscape attributes) and biotic factors (host density).

During the first survey from 2006 through 2010 of *T. gondii* in feral swine, antibodies were found in 17.7% of 3247 pigs; sera were tested using a commercial ELISA kit. In the follow up study of *T. gondii* in feral swine tested in 2012–2013, seroprevalence was higher using the MAT test (28%). The difference seen may be related to the difference in serological tests used during the two time periods, the duration period of the surveys (5 years—2006–2010, versus 1 year—2012–2013, so more animals were surveyed during part one of the study). Further, the predominant locations from which the feral swine were collected differed between the two surveys; in the follow up study, 75% of the feral pigs were collected from the South. Feral pigs from this region had a higher rate of seropositivity for both *T. gondii* and *Trichinella* than other regions of the country in the 2006–2010 surveys. Whatever the reason for these different seroprevalence the results indicate stable rate of *T. gondii* exposure of feral swine in the USA over 7 years.

Sport and game hunters that target feral swine 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 undercooked 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* in various infective stages. To prevent infection of humans by *T. gondii*, thorough washing of hands with soap and water after handling raw meat is essential for killing the parasite. All cutting boards, sink tops, knives, and other utensils coming in contact with uncooked meat should be washed thoroughly with soap and water. *Trichinella* spp. larvae and *T. gondii* organisms in meat can be killed by heating throughout to 67 °C for at least 4 min before consumption, or cooling to −13 °C for three days (Kotula et al., 1983, 1990; Gamble et al., 2000; Hill et al., 2009). Tasting meat prior to cooking should be avoided. Adherence to good hygienic measures and safe handling and processing of meats is the most practical and effective method available to minimize transmission of *Trichinella* spp. (Gamble et al., 2014) and *T. gondii* to humans from feral swine meat.

Feral swine also pose a significant risk for introduction of *Trichinella* and *T. gondii* into non-biosecure domestic pigs 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 USA. Rearing of pigs outdoors has been identified as a major risk factor for domestic pig infection with both *Trichinella* and *T. gondii* due to increased exposure to potentially infected reservoir hosts, as well as exposure to oocyst contaminated soil in the case of *T. gondii* (Gamble et al., 2000, 2001; Pyburn et al., 2005; Hill et al., 2010). Transmission of *T. spiralis* and *T. gondii* has been observed to occur due to cannibalism in free-ranging pigs (Hanbury et al., 1986; Dubey et al., 1986a; Hill et al., 2010). Tail biting is common in pigs and *T. gondii* is known to encyst in musculature of pig tails (Dubey et al., 1986b). Consumer demand for 'organically raised', 'humanely raised' and 'free range' pork products has resulted in increasing numbers of pigs being raised in non-confinement systems (Honeyman et al., 2006). Pig 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 *T. gondii*. Burke et al. (2008) identified pastured pig

operations in the eastern USA; there is close proximity between some of these operations and the locations of collection spots for *Trichinella* and *T. gondii* positive feral pigs identified in this study (Fig. 4). The practice of field dressing hunted feral pigs and aerial hunting which leaves carcasses in the field should be discouraged, as feral pig carcasses and offals can serve as sources of infection for grazing domestic pigs and for sylvatic carnivores that serve as reservoirs of infection for both parasites. Hunters should be encouraged to wear gloves when dressing carcasses and educated in the potential hazards of contact with these parasites.

5. Conclusions

Contact between feral swine and domestic pigs should be prevented. Increased surveillance efforts coupled with efforts to reduce or eliminate feral swine populations should be focused in regions with significant numbers of pasture raised pigs to prevent introduction of these parasites into domestic animals destined for human consumption.

Conflict of interest

None.

Acknowledgments

The authors would like to thank Jorrell Fredericks and Dominique Carter, Tuskegee University, Marc K. Kouam, Agricultural University of Athens, Greece Kelsey Zarlenga, Towson University, and Wildlife Services, state directors and wildlife disease biologists in the 32 participating states, and Sarah Goff at the National Wildlife Disease Program Feral Swine Tissue Archive.

This work was funded in part by grant # 08-216 from the National Pork Board, Des Moines, Iowa.

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