

## A Serosurvey for *Brucella suis*, Classical Swine Fever Virus, Porcine Circovirus Type 2, and Pseudorabies Virus in Feral Swine (*Sus scrofa*) of Eastern North Carolina

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**ABSTRACT:** As feral swine (*Sus scrofa*) populations expand their range and the opportunity for feral swine hunting increases, there is increased potential for disease transmission that may impact humans, domestic swine, and wildlife. From September 2007 to March 2010, in 13 North Carolina, USA, counties and at Howell Woods Environmental Learning Center, we conducted a serosurvey of feral swine for *Brucella suis*, pseudorabies virus (PRV), and classical swine fever virus (CSFV); the samples obtained at Howell Woods also were tested for porcine circovirus type 2 (PCV-2). Feral swine serum was collected from trapped and hunter-harvested swine. For the first time since 2004 when screening began, we detected *B. suis* antibodies in 9% (9/98) of feral swine at Howell Woods and <1% (1/415) in the North Carolina counties. Also, at Howell Woods, we detected PCV-2 antibodies in 59% (53/90) of feral swine. We did not detect antibodies to PRV ( $n=512$ ) or CSFV ( $n=307$ ) at Howell Woods or the 13 North Carolina counties, respectively. The detection of feral swine with antibodies to *B. suis* for the first time in North Carolina warrants increased surveillance of the feral swine population to evaluate speed of disease spread and to establish the potential risk to commercial swine and humans.

**Key words:** *Brucella suis*, classical swine fever, feral swine, North Carolina, porcine circovirus type 2, pseudorabies virus, *Sus scrofa*.

In the USA, the feral swine population has quadrupled over the past 10 yr and is estimated to be approximately 4 million animals distributed across at least 37 states (Clay, 2007; Southeastern Cooperative Wildlife Disease Study, 2011). As the feral swine population expands and feral swine hunting increases, there is increased interaction and greater potential for disease

transmission among feral swine, humans, commercial swine, and wildlife (Corn et al., 2009; Wyckoff et al., 2009).

The National Wildlife Disease Program within the US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (USDA-APHIS-WS) routinely screens feral swine for antibodies to classical swine fever virus (CSFV), pseudorabies virus (PRV), and *Brucella suis*. Currently, these pathogens do not occur in US domestic swine operations. Classical swine fever, formerly called hog cholera, was eradicated from the US in 1976 (US Department of Agriculture, 2005), and active surveillance programs focus primarily on domestic swine and pork products. Porcine circovirus type 2 (PCV-2) is commonly found in domestic swine throughout North America and is associated with postweaning multisystemic wasting syndrome (PMWS; Ellis et al., 1998). Corn et al. (2009) reported that PCV-2 antibody is common in feral swine in North and South Carolina, USA. Historically, in eastern North Carolina, feral swine have been antibody-negative for *B. suis* and PRV (Zygmunt et al., 1982; Cavendish et al., 2008; Corn et al., 2009), but continued surveillance is important because of the high numbers of domestic swine produced in North Carolina. We screened feral swine on a privately owned property and throughout the high pork production counties in eastern North Carolina for antibodies to CSFV, PRV, *B. suis*, and

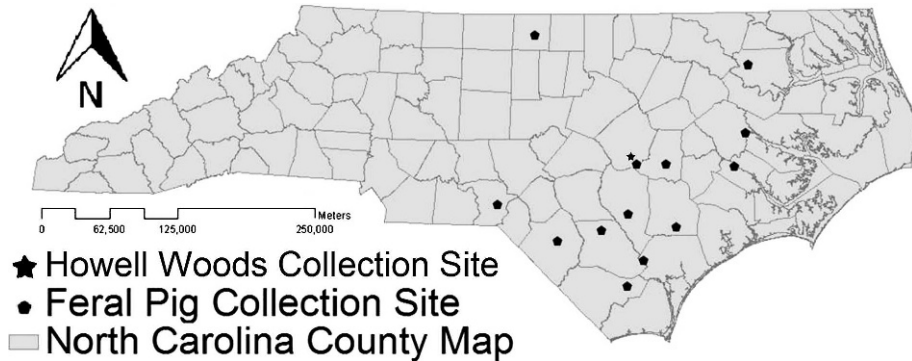


FIGURE 1. Sites within 13 North Carolina, USA, counties where feral pigs (*Sus scrofa*) were collected for serologic testing, September 2007–May 2010.

PCV-2, all of which have the potential to negatively affect the domestic swine industry and human health.

From 2007 to 2010, we conducted a serosurvey of feral swine at sites in 13 North Carolina counties: Bertie, Bladen, Caswell, Columbus, Craven, Duplin, Johnston, Pender, Pitt, Richmond, Robeson, Sampson, and Wayne (Fig. 1). All counties are in eastern North Carolina where the majority of the state's commercial swine production occurs, except for Caswell County in north central North Carolina and Richmond County in south central North Carolina. We also conducted our research at Howell Woods Environmental Learning Center (35°22'14.7"N, 78°18'23.4"W), an 11-km<sup>2</sup> private property, in Johnston County, in eastern North Carolina.

In the 13 counties, feral swine were collected January 2007–May 2010 on private properties by using walk-in drop door traps (1.3×2×1-m box-style traps and 6×6×2-m corral traps) baited with corn (*Zea mays*), or shot with the aid of spotlights at night. We collected 1–3 cc of whole blood via heart puncture; serum was obtained by centrifugation (Mobile-spin model 128 centrifuge, Vulcon Technologies, Grandview, Missouri, USA) and stored at –22 C.

At Howell Woods, feral swine were hunted September 2007–March 2010, during 45 hunting sessions of 4 days.

During each hunting session, approximately 20 hunters harvested feral swine from tree stands overlooking an automated feeder programmed to dispense corn at 4:30 PM daily. Harvested swine were transported to a central processing site for cleaning; we determined weight, sex, and age (based upon dental characteristics; Matschke, 1967), and we divided swine into three age classes: juvenile ( $\leq 5$  mo), subadult (5–8 mo), and adult ( $> 8$  mo). We collected 1–3 cc of whole blood via heart puncture, cranial sinus puncture, or from the wound site; and then we centrifuged at  $1100 \times G$  for 10–15 min and stored the samples at –80 C until testing.

Feral swine hunting at Howell Woods did not occur from April to August in any year of the study; therefore, feral swine were grouped into three time periods; season 1 (September 2007–March 2008), season 2 (September 2008–March 2009), and season 3 (September 2009–March 2010). Only swine collected from Howell Woods were screened for antibodies to PCV-2. Serum samples were sent to Rollins Animal Disease Diagnostic Laboratory, Raleigh, North Carolina, USA, and analyzed using a SERELISA<sup>TM</sup> PCV2 Ab mono blocking kit (Synbiotics Europe, Lyon, France; Corn et al., 2009). Samples with a negative corrected ratio of  $\leq 0.50$  were considered positive for PCV-2 antibodies in serum, and samples with a ratio

of  $>0.50$  were considered negative. In 2007 and 2008, feral swine sera tested for PRV and *B. suis* were sent to the Rollins Animal Disease Diagnostic Laboratory. In 2009, samples were sent to the USDA–APHIS–Veterinary Services (VS) Eastern Region Federal Brucellosis Laboratory. *Brucella suis* testing included three sequential analyses: the buffered acidified plate antigen (BAPA) test, card test (Rose Bengal), and fluorescence polarization assay (FPA). The PRV serology tests were the Autolex™ Anti-PRV Screen (Viral Antigens, Inc., Memphis, Tennessee, USA) and the HerdChek™ Anti-PRV gpI (IDEXX Laboratories, Inc., Westbrook, Maine, USA). Positive samples were subsequently screened for PRV gpI antibody to distinguish between field strains and vaccine strains lacking gpI. Feral swine sera were sent to the USDA–APHIS–VS Foreign Animal Disease Diagnostic Laboratory and screened for CSFV antibodies. Tests included an enzyme-linked immunosorbent assay followed by an immunoperoxidase test and finally virus neutralization.

Between 2007 and 2010, there were 488 feral swine harvested from the 13 counties and 140 harvested at Howell Woods (Tables 1 and 2). Due to variation in the amount of serum collected, not all feral swine harvested were tested for antibodies to each disease. There were 513 swine tested for *B. suis*, 512 tested for PRV, 307 tested for CSFV, and 90 (from Howell Woods) tested for PCV-2 (Table 2).

No feral swine had antibodies to CSFV or PRV at any of the collection sites. Classical swine fever is a foreign animal disease, so negative results were not unusual. Since 2004, feral swine sampled in eastern North Carolina have consistently been negative for antibodies to PRV, but feral swine in western North Carolina have been found with antibodies to PRV since 2005 (Cavendish et al., 2008). Corn et al. (2009) suggested that feral swine populations in North Carolina, unlike their counterparts in South Carolina,

became established after active eradication of PRV in commercial swine during the 1990s. However, there has been a continuous presence of feral swine in Howell Woods for  $>50$  yr, which suggests that the population was established originally with uninfected swine before any disease eradication programs.

During two hunting seasons, the prevalence of antibody to PCV-2 at Howell Woods was 59% (53/90), which is similar to previous surveys of feral swine in Johnston County where 60% ( $n=45$ ) were antibody-positive for PCV-2 (Corn et al., 2009). The impact of PCV-2 on feral swine is unknown. Even in domestic swine, pathogenesis is complex and ranges from unapparent to severe PMWS epidemics with postweaning mortality rates 3–4 times normal levels (Harding, 2004). Currently, it is not possible to determine whether PCV-2 is maintained in feral swine populations in the absence of nearby domestic pig operations. More research on spatial and temporal relationships between feral and domestic swine is necessary to understand the relative contributions of the two populations to maintaining PVC-2 in their respective environments.

Since screening for *Brucella* antibodies in North Carolina feral swine began in 2004, no positive animals were detected until the second season (September 2008–March 2009) of hunting at Howell Woods when 22% (6/27) of the harvested animals were antibody-positive, suggesting a recent introduction of *Brucella* into the population. Antibody prevalence to *B. suis* during the following hunting seasons at Howell Woods was 8% (3/36) and, over the 3-yr period, 9% (9/98) had antibodies to *B. suis*. In the 13 North Carolina counties, during routine surveillance conducted by USDA–APHIS–WS, one feral swine from Bladen County had antibodies to *B. suis*. It is believed that feral swine are being moved by humans into and around North Carolina for recreational hunting, with the source of these swine probably in South Carolina. South Carolina has a large

TABLE 1. Age and gender for feral swine (*Sus scrofa*) collected in 13 North Carolina, USA, counties and at Howell Woods Environmental Learning Center, Howell Woods, Four Oaks, North Carolina, 2007–2010.

Year	Adult	Subadult	Juvenile	Unknown	Male	Female
2007	77	3	170	0	126	124
2008	57	93	28	7	95	90
2009	36	42	24	0	49	53
2010	34	31	26	0	47	44
Total	204	169	248	7	317	311

feral swine population in which antibodies to *B. suis* are routinely detected (Stoffregen et al., 2007; Corn et al., 2009).

The introduction of *B. suis* into a feral swine population that is routinely hunted raises concern about disease transmission to humans. Recent cases of *B. suis* infection in feral swine hunters were linked to butchering of swine but not to consumption of the meat (CDC, 2009). Howell Woods has facilities for processing harvested feral swine on-site, and although gloves are worn, extra care, attention, and hunter education are warranted. Hunters need to be aware that clinical signs are nonspecific and can develop weeks to months after exposure.

North Carolina's domestic swine industry is second in the nation earning US\$2 billion a year (North Carolina Department of Agriculture and Consumer Services, 2005), and *B. suis* spillover into the domestic population can adversely impact profits. Transmission of *B. suis* between feral and domestic swine can occur, and infected domestic swine would pose a greater risk to pork processing plant

workers (CDC, 1994) and consumers. Howell Woods is in a 5.2-km<sup>2</sup> area containing approximately 13,320 domestic swine, and studies in the Southeast have estimated feral swine home ranges to be 1.2–11.6 km<sup>2</sup> (Wood and Brenneman, 1980; Hayes et al., 2009), making contact with domestic swine reasonable. Although the majority of domestic swine are raised in confinement facilities with biosecurity measures to prevent contact with or contamination from feral swine, transitional or free-ranging operations do little to reduce contact with feral swine and are at risk for introduction of infectious diseases (van der Giessen et al., 2007).

This study was funded by the Fisheries, Wildlife, and Conservation Biology Program and Department of Forestry and Environmental Resources at North Carolina State University; Howell Woods Environmental Learning Center; and USDA–APHIS–WS National Wildlife Disease Program. We thank T. Ray; C. Barker; the staff of Howell Woods; and all project volunteers, including J. Sasser, M. Rose, J. Parker, C. Ayers, and S. Allen.

TABLE 2. Number tested (test) and number positive (pos) for feral swine (*Sus scrofa*) from Howell Woods, Four Oaks, North Carolina, USA, tested for antibody to four swine pathogens 2007–2010.<sup>a</sup>

Season <sup>b</sup>	BS test	BS pos	PRV test	PRV pos	CSFV test	CSFV pos	PCV-2 test	PCV-2 pos
1	35	0	34	0	— <sup>c</sup>	—	50	33
2	27	6	27	0	20	0	40	20
3	36	3	36	0	36	0	—	—
Total	98	9	97	0	56	0	90	53

<sup>a</sup> BS = *Brucella suis*; PRV = pseudorabies virus; CSFV = classical swine fever virus; PCV-2 = porcine circovirus type 2.

<sup>b</sup> See text for explanation of seasons.

<sup>c</sup> — = none tested.

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*Submitted for publication 11 March 2011.*

*Accepted 3 November 2011.*