Veterinary Parasitology 325 (2024) 110090

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Distribution and prevalence of antibodies to *Trichinella* spp. and *Toxoplasma gondii* in wild pigs (*Sus scrofa*) in the United States

Christopher A. Cleveland [a,b,*,1], Ellen Haynes [a,**,1], Katherine C. Callaghan [c], Alinde Fojtik [a], Sarah Coker [a], Emily Doub [a], Vienna R. Brown [d], Ania A. Majewska [b,e], Michael J. Yabsley [a,b,*]

[a] Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA 30602, USA
[b] Center for Ecology of Infectious Diseases, University of Georgia, Athens, GA 30602, USA
[c] Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602, USA
[d] USDA APHIS Wildlife Services, National Feral Swine Damage Management Program, Fort Collins, CO 80521, USA
[e] Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

ARTICLE INFO

Keywords:
Invasive species
Geographic distribution
Pathogens
Seroprevalence
Toxoplasma gondii
Trichinella spp.
Wild pigs

ABSTRACT

Invasive wild pigs (*Sus scrofa*) are a reservoir for over 100 viral, bacterial, and parasitic pathogens that are transmissible to humans, livestock, domestic animals, and wildlife in North America. Numerous historical local surveys and results from a nation-wide survey (2006–2010) indicated that wild pigs in the United States act as reservoirs for *Trichinella* spp. and *Toxoplasma gondii*, two zoonotic pathogens of importance for human and animal health. Since that time, wild pig populations have expanded and increased in density in many areas. Population expansion of wild pigs creates opportunities for the introduction of pathogens to new areas of the country, increasing health risks. The goal of this study was to investigate the current geographic distribution and prevalence of *Trichinella* spp. and *T. gondii* antibodies in wild pigs using serum samples collected from 2014 to 2020. Serum samples from 36 states were tested for antibodies to *Trichinella* spp. (n = 7467) and *T. gondii* (n = 5984) using commercially available enzyme-linked immunosorbent assays. Seroprevalence for *Trichinella* spp. (12.4%, 927/7467) and *T. gondii* (40.8%, 2444/5984) are significantly higher compared to a previous 2006–2010 study across all regions. Results from this study also showed a lower seroprevalence (4.8%) for *Trichinella* spp. in the West region compared to the other regions (South: 13.4%; Midwest: 18.4%; Northeast: 19.1%). There were new detection records for antibodies to *Trichinella* spp. in 11 states, mostly in the West, Midwest, and Northeast regions compared to a previous study in 2014. Males and juveniles were less likely to be positive for *Trichinella* spp. antibodies, compared to females and older animals, respectively. Seroprevalence was similar for *T. gondii* across the regions (31.8–56%) with some states having particularly high seroprevalence (e.g., Hawaii 79.4% and Pennsylvania 68%). There were new *T. gondii* antibody detection records for 12 states, mostly in the West, Midwest, and Northeast regions. Adults were more likely than juveniles and subadults to be seropositive. These data confirm that the distribution and prevalence of antibodies for *Trichinella* spp. and *T. gondii* are increasing in the United States, likely driven by wild pig population growth and range expansion.

1. Introduction

Wild pigs (*Sus scrofa)* are a non-native invasive species in the United States that serve as reservoirs for many pathogens transmissible to humans, livestock, and wildlife species (Meng et al., 2009, Barrios-Garcia and Ballari, 2012; Bevins et al., 2014). Two types of zoonotic pathogens circulating among wild pig populations in the United States, *Trichinella* spp. and *Toxoplasma gondii,* are parasites with significant economic and human health consequences (Hill et al., 2014). Due to high reproduction rates and anthropogenic movement to benefit hunters (i.e., humans stocking new areas of the country with wild pigs for sport hunting), the distribution of wild pigs in the United States has expanded, with wild pigs currently occupying 35 states at an estimated population size of 6 million individuals (Corn and Jordan, 2017; Tabak

* Corresponding authors at: Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA 30602, USA.
** Corresponding author.
E-mail addresses: ccleve@uga.edu (C.A. Cleveland), ellen.haynes@uga.edu (E. Haynes), myabsley@uga.edu (M.J. Yabsley).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.vetpar.2023.110090
Received 30 May 2023; Received in revised form 21 November 2023; Accepted 24 November 2023
Available online 28 November 2023

et al., 2017; Beasley et al., 2018). Therefore, a better understanding of the current distribution of key pathogens in wild pig populations is needed to assess threats to human, wildlife, and domestic animal health.

Trichinella spp. are parasitic nematodes transmitted to definitive hosts through the ingestion of infected carcasses or meat products (Zarlenga et al., 2020). *Trichinella* spp. can be transmitted to humans by ingestion of undercooked meat containing infectious larvae (Capo and Despommier, 1996). Currently, most human cases of infection with *Trichinella* spp. are linked to consumption of infected, undercooked wild game in industrialized countries (Diaz et al., 2020). While multiple species of *Trichinella* spp. are found in the United States, *T. spiralis* and *T. pseudospiralis* are the two species for which wild pigs serve as a main source of infection for humans (Gottstein et al., 2009; Zarlenga et al., 2020).

Toxoplasma gondii, the causative agent of toxoplasmosis, is a protozoan parasite capable of infecting nearly all warm-blooded animals, including humans (Hill and Dubey, 2002; Dubey et al., 2005; Dubey, 2016). Toxoplasmosis is one of the most common human infections in the world. In the United States, it is considered a leading cause of death due to foodborne illness, and the Centers for Disease Control and Prevention considers it 1 of 5 neglected parasitic infections targeted for public health action (Togerson and Macpherson, 2011; CDC, 2023). Infection occurs when felid definitive host ingests cysts, which can be found in tissues of previously infected intermediate hosts. *Toxoplasma gondii* oocysts are shed from felid fecal matter and are known to persist in moist soil for up to 18 months, presenting an increased transmission risk when environmental conditions are suitable (Shapiro et al., 2019). Wild pigs can ingest *T. gondii* cysts in infected intermediate host tissues or oocysts from soil or water through behaviors such as rooting and wallowing (Beral et al., 2012).

Wild pigs in the United States can establish populations in novel areas, interact with domestic pets and livestock, and be harvested through liberal hunting regulations (Massei et al., 2011; Tabak et al., 2017). Each of these factors can increase the likelihood of parasite maintenance and transmission. Previous studies in 2006–2010 investigated 3247 wild pig serum samples from 26 states and found the prevalence of antibodies to *Trichinella* spp. and *T. gondii* to be 3.0% and 17.7%, respectively (Hill et al., 2014). A follow up survey of 984 individuals in 26 states from 2012 to 2013 found that 2.9% of wild pigs were seropositive for *Trichinella* spp. and 28.4% were seropositive for *T. gondii* (Hill et al., 2014). The primary objective of the current study was to provide contemporary data on the seroprevalence of *Trichinella* spp. and *T. gondii* in wild pigs in some areas of the United States and determine if there has been a change in the prevalence and spatial distribution of antibodies to these pathogens within the past decade.

2. Methods

2.1. Sample collection

Between 2014 and 2020, United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) Wildlife Services field biologists collected samples from wild pigs that were removed opportunistically to protect agricultural operations, natural resources, private property, and human health and safety. All collection efforts for this study adhered to the United States Department of Agriculture's Animal and Plant Health Inspection Services Wildlife Service Directive 2.505 (05/18/11) providing guidelines for the ethical lethal control of animals (USDA Wildlife Services Feral Swine Damage Management Program, Fort Collins, CO). The primary route of blood collection was cardiac venipuncture. Pig age-class (juvenile – nursing; sub-adult – no longer nursing but not reproductively active; or adult – reproductively active) and sex (male or female) were recorded for each sample. Blood was placed in serum-separating Vacutainer tubes (Greiner Bio-one GmbH, Kremsmünster, Austria) and allowed to clot prior to centrifugation. Serum was transferred into uniquely barcoded 2 mL Cryovials (Corning Incorporated, Corning, NY, United States), shipped on ice packs to the National Wildlife Research Center (NWRC-Fort Collins, CO, United States) within three days of collection, and stored at 4 °C. Serum samples from 36 states were shipped frozen to the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia (Athens, GA, United States) and stored at − 20 °C until serological assays were performed.

Sampled states were organized into regions (Southeast, Northeast, Midwest, West) based on USA Census Bureau (www.census.gov) designations for comparison to previous studies (Hill et al., 2014). Wild pig serum samples collected from 2014 to 2020 from 36 states were tested for *Trichinella* spp. (n = 7467) and *T. gondii* (n = 5984). Wild pig serum samples were tested from 15 states in the Southern region (Alabama (AL), Arkansas (AR), Florida (FL), Georgia (GA), Kentucky (KY), Louisiana (LA), Mississippi (MS), North Carolina (NC), Oklahoma (OK), Puerto Rico (PR), South Carolina (SC), Tennessee (TN), Texas (TX), Virginia (VA), West Virginia (WV)); 8 states in the Midwest region (Iowa (IA), Illinois (IL), Indiana (IN), Kansas (KS), Michigan (MI), Missouri (MO), Ohio (OH), Wisconsin (WI)); 8 states in the Western region (Arizona (AZ), California (CA), Colorado (CO), Hawaii (HI), New Mexico (NM), Nevada (NV), Oregon (OR), Utah (UT)); and 5 states in the Northeast region (Maine (ME), New Hampshire (NH), New York (NY), Pennsylvania (PA), Vermont (VT)). A total of 5930 samples were tested for antibodies to both *Trichinella* spp. and *T. gondii*.

2.2. Serology

The testing of serum collected by other researchers for pathogen surveillance has been reviewed and approved by the University of Georgia IACUC (Protocol #A2020 11–010). Serum samples were tested using two validated commercially available enzyme-linked immunosorbent assay (ELISA) kits: PrioCHECK™ Porcine *Trichinella* Ab Strip Kit and PrioCHECK™ Porcine *Toxoplasma* Ab Strip Kit (Applied Biosystems, ThermoFisher Scientific, Lelystad, The Netherlands) to screen for antibodies to *Trichinella* spp. and *Toxoplasma gondii*, respectively. The *Trichinella* ELISA uses excretion/secretion (E/S) antigen, while the *Toxoplasma* ELISA uses cell culture-derived tachyzoite antigen. The manufacturer's protocols were followed for both kits.

Briefly, for the *Trichinella* spp. ELISA, sera were diluted 1:50 (specificity (Sp), 100%; sensitivity (Sn), 100%); for the *T. gondii* ELISA, sera ware diluted 1:50 (Sp= 99.1%; Sn = 97.9%). Positive and negative controls provided by the manufacturer for each parasite were used on each plate, and all ELISAs were performed using an EMax® Plus microplate reader (EMax Plus®, Molecular Devices, San Jose, CA, United States) with SoftMax® Pro software (Molecular Devices). To validate the results, all criteria for optical density (OD) of the positive and negative controls were followed per the manufacturer's protocol. For the *Trichinella* spp. test, OD of the positive controls must be greater than 1.0, the mean OD of the negative controls must be less than 0.2, and weak positive controls mean percentage of positivity (PP) must be greater than 35%. When interpreting the results, any results greater than 15 PP were considered positive, and any below 15 PP were considered negative, based on the manufacturer's guidelines for interpretation of results. For the *T. gondii* test, the mean OD of positive controls must be greater than 1.2, the mean OD of the negative controls must be less than 0.15, and the mean PP of weak positives must be greater than 35%. Any results greater than 20 PP were considered positive, and any below 20 PP were considered negative, based on the manufacturer's guidelines for interpretation of results.

2.3. Statistical analyses

All analyses were performed using R (R Core Team, 2022). To investigate whether a positive result for antibodies to *Trichinella* spp. or *T. gondii* was impacted by pig age class, sex, and geographic region of collection, generalized linear models (GLMs) with binomial errors were

employed. The response variable was the test result (0: negative; 1: positive), and age class, sex, and region served as fixed effects. Additionally, investigation into whether antibody status for *Trichinella* spp. was related to positive antibody status for *T. gondii* occurred, incorporating age class, sex, and region to assess the impact of these factors on exposure to both parasites.

Comparison of results with those reported by Hill et al. (2014) to assess seroprevalence differences between studies involved a generalized linear regression model with binomial distribution (see Table 1). The response variable was the number positive and negative in each state examined, and the explanatory variable was the study (current study vs Hill et al. 2014).

3. Results

3.1. *Trichinella spp*

The overall *Trichinella* spp. seroprevalence from screened samples was 12.4% (927/7467; 95% confidence interval (CI) = 11.7–13.2%). When evaluated by region, the seroprevalence was 13.4% (763/5694; 95% CI = 12.5–14.3%) in the South, 18.4% (98/532; 95% CI = 15.2–22.0%) in the Midwest, 4.8% (57/1194; 95% CI = 3.6–6.1%) in the West, and 19.1% (9/47; 95% CI = 9.2–33.3%) in the Northeast (Table 1, Fig. 1). Among states with sample sizes of individual animals greater than 10, NH had the highest prevalence at 45.5% (5/11; 95% CI = 16.8–76.6%), followed by MO at 31.3% (30/96; 95% CI = 22.2–41.5%), and prevalence > 15% was reported from AR, NC, SC, TN, and VA in the Southern region; IN, MO, and OH in the Midwest; and NH in the Northeast region. Several states and territories had no detections including: PR, IA, IL, WI, CO, NV, UT, ME (Table 1).

The probability of detecting *Trichinella* spp. antibodies was significantly lower for males compared to females ($z = -5.23$, $p < 0.01$) and for juveniles compared to sub-adults and adults ($z = -2.89$, $p < 0.01$, Table 2, Fig. 1). Seroprevalence was lower in the Western and Southern regions compared to Northeast and Midwest (Western: $z = -7.89$, $p < 0.01$; Southern: $z = -3.00$, $p < 0.01$, Table 2, Table S1). For full model outputs see supplementary data (Table S1). Seroprevalence was higher than previously reported by Hill et al. (2014) for all regions and animals seropositive for *Trichinella* spp. were newly detected from 11 states (previous 0% seroprevalence or surveillance not conducted in that state): CA, HI, IN, KY, MI, NY, OH, OR, PA, SC, and VT (Table 1; Hill et al., 2014). Among states with sample sizes > 10 for both this study and Hill et al. (2014), the seroprevalence of *Trichinella* spp. was significantly higher ($p < 0.05$) in the current study in 11 states: AR, CA, FL, HI, KS, MO, NC, NH, OK, TN, and TX (Table 1, Fig. 2). Analyses comparing our results to those reported by Hill et al. (2014) indicated that the probability of detecting antibodies to *Trichinella* spp. at the state level was higher in the current study ($z = 13.77$, $p < 0.01$, Fig. 2).

3.1.1. *Toxoplasma gondii*

The overall *T. gondii* seroprevalence from screened samples was 40.8% (2444/5984; 95% CI = 39.6–42.1%). When evaluated by region, the seroprevalence was 41% (1905/4650; 95% CI = 39.6–42.4%) in the South, 56% (243/434; 95% CI = 51.2–60.7%) in the Midwest, 31.8% (271/853; 95% CI = 28.6–35.0%) in the West, and 53.2% (25/47; 95% CI = 38.1–67.9%) in the Northeast (Table 1, Fig. 2). Among states with sample sizes greater than 10, HI had the highest seroprevalence at 79.4% (135/170; 95% CI = 72.6–85.2%), followed by PA at 68% (17/25; 95% CI = 46.5–85.0%), and seroprevalence > 15% was reported from all states except WV in the Southern region; all states except WI in the Midwest; CA, HI, and OR in the Western region; and PA in the Northeast region. Only three states had no antibody detections: WI, NV, and ME (Table 1).

Probability of detecting antibodies for *T. gondii* was similar across sexes ($z = 0.82$, $p = 0.41$), but significantly lower in juveniles and sub-adults compared to adults (juvenile: $z = -8.28$, $p < 0.01$; sub-adult:

Table 1
Results from current study showing the comparison of *Trichinella* spp. and *Toxoplasma gondii* seroprevalence from wild pigs (*Sus scrofa)* with data from Hill et al. 2014.

		Trichinella spp. Prevalence (# positive/ total samples)		*Toxoplasma gondii* Prevalence (# positive/ total samples)	
		Current Study	Hill et al. 2014	Current Study	Hill et al. 2014
Southern Region	AL^	9.7% (67/691)	3.8% (3/79)	66.4% (166/250)	21.5% (17/79)
	AR*^	16.0% (115/721)	2.6% (1/38)	56.1% (152/271)	34.2% (13/38)
	FL*^	12.0% (87/724)	5.6% (16/288)	38.0% (251/660)	17.0% (49/288)
	GA^	13.6% (51/374)	13.4% (11/82)	45.5% (170/374)	13.4% (11/82)
	KY	8.4% (8/95)	0% (0/8)	54.0% (27/50)	0% (0/8)
	LA	13.7% (29/212)	–	34.4% (73/212)	–
	MS	12.3% (23/187)	4.3% (2/47)	46.5% (87/187)	19.1% (9/47)
	NC*^	20.6% (73/355)	8.3% (15/180)	49.7% (190/382)	13.9% (25/180)
	OK*^	8.1% (36/446)	1.4% (6/425)	41.5% (185/446)	19.5% (83/425)
	PR	0% (0/2)	–	50.0% (1/2)	–
	SC	26.5% (69/260)	–	66.5% (173/260)	–
	TN*^	19.4% (41/211)	4.3% (2/47)	51.7% (107/211)	10.6% (5/47)
	TX*^	10.7% (129/1207)	3.3% (27/814)	23.8% (289/1212)	12.4% (101/814)
	VA	17.9% (28/156)	10.3% (3/29)	27.9% (31/111)	27.6% (8/29)
	WV	13.2% (7/53)	5.6% (1/18)	13.6% (3/22)	22.2% (4/18)
	Regional total	**13.4% (763/5694)**	**4.2% (87/2055)**	**41.0% (1905/4650)**	**15.8% (325/2055)**
Midwest Region	IA	0% (0/13)	0% (0/2)	46.2% (6/13)	100% (2/2)
	IL	0% (0/19)	–	63.2% (12/19)	–
	IN	30.1% (34/113)	–	67.0% (59/88)	–
	KS*^	7.3% (11/150)	1% (4/413)	56.0% (84/150)	21.5% (89/413)
	MI^	5.9% (1/17)	0% (0/34)	35.3% (6/17)	11.8% (4/34)
	MO*^	31.3% (30/96)	3.1% (7/223)	52.1% (50/96)	20.2% (45/223)
	NE	-	0% (0/20)	-	20% (4/20)
	OH	17.9% (22/123)	0% (0/7)	52.0% (26/50)	42.9% (3/7)
	WI	0% (0/1)	–	0% (0/1)	–
	Regional total	**18.4% (98/532)**	**1.5% (11/699)**	**56.0% (243/434)**	**21.0% (147/699)**
Western Region	AZ	0% (0/112)	0% (0/17)	12.0% (9/75)	5.9% (1/17)
	CA*^	4.7% (34/727)	0% (0/176)	24.2% (105/433)	4.0% (7/176)
	CO	0% (0/10)	0% (0/7)	20.0% (2/10)	0% (0/7)
	HI*^	10% (17/170)	0% (0/234)	79.4% (135/170)	39.7% (93/234)
	NM	1.8% (2/114)	2.5% (1/40)	4.4% (5/114)	2.5% (1/40)
	NV	0% (0/4)	–	0% (0/4)	–
	OR	7.4% (4/54)	–	31.8% (14/44)	–

(*continued on next page*)

Table 1 (*continued*)

		Trichinella spp. Prevalence (# positive/ total samples)		*Toxoplasma gondii* Prevalence (# positive/ total samples)	
		Current Study	Hill et al. 2014	Current Study	Hill et al. 2014
	UT	0% (0/3)	–	33.3% (1/3)	–
	Regional total	**4.8% (57/1194)**	**0.21% (1/474)**	**31.8% (271/853)**	**21.5% (102/474)**
Northeast Region	ME	0% (0/2)	–	0% (0/2)	–
	NJ	-	0% (0/7)	-	14.3% (1/7)
	NH*	45.5% (5/11)	8.3% (1/12)	9.1% (1/11)	0% (0/12)
	NY	16.7% (1/6)	–	100% (6/6)	–
	PA	8.0% (2/25)	0% (0/2)	68.0% (17/25)	50.0% (1/2)
	VT	33.3% (1/3)	–	33.3% (1/3)	–
	Regional total	**19.1% (9/47)**	**4.7% (1/21)**	**53.2% (25/47)**	**9.5% (2/21)**
TOTAL		**12.4% (927/7467)**	**3.0% (100/3249)**	**40.8% (2444/5984)**	**17.7% (576/3249)**

* Indicates a significantly higher prevalence of *Trichinella* spp. in the current study, compared to Hill et al. (2014); ^ indicates a significantly higher prevalence of *Toxoplasma gondii* in the current study, compared to Hill et al. (2014). State abbreviations are: Southern region (Alabama (AL), Arkansas (AR), Florida (FL), Georgia (GA), Kentucky (KY), Louisiana (LA), Mississippi (MS), North Carolina (NC), Oklahoma (OK), Puerto Rico (PR), South Carolina (SC), Tennessee (TN), Texas (TX), Virginia (VA), West Virginia (WV)); Midwest region (Iowa (IA), Illinois (IL), Indiana (IN), Kansas (KS), Michigan (MI), Missouri (MO), Ohio (OH), Wisconsin (WI)); Western region (Arizona (AZ), California (CA), Colorado (CO), Hawaii (HI), New Mexico (NM), Nevada (NV), Oregon (OR), Utah (UT)); Northeast region (Maine (ME), New Hampshire (NH), New York (NY), Pennsylvania (PA), Vermont (VT)).

$z = -7.39$, $p < 0.01$, Table 2, Fig. 1). Seroprevalence was lower in the Western and Southern regions compared to Northeast and Midwest (Western: $z = -7.11$, $p < 0.01$; Southern: $z = -5.25$, $p < 0.01$, Table 2, Table S1). For full model outputs see supplementary data (Table S1). Seroprevalence was higher than previously reported for all regions and animals seropositive for *T. gondii* were newly detected from 12 states (previous 0% prevalence or surveillance not conducted in that state): CO, IL, IN, KY, LA, NH, NY, OR, PR, SC, UT, and VT (Table 1; Hill et al. 2014). Among states with sample sizes > 10 for both this study and Hill et al. (2014), the seroprevalence of *Toxoplasma gondii* was significantly higher ($p < 0.05$) in the current study in 14 states: AL, AR, CA, FL, GA, HI, KS, MI, MO, MS, NC, OK, TN, and TX (Table 1, Fig. 2). Analyses comparing our results to those reported by Hill et al. (2014) indicated that the probability of detecting antibodies to *T. gondii* at the state level was higher in the current study ($z = 21.96$, $p < 0.01$, Fig. 2). (Fig. 3).

3.2. Co-seropositivity

A total of 390 samples were seropositive for both *Trichinella* spp. and *T. gondii* out of 5930 samples tested for both pathogens. Co-seropositive probability was lower for males compared to females ($z = -2.14$, $p = 0.03$) and lower for juveniles and sub-adults compared to adults (juvenile: $z = -4.02$, $p < 0.01$; sub-adult: $z = -3.10$, $p < 0.01$). Co-seropositivity was lower in the Western and Southern regions compared to Northeast and Midwest (Western: $z = -5.80$, $p < 0.01$; Southern: $z = -3.46$, $p < 0.01$, Table S1). For full model outputs see supplementary data (Table S1).

4. Discussion

This study provides contemporary nationwide data on the seroprevalence in wild pigs of two types of pathogens of importance to human, livestock, and wildlife health: *Trichinella* spp. and *T. gondii*. By capitalizing on serum samples collected across broad regions of the United States, the antibody distribution of these parasites was assessed, which allowed the investigation of changes in overall prevalence among sampled wild pig populations. The hypothesis of this study was that seroprevalence of *Trichinella* spp. and *T. gondii* would increase among sampled wild pig populations due to increasing population numbers and increasing geographical range of wild pigs.

As a result of an increase in efforts to decrease wild pig populations, notable features of this study were the increased overall sample size ($n = 5829$) compared to previous studies ($n = 3247$, Hill et al. 2014), as well as more states being included in this study ($n = 36$) compared to previous work ($n = 26$; Hill et al. 2014). Controlling for sample sizes, an increase in the seroprevalence of both parasites, as well as an increase in the geographical range of antibodies were detected. In some areas, wild pig populations are increasing in number and distribution, despite control efforts. This population expansion may increase pathogen transmission; however, additional research must be done to show that the prevalence of both *Trichinella* spp. and *T. gondii* will increase with higher numbers of susceptible individuals. Therefore, establishing continuous long-term surveillance for these pathogens and their spatial distribution is necessary, especially in the face of ongoing climate and landscape change.

The seroprevalence of both *Trichinella* spp. and *T. gondii* was highest in the Northeast and Midwest regions, while previous work reported higher seroprevalence of *Trichinella* spp. in the Southeast and highest seroprevalence of *T. gondii* in the Southeast, Northeast, and Midwest regions (Hill et al. 2014). This may be attributed in part to differences in sampling approaches in the Northeast and Midwest regions, with more states sampled in these regions in the current study. Interestingly, 11 states that were previously negative or untested for *Trichinella* spp. antibodies had positive wild pigs in this study and 12 states previously negative for *Toxoplasma gondii* antibodies had positive pigs. Only AZ, CO, and IA continued to have all sampled pigs testing seronegative for *Trichinella* spp., and in the current study, all states tested had pigs seropositive for *Toxoplasma gondii*. However, sample sizes in some of these states were low, indicating a need for additional surveillance. While seroprevalence of *T. gondii* increased across all study regions, a lower seroprevalence in WV was detected, but this decrease was not statistically significant (13.6% vs. 22.2%) and both studies had low samples sizes for WV. These results emphasize that long-term surveillance with large sample sizes is necessary to monitor changes in seroprevalence over time across the United States and to assess risk to domestic animals and humans.

The likelihood of detecting *Trichinella* spp. antibodies was higher for females compared to males, and higher for sub-adults and adults compared to juveniles. Previous work found a similar association between sex and *Trichinella* spp. exposure, with females being more likely to have antibodies, and that sub-adult pigs were most likely to be exposed to *Trichinella* spp. (Hill et al., 2014). Additionally, the likelihood of detecting *T. gondii* antibodies was higher in adults compared to juveniles and sub-adults. Hill et al. (2014) also found that adults were more likely to be exposed to *T. gondii* and other work in wild pigs found that *T. gondii* seroprevalence increased with age, supporting postnatal transmission (Dubey, 2009). Conversely, a study of 376 swine from Texas abattoirs found no associations between age class or sex and seropositivity for either *T. spiralis* or *T. gondii* (Pedersen et al., 2017). For both pathogens, the higher seroprevalence in older animals can be explained by these animals having more opportunities to be exposed and the potential for both parasites to persist for a long time in the host. For *Trichinella* spp., the time to seroconversion has been shown to depend on the parasite species, as well as the larval burden in the muscle, and can

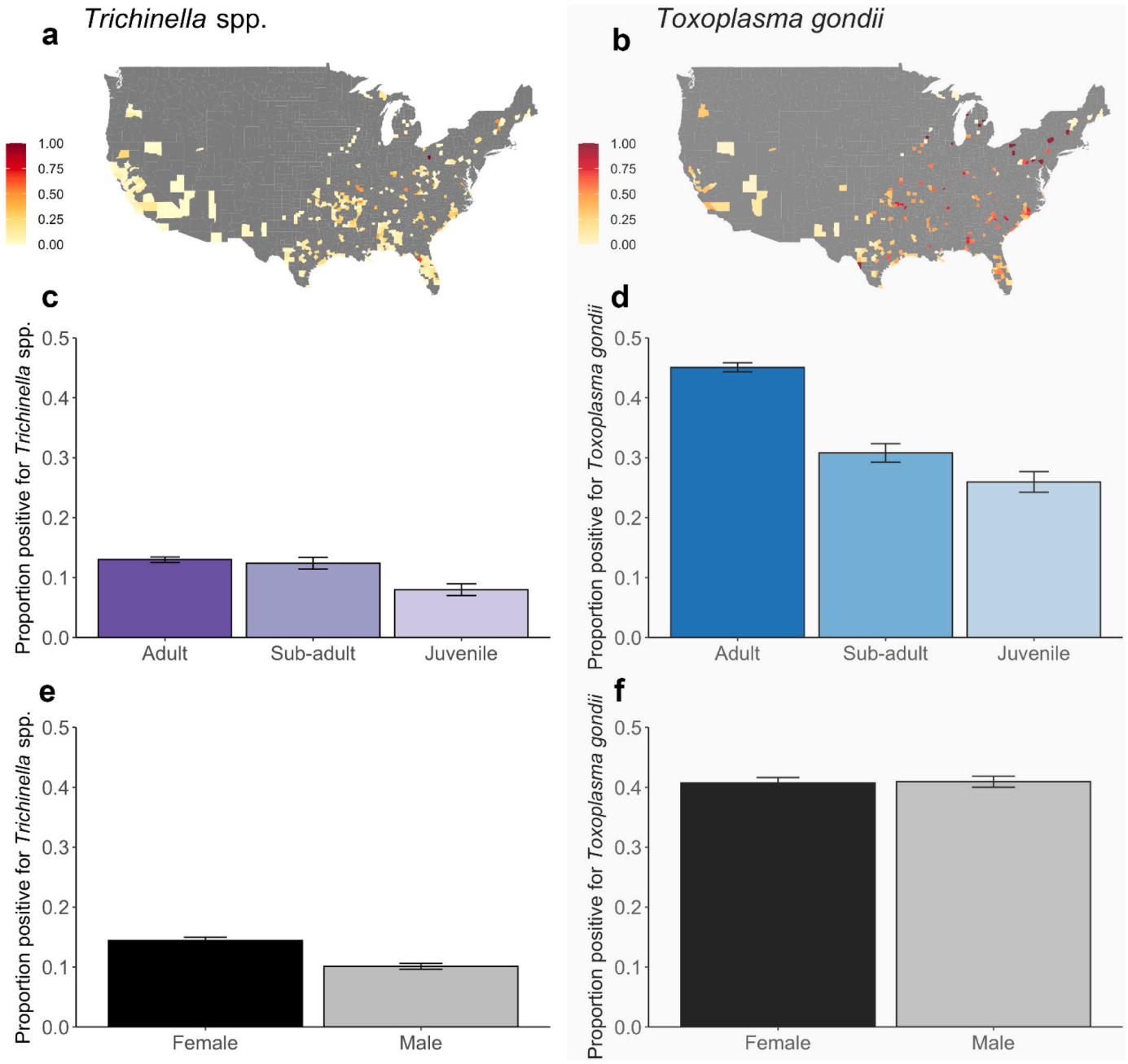

Fig. 1. Proportion of positive individuals (0%−100%) for *Trichinella* spp. across counties in USA (a), in relation to age class (c), and sex (e). Proportion of positive individuals (0%−100%) for *Toxoplasma gondii* across counties in USA (b), in relation to age class (d), and sex (f). Error bars represent standard error (SE).

be as short as 2–3 weeks, with antibodies persisting for between 12 and > 24 months, or potentially indefinitely (Gottstein et al., 2009; Pozio et al., 2020). Additionally, antibodies to *T. gondii* are also thought to persist for the life of the animal (Castillo-Cuenca et al., 2021).

For decades, there has been a concerted effort to eliminate these two parasites from the commercial swine industry. A recent study found that prevalence of antibodies to *T. gondii* (determined by ELISA) among 20,209 samples from 22 slaughter plants in the United States was 0.74%, with sows having a seroprevalence of 1.03% (Fredericks et al., 2021). A previous estimate of *T. gondii* seroprevalence among domestic pigs in the U.S. was 2.6%, based on sera collected through the USDA National Animal Health Monitoring System (NAHMS) from swine in 2006 (Hill et al., 2010). The notable decline in *T. gondii* seroprevalence from surveys in 1990 (20% seroprevalence in sows, (Patton et al., 1996)), 1995 (15% in sow/breeder facilities and 3.2% in grower/finisher facilities, (Patton et al., 1996)), and 2000 (6% in sow/breeder facilities and 0.9% in grower/finisher facilities, (Patton et al., 2002)) in the U.S. sow population has been attributed to movement of the swine industry toward total confinement rearing and increased facility biosecurity (Pyburn et al., 2005). In these studies, the higher seroprevalence in sows is thought to be due to breeding pigs living longer and therefore being more likely to be exposed to *T. gondii* in the environment (Patton et al., 2002; Dubey, 2009). Seroprevalence for *T. spiralis* has been monitored through the USDA NAHMS in 1990 (5 positive sows out of 3048 samples), 1995 (1 positive sow out of 7987 samples), 2000 (no positives out of 14,328 samples), 2006 (no positives out of, 6238 samples), and 2012 (1 positive sample out of 5705 samples). The downward trend in seroprevalence is attributed to declines in the use of cats for rodent control and decreased outdoor access for breeding females and finishers (Services, 2018).

Table 2
Demographic and geographic variables associated with seroprevalence of *Trichinella* spp. and *Toxoplasma gondii* antibodies, separately and co-infected, in wild pigs in the United States.

Factor	*Trichinella* spp.		*Toxoplasma gondii*		Coinfection with *Trichinella* spp. and *T. gondii*	
	n	%	n	%	n	%
Sex						
Male	358/3538	10.1	1165/2846	40.9	160/2819	5.7
Female	560/3887	14.4	1269/3115	40.7	228/3092	7.4
Unknown	3/32	9.4	10/22	45.4	2/19	10.5
Age						
Adult	719/5537	13.0	1994/4422	45.1	335/4396	7.6
Subadult	140/1131	12.4	276/896	30.8	40/883	4.5
Juvenile	61/766	8.0	169/651	26.0	14/640	2.2
Unknown	1/23	4.3	5/14	35.7	1/11	9.1
Region						
Northeast	9/47	19.1	25/47	53.2	4/47	8.5
Midwest	98/532	18.4	243/434	56.0	51/434	11.8
South	763/5694	13.4	1905/4649	41.0	311/4616	6.7
West	57/1194	4.8	271/853	31.8	24/833	2.9
Total	**927/7467**	**12.4**	**2444/5983**	**40.8**	**390/5930**	**6.6**

One recommendation to reduce the prevalence of *Trichinella* spp. is to discourage hunters from leaving animal carcasses in the field, which would reduce the probability of transmission to new hosts (Gottstein et al., 2009). Additionally, hunters should be educated about the risks of consuming raw or semi-raw meat from wild pigs and about effective methods for inactivating *Trichinella* spp. larvae and *T. gondii* tissue cysts in meat, specifically cooking to reach a core temperature of 71 °C for at least one minute, freezing, and irradiation (Gottstein et al., 2009; Jones and Dubey, 2012). While risk of *Trichinella* spp. exposure is low for domestic pigs raised in systems with high biosecurity, the risk remains when such biosecurity breaks down or pigs are raised in open environments with access to rodents and wildlife, with risk increasing as infection levels in wildlife increase (Gamble, 2022).

The comparison of results from this study to those of other studies is limited by the use of ELISA kits from different manufacturers, specifically between studies by Hill et al. (2014), Pedersen et al. (2017), and the current study. Although the same sample type (serum) was used for all testing, and was paired with confirmed positive controls from wild pigs, these kits have variable sensitivity and specificity and there may have been additional differences in sample collection and processing that impacted the results. Future studies should standardize sample testing protocols and compare the performance of various assays so that comparisons can be made with greater confidence.

Finally, because *T. gondii* is capable of infecting a wide range of hosts, it is important to consider the potential impacts of *T. gondii* infections on wildlife that share habitat with wild pigs. Dubey et al. (2011) investigated the genetic characterization of *T. gondii* among several species of wildlife in North America, highlighting the diversity of wildlife species that may be affected by infection. Additionally, a serosurvey of *T. gondii* in wildlife from the southeastern United States showed high seroprevalence of *T. gondii* in white-tailed deer (*Odocoileus virginianus*), wild pigs, raccoons (*Procyon lotor*) and coyotes (*Canis latrans*) (Gerhold et al., 2017). Wild birds that feed from the ground (e.g., wild turkeys (*Meleagris gallopavo*)) are also epidemiologically relevant, as a recent study has shown wild turkeys are commonly infected with *T. gondii,* despite rarely showing clinical disease (Cerquiera-Cézar et al., 2019). Importantly, this indicates that ingestion of skeletal or cardiac muscle from infected turkeys can be a source of *T. gondii* for wildlife, domestic animals, and

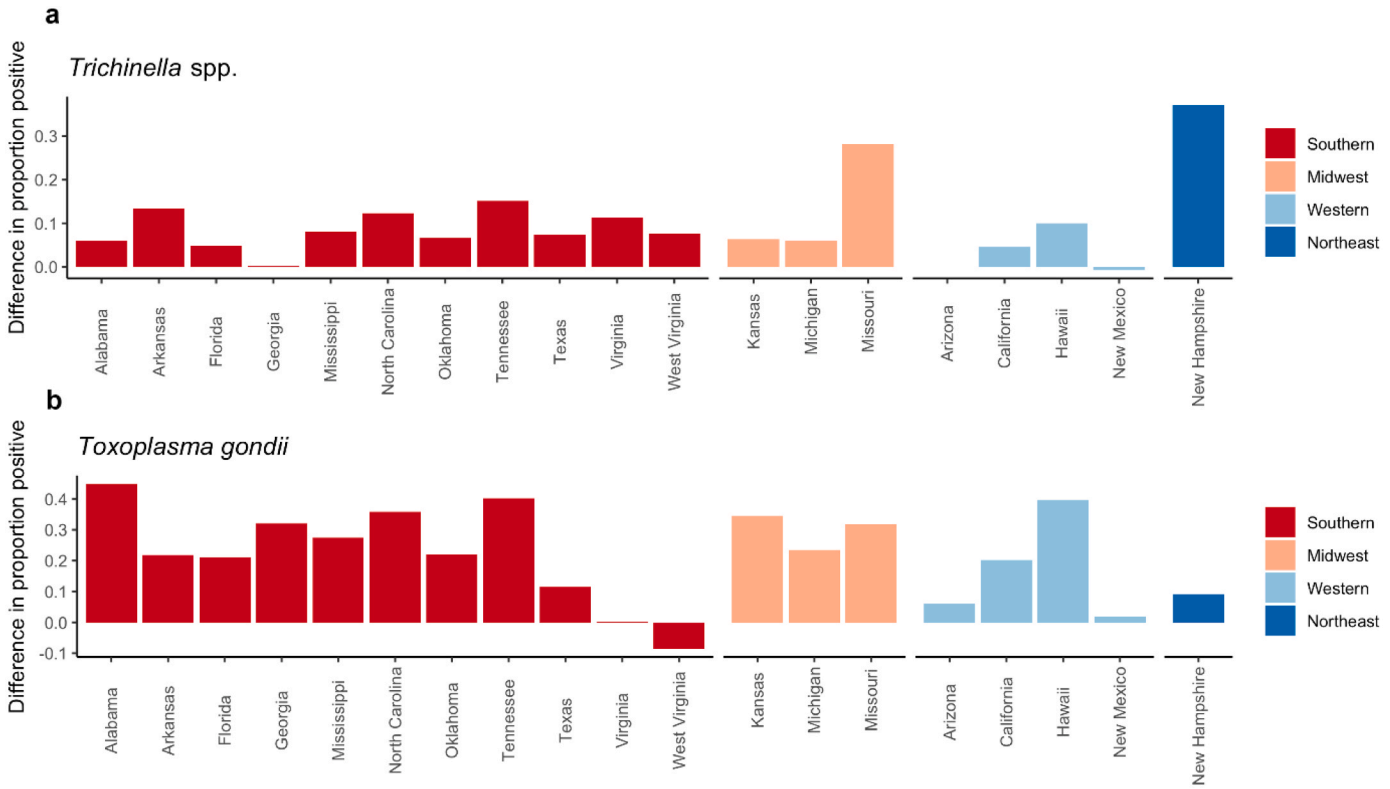

Fig. 2. Difference in the proportion of positive detections between this study and Hill et al. 2014. Only states for which at least 10 individuals sampled are shown. Positive values indicate that the current study reports higher proportion of positives than Hill et al. 2014 and negative values indicate that the current study reports lower proportion positive than Hill et al. 2014. Two-sample test for equality of proportions indicated that (a) *Trichinella* spp. detections were higher ($p < 0.05$) in this study compared to Hill et al. 2014, except for Michigan, Georgia, Mississippi, Alabama, West Virginia, Virginia, New Mexico, and Arizona. For (b) *Toxoplasma gondii*, rates did not differ for West Virginia, Virginia, New Hampshire, New Mexico, and Arizona. Two-sample test results reported in Table S1.

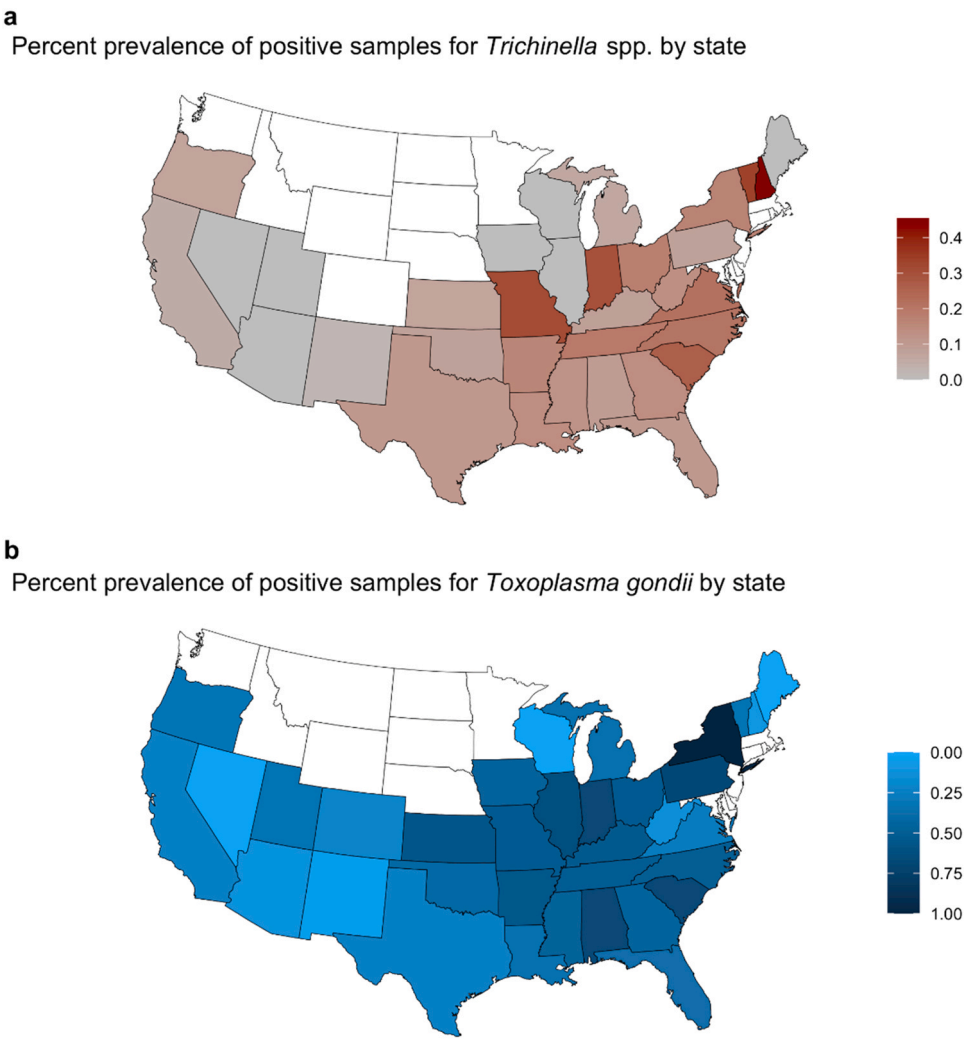

Fig. 3. Percent prevalence of positive samples (0%–100%) for (a) *Trichinella* spp. and (b) *Toxoplasma gondii* by state on mainland USA.

humans (Cerquiera-Cézar et al., 2019). Therefore, wild pigs may be a useful sentinel for detection of *T. gondii* in the environment and in spatially co-occurring wildlife.

5. Conclusion

The results of this work highlight an increase in the seroprevalence of two zoonotic pathogens found in a widely distributed invasive species in North America. Wild pig populations continue to increase in number and geographic distribution, despite considerable population management efforts. The potential for frequent interactions between wild pigs and other wildlife species, livestock, and humans illustrates a quintessential One Health challenge with pathogen transmission. Wild pigs are known reservoirs for a variety of zoonotic pathogens (Meng et al., 2009), have the capacity to be involved in emerging infectious diseases with serious implications for human health such as Japanese Encephalitis Virus (Williams et al., 2022), and may be a useful sentinel system for detection of *T. gondii* and *Trichinella* spp. Therefore, these findings support the need for continued surveillance of pathogens in wild pigs alongside management and control efforts.

CRediT authorship contribution statement

Majewska Ania A: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation. **Brown Vienna R.:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Doub Emily:** Writing – original draft, Methodology. **Coker Sarah M:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis. **Fojtik Alinde:** Writing – original draft, Methodology. **Callaghan Katherine C.:** Writing – original draft, Investigation, Formal analysis. **Haynes Ellen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Cleveland Christopher A.:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yabsley Michael J:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christopher A. Cleveland reports financial support was provided by USDA-APHIS Wildlife Services.

Acknowledgements

We would like to thank the numerous United States Department of Agriculture, Animal and Plant Health Inspection Service biologists for their efforts in sample collection. Funding for this work was provided by

the United States Department of Agriculture, Animal and Plant Health Inspection Service's Feral Swine Damage Management Program. Additional financial support was provided by the wildlife management agencies of the Southeastern Cooperative Wildlife Disease Study member states through the Federal Aid to Wildlife Restoration Act (50 Stat. 917).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2023.110090.

References

Barrios-Garcia, M.N., Ballari, S.A., 2012. Impact of wild boar (*Sus scrofa*) in its introduced and native range: a review. Biol. Invasions 14, 2283–2300.

Beasley, J.C., Ditchkoff, S.S., Mayer, J.J., Smith, M.D., Vercauteren, K.C., 2018. Research priorities for managing invasive wild pigs in North America. J. Wildl. Manag. 82, 674–681.

Beral, M., Rossi, S., Aubert, D., Gasqui, P., Terrier, M.E., Klein, F., Villena, I., Abrial, D., Gilot-Fromont, E., Richomme, C., Hars, J., Jourdain, E., 2012. Environmental factors associated with the seroprevalence of *Toxoplasma gondii* in wild boars (*Sus scrofa*), France. EcoHealth 9, 303–309.

Bevins, S.N., Pedersen, K., Lutman, M.W., Gidlewski, T., Deliberto, T.J., 2014. Consequences associated with the recent range expansion of nonnative feral swine. Bioscience 64, 291–299.

Capo, V., Despommier, D.D., 1996. Clinical aspects of infection with *Trichinella* spp. Clin. Microbiol. Rev. 9, 47–54.

Castillo-Cuenca, J.C., Martinez-Moreno, A., Diaz-Cao, J.M., Entrena-Garcia, A., Fraga, J., Arias, P.C., Almeria, S., Garcia-Bocanegra, I., 2021. Seroprevalence of *Toxoplasma gondii* and associated risk factors in domestic pigs raised from Cuba. Parasitol. Res. 120, 2897–2903.

CDC. 2023. Toxoplasmosis. ⟨https://www.cdc.gov/parasites/toxoplasmosis/index.html#:~:text=Toxoplasmosis%20is%20considered%20to%20be,the%20parasite%20from%20causing%20illness⟩. Accessed on: September 5, 2023.

Corn, J.L., Jordan, T.R., 2017. Development of the national feral swine map, 1982-2016. Wildl. Soc. Bull. 41, 758–763.

Diaz, J.H., Warren, R.J., Oster, M.J., 2020. The disease ecology, epidemiology, clinical manifestations, and management of Trichinellosis linked to consumption of wild animal meat. Wilderness Environ. Med. 31, 235–244.

Dubey, J.P., 2009. Toxoplasmosis in pigs-The last 20 years. Vet. Parasitol. 164, 89–103.

Dubey, J.P., 2016. Toxoplasmosis of animals and humans. CRC Press, Boca Raton, p. 313.

Dubey, J.P., Hill, D.E., Jones, J.L., Hightower, A.W., Kirkland, E., Roberts, J.M., Marcet, P.L., Lehmann, T., Vianna, M.C.B., Miska, K., Sreekumar, C., Kwok, O.C.H., Shen, S.K., Gamble, H.R., 2005. Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: risk assessment to consumers. J. Parasitol. 91, 1082–1093.

Dubey, J.P., Velmurugan, G.V., Rajendran, C., Yabsley, M.J., Thomas, N.J., Beckmen, K. B., Sinnett, D., Ruid, D., Hart, J., Fair, P.A., McFee, W.E., 2011. Genetic characterisation of *Toxoplasma gondii* in wildlife from North America revealed widespread and high prevalence of the fourth clonal type. Int. J. Parasitol. 41 (11), 1139–1147.

Fredericks, J., Hill, D.E., Hawkins-Cooper, D.S., Fournet, V.M., Calero-Landa, J., Adams, B., Johnson, A., Barrow, M., Aquino, J., Mahmoud, T., Murphy, V., Barlow, A., Patel, P., George, M., Chehab, N., Kramer, M., Bauer, N.E., 2021. Seroprevalence of *Toxoplasma gondii* in market hogs collected from US slaughterhouses. J. Parasitol. 107, 404–410.

Gamble, H.R., 2022. *Trichinella* spp. control in modern pork production systems. Food Water Parasit. 28, e00172 https://doi.org/10.1016/j.fawpar.2022.e00172.

Gerhold, R.W., Saraf, P., Chapman, A., Zou, X., Hickling, G., Stiver, W.H., Houston, A., Souza, M., Su, C., 2017. *Toxoplasma gondii* seroprevalence and genotype diversity in select wildlife species from the southeastern United States. Parasites Vectors 10, 1–8.

Gottstein, B., Pozio, E., Nockler, K., 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin. Microbiol. Rev. 22, 127–145.

Hill, D., Dubey, J.P., 2002. *Toxoplasma gondii*: transmission, diagnosis and prevention. Clin. Microbiol. Infect. 8, 634–640.

Hill, D.E., Haley, C., Wagner, B., Gamble, H.R., Dubey, J.P., 2010. Seroprevalence of and risk factors for *Toxoplasma gondii* in the US swine herd using sera collected during the National Animal Health Monitoring Survey (Swine 2006). Zoonoses Public Health 57, 53–59.

Hill, D.E., Dubey, J.P., Baroch, J.A., Swafford, S.R., Fournet, V.F., Hawkins-Cooper, D., Pyburn, D.G., Schmit, B.S., Gamble, H.R., Pedersen, K., Ferreira, L.R., Verma, S.K., Ying, Y., Kwok, O.C., Feidas, H., Theodoropoulos, G., 2014. Surveillance of feral swine for *Trichinella* spp. and *Toxoplasma gondii* in the USA and host-related factors associated with infection. Vet. Parasitol. 205, 653–665.

Jones, J.L., Dubey, J.P., 2012. Foodborne toxoplasmosis. Clin. Infect. Dis. 55, 845–851.

Massei, G., Roy, S., Bunting, R., 2011. Too many hogs? A review of methods to mitigate impact by wild boar and feral hogs. Hum. -Wildl. Interact. 5, 79–99.

Meng, X.J., Lindsay, D.S., Sriranganathan, N., 2009. Wild boars as sources for infectious diseases in livestock and humans. Philos. T R. Soc. B 364, 2697–2707.

Patton, S., Zimmerman, J., Roberts, T., Faulkner, C., Diderrich, V., AssadiRad, A., Davies, P., Kliebenstein, J., 1996. Seroprevalence of *Toxoplasma gondii* in hogs in the National Animal Health Monitoring System (NAHMS). J. Eukaryot. Microbiol. 43, S121. -S121.

Patton, S., Faulkner, C., Anderson, A., Smedley, K., Bush, E. 2002. *Toxoplasma gondii* infection in sows and market weight pigs in the United States and its potential impact on consumer demand for pork. ⟨https://porkcheckoff.org/wp-content/uploads/2021/02/00–130-PATTONUofTenn.pdf⟩. Accessed 10 March 2023.

Pedersen, K., Bauer, N.E., Rodgers, S., Bazan, L.R., Mesenbrink, B.T., Gidlewski, T., 2017. Antibodies to various zoonotic pathogens detected in feral wwine (*Sus scrofa*) at abattoirs in Texas, USA. J. Food Prot. 80, 1239–1242.

Pozio, E., Merialdi, G., Licata, E., Della Casa, G., Fabiani, M., Amati, M., Cherchi, S., Ramini, M., Faeti, V., Interisano, M., Ludovisi, A., Rugna, G., Marucci, G., Tonanzi, D., Gómez-Morales, M.A., 2020. Differences in larval survival and IgG response patterns in long-lasting infections by *Trichinella spiralis*, *Trichinella britovi* and *Trichinella pseudospiralis* in pigs. Parasites Vectors 13, 520.

Pyburn, D.G., Gamble, H.R., Wagstrom, E.A., Anderson, L.A., Miller, L.E., 2005. Trichinae certification in the United States pork industry. Vet. Parasitol. 132, 179–183.

Services, U.A.V. 2018. *Trichinella* antibody seroprevalence in US swine, 1990–2012. ⟨https://www.aphis.usda.gov/animal_health/nahms/swine/downloads/swine2012/Swine2012_is_Trich_1.pdf⟩. Accessed 10 March 2023.

Shapiro, K., Bahia-Oliveira, L., Dixon, B., Dumetre, A., de Wit, L.A., VanWormer, E., Villena, I., 2019. Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. Food Water Parasit. 15, e00049 https://doi.org/10.1016/j.fawpar.2019.e00049.

Tabak, M.A., Piaggio, A.J., Miller, R.S., Sweitzer, R.A., Ernest, H.B., 2017. Anthropogenic factors predict movement of an invasive species. Ecosphere 8, e01844. https://doi.org/10.1002/ecs2.1844.

Williams, C.R., Webb, C.E., Higgs, S., van den Hurk, A.F., 2022. Japanese Encephalitis Virus emergence in Australia: public health importance and implications for future surveillance. Vector-Borne Zoonotic Dis. 22, 529–534.

Zarlenga, D., Thompson, P., Pozio, E., 2020. *Trichinella* species and genotypes. Res. Vet. Sci. 133, 289–296.