# **PEER-REVIEWED**

# **BRIEF COMMUNICATION**

# Antibody evidence of porcine reproductive and respiratory syndrome virus detected in sera collected from feral swine (*Sus scrofa*) across the United States

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#### Summary

Feral swine sera from across the United States were tested for antibodies to porcine reproductive and respiratory syndrome virus. Antibodies to the virus were detected in 1.2% (68 of 5506) of the samples tested, suggesting that feral swine are unlikely to be an important source of spillback into domestic swine.

Keywords: swine, disease, feral swine, porcine reproductive and respiratory syndrome, *Sus scrofa* 

Received: February 2, 2017 Accepted: June 13, 2017

F eral swine (*Sus scrofa*) are an invasive and destructive species in the United States. Although originally introduced into the United States in the early 1500s by Spanish explorers,<sup>1</sup> their more recent range Resumen - Evidencia de anticuerpos contra el virus del síndrome reproductivo y respiratorio porcino detectado en sueros de cerdos silvestres (*Sus scrofa*) a lo largo de los Estados Unidos

Se analizaron sueros de cerdos silvestres a lo largo de los Estados Unidos en busca de anticuerpos contra el virus del síndrome reproductivo y respiratorio porcino. Se detectaron anticuerpos contra el virus en 1.2% (68 de 5506) de las analizadas, lo que sugiere que es poco probable que los cerdos silvestre sean una fuente importante de fuga hacia la población porcina doméstica.

expansion and rapidly increasing populations have led to concern not only because of the damage they cause to agricultural crops and ecosystems<sup>2</sup> through their rooting behavior, but also because of the numerous

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This article is available online at http://www.aasv.org/shap.html.

Pedersen K, Miller RS, Musante AR, et al. Antibody evidence of porcine reproductive and respiratory syndrome virus detected in sera collected from feral swine (*Sus scrofa*) across the United States. *J Swine Health Prod.* 2018;26(1):41–44.

#### Résumé - Présence d'anticorps contre le virus du syndrome reproducteur et respiratoire porcin dans des échantillons de sérum prélevés de porcs sauvages (*Sus scrofa*) à travers les États-Unis

Des échantillons de sérum prélevés de porcs sauvages à travers les États-Unis ont été testés pour la présence d'anticorps contre le virus du syndrome reproducteur et respiratoire porcin. Des anticorps contre le virus ont été détectés dans 1,2% des échantillons testés (68 des 5506), suggérant ainsi que les porcs sauvages ne seraient pas une source importante de reflux du virus vers les porcs domestiques.

pathogens they carry that are infectious to humans and livestock.<sup>3</sup> While populations are concentrated in the southeastern part of the United States, the increasing geographic distribution of feral swine into northern regions of the country signifies a concurrent risk of the potential for increased pathogen transmission.<sup>4</sup> Porcine reproductive and respiratory syndrome (PRRS) virus is of particular economic importance to the US commercial swine industry. The disease has been estimated to cost \$664 million annually or \$1.8 million per day in combined productivity losses to breeding and growing pig herds.<sup>5</sup> First identified in the United States in 1987,<sup>6</sup> PRRS is an important cause of late-term reproductive losses, severe pneumonia, reduced growth rates, and increased mortality.<sup>7</sup> Although it may have been introduced from Europe by imported wild boar,<sup>8</sup> the role of feral swine and wild boar in the transmission and maintenance of PRRS in the United States is uncertain. Previous small-scale surveys for PRRS, conducted in feral swine in Alabama, Arkansas, California, Florida, Georgia, Hawaii, Kansas, Louisiana, Michigan, Mississippi, Missouri, New

Mexico, North Carolina, Oklahoma, South Carolina, and Texas estimated the antibody prevalence as 1% to 3%<sup>2,9-12</sup> However, there has been no national-level surveillance conducted for the disease in feral swine in the United States. Our objective was to fill this gap by establishing baseline antibody data for feral swine across the United States that could be used to identify areas of risk of pathogen transmission between domestic swine and feral swine.

# Materials and methods

The United States Department of Agriculture, Animal and Plant Health Inspection Services' Wildlife Services removes feral swine for damage management purposes. Feral swine are lethally removed following the American Veterinary Medical Association Guidelines on Euthanasia. Damage is defined as destruction of agricultural crops, damage to urban areas, and impacts to native wildlife, in addition to transmission of pathogens to livestock, including domestic swine. Various pathogens have been documented in feral swine that can be transmitted to domestic swine.<sup>3,4</sup> Sera collected from feral swine targeted for removal were tested for exposure to various pathogens, including PRRS virus (PRRSV). Samples were submitted to any one of eight accredited veterinary diagnostic laboratories in the United States for testing with an enzyme-linked immunosorbent assay (ELISA: PRRS X3 Antibody Test Kit; IDEXX Laboratories, Inc, Westbrook, Maine) according to the manufacturer instructions.

A hierarchical Bayesian model<sup>13,14</sup> was used to estimate national- and state-level antibody prevalence in feral swine. Previous work has determined that PRRS antibody prevalence in feral swine varies regionally by the amount of domestic swine production.<sup>15</sup> To account for this variation and to determine potential risks to domestic swine production, the antibody prevalence was estimated nationally for each state, and separately for states with large and small swine farms. Nationally, the median (50<sup>th</sup> percentile) number of domestic pig farms by state was 1200 farms. This number was used to distinguish states with large swine industries  $(\geq 1200 \text{ farms})$  from states with small swine industries (< 1200 farms). Samples collected in the same county were assumed to originate from the same feral swine population, and samples collected in the same month and year were considered a single sampling

event. The ELISA used for detection has an estimated sensitivity (SN) of 98.8% and specificity (SP) of 99.9%.<sup>16</sup> Uncertainty regarding the test performance in feral swine and between the eight testing laboratories was accounted for by using beta distributed priors for SN ( $\alpha$  = 35.55,  $\beta$  = 1.42) and SP  $(\alpha = 28.9, \beta = 1.03)$  assuming 95% certainty that the ELISA SN and SP were greater than 90%. On the basis of previous studies,<sup>10,11</sup> the prevalence was assumed to be below 10% with 95% certainty, and a moderately informative beta prior for prevalence ( $\alpha = 1.45$ ,  $\beta = 35.98$ ) was utilized. Posterior inference used 100,000 iterations from three Markov chain Monte Carlo (MCMC) simulations, with the first 20,000 iterations discarded as burn-in. Convergence was confirmed by using autocorrelation among samples and the Brooks-Gelman-Rubin convergence statistic.<sup>17</sup> The highest posterior density (HPD) was used as an estimate of the expected national prevalence. Multivariate generalized linear model with a logit link, sometimes referred to as a fractional logit,<sup>18</sup> was used to investigate the mean potential associations between state prevalence, the density of domestic swine production, and the size of domestic swine farms. The predicted HPD prevalence (response variable) for each contiguous state was regressed against National Agricultural Statistics Service (NASS) data reporting the total number of domestic swine farms, number of small farms (< 100 animals), number of large farms ( $\geq 2000$  animals), and total inventory of swine. Differences in state prevalence were compared using the amount of posterior overlap and calculated the probability that the posterior distributions were different than the national prevalence. Bayesian models were fit using MCMC techniques and implemented in R (R Project for Statistical Computing, Vienna, Austria) and JAGS software (Just Another Gibbs Sampler, Vienna, Austria), and regression analysis was conducted in R.

### Results

From October 1, 2013, through September 30, 2015, we submitted 5506 sera collected from feral swine in 316 counties of 26 states for PRRS antibody testing. At least one positive was detected in 43 counties of 14 states (Table 1), and the national antibody prevalence estimated by the Bayesian model was 1.9% (95% HPD interval = 0.3 to 7.2; Table 1). State level prevalence estimates varied from 0.8% (95% HPD interval = 0.09 to 4.1) in Kansas to 4.1% (95% HPD interval = 0.8 to 9.5) in Michigan. Antibody prevalence in states with  $\geq 1200$  farms was 2.2% (95% HPD interval = 1.2 to 3.7) and was higher than in states with < 1200 farms (1.6%; 95% HPD interval = 1.0 to 2.4) with a moderate probability (Pr = 0.51) of being different. State antibody prevalence was positively associated with the total number of farms (log odds = 1.10; 95% confidence interval (CI) = 1.06-1.14; P < .001), but not associated with the number of domestic swine ( $\log \text{ odds} = 0.99$ ; 95% CI = 0.98-1.0;  $P \ge .05$ ). Farm size was a significant predictor of prevalence, with small farms being positively associated with prevalence (log odds = 1.11; 95% CI = 1.06-1.16; *P* < .001). Large farms were not associated with state prevalence ( $\log \text{ odds} = 1.03$ ; 95% CI = 0.43-2.5;  $P \ge .05$ ). When considered alone, the total number of small domestic swine farms explained the majority of the variance in PRRS prevalence in feral swine with an adjusted R<sup>2</sup> of 63%. Every additional 100 small farms in a state was associated with an 11% increase in state prevalence.

# Discussion

Similar to our findings, in France the antibody prevalence of PRRS in feral swine was approximately 3.5%, and all positive feral swine were identified in areas with a high density and prevalence of infection in domestic swine.<sup>15</sup> However, no antibodies to PRRSV were detected in feral swine in Spain<sup>19</sup> or Slovenia,<sup>20</sup> which may be due to the relatively small sample sizes (78 in Spain and 178 in Slovenia) in those studies or attributed to a difference in herd structure and management.<sup>6</sup>

Transmission of PRRS occurs through direct contact, contaminated fomites, or aerosolized particles.<sup>21,22</sup> Direct contact between domestic swine and feral swine has been documented<sup>11</sup> and suggests that there is a potential for pathogen transmission to occur via this route. PRRS is common in US domestic swine, with antibody prevalence in unvaccinated animals ranging from 20.0% to 69.6%.<sup>23-25</sup> Since the antibody prevalence of PRRS virus detected in feral swine in this study was so low in comparison, and the antibody prevalence in feral swine increased with the number of domestic swine farms in the state, the risk of feral swine transmitting PRRS to domestic swine remains low as reported previously.<sup>12</sup> It also suggests that feral swine acquired the infection from domestic swine. However, it remains unclear if feral

**Table 1:** Apparent antibody prevalence with 95% confidence intervals (CI) and Bayesian estimated true prevalence with 95% credible intervals (CrI) of feral swine serum samples collected from across the United States from October 1, 2013, through September 30, 2015, and tested for exposure to porcine reproductive and respiratory syndrome with an enzyme-linked immunosorbent assay

State (n)	Apparent prevalence (95% CI)	True prevalence (95% CrI)	Pr* prevalence ≠ national
National (5506)	1.2 (0.01-1.5)	1.9 (0.3-7.2)	NA
Alabama (194)	0.5 (0.09-2.9)	2.1 (0.4-7.8)	0.05
Arizona (44)	0 (0-8.0)	1.7 (0.3-6.1)	0.14
Arkansas (323)	0 (0-1.2)	1.6 (0.3-3.8)	0.34
California (479)	1.3 (0.6-2.7)	1.5 (0.4-6.2)	0.08
Florida (584)	2.6 (1.6-4.2)	3.5 (0.4-7.4)	0.16
Georgia (320)	2.2 (1.1-4.5)	2.0 (0.4-8.2)	0.10
Hawaii (297)	3.4 (1.8-6.1)	3.5 (2.0-5.5)	0.48
Illinois (21)	0 (0-15.4)	3.3 (0.7-7.6)	0.16
Indiana (12)	0 (0-24.3)	3.2 (0.8-8.5)	0.19
Kansas (195)	0 (0-1.9)	0.9 (0.1-4.1)	0.52
Kentucky (20)	0 (0-16.1)	3.1 (0.9-8.4)	0.20
Louisiana (276)	0.7 (0.2-2.6)	2.0 (0.3-7.2)	0.08
Michigan (16)	0 (0-19.4)	4.1 (0.8-9.6)	0.30
Mississippi (256)	0.4 (0.1-2.2)	2.9 (0.6-7.9)	0.13
Missouri (114)	0 (0-3.3)	1.9 (0.2-5.5)	0.18
New Mexico (97)	0 (0-3.8)	2.5 (0.8-8.1)	0.19
New York (11)	0 (0-25.9)	1.4 (0.4-7.3)	0.05
North Carolina (245)	1.2 (0.4-3.5)	1.3 (0.3-7.0)	0.09
Ohio (72)	0 (0-5.1)	1.2 (0.2-4.9)	0.31
Oklahoma (467)	0.9 (0.3-2.2)	1.7 (0.4-7.6)	0.07
Oregon (49)	0 (0-7.3)	2.6 (0.5-6.3)	0.10
South Carolina (274)	3.3 (1.7-6.1)	1.0 (0.3-8.8)	0.22
Tennessee (125)	0 (0-3.0)	1.1 (0.2-5.8)	0.24
Texas (889)	0.9 (0.5-1.8)	2.0 (0.4-7.3)	0.05
Virginia (86)	2.3 (0.6-8.1)	1.7 (0.4-8.6)	0.15
West Virginia (40)	0 (0-8.8)	1.8 (0.3-5.7)	0.14

\* Probability

NA = not applicable.

swine are important sources of virus spillback into domestic swine or for long-term maintenance of the virus, since direct contact or high densities would be required for this to occur. Given the relatively high PRRS prevalence in domestic swine, areas with high densities of feral swine or poor biosecurity (ie, feral swine access to domestic swine) may increase the likelihood of PRRS transmission between domestic and feral swine in localized areas. Small swine farms (< 100 animals) were associated with increased prevalence and may be at higher risk for contact and transmission of PRRS and other pathogens due to poor biosecurity compared to that in commercial swine operations. Thus, we recommend additional studies to quantify the risk to both small swine farms and to large swine operations. Although feral swine populations were reported in 17 states in 1988, they now exist in at least 35 states and exceed 5 million individuals.<sup>4</sup> Relative to the distribution and size of feral swine populations in the United States, our sample size was small and may have missed local areas of higher prevalence. Consequently, this study

should be considered an initial investigation into national scale PRRS prevalence. Since antibody prevalence is not equivalent to viral shedding, it is unclear whether the feral swine tested in this study were infectious at the time they were sampled. Additional surveillance in feral swine is warranted to quantify the frequency with which feral swine shed virus and to determine if areas with higher prevalence are associated with certain swine production practices such as pastureraised swine or organic production. These practices may result in more opportunities for pathogen transmission. Surveillance and longitudinal studies to investigate PRRS prevalence and strain diversity in areas where feral and domestic swine overlap are recommended to provide better information on transmission and the role of feral swine in the epidemiology of PRRS.

# Implications

- Although feral swine may become infected with PRRSV, it is unclear if they are an important reservoir and source of spillback to domestic swine or involved in local area spread of PRRS.
- The relatively low prevalence of PRRS in feral swine combined with increased antibody prevalence in areas where domestic swine farms exist suggest that the risk of transmission from feral swine to domestic swine is low.

#### Acknowledgements

We thank all of the wildlife biologists and technicians who participated in collecting the samples included in this study and spending many hours trapping feral swine and preparing samples for testing. Mention of trade names or commercial products in this work is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

#### Conflict of interest

None reported.

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