## ABSTRACT

SANDFOSS, MARK ROBERT. The Serosurvey of Feral Pigs (*Sus scrofa*) in Eastern North Carolina. (Under the direction of Christopher S. Deperno.)

Feral pigs (*Sus scrofa*) survive in many climates, reproduce year-round, and are dietary generalists. In the United States, the size and range of the feral pig population has expanded, resulting in greater interaction with humans and domestic swine and increased potential for disease transmission. I conducted a serosurvey in feral pigs from eastern North Carolina to determine exposure to the zoonotic parasites, *Toxoplasma gondii* and *Trichinella* spp. Between September 2007 and March 2009, blood serum was collected from 83 feral pigs harvested at Howell Woods Environmental Learning Center, Four Oaks, North Carolina. We used a modified agglutination test (MAT) to test for *T. gondii* antibodies and an enzyme-linked immunosorbent assay (ELISA) to test for *Trichinella* spp. antibodies. The seroprevalence of antibodies to *T. gondii* and *Trichinella* spp. was 27.7% and 13.3%, respectively, and three pigs, 3.6% had antibodies to both diseases. We detected an increased risk of *T. gondii* antibodies with age ($\chi^2_2 = 6.89$, P = 0.032), whereas the risk of exposure to *T. gondii* across years ($\chi^2_1 = 1.79$, P = 0.181) and sex ($\chi^2_1 = 0.001$, P = 0.939) were similar. In eastern North Carolina, feral pigs have been exposed to *T. gondii* and *Trichinella* spp. and may pose a health risk to domestic swine and humans.

To further investigate the health risk feral pigs pose we conducted a serosurvey for antibodies to porcine circovirus type 2 (PCV-2), *Brucella suis*, pseudorabies virus (PRV), and classical swine fever (CSF) in 13 North Carolina counties and Howell Woods from September 2007 to May 2009. Feral pigs were collected by trapping and hunter harvest. At Howell

Woods, we detected PCV-2 antibodies in 58.9% (53/90) of feral pigs that differed between collection years ($\chi^2_1 = 6.08$, $P = 0.01$) but was similar across age classes ($\chi^2_2 = 2.62$, $P = 0.27$) and sexes ($\chi^2_1 = 0.39$, $P = 0.53$); no feral pigs collected in the 13 North Carolina counties were screened for PCV-2 for this study. We detected *B. suis* antibodies in 7.5% (6/80) of feral pigs at Howell Woods which differed between collection years ($P = 0.005$, Fisher's exact test), and 0/265 in the ten North Carolina counties. We did not detect antibodies for PRV (n = 61, 264) or CSF (n = 40, 130) at Howell Woods or the 13 North Carolina counties, respectively. The detection of feral pigs with antibodies to *B. suis* for the first time in North Carolina warrants increased surveillance of the feral pig population surrounding areas to evaluate how quickly the disease spreads and to establish the potential risk to commercial pig producers.

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A Serosurvey of Feral Pigs (*Sus scrofa*) in Eastern North Carolina

by
Mark Robert Sandfoss

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APPROVED BY:

| | |
|---|---|
| Richard A. Lancia | Kevin Gross |
| Suzanne Kennedy-Stoskopf | Christopher S. DePerno<br>Chair of Advisory Committee |

## DEDICATION

In dedication to what many might read as the usual people, my supportive parents and family, friends, professors, dog, and of course my wife, but I insist these people are by no means "usual" and that has made all the difference.

## BIOGRAPHY

Mark Sandfoss was born in Fort Thomas, Kentucky to parents Steve and MaryAnn. He received his Bachelor's degree in 2006 in Wildlife Biology from Murray State University in Murray, Kentucky.

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acknowledge the scientific achievements of Aldo Leopold who made watching and catching animals all day a science and a valid career and Charles Darwin, whose brilliance and ideas continue to inspire us all. Last but not least, I want to thank my wife, Carolina, who supports me and shares my passion for biology and life.

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## STUDY INTRODUCTION

The European wild boar (*Sus scrofa*) was first introduced as free-range livestock, in Florida, during the mid 16th century by early European explorers (Towne and Wentworth, 1950). Since their original introduction to the United States, pigs have increased in numbers and distribution, with large populations in California, Texas, and the Southeast (Clay, 2007). Throughout the United States the feral pig population is estimated to be ~ 5 million animals (Clay, 2007). The history and origins of the wild pigs of North Carolina are not clear; there are pure wild boar, escaped domestic "feral" pigs, and mixes of the two. In North Carolina, pure European wild boar of German, Polish, or Russian origin (Bratton, 1977) were introduced to Graham County by the Whiting Manufacturing Company in 1912, for the purpose of a game preserve (Jones, 1959). These pigs have expanded their range at approximately 2.5 km/year (Singer, 1981) and are now concentrated in 6 western counties where they have been classified by the state as a "game animal," and have a regulated harvest and protection outside of season (Wood and Barrett, 1979). All other wild pigs in North Carolina are classified as "feral" pigs and have no game status, regulated harvest, or protection. These pigs are most likely products of escaped domestic pigs and/or are transplanted feral pigs from other parts of the Southeast. The pigs at our study site, Howell Woods Environmental Learning Center (hereafter Howell Woods), located in eastern North Carolina, are "feral pigs" and have no game status or protection.

The spread of feral pigs throughout the United States is primarily due to the movement of feral pigs by humans for recreational harvest as opposed to natural dispersal (Wood and Barrett, 1977; Gipson et al., 1997; Waithman et al., 1999). New populations are established by releases of wild pigs for hunting, escape of wild pigs from shooting preserves, dispersal from

established populations, and domestic pigs avoiding capture in free-range commercial operations (Gipson et al., 1997). Feral pig hunting popularity has increased because of, or in concert with, the spread of populations throughout the U.S. In California where feral pigs have game status, feral pig hunting has eclipsed deer (*Odocoileus virginianus*) hunting in popularity (Barrett, 1993). It is difficult to track feral pig hunting statistics in states where feral pigs have no regulated harvest. Feral pig hunting popularity has increased because feral pig hunting can be undertaken year-round with no bag limits or fees, and the desire of landowners to manage feral pig populations to control pig damage.

The reason feral pigs have been successfully transplanted throughout the U.S. is their ability to adapt in many climates, reproduce year-round, and survive on a varied diet (Wilcox and Van Vuren, 2009). The species is the most abundant wild, exotic ungulate in the U.S. (McKnight, 1964; Decker, 1978) and possesses the highest reproductive potential of any North American large mammal (Wood and Barrett, 1979). Reproductive production varies due to nutrition, but under good conditions feral pigs can produce large litters (4-8 piglets) twice a year (Taylor et al., 1998).

The spread of feral pigs is a serious ecological threat as pigs cause extensive environmental damage. Feral pigs damage seedlings, agricultural crops, natural vegetation (Singer et al., 1984; Tate, 1984; Cushman et al., 2004; Seward et al., 2004; Engeman et al., 2007a), and ecologically sensitive areas (Engeman et al., 2004). Also, feral pigs cause soil leaching (Tate, 1984), predate and compete with native wildlife for resources (Adkins and Harveson, 2007; Kaller et al., 2007; Mersinger and Silvy, 2007; Jolley et al., 2010), and transmit pathogens to native wildlife, livestock, and humans (Corn et al., 2004; Wood et al.,

1976). Pimentel et al. (2000) estimated feral pig damage in the U.S. to be US$ 800 million annually and that amount has certainly increased over the past 10 years.

As feral pigs have spread throughout the world, with populations in all seven continents with the exception of Antarctica, their ecological impacts have received more attention and there has been increased research on methods of control and eradication. Due to the increased size of the feral pig population in North Carolina and the threats they pose, the North Carolina House of Assembly passed House Bill 1118 in 2009, which advocated the study of feral pig importance in the state.

This study focused on the importance of feral pigs in North Carolina as disease reservoirs. Little research has focused on evaluating feral pigs as potential reservoirs of *Toxoplasma gondii* (Diderrich et al., 1996; Gresham et al., 2002; Blumenshine et al., 2009). Recently, the role of feral pigs as reservoirs of *Trichinella spiralis* has been investigated as many countries attempt to demonstrate free status for international pig production (Gamble et al., 2005; Antolova et al., 2006; Nockler et al., 2006). Nevertheless, the numbers and range of feral pigs has expanded, resulting in greater interaction with humans and domestic swine, and increased potential for parasite transmission. Hence, an objective of my study was to investigate antibody prevalence in feral pigs in eastern North Carolina to *Trichinella* spp. and *T. gondii*.

Another objective of my study was to evaluate hunter harvest demographics over a 2-year period on a privately owned property; we collected serum samples from hunter-killed feral pigs screened for antibodies to CSF, pseudorabies, *B. suis*, and PCV-2. My objective was to evaluate antibody prevalence in more feral swine from a smaller geographic area (2800 acres) and compare to routine seroprevalence data from feral pigs sampled throughout North Carolina.

My final objective was to examine PCV-2 seroprevalence in an established feral pig population over time.

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**Seroprevalence of *Toxoplasma gondii* and *Trichinella* spp. in Feral Pigs (*Sus scrofa)* of Eastern North Carolina.**


## ABSTRACT

Feral pigs (*Sus scrofa*) survive in many climates, reproduce year-round, and are dietary generalists. In the United States, the size and range of the feral pig population has expanded, resulting in greater interaction with humans and domestic swine and increased potential for disease transmission. A serosurvey was conducted in feral pigs from eastern North Carolina to determine exposure to the zoonotic parasites, *Toxoplasma gondii* and *Trichinella* spp. Between September 2007 and March 2009, blood serum was collected from 83 feral pigs harvested at Howell Woods Environmental Learning Center, Four Oaks, North Carolina. We used a modified agglutination test (MAT) to test for *T. gondii* antibodies and an enzyme-linked immunosorbent assay (ELISA) to test for *Trichinella* spp. antibodies. The seroprevalence of antibodies to *T. gondii* and *Trichinella* spp. was 27.7% and 13.3%, respectively, and three pigs, 3.6% had antibodies to both diseases. We detected an increased risk of *T. gondii* antibodies with age ($\chi^2_2 = 6.89$, P = 0.032), whereas the risk of exposure to *T. gondii* across years ($\chi^2_1 = 1.79$, P = 0.181) and sex ($\chi^2_1 = 0.001$, P = 0.939) were similar. In eastern North Carolina, feral pigs have been exposed to *T. gondii* and *Trichinella* spp. and may pose a health risk to domestic swine and humans.


## INTRODUCTION

Since their original introduction to the United States from Europe, in the mid 16$^{th}$ century, pigs (*Sus scrofa*) were raised as domestic livestock and pursued as a game animal

(Towne and Wentworth, 1950). However, because of their ability to adapt in many climates, reproduce year-round, and survive on a varied diet (Wilcox and Van Vuren, 2009), feral pigs have expanded their range and increased in numbers. Today, the United States' feral pig population is estimated to be ~4 million animals across 39 states, with large populations in California, Texas, and the Southeast (Clay, 2007). The increasing feral pig population has resulted in greater feral pig interactions with domestic swine and humans and increased risk of zoonotic disease transmission, including the parasites *Toxoplasma gondii* (Dubey and Beattie, 1988) and *Trichinella spiralis* (Campbell, 1983).

*Toxoplasma gondii* is a protozoan parasite that infects domestic animals, wildlife, and humans, through the uptake of an infective stage of the *T. gondii* life cycle (Dubey and Beattie, 1988). *Toxoplasma gondii* oocysts, the infective stage, are shed into the environment by the definitive host, members of the family Felidae. Oocysts can persist in the environment from 46 to 410 days (Yilmaz and Hopkins, 1972) and survive in water up to 54 months (Benenson et al., 1982; Bowie et al., 1997; Dubey, 2004). If oocysts are ingested by a non-felid host, including humans, the parasite will invade and encyst in muscle tissue and organs. Further, transmission of *T. gondii* may occur by consumption of parasite stages encysted in muscle tissue, including improperly cooked meat (Dubey and Beattie, 1988). In feral pigs, it is unclear if infections primarily occur by ingestion of oocysts from the environment or from ingesting muscle cysts in prey or carrion.

There have been seven described species of nematode within the genus *Trichinella* (*Trichinella britovi, Trichinella murrelli, Trichinella nativa*, *Trichinella nelson, Trichinella papuae, Trichinella pseudospiralis,* and *Trichinella spiralis*) (Pozio et al., 1992, 1999; Nagano et al., 1999; La Rosa and Pozio, 2000; Pozio and La Rosa, 2000), two of which have been

predicted to occur in eastern North Carolina, *T. spiralis* and *T. murrelli* (Pozio, 2000; Masuoka et al., 2009). *Trichinella spiralis* is a widely distributed nematode parasite which has a direct life cycle and can be transmitted interspecifically to mammals and humans (Campbell, 1983). Infection of *T. spiralis* in humans is commonly associated with ingestions of raw or undercooked game meat (Gamble et al., 1999) and may become clinical, potentially leading to human fatalities. Similarly, domestic pigs may become infected by ingesting *T. spiralis* laden tissue of other omnivorous or carnivorous species (Zimmermann et al., 1962), feces containing gravid intestinal worms (Hill, 1968), or cannibalism (Leighty, 1983). *Trichinella murrelli*, has been widely detected in wildlife within the United States, but the complete distribution is yet to be defined (Pozio and La Rosa, 2000).

Little research has focused on evaluating feral pigs as potential reservoirs of *T. gondii* (Diderrich et al., 1996; Gresham et al., 2002; Blumenshine et al., 2009). However, the role of feral pigs as reservoirs of *T. spiralis* has been investigated as many countries attempt to demonstrate free status for international pig production (Gamble et al., 2005; Antolova et al., 2006; Nockler et al., 2006). The objective of this study was to investigate antibody prevalence to *Trichinella* spp. and *T. gondii* in feral pigs in eastern North Carolina, where domestic swine farms are concentrated.

## METHODS

*Study site and data collection*

Our research was conducted between September 2007 and March 2009 at Howell Woods Environmental Learning Center in eastern North Carolina (35.22'16.30N, 78.18'23.43W). Howell Woods encompassed 11 $km^2$ with elevations ranging from 32 to 50 m.

The climate was temperate with an average rainfall of 120.4 cm and the average maximum temperatures in July and January were 32.0° C and 11.0° C, respectively. Howell Woods was primarily comprised of bottomland hardwood forest including red maple (*Acer rubrum*), willow oak (*Quercus phellos*), loblolly pine (*Pinus taeda*), and sweetgum (*Liquidambar styraciflua*). The understory consisted of giant river cane (*Arundinaria gigantean*) and possumhaw (*Ilex deciduas*). Howell Woods was located within 5 $km^2$ of ~13,320 domestic pigs.

Feral pigs were hunted at Howell Woods from September 2007 to March 2009. A total of 30 hunts were conducted consisting of ≤ 20 hunters for ≤ 4 consecutive days. All hunting was conducted from tree stands overlooking automated feeders programmed to dispense corn at 1630 hours. Feral pig hunting did not occur from April to August in any year of the study; therefore, pigs were grouped into two time periods: year one (September 2007 to March 2008) or year two (September 2008 to March 2009).

Feral pigs killed by hunters were transported to a central processing site for field dressing. Once at the processing site, pigs were weighed, sexed, aged, and blood was collected by heart puncture, cranial sinus puncture, or directly from the wound site. Pigs were aged based upon dental characteristics (Matschke, 1967), and divided into three age-classes: juvenile (≤ 5 months), sub-adult (5-8 months), and adult (7-11+ months). Blood was centrifuged (Vulcon Technologies Mobilespin Model #128 centrifuge, 718 Main St., Grandview, MO, USA) at 3082 rpm for 10-15 minutes and stored at -80°C until tested. Serum samples were tested for antibodies to *T. gondii* and *Trichinella* spp. by the Clinical Parasitology Diagnostic Service at the University of Tennessee, College of Veterinary Medicine.

*Serology*

Serum was screened for *Toxoplasma gondii* IgG using the modified agglutination test , MAT, (Toxo-Screen DA, Biomerieux SA, Capital 12 029 370 EUR, 69280 Marcy-l'Etoile/ France, RCS Lyon B) as previously described (Desmonts and Remington, 1980; Patton et al., 1991; Smith et al., 1992; Assadi-Rad et al., 1995; Dubey et al., 1995). Dubey et al., (1995) concluded the sensitivity and specificity of the MAT for *T. gondii* antibodies in pigs was 82.9% and 90.2% respectively. Also, the MAT detected antibody at titers of at least 1:80 in all pigs recently infected as confirmed by bioassay (the gold standard) with low numbers of *T. gondii* (Dubey et al., 1995. Serum was screened for *T. gondii* IgG antibodies at 1:16, 1:32, and 1:512 dilutions, and any serum with an IgG titer ≥ 1:32 was considered positive for *T. gondii*.

Serum antibodies (IgG) to *Trichinella* species were determined using a validated commercial kit (Safepath Laboratory, Carlsbad, CA, USA now marketed by Bio-Rad) which is a USDA-licensed serology enzyme-linked immunosorbent assay (ELISA) (Gamble, 1993; Davies et al., 1998) and the recommended test for swine (OIE, 2000, Gamble et al., 2004). Test sera was added to wells that came coated with excretory-secretory (ES) antigen derived from *Trichinella* in the muscle of infected pigs. Sera were tested at a 1:200 dilution as recommended by the manufacturer and positive and negative control sera were incubated on each plate. According to the manufacturer, the ELISA values were considered positive if the optical density (OD) exceeded 0.300 after subtraction of the negative control well. The test is 98.4% sensitive and 100% specific.

*Statistical analyses*

Seropositivity of *T. gondii* was analyzed by age, sex, and year using a likelihood ratio test. A Fisher's exact test (2-tailed) was used to compare years for *Trichinella* spp. All

statistical analyses were conducted using SAS's JMP 7.0.2 (SAS Institute, Inc., 100 SAS Campus Drive, Cary, North Carolina, USA) and alpha was set at 0.05.

## RESULTS

Forty-three and 40 feral pigs were tested during year one (2007-2008) and year two (2008-2009), respectively, for *Trichinella* spp. and *T. gondii* antibodies. In year one, 13 out of 43 (30.2%) feral pigs were positive for antibodies to *T. gondii* and eight out of 43 (18.6%) feral pigs were positive for antibodies to *Trichinella* spp. In year two, 10 out of 40 (25%) feral pigs had antibodies to *T. gondii* and three out of 40 (7.5%) feral pigs had antibodies to *Trichinella* spp. When combined across years, the seroprevalence was 27.7% for *T. gondii* and 13.3% for *Trichinella* spp.. In year one, three feral pigs (7%) had antibodies for both parasites.

We detected an increased risk of *T. gondii* antibodies with age ($\chi^2_2 = 6.89$, P = 0.032); older feral pigs were more likely to be infected than younger. No effect of year ($\chi^2_1=1.79$, P = 0.181) or sex ($\chi^2_1 = 0.001$, P = 0.939) was detected on the presence of *T. gondii* or *Trichinella* spp. antibodies. Further, the probability of feral pigs being infected in year one compared to year two was similar (P = 0.198).

## DISCUSSION

We detected antibodies to *T. gondii* and *Trichinella* spp. in feral pigs at Howell Woods in eastern North Carolina. In our study, the seroprevalence (27.7%, n = 83) of antibodies to *T. gondii* in feral pigs was similar to other studies (0.5-38%; Dubey et al. 1991, 1997; Diderrich et al., 1996; Davies et al., 1998; Gauss et al., 2005). Currently in the United States, the prevalence of *T. gondii* within domestic swine is reportedly zero, which is reduced from

previous levels, due to the implementation of modern biosecurity on commercial production farms (Lubroth et al., 1983; Dubey and Weigel, 1996). However, as there has been a decrease in the prevalence of *T. gondii* in domestic pigs, there has not been a corresponding drop in human exposure to *T. gondii* based upon seropositivity, which remains around 25% of the adult population (Jones et al., 2003). Hence, it is believed that human exposure is being maintained from an underestimated or increasing oocyst presence in the environment and not from domestic pork consumption (Conrad et al., 2005).

Feral cats have been trapped and removed from Howell Woods but none have been tested for *T. gondii* antibodies. A seroprevalence study of feral cats in a central North Carolina county detected antibodies in 63% of the cats tested (Nutter et al., 2004). In the United States, surveys of domestic cats have detected *T. gondii* seroprevalences ranging from 8 to 74% (Conrad et al., 2005). Further, up to 2% of feral cats may be shedding oocysts at any time (Dubey, 1973; Christie et al., 1976; Guterbock and Levine, 1977) and an infected cat can shed more than 100 million oocysts in its feces (Dubey et al., 1970; Dubey and Frenkel, 1972; Tenter et al., 2000). Although only felids shed oocysts, a number of native wildlife species are known to have antibodies to *T. gondii* and may serve as potential intermediate hosts including: raccoons (*Procyon lotor*) (Smith et al., 1992), white-tailed deer (*Odocoileus virginianus*) (Humphreys et al., 1995), shrews and mice (Kijlstra et al., 2008), striped skunk (*Mephitis mephitis*) (Smith et al., 1992), opossum (*Didelphis virginianus*) (Smith et al., 1992), and birds (Dubey, 2002). Notably, feral pigs may consume all of these species as prey or carrion, thus ingesting infective *T. gondii* cysts.

During this study, we detected a 13.3% seroprevalence of antibodies to *Trichinella* spp. in feral pigs in eastern North Carolina. The pigs positive for antibodies could have been

infected by one of the three species of the *Trichinella* genus predicted to occur within eastern North Carolina (*T. murrelli*, *T. pseudospiralis*, and *T. spiralis)* as the ELISA test is not species specific. Although, previous research within North Carolina has detected *T. spiralis* infection within domestic swine (Davies et al., 1998), feral pigs have a slightly higher incidence of antibodies to *T. spiralis* (1.3%) than domestic pigs (0.4%) (Gamble et al., 1999).

Modern market farm production practices have nearly eliminated *T. gondii* and *T. spiralis* prevalence (Davies et al., 1998); however, the recent trend towards "organic" and free-ranging pig production has increased domestic pigs' exposure to infection and the possibility of human infection through pork consumption (Kijlstra et al., 2004; Schulzig and Fehlhaber, 2006; van der Giessen et al., 2007; Gebreyes et al., 2008). Further, the importance of feral pigs as sources of infection to humans and domestic swine has increased (Nelson et al., 1961; Schultz, 1970; Bessonov, 1979; Dubey and Jones, 2008). As feral pig range and population size expands, either naturally or with human assistance, the opportunity for feral pig hunting increases. We recommend education programs be conducted for hunters to understand the risk of exposure to zoonotic diseases during the cleaning process and meat consumption.

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**A serosurvey of *Brucella suis,* classical swine fever, porcine circovirus type 2, and pseudorabies in feral pigs (*Sus scrofa)* of eastern North Carolina.**


## ABSTRACT

As feral pig (*Sus scrofa*) populations expand their range and the popularity of feral pig hunting increases, there is increased potential for disease transmission that may impact humans, domestic swine, and wildlife. In the United States, North Carolina is the second largest producer of pork, valued at US$ 2 billion dollars annually. From September 2007 to May 2009, in 13 North Carolina counties and at Howell Woods Environmental Learning Center (hereafter Howell Woods), we conducted a serosurvey of feral pigs for antibodies to porcine circovirus type 2 (PCV-2), *Brucella suis*, pseudorabies virus (PRV), and classical swine fever (CSF). Feral pigs were collected by trapping and hunter harvest. At Howell Woods, we detected PCV-2 antibodies in 58.9% (53/90) of feral pigs that differed between collection years ($\chi^2_1 = 6.08$, $P = 0.01$) but was similar across age classes ($\chi^2_2 = 2.62$, $P = 0.27$) and sexes ($\chi^2_1 = 0.39$, $P = 0.53$); no feral pigs collected in the 13 North Carolina counties were screened for PCV-2 for this study. We detected *B. suis* antibodies in 7.5% (6/80) of feral pigs at Howell Woods which differed between collection years ($P = 0.005$, Fisher's exact test), and 0/265 in the ten North Carolina counties. We did not detect antibodies for PRV (n = 61, 264) or CSF (n = 40, 130) at Howell Woods or the 13 North Carolina counties, respectively. The detection of feral pigs with antibodies to *B. suis* for the first time in North Carolina warrants increased surveillance of the feral pig population surrounding areas to evaluate how quickly the disease spreads and to establish the potential risk to commercial pig producers.

## INTRODUCTION

In the mid 16th century, pigs (*Sus scrofa*) were brought from Europe to mainland United States, maintained in captive facilities, and released into the wild as free-range livestock (Towne and Wentworth, 1950). Feral pigs have increased in numbers and expanded their range making them the most abundant free-ranging, exotic ungulate in the United States (McKnight, 1964; Decker, 1978). In the United States, the feral pig population has quadrupled over the last 10 years and is estimated to be ~4 million animals distributed across 39 states (Nettles and Erickson, 1984; Clay, 2007). This increase in feral pig numbers has resulted from intentional or accidental introductions including 1) translocation to establish populations for hunting, 2) escapes from shooting preserves or confinement operations, 3) avoidance of capture by domestic pigs in free-ranging livestock operations, 4) abandonment by their owners, and 5) dispersal from established feral populations (Gipson et al., 1997; Witmer et al., 2003; Seward et al., 2004). As the feral pig population expands and the popularity of feral swine hunting increases, there is increased feral pig interaction with domestic swine and humans, respectively. The potential for disease transmission that can affect humans, commercial pigs, and wildlife thus elevates (Evans, 1947; Capua et al., 1997; Starnes et al., 2004; Ruiz-Fons et al., 2008a).

The National Wildlife Disease Program within USDA-APHIS routinely screens feral pigs for antibodies to classical swine fever (CSF), pseudorabies (PRV), and *Brucella suis*. These diseases currently do not occur in U.S. commercial pig operations. Classical swine fever, formerly called hog cholera, was eradicated from the U.S. in 1976 (USDA, 2005). Active surveillance for CSF focuses primarily on domestic pigs and pork products because reintroduction of this highly contagious *Pestivirus* into the country would most likely occur

through commercial sources. Surveillance of feral pigs, however, is important to reinforce the country's CSF free status. *Brucella suis* and pseudorabies do occur in feral pig populations in the U.S. (Wood et al., 1976; Zygmont et al., 1982; Corn et al., 1986; Pirtle et al., 1989; van der Leek et al., 1993; Gresham et al., 2002; Stoffregen et al., 2007; Cavendish et al., 2008); although historically, North Carolina feral pigs have been seronegative for *B. suis* (Corn et al., 2009; Erickson pers comm.). Pseudorabies has been detected in feral pigs in the western portion of the state since 2005 (Cavendish et al., 2008).

Porcine circovirus type 2 is commonly found in domestic pigs in North America and is associated with a variety of clinical presentations (Desrosiers, 2007). One of the more problematic is post-weaning multisystemic wasting syndrome (PMWS; Ellis et al., 1998). Until recently, there has been little information on the seroprevalence of PCV-2 in feral pigs within the U.S. Corn et al. (2009) reported that PCV-2 seroprevalence is common in feral pigs in North and South Carolina (66.7% and 59.2%, respectively).

Surveying feral pigs annually for select infectious diseases provides information on whether populations have become established reservoirs and could potentially pose a risk to domestic pigs. Sampling feral pigs can be labor intensive and costs of tests prohibit large numbers of pigs being screened annually. In North Carolina, most of the sampling effort is in eastern North Carolina where commercial pig operations are concentrated. Approximately 100-200 feral pigs are screened each year with sampling effort distributed over multiple counties resulting in smaller numbers of pigs per area. Consequently, prevalence of disease exposure would have to be relatively high before positive animals are detected.

As part of a study to evaluate hunter harvest demographics over a 2-year period in a privately owned property, we collected serum samples from hunter-killed feral pigs and

screened for antibodies to CSF, pseudorabies, *B. suis*, and PCV-2. Our first objective was to evaluate antibody prevalence in more feral swine from a smaller geographic area (2800 acres) and compare to routine seroprevalence data from feral pigs sampled throughout North Carolina. The second objective was to examine PCV-2 seroprevalence in an established feral pig population over time.

## METHODS

*Study Area*

From 2007 to 2009, we conducted a serosurvey of feral pigs. Our research was conducted at sites within ten North Carolina counties including Bertie (36°01'28.3"N, -76°57'51.5"W), Bladen (34°35'17.7"N, -78°33'57.9"W), Caswell (36°23'09.7"N, -79°17'24.8"W), Columbus (34°15'17.5"N, -78°44'51.4"W), Craven (35°05'16.0"N, -77°03'23.2"W) , Duplin (34°53'02.4"N, -78°01'10.3"W), Johnston (35°26'25.3"N, -78°23'03.2"W), Pender (34°31'01.5"N, -77°50'12.2"W), Pitt (35°33'32.1"N, -77°25'27.5"W), Richmond (35°00'11.0"N, -79°47'02.4"W), Robeson (34°38'18.2"N, -79°06'34.9"W), Sampson (34°55'11.1"N, -78°23'03.2"W), and Wayne (35°21'23.6"N, -77°58'25.9"W) (Figure 1). All counties were in eastern North Carolina where commercial pig production occurs except Caswell County located at the border with Virginia. In 2007, 200 pigs were culled in this county as a depopulation effort. Our research was also conducted at Howell Woods (35°22'14.7"N, -78°18'23.4"W), an 11 $km^2$ private property, located within Johnston County, North Carolina.

In the 13 North Carolina counties, feral pigs were collected from January 2007 to May 2009 on private agricultural properties using walk-in drop door traps (i.e., 1.3x2x1-m box-style

traps and 6x6x2-m corral type traps) baited with corn, or shot with the aid of spotlights at night. Feral pigs collected via traps and night-hunting were necropsied in the field. Between 1-3 cc of whole blood was collected via heart puncture and serum was obtained by centrifugation, frozen, and stored at -22°C.

At Howell Woods, feral pigs were hunted from September 2007 to March 2009, during 30 hunting sessions, each lasting four days. During each hunting session, there were 20 hunters and all hunting was conducted from tree stands overlooking an automated feeder programmed to dispense corn once daily at 1630 hours. At Howell Woods, feral pigs harvested by hunters were transported to a central processing site for cleaning. Once at the processing site, feral pigs were weighed, sexed, aged, and blood was collected by heart puncture, cranial sinus puncture, or from the wound site. Blood was centrifuged (Vulcon Technologies Mobilespin Model #128 centrifuge) at 3082 rpm for 10-15 minutes and stored at -80°C until tested.

Feral pig hunting at Howell Woods did not occur during the months April to August in any year of the study; therefore, feral pigs were grouped into two time periods as either season one (September 2007 to March 2008) or season two (September 2008 to March 2009). Additionally, all feral pigs collected were aged based upon dental characteristics (Matschke, 1967), and divided into three age-classes: juvenile (≤ 5 months), sub-adult (5-8 months), and adult (7-11+ months).

*Serum Analyses*

Only feral pigs collected from Howell Woods were screened for antibodies to PCV-2 in this study. Serum samples were sent to Rollins Animal Disease Diagnostic Laboratory (Rollins Animal Disease Diagnostic Laboratory, Raleigh, North Carolina, USA) and analyzed using a SERELISA$^{TM}$ PCV2 Ab mono blocking kit (Synbiotics Europe, Lyon, France) (Corn et al.,

2009). The test kit uses a single well blocking immunoenzymatic technique for the detection of anti-PCV-2 in serum. Samples with a negative corrected ratio of ≤ 0.50 are considered positive for the presence of PCV-2 antibodies in serum and samples with a ratio of > 0.50 are considered negative.

In 2007 and 2008, feral pig sera tested for PRV and *B. suis* were sent to the Rollins Animal Disease Diagnotic Laboratory, whereas in 2009, samples were sent to the USDA-APHIS-VS Eastern Region Federal Brucellosis Laboratory. *Brucellosis suis* test procedures include three sequential analyses: the buffered acidified plate antigen (BAPA) test, card test (Rose Bengal) and fluorescence polarization assay (FPA). Due to the high sensitivity of these tests any negative result interrupted the test sequence whereas a positive result was analyzed by all three tests.

The BAPA test is a latex agglutination assay that detects *Brucella* spp antibodies and is used as the initial screening test for *B. suis*. Positive reactions are then followed by the card test, another latex agglutination assay with roughly the same sensitivity and specificity as the BAPA. The FPA is a qualitative test that detects antibodies to *B. abortus* O-polysaccharide which is covalently linked with a fluorescein isothiocyanate tracer molecule. If antibody is present in a serum sample, the resulting antibody-antigen complex reduces the rotation of the fluorescein tracer and increases polarization of emitted light. Serum samples with polarization values 20 above the negative control are considered positive for *B. suis*. The fluorescence polarization assay is highly specific and used as a confirmatory test for *B. suis*.

The PRV serology tests were the Autolex™ Anti-PRV Screen (Viral Antigens, Inc.) and the HerdChek™ Anti-PRV gpI (IDEXX). The Autolex™ is a highly specific, semi-automated latex agglutination immunoassay for the detection of antibodies to pseudorabies

virus in swine serum. A negative reaction suspended further examinations with high confidence. Positive reaction samples were subsequently screened for PRV gpI antibody to distinguish between field strains and vaccine strains lacking gpI.

Feral pig sera were sent to the United States Department of Agriculture Veterinary Services, Foreign Animal Disease Diagnostic Laboratory and screened for CSF antibodies. Tests included an ELISA followed by an immunoperoxidase test and finally virus neutralization. Any negative reaction stopped further testing.

*Statistics*

We analyzed seropositivity of PCV-2 by age, sex, and year using a likelihood ratio test. We used a Fisher's exact test (2-sided, $\alpha = 0.05$) to compare *B. suis* across years. Due to no detection of CSF and PRV antibodies, statistical analyses were not conducted. All statistical analyses were conducted using SAS's JMP 7.0.2 (SAS Institute, Inc., 100 SAS Campus Drive, Cary, North Carolina, 27513, USA) and alpha was set at 0.05.

## RESULTS

Between 2007 and 2009, there were 433 feral pigs harvested from the 13 North Carolina counties and 104 harvested at Howell Woods (Table 1). Due to the variation in the amount of serum collected, not all feral pigs harvested were tested for every disease. There were 422 feral pigs tested for *B. suis*, 421 feral pigs tested for PRV, 216 feral pigs tested for CSF (Table 2) and 90 feral pigs from Howell Woods tested for PCV-2 (Table 3). No feral pigs had antibodies to CSF ($n = 196$) or PRV ($n = 360$). In the 13 North Carolina counties, excluding Howell Woods, no feral pigs had antibodies to *B. suis* ($n = 360$).

Due to serum sample hemolysis, four samples from Howell Woods were excluded from all laboratory analyses during year one and 10 during year two. In season one, 0/35 feral pigs had antibodies to *B. suis*, whereas in season two 6/27 (22.2%) feral pigs had antibodies, which differed between seasons ($P = 0.005$). During season one, 33/54 (66%) feral pigs tested positive for antibodies to PCV-2 and 20/50 (40%) feral pigs tested positive for antibodies to PCV-2 during season two. Presence of PCV-2 antibodies was significantly different between seasons ($\chi^2_1 = 6.08$, $P = 0.01$) but similar across age classes ($\chi^2_2 = 2.62$, $P = 0.27$) and sexes ($\chi^2_1 = 0.39$, $P = 0.53$).

## DISCUSSION

North Carolina's domestic swine industry is ranked second in the nation earning US$ 2 billion a year (NCACS, 2005). While the majority of pigs are raised in confinement facilities with biosecurity measures to prevent contact with or contamination from feral pigs, there is a growing shift to free-range operations that place domestic pigs at greater risk to diseases carried by feral pigs as suggested for PRV by Ruiz-Fons et al. (2008b) and has been demonstrated with *Trichinella spiralis* and *Toxoplasma gondii* (Kijlstra et al., 2004; van der Giessen et al., 2007). No feral pigs had antibodies to CSF and PRV at any of the collection sites in North Carolina, including Howell Woods. Classical swine fever is a foreign animal disease so negative findings are expected. Absence of antibodies to PRV has been consistently seen in feral pigs in eastern North Carolina since routine surveillance began in 2004, but has been detected yearly in western North Carolina since 2005 (Cavendish et al., 2008) and is present in feral pigs of other states (Nettles and Erickson, 1984; Corn et al., 1986; Pirtle et al., 1989; van der Leek et al., 1993).

Swine are the natural host and reservoir of suid herpesvirus 1, etiology of pseudorabies. Although a wide range of domestic and wildlife species are susceptible to PRV, they are considered dead-end hosts because they do not live long enough to establish an effective reservoir (Trainer and Karstad, 1963; Kirkpatrick et al., 1980, Wright and Thawley, 1980, Stallknecht and Howerth, 2001). In 1989, a joint private and public program was initiated to eradicate PRV from domestic swine herds in the United States, which has proven successful for many commercial operations (Romero et al., 1997), including those in North Carolina. No such effort, however, was made to eradicate PRV from feral pigs, which presumably could have maintained the infection in the state. Corn et al. (2009) suggested that feral pig populations in North Carolina, unlike their counterparts in South Carolina, became established following active eradication of PRV in commercial pigs during the '90's. However, there has been continuous presence of feral pigs in Howell Woods for over 50 years (Betsill pers. comm.), which makes the alternative explanation that the population was established originally with uninfected pigs before any disease eradication programs were initiated more plausible.

Over the two collection seasons at Howell Woods, the seroprevalence of antibodies to PCV-2 in feral pigs was 58.9% (n = 90). This is similar to a previous survey of feral pigs in Johnston County where 60% (n = 45) were seropositive for PCV-2 (Corn et al., 2009). However, there was a 26% decrease in PCV-2 seropositive animals in the second collection season of our study. The significance of this is difficult to interpret. The impact of PCV-2 on feral pigs is unknown. Even in domestic pigs, disease pathogenesis is complex and ranges from inapparent to severe PMWS epidemics increasing post-weaning mortality rates 3-4 times normal levels (Harding, 2004). Co-infections, age, management practices, and genetics have

all been implicated in contributing to PCV-2 associated clinical disease expression in domestic pigs (Dorr et al., 2007; Desrosiers, 2007). Porcine circovirus type 2 antibodies have previously been found in wild boar in Europe (Sanchez et al., 2001) and Ellis et al., (2003) concluded that PCV-2 was associated with PMWS in wild boar that experienced poor body condition, diarrhea, and rapid death. However, these animals were farm-reared and may have experienced other co-factors relative to captivity that contributed to the mortalities. At this point, it is not possible to determine whether PCV-2 is maintained in feral pig populations in the absence of close proximity to domestic pig operations. Seroprevalence studies would need to be conducted in feral pigs isolated from farm and commercial operations.

No *Brucella* antibodies were detected in any feral pig samples from North Carolina with the exception of the second collection season at Howell Woods. Six samples out of 27 (22%) were seropositive for *B. suis*. The feral pig population at Howell Woods has been tested for *B. suis* since 2004 with no positive individuals until this study. It is unknown how *B. suis* was introduced to the population. Possibly the founder population had infected pigs that were missed during previous disease screenings; but more likely, there were recent immigrations or introductions of carrier pigs, transitional or feral, to the population. It is believed that feral pigs were being moved by humans into and around the state for recreational hunting with the source of these pigs most likely South Carolina, which has a large feral pig population positive for *B. suis* (Stoffregen et al., 2007; Corn et al., 2009).

Howell Woods is ~ 134 km from the South Carolina state line making feral pig natural movement unlikely. Further feral pig populations in South Carolina are greater in counties that border North Carolina counties in the far west of the state and not the east (Corn et al., 2009). Among the eastern North Carolina counties where feral pig samples were

collected for this study, Robeson and Columbus counties border South Carolina and Bladen and Sampson counties are juxtaposed between those counties and Johnston County. A total of 39 serum samples were collected from these 4 counties situated between South Carolina and Howell Woods and none were positive for *Brucella* (Table 2). Although sampling effort in each county was relatively low, we can be 95% confident that the prevalence of *B. suis* in the 4 county population was not greater than 7.7% (Hanley and Lippman-Hand, 1983) which is much lower than the 22% in Howell Woods suggesting that pigs were recently transplanted here.

The recent introduction of *B. suis* into a feral pig population that is routinely hunted raises concern about zoonotic transmission. Howell Woods has facilities for processing carcasses on-site, and although gloves are worn, extra care and attention are warranted. Recent cases of *B. suis* in feral pig hunters were linked to butchering of pigs and not consumption of the meat (CDC, 2009). In one instance, a hunter cut his hand during field dressing and was not wearing gloves. Hunters need to be aware that clinical signs can develop weeks to months after exposure and are relatively non-specific. It is important that hunters who develop febrile illnesses inform their physicians of their activities so appropriate differentials, like *B. suis*, can be included and screened.

The impact of *B. suis* on feral pig populations is not well known, but is probably negligible. Despite the ability of *B. suis* to cause sterility in boars and abortions in sows, feral pig populations with *B. suis* are able to maintain a high level of reproductive productivity (Stoffregen et al., 2007). The problem is spillover into the domestic population where sterility and abortions adversely impact profit margins, transmission can occur more readily because there are more animals in closer proximity, and infected animals pose a greater zoonotic risk to pork processing plant workers (CDC, 1994) and potentially consumers.

Howell Woods is located within 5.2 $km^2$ of ~ 13320 domestic pigs with the average distance of a *B. suis* seropositive feral pig to a swine farm was 4.3 km (range 1.5 – 7.8 km) and feral pig home ranges in the Southeast have been estimated from 1.2 $km^2$ to 11.6 $km^2$ (Wood and Brenneman, 1980; Hayes et al., 2009). Farm operations included commercial confinement and transitional. Transmission of *B. suis* is primarily by ingestion of infected tissues or fluids, although sexual transmission is possible. Transitional or free-range operations are at greater risk for the introduction of *B. suis* commercial confinement operations because direct contact is necessary for effective transmission.

The introduction of *B. suis* into southeastern Johnston County could have been easily missed for years with traditional, limited sampling of feral pigs in multiple counties with swine production facilities. *Brucella suis* seropositive pigs were found in the following hunting season at Howell Woods. With the continual movement of feral pigs with the aid of humans (Gipson et al., 1997) and the indication from this study of an established feral pig population recently infected with a zoonotic disease, increased surveillance of the feral pig population in surrounding areas is necessary to evaluate the speed of disease spread and to establish the potential risk to commercial pig producers. Surveillance programs need to standardize their effort and sample more pigs from each population to increase the likelihood of detecting the presence of a disease within the population.

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Table 2.1. Age and gender of all feral pigs collected in North Carolina*, 2007-2009.

| Year | Adult | Sub-adult | 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 |
| Total | 170 | 138 | 222 | 7 | 270 | 267 |

*Includes feral pigs from Howell Woods.

Table 2.2. Summary of feral pigs tested for antibodies organized by year, county, and disease, North Carolina 2007-2009.

| Year | County | SB[x] test | SB[x] pos | PRV test | PRV pos | CSF test | CSF pos |
|---|---|---|---|---|---|---|---|
| 2007 | Bertie | 1 | 0 | 1 | 0 | 1 | 0 |
| | Bladen | 11 | 0 | 11 | 0 | 11 | 0 |
| | Caswell | 195 | 0 | 195 | 0 | 0 | 0 |
| | Duplin | 19 | 0 | 19 | 0 | 17 | 0 |
| | Johnston* | 6 | 0 | 6 | 0 | 6 | 0 |
| | Pitt | 5 | 0 | 5 | 0 | 5 | 0 |
| | Wayne | 9 | 0 | 9 | 0 | 9 | 0 |
| 2008 | Bertie | 13 | 0 | 13 | 0 | 14 | 0 |
| | Bladen | 47 | 0 | 47 | 0 | 52 | 0 |
| | Caswell | 17 | 0 | 17 | 0 | 0 | 0 |
| | Columbus | 10 | 0 | 10 | 0 | 14 | 0 |
| | Johnston* | 23 | 0 | 23 | 0 | 22 | 0 |
| | Richmond | 2 | 0 | 2 | 0 | 0 | 0 |
| 2009 | Bladen | 19 | 0 | 18 | 0 | 19 | 0 |
| | Columbus | 7 | 0 | 7 | 0 | 7 | 0 |
| | Craven | 5 | 0 | 5 | 0 | 6 | 0 |
| | Johnston* | 8 | 0 | 8 | 0 | 8 | 0 |
| | Pender | 12 | 0 | 12 | 0 | 12 | 0 |
| | Robeson | 2 | 0 | 2 | 0 | 2 | 0 |
| | Sampson | 11 | 0 | 11 | 0 | 11 | 0 |
| Totals | | 422 | 0 | 421 | 0 | 216 | 0 |

*Howell Woods' is located within Johnston County but the results from Howell Woods are reported separately.

[x]SB refers to *Brucella suis*.

Table 2.3. Serosurveillance results of feral pigs harvested from Howell Woods, Four Oaks, North Carolina 2007-2009.

| Season | Harvest Site | SB test | SB pos | PRV test | PRV pos | CSF test | CSF pos | PCV-2 test | PCV-2 pos |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Howell Woods | 35 | 0 | 34 | 0 | - | - | 50 | 33 |
| 2 | Howell Woods | 27 | 6 | 27 | 0 | 20 | 0 | 40 | 20 |
| Total | | 62 | 6 | 61 | 0 | 20 | 0 | 90 | 53 |

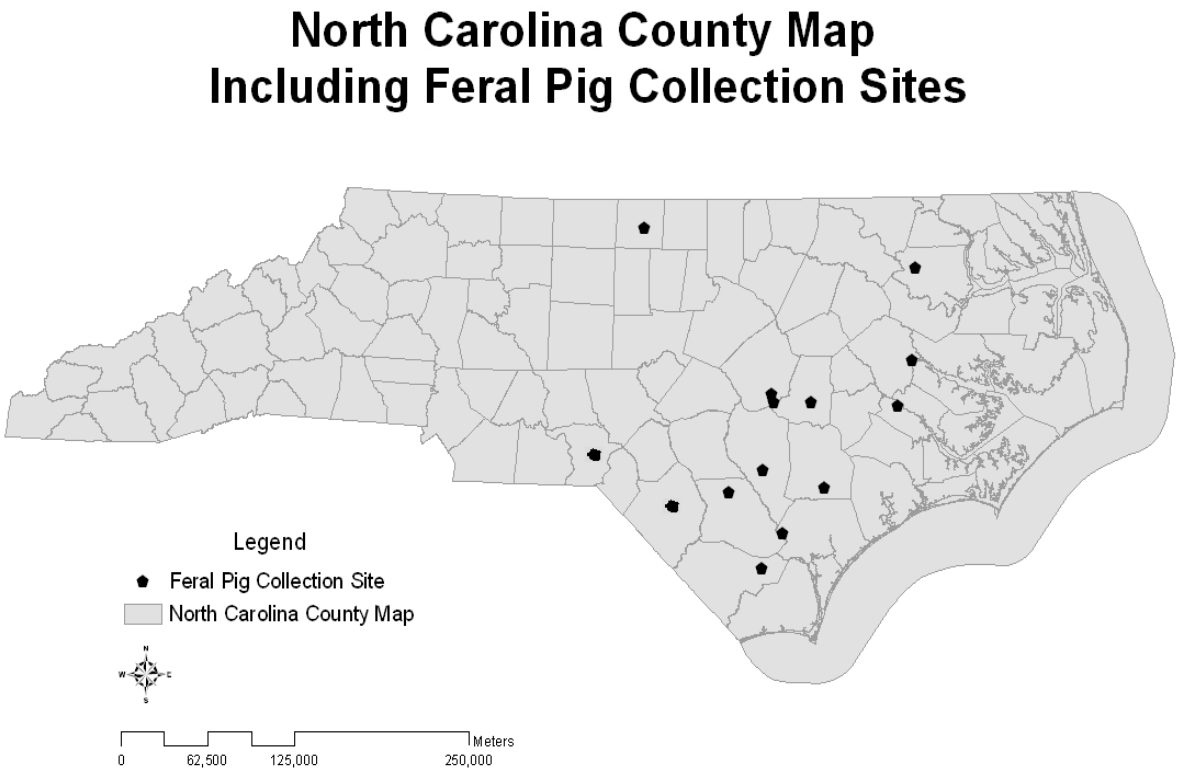


Figure 2.1. Feral pig collection sites within North Carolina 13 counties from September 2007 to May 2009.