

Pseudorabies Virus and *Brucella abortus* from an Expanding Wild Pig (*Sus scrofa*) Population in Southern Oklahoma, USA

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ABSTRACT: Wild pigs (*Sus scrofa*) are causing increasing ecologic and economic damage at a global scale. Because wild pigs can carry ≥ 65 diseases that affect livestock, their widespread expansion threatens native wildlife and livestock. We screened wild pigs from south-central Oklahoma, US for antibodies against *Brucella abortus*, pseudorabies virus (PRV), and porcine reproductive and respiratory syndrome virus (PRRS). These pathogens were chosen because they are part of eradication programs in the US and could have large economic impacts on domestic livestock if transmitted from wild animals. We tested 282 serum samples during spring 2010 ($n=149$) and 2011 ($n=133$) and found an overall exposure rate to PRV of 24.1% ($n=68$); PRV was detected at two of three study sites. Two wild pigs had detectable antibody to *B. abortus*, and one had detectable antibody to PRRS. On average, 27% of wild pigs within a sounder were positive for PRV antibody, with 44% of the sounders (16/36) having at least one positive individual. These data highlight that wild pigs could carry pathogens that affect domestic livestock. Because the US is free of these pathogens in commercial livestock operations, continued surveillance and vaccination of domestic livestock are needed. Commercial livestock producers at the wildlife-livestock interface may benefit from spatial prioritization of risk zones to facilitate strategic control efforts.

Key words: Brucellosis, feral swine, livestock, pathogen transmission, porcine reproductive and respiratory syndrome virus, pseudorabies virus.

In the US, wild pigs (*Sus scrofa*) are considered an exotic and invasive species, currently causing great concern at a global scale. Their ability to rapidly expand their distribution is in part due to their high reproductive potential (Taylor et al. 1998) and generalist food habits, which allow them to inhabit a diverse array of vegetation types (Ilse and Hellgren 1995; Mersinger and Silvy 2007). Like many other exotic and invasive

species, wild pigs serve as reservoirs for disease spread (Meng et al. 2009), particularly to domestic livestock and native wildlife. With increasing distribution and population density, wild pigs will come into more frequent contact with livestock and humans (Witmer et al. 2003), threatening human health and safety because wild pigs serve as reservoirs for pathogens and spread pathogens in the environment (e.g., water and soil; Wyckoff et al. 2009). Wild pigs can be infected with more than 65 pathogens that affect livestock (Cooper et al. 2010). Common pathogens occurring in wild pig populations in Oklahoma are *Brucella*, pseudorabies virus (PRV), and porcine reproductive and respiratory syndrome virus (PRRS; Gaskamp 2012). These pathogens can be spread from wild hosts to livestock, are part of national eradication programs (Miller et al. 2013), and could cause devastating impacts (e.g., decreased production, animal deaths, quarantine) if infections reach commercial livestock operations and result in economic burdens to producers and consumers. Because of the environmental problems associated with wild pigs (Stevens 2010), diseases that they harbor, and the paucity of data related to diseases of wild pigs in Oklahoma (Saliki et al. 1998), we undertook a serologic survey of wild pigs in south-central Oklahoma. We tested for antibodies against *Brucella abortus*, PRV, and PRRS.

Our 2-yr study (2010 and 2011) was conducted at three study sites in Love County, Oklahoma, US: Oswalt Road Ranch (2,093 ha; 33°59'N, 97°15'W), Coffey Ranch (1,024 ha; 33°53'N, 97°16'W), and Hoffman Ranch (930 ha; 34°2'N, 97°28'W). All study sites are within the Cross Timbers and Prairies eco-

region (Gee et al. 2011). Prior to the start of this study, wild pig control consisted of trapping using drop nets and corral traps on Oswalt Road Ranch, corral traps on Coffey Ranch, and hunting on Hoffman Ranch; all hunting and trapping were prohibited at the three study sites for 1 yr prior to the start of the study. Each ranch was divided into two units and assigned to one of three treatments: corral traps, drop nets, or control. Corral traps and drop nets were used on Oswalt Road Ranch, drop nets and a control unit were assigned to Coffey Ranch, and corral traps and a control unit were implemented on Hoffmann Ranch. The drop net was capable of catching entire sounders (Gaskamp 2012), but whole sounders were not targeted in that any pig, or any number of pigs, were captured once under the net.

We conducted trapping from January to April. We prebaited trap sites with whole kernel corn for 7 d, and if pigs used the bait site, a trap was erected at the site. Upon capture, we collected whole blood (≥ 10 mL) from the heart of euthanized pigs via cardiac puncture. Animal capture and euthanasia techniques were in accordance with Animal Use Protocol 2008-160 issued by Texas A&M University. Whole blood was centrifuged at $1,000 \times G$ for 20 min (IEC Centra CL2, International Equipment Company, Needham Heights, Massachusetts, USA), and serum was separated and stored in a freezer (-10 C) until testing. Each sample was given an identifying number to cross-reference with capture data that included: date, sex, weight, location (ranch), and sounder size. We tested serum at the National Animal Disease Center in Ames, Iowa, US. Serum samples were screened using the *B. abortus* plate agglutination (BAPA) obtained from the National Veterinary Services Laboratories (NVSL; Ames, Iowa, USA). If samples were positive by BAPA, they were retested using the *Brucella* Rivanol precipitation assay (RIV; NVSL). If samples were positive by RIV, they were retested using *B. abortus* fluorescent polarization assay (FPA; Diachemix, Milwaukee, Wisconsin, USA). To detect PRV antibodies, we screened serum using the PRV-gB

enzyme-linked immunosorbent assay (ELISA; Idexx Laboratories, Westbrook, Maine, USA). Samples positive for PRV were retested in duplicate using the PRV-g1 ELISA (Idexx Laboratories). To detect PRRS antibodies, we screened serum using the PRRSX3 virus ELISA (Idexx Laboratories). If samples indicated positive by ELISA, they were retested twice again using the PRRSX3 ELISA (Idexx Laboratories). The probability of having antibodies (dependent variable: positive or negative) against PRV was tested using logistic regression in SAS[®] 9.3 (SAS Institute, Inc., Cary, North Carolina, USA) to examine difference among years, ranches, and sexes.

From 282 serum samples collected from wild pigs during spring 2010 and 2011 in Love County, we found an overall exposure rate to PRV of 24.1% using PRV-gB ELISA (Table 1). The 68 positive samples were retested in duplicate using the PRV-g1 ELISA, and 65 were positive. The probabilities of PRV exposure varied by ranch and year but were similar between sexes (Table 1). Antibodies against PRV were detected in 54.5% and 8.5% of pigs sampled on Oswalt and Coffey Ranches, respectively; antibodies to PRV were not detected at Hoffman Ranch (Table 1). Prevalence of PRV antibodies was greater in 2011 than in 2010 (Table 1). The prevalence of *B. abortus* antibody was 0.35% ($n=2$) by BAPA; both positive pigs were from Oswalt Ranch (1 male, 1 female) in 2010. Retesting of both positive samples by RIV and FPA detected *B. abortus* antibody in only one sample (female). One female from Oswalt Ranch in 2010 was positive by PRRSX3 ELISA. The same sample was positive when retested twice using the PRRSX3 ELISA.

Our serosurvey for three pathogens in wild pigs from Love County, Oklahoma, showed high prevalence of PRV antibodies. Of the 43 capture events, 36 whole or partial (≥ 2 pigs) sounders were captured, of which 44% ($n=16$) had at least one individual positive for PRV antibodies. In sounders positive for PRV antibody, 27% of individuals were positive. One sounder captured in 2011, composed of four adult females and 21 juveniles, had a 100% prevalence of antibodies to PRV. The

TABLE 1. Exposure rates and results of logistic regression analysis in female and male wild pigs (*Sus scrofa*) for pseudorabies virus (PRV) on three ranches in Love County, Oklahoma, USA, during 2010 and 2011. Probability of infection with PRV due to year, ranch, and sex was tested using logistic regression.

Ranch ^a	Sex ^b	2010 ^c		2011 ^c		Total	
		No. tested	Positive, n (%)	No. tested	Positive, n (%)	No. tested	Positive, n (%)
Coffey	F	44	1 (2.3)	47	8 (17.0)	91	9 (9.9)
	M	37	3 (8.1)	36	2 (5.6)	73	5 (6.8)
Hoffmann	F	6	0 (0.0)	6	0 (0.0)	12	0 (0.0)
	M	2	0 (0.0)	5	0 (0.0)	7	0 (0.0)
Oswalt	F	31	13 (41.9)	23	17 (73.9)	54	30 (55.6)
	M	29	11 (37.9)	16	13 (81.3)	45	24 (53.3)
Total		149	28 (18.8)	133	40 (30.1)	282	68 (24.1)

^a $\chi^2=56.72$, $df=2$, $P<0.001$.

^b $\chi^2=0.15$, $df=1$, $P=0.7$; F = female, M = male.

^c $\chi^2=13.72$, $df=1$, $P<0.001$.

juveniles (<10 kg) may have acquired antibodies as neonates through milk or colostrum from lactating females or as fetuses via transplacental transmission (Bouma et al. 1997; Pomeranz et al. 2005). Thus, prevalence could have been biased by the presence of maternal antibody in uninfected juveniles.

Although we found antibodies to PRRS in only one individual, the pathogen could impact domestic swine facilities if positive wild pigs come into contact with livestock in these facilities. The most common route of transmission is by direct contact with other pigs or with mammary or nasal secretions, urine, semen, or feces. Similar to PRRS, *B. abortus* antibody prevalence was low (only two individuals). *Brucella suis* is the most common species to infect domestic swine (*Sus scrofa domesticus*), but on many rangelands managed for cattle (*Bos taurus*), *B. abortus* is of greater concern. Vaccination of cattle for brucellosis is routine, but at a high cost (>\$3.5 billion since 1951; Richey and Harrell 1997). Because wild pigs and wildlife can be carriers of *B. abortus*, cattle producers should consider that infection could occur even in previously disease-free herds, so continued vaccination will be critical to maintaining brucellosis-free herds.

We found that drop nets could capture whole sounders (Gaskamp 2012), allowing for disease testing of all individuals in the

sounder. As described earlier, as many as 25 individuals within a single sounder were positive for PRV antibody. The capture of whole sounders will add a new level of resolution to disease sampling to help determine potential route of exposure (i.e., acquired versus infected) and within-sounder dynamics.

Control strategies should focus on all segments of wild pig populations, including boars, because they range over long distances (McIlroy et al. 1989; Stevens 2010). Knowledge about the pathogens that reside in wild pig populations, how pigs traverse the landscape, and how each pathogen is transmissible will allow spatial prioritization of risk zones to facilitate strategic control efforts. Though control may not reduce prevalence of several pathogens, it can reduce the density of positive animals that could come into contact with domestic livestock or food animals. In the US, domestic swine populations are free of PRV (Hahn et al. 2010), accentuating the need to maintain disease-free domestic livestock. This may be difficult at the wildlife-livestock interface where native and feral wildlife that are carriers of pathogens can come into contact with domestic livestock. However, wild pigs often do not travel large distances out of traditionally used ranges (Wyckoff et al. 2009), offering the opportunity to trap and remove local populations that pose

a threat to livestock facilities. Efficient capture at the wildlife-livestock interface also is possible through the capture and removal of whole sounders using techniques such as the drop net.

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