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Infectious agents in feral swine in Ohio, USA (2009-2015): A low but evolving risk to agriculture and public health

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ABSTRACT

As highly mobile and prolific animals, feral swine threaten public and livestock health. To quantify these threats, we analyzed disease surveillance samples to estimate the prevalence of key pathogens and parasites in feral swine captured in Ohio. Between 2009 and 2015, samples from 205 feral swine were tested for up to 13 pathogens. Methicillin susceptible *Staphylococcus aureus* was cultured from 29 of 138 (21%) individuals and antibodies to *Leptospira* spp. (16/69; 23%), *Toxoplasma gondii* (11/76; 15%), *Trichinella spiralis* (4/69; 6%), hepatitis E virus (1/32; 3%), *Brucella* spp. (2/138; 1%), and influenza A virus (2/176; 1%) were also detected. Lungworms (*Metastrongylus* spp.) were frequently identified (46/55; 84%). Evidence of infection or exposure was not detected to *Mycobacterium bovis*, classical swine fever, pseudorabies, and porcine reproductive and respiratory syndrome. All positive *Brucella* and hepatitis E virus samples and 63% (10/16) of the positive *Leptospira* spp. samples came from individuals identified as illegal out-of-state feral swine introductions. Results indicated an overall low prevalence of pathogens in feral swine in Ohio; however, the importation of live feral swine from other states remained an important concern for pathogen introduction and spread.

1. Introduction

The United States of America feral swine (*Sus scrofa*) population is estimated at over 6 million individuals (USDA-APHIS-WS, 2015a). The population in Ohio state is comparably low, estimated at fewer than 2,000 individuals. These swine likely originated from hunting preserve escapees or from illegal interstate transportation, with continued population growth through breeding and on-going illegal importation. As of 2015, established breeding populations of feral swine were present in at least nine Ohio counties and sporadic reports of feral swine sightings recorded in an additional eight counties across the state (Fig. 1).

Feral swine are potential carriers of over 60 viral, bacterial and parasitic pathogens, many of which are transmissible to other animals including livestock, domestic pets, wildlife and humans (Davidson, 2006). Additionally, they are carriers of external parasites such as ticks and lice, many of which are vectors for numerous pathogens (Davidson, 2006). Despite their low numbers, the ability of feral swine to carry a variety of pathogens and their potential interactions with wildlife, domestic animals, and people make them potentially important infectious vectors in the state.

Since 2009, Ohio Wildlife Services (OHWS), a program of the United States Department of Agriculture's Animal and Plant Health Inspection Service, has performed disease surveillance in feral swine in conjunction with a population elimination program. Known feral swine populations are carefully monitored with an end goal of elimination. New populations are identified through reports by the public and partnerships with local and regional government agencies. New populations are investigated by wildlife personnel to determine the likely source (e.g., local release, inter-state importation) and monitored, again with an end goal of elimination. The present study analyzed the results of biological samples collected from feral swine taken during elimination efforts by OHWS from April 2009 to September 2015.

2. Material and methods

All procedures for sample collection and processing were performed in accordance with standard guidelines set forth by the USDA Wildlife Services National Wildlife Disease Program in the annual “Wildlife Services' Comprehensive Feral Swine Disease Surveillance Procedures Manual” for the years 2009-2015 (USDA-APHIS-WS, 2015b). During

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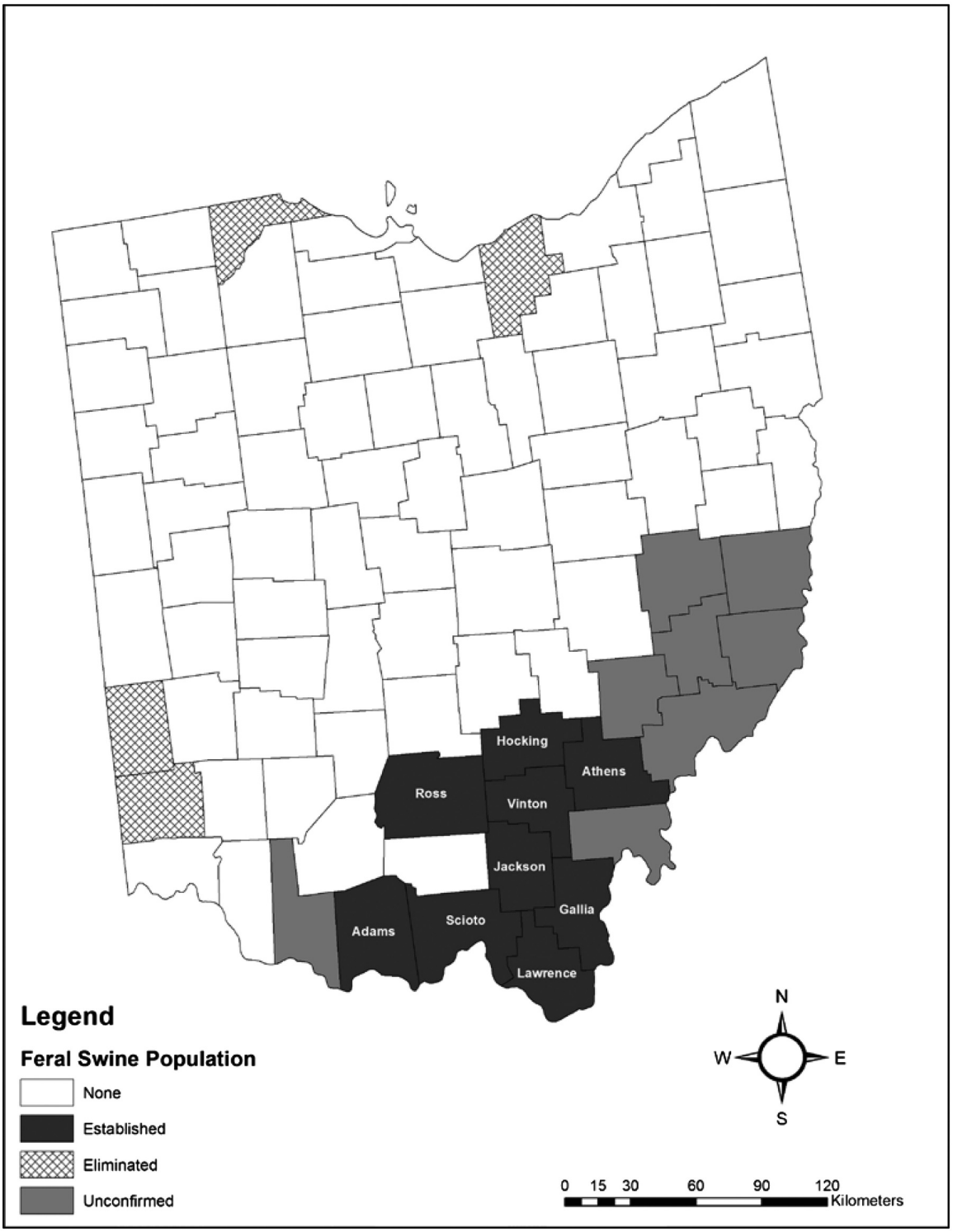

Fig. 1. Feral swine status in Ohio counties (as of September 2015) based on data and observations of the Ohio Program of USDA-APHIS-Wildlife Services in Groveport, Ohio. Established: A sustainable breeding population consisting of at least one adult male and one adult female swine with offspring occupying a county for more than one year.

that time, there was some variation in sample type, sample processing, and specific laboratories due to funding changes and specific disease interest at the national level. Between 2009-2012, sample collection occurred irrespective of animal age and was based on availability of captured individuals. This approach was replaced in 2013 by targeted sampling of adults, when available, to maximize pathogen detection as seroprevalence of some pathogens has been shown to be increased in older feral swine (Hill et al., 2014).

During necropsy, the heart, liver, lungs, and trachea were grossly examined and the presence of lung worms (*Metastrongylus* spp.) was noted. Samples (n = 1244; serum, axilla and nasal swabs, feces, bronchial alveolar lavage samples) were shipped to collaborating laboratories for pathogen testing. A combination of serological, polymerase chain reaction (PCR) and culture techniques were used to detect

Table 1
Test method and prevalence of pathogens and parasites from 205 feral swine captured in Ohio, USA (April 2009 - September 2015).

Pathogen/ Parasite	Test Method(s)	Sample Location/ Type	Positive/ Negative Frequency	Prevalence (95% CI)
Metastrongylus spp.	Visual inspection during necropsy	Heart, liver, lungs, trachea	46/55	84 (74-94)
Leptospira spp.	ELISA, Microscopic Agglutination Test	Serum	16/69	23 (13-33)
Methicillin susceptible *Staphylococcus aureus*	Bacterial isolation	Axilla, nasal swabs	29/138	21 (15-29)
Toxoplasma gondii	ELISA, Modified Agglutination Test	Serum	11/76	15 (8-24)
Trichinella spiralis	ELISA	Serum	4/69	6 (2-14)
Hepatitis E virus	ELISA	Serum	1/32	3 (0.5-16)
Porcine epidemic diarrhea virus	ELISA, IFA, PCR	Serum, feces	1/45	2 (0.4-12)
Brucella spp.	Card Test, FPA	Serum	2/138	1 (0.4-5)
Influenza-A virus	ELISA, RT-PCR (matrix), virus isolation	Serum, nasal swab, bronchial alveolar lavage	2/176	1 (0-4)
Methicillin resistant *Staphylococcus aureus*	Bacterial isolation	Axilla, nasal swabs	0/138	0 (0-3)
Mycobacterium bovis	ELISA	Serum	0/13	0 (0-23)
Classical swine fever virus	ELISA	Serum	0/139	0 (0-3)
Porcine reproductive and respiratory syndrome virus	ELISA	Serum	0/72	0 (0-5)
Pseudorabies virus	gB ELISA	Serum	0/139	0 (0-3)

FPA: Fluorescence polarization assay.
ELISA: Enzyme-linked immunosorbent assay.
IFA: Indirect fluorescence assay.

pathogens or evidence of exposure, following established protocols unless otherwise stated (Table 1). Detection of *Toxoplasma gondii* (ELISA and modified agglutination test) and *Trichinella spiralis* (ELISA) antibodies were performed on serum as previously described (Hill et al., 2014). Samples with titers of 1:25 and greater were considered positive for *T. gondii*. Detection of *Leptospira* spp. serovar antibodies (ELISA, microscopic agglutination test) was performed on serum samples, considering samples positive with titers of 1:200 and greater (Pedersen et al., 2015). Serum samples were tested by ELISA for antibodies against *Mycobacterium bovis* (Pedersen, Miller, et al., 2017), classical swine fever (CSF; Swafford, Schmit, Pedersen, Lutman, & Deliberto, 2009), pseudorabies (Pedersen et al., 2013), hepatitis E virus (HEV), and porcine reproductive and respiratory syndrome (PRRS) virus (Pedersen et al., 2018). *Brucella* spp. antibodies were detected in serum (card test) and, if positive, followed by fluorescence polarization assay (Pedersen et al., 2014). Porcine epidemic diarrhea virus (PEDV) was detected in feces (PCR) and antibodies in serum (ELISA and IFA; Jung et al., 2014).

For *Staphylococcus aureus*, samples from the nasal cavity and axilla were acquired by using a BD BBL Culture Swab™ to gently swab with a circular motion to cover as much surface as possible, then placing in a sterile vial. Samples were processed according to previously described protocols, differentiating between methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA; van Balen et al., 2014). Influenza-A virus (IAV) testing was performed on serum (ELISA; Martin et al., 2017) and feral swine bronchial alveolar lavage samples and nasal swabs using the VetMAX-Gold SIV Detection Kit (Thermo Fisher Scientific) as previously described (Bliss, Nelson, Nolting, & Bowman, 2016). JMP software MP® 11.0.0 was used to calculate 95% confidence intervals for the estimated prevalence/seroprevalence of each pathogen.

3. Results and discussions

A total of 205 feral swine were tested for one or more of 13 pathogens and examined for the presence of *Metastrongylus* spp. (Table 1). Overall, both sexes were sampled in approximately equal numbers (108 females and 97 males), with adults (> 12 months) most frequently sampled (n = 87) followed by sub-adults (2-12 months; n = 67) and juveniles (< 2 months; n = 51). Given year-to-year changes in federal testing priorities and availability of feral swine, the number of feral swine sampled and the pathogens tested varied annually.

Seven zoonotic pathogens (or their associated antibodies) were detected: *Leptospira* spp., MSSA, *T. gondii, T. spiralis*, HEV, IAV, and *Brucella* spp. (Table 1). Multiple pathogens were identified in eight individuals: *T. gondii* and *T. spiralis* (n = 1), *T. spiralis* and *Leptospira* spp. (n = 1), *T. gondii* and *Leptospira* spp. (n = 1) and *Leptospira* spp. and MSSA (n = 5).

Antibodies to *Leptospira* spp. were identified in 16 feral swine (16/69; 23%), with some individuals having elevated titers to multiple serovars. The most common servovars detected were *L. Bratislava* (9/69; 13%), followed by *Icterohaemorrhagiae* (6/69; 9%), *Grippotyphosa* (5/69; 7%) and *Canicola* (2/69; 3%). Serovars *Pomona and Hardjo* were not detected. *Staphylococcus aureus* was detected in 21% (29/138) of the samples; all were MSSA. Antibodies to *T. gondii* were identified in 15% of samples (11/76), while antibodies to *T. spiralis* were found in 6% of samples (4/69). Two samples tested positive for *Brucella* spp. (2/138; 1%). One sample was positive for HEV (1/32; 3%) and two samples were positive for IAV antibodies (2/176; 1%).

Presence of (or antibodies to) *M. bovis*, CSF, PRV, and PRRSV were not detected. One of the 45 pigs tested for PEDV had test results interpreted as suspect positive (i.e., result between negative and positive ranges). *Metastrongylus* spp. were noted in 84% (46/55) of the pigs examined.

During the study period, legal investigations into new feral swine populations indicated some of the pathogen-positive samples had come from individuals illegally imported into the state and intentionally released (Table 2). Specifically, all positive *Brucella* spp. and HEV samples and 63% (10/16) of the positive *Leptospira* spp. samples were from feral swine populations determined to be illegally imported from other states (reportedly Georgia and Louisiana). Previous studies have documented an elevated prevalence of *Brucella* spp. in feral swine from the southern US (Pedersen et al., 2012).

Feral swine are a potential reservoir of zoonotic and non-zoonotic pathogens and parasites, and as long as they are present in a region, the risk of infectious disease transmission and/or re-emergence of diseases should not be ignored. From a public health standpoint, our results indicate feral swine pose a risk, albeit overall low, for zoonotic pathogen transmission in Ohio. The risk to the public is perhaps greatest for hunters, biologists and other professionals who have close contact with these animals or those who consume their meat. Based on our results, the southern counties of Ohio had the greatest chance of contact

Table 2
Positive test results by pathogen, county, and year of Ohio feral swine samples (2009 - 2015).

Pathogen	Year					
	2009	2010	2012	2013	2014	2015
Toxoplasma gondii		Lucas[a]	Vinton[b]			
Trichinella spiralis		Lucas[a]	Vinton[b]			
Methicillin susceptible *Staphylococcus aureus*			Jackson[b]	Lorain[c], Vinton[b]	Vinton[b]	Vinton[b]
Leptospira spp.	Gallia[b]		Vinton[b], Jackson[b]	Lorain[d], Vinton[b]	Lorain[d], Vinton[b]	
Brucella spp.			Lorain[c]	Lorain[c]		
Influenza-A virus			Vinton[b], Lorain[c]			
Hepatitis E virus			Scioto[d]			

[a] Intentional release reported 2010, origin of feral swine unknown. Population eliminated.
[b] Accidental escapees from hunting preserves first noted around 1980, Eurasian lineage and descendants. Established populations.
[c] Intentional release reported 2012, feral swine from (reportedly) Georgia. All individuals removed.
[d] Intentional release reported 2011, feral swine from (reportedly) Louisiana. Several individuals not captured.

between infected/exposed feral swine, livestock, domestic swine and humans (Fig. 1, Table 2); the presence of backyard livestock and facilities with limited biosecurity systems likely elevated this risk.

Our results are similar to the estimated national prevalence of pathogens in feral swine (e.g., 13% positive for *Leptospira* spp.; Pedersen et al., 2015) yet considerably lower than some regional studies (e.g., 49% positive for *Leptospira spp*. in a Texas study; Pedersen, Bauer, Rodgers et al., 2017). It is interesting to note that MSSA was recovered from 21% of individuals, suggesting this human and animal pathogen was circulating in the feral swine population. The high prevalence of *Metastrongylus* spp. we noted is consistent with the limited previous reports of this parasite in feral swine (Shender, Botzler, & George, 2002) and poses an important concern for the domestic swine industry.

Given that pork production is a USD 15 billion industry in the United States and as Ohio is the eighth highest US state for swine inventory (NPB, 2016), introduction of one or more of these pathogens into an Ohio swine facility could result in significant economic impact. Fortunately, most of the commercial swine production in Ohio is located to the northwestern region of the state, and most established feral swine populations remain in the southeastern reaches of the state. The cases of *Brucella* spp. and *Leptospira* spp. found in Lorain County (north central Ohio) came from the same intentional release; this Lorain County population was completely eliminated in 2015.

This study had several limitations. The number of samples available for testing was low and opportunistic, which may have affected prevalence estimates and precluded analysis by subgroups (e.g., age). As this work was part of a larger elimination and surveillance program, sample numbers (including number of feral swine tested) was based on availability and national program priorities. However, the total number of feral swine tested (n = 205) provided an adequate sample size to ensure reasonable pathogen testing breadth and depth. Specifically, all individuals were tested for one or more of 13 pathogens with most samples per pathogen exceeding 50 [number needed to detect a pathogen with a low (i.e. 5%) prevalence with 95% confidence, as well as provide a reasonable estimate for a relatively common pathogen (i.e. 15% prevalence ± 10% with 95% confidence)]. Additionally, some testing modalities have been shown to underestimate true prevalence in feral swine (e.g., antibody testing for *B. suis*), therefore actual prevalence may be greater than we estimated (Pedersen,Bauer, Olsen et al., 2017). Although samples were tested for 13 pathogens, many were not included, such as pathogens carried by ectoparasites. The tick vector *Ixodes scapularis*, along with associated disease-causing pathogens (e.g., *Borrelia burgdorferi*), are emerging in Ohio (Wang et al., 2014). Ticks are frequently found on feral swine, potentially increasing risk of tick bite-associated diseases for humans with feral swine contact. Future studies are encouraged to improve our understanding of feral swine population dynamics and pathogen presence in Ohio.

This study serves as an important step in recognizing feral swine risks in Ohio and assisting in prioritizing future surveillance, pathogen testing, and control strategies. Illegal introductions of animals carrying pathogens remain a critical threat to Ohio's swine industry.

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Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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