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Surveillance of feral swine for *Trichinella* spp. and *Toxoplasma gondii* in the USA and host-related factors associated with infection

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ABSTRACT

Trichinella spp. and *Toxoplasma gondii* are important zoonotic parasites that infect warm blooded animals and humans worldwide. Among domesticated food animals, pigs are the main host for *Trichinella spiralis*. Pigs, chickens, sheep, and goats are known to be infected with *T. gondii* at varying rates, depending on husbandry. Infections in wildlife with these parasites are generally higher than in domesticated species. Feral swine act as reservoirs of infection in the sylvatic ecosystem for *Trichinella* spp. and *T. gondii*, acting as sources of infection for peridomestic carnivores whose home ranges overlap with domestic pigs. Feral swine can have direct contact with non-biosecure domestic pigs, presenting opportunity for direct disease transmission through cannibalistic behavior. Determination of the prevalence of *Trichinella* spp. and *T. gondii* infection in feral swine is needed to understand the risk of transmission of these parasites to domestic pigs. A cross-sectional serological survey was conducted between 2006 and 2010 to estimate the antibody prevalence of *Trichinella* spp. and *T. gondii* and risk factors associated with infection in feral swine in the USA. Serum samples were tested from 3247 feral pigs from 32 states; results are reported from 26 states. Maximum entropy ecological niche modeling and spatial scan statistic were utilized to predict the geographic range and to examine clusters of infection of *Trichinella* spp. and *T. gondii* in feral pigs. The seroprevalence of antibodies to *Trichinella* spp. and *T. gondii* was 3.0% and 17.7%, respectively. Species distribution modeling indicated that the most probable distribution areas for both parasites was similar, concentrated primarily in the South and the Midwest regions of the USA. A follow up survey conducted during 2012–2013 revealed that 2.9% of 984 sampled feral swine were seropositive for

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Trichinella spp., and 28.4% were seropositive for *T. gondii*. Three hundred and thirty (330) tongues were collected from the 984 sampled animals during 2012–2013; 1.81% were tissue positive for *T. spiralis* muscle larvae; no other genotypes were found. The potential exists for introduction of these pathogens into domestic herds of non-biosecure domestic pigs as a result of increasing overlap of the range of feral pigs with non-biosecure domestic pigs production facilities in the USA.

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1. Introduction

The USA feral swine population is estimated at five million and is growing rapidly. Feral swine are now found in at least 39 states due to natural range expansion, accidental or intentional release, and illegal movement of animals for hunting opportunities (USDA, 2011, APHIS Bulletin # 2086). Feral swine approach domestic pig facilities that overlap their home range due to the presence of breeding sows, access to food resources, and commingling. As a result, localized populations of feral swine pose an increasing risk to non-biosecure domestic pig facilities by serving as reservoirs for pathogens which might be transmitted to domestic pigs. *Trichinella* spp. and *Toxoplasma gondii* are important parasites worldwide which have been largely eliminated from domestic pigs in the USA (Pyburn et al., 2005; Hill et al., 2010). Human trichinellosis results from ingestion of larvae in raw or undercooked meat (Gottstein et al., 2009); *T. gondii* infection results from congenital infection, accidental ingestion of oocysts in the environment, or from ingestion of tissue cysts in raw or undercooked meat (Dubey et al., 2005). Among domesticated food animals, pigs are most commonly infected with *Trichinella* spp., while chickens, sheep, goats, and pigs are known to be infected with *T. gondii* at varying rates, depending on husbandry (Gamble et al., 1999; Dubey et al., 2002; Hill et al., 2010). Infection rates in wildlife with these parasites are generally higher than in domestic species (Zarnke et al., 2000; Dubey et al., 2009; Aubert et al., 2010).

Despite the zoonotic potential of trichinellosis and toxoplasmosis, very little is known of the prevalence of *Trichinella* spp. and *T. gondii* in feral swine in the USA. Also of concern is the potential for direct contact of feral swine with non-biosecure domestic pigs, which has been documented, and which presents opportunity for disease transmission (Wychoff et al., 2009). Transmission of *Trichinella* and *T. gondii* has been observed to occur due to cannibalism in free-ranging pigs (Dubey et al., 1986a; Hanbury et al., 1986; Hill et al., 2010), and commingling of feral swine and non-biosecure domestic swine provides an ideal circumstance for this behavior to occur. Hunters commonly field dress feral swine, resulting in partial carcasses being left in the field and available for scavenging by free-ranging hogs (Giurgiutiu et al., 2009). In addition, some states allow aerial hunting of feral swine, leaving entire carcasses in the field for scavenging (Moarthland, 2011).

A cross-sectional, serological survey was conducted from 2006 to 2010 to determine the seroprevalence of *Trichinella* spp. and *T. gondii* in feral swine in the USA. In addition, a predictive map based on environmental conditions using the maximum entropy (Maxent) approach to species distribution modeling (Phillips et al., 2006) was created for both parasites to highlight the geographical areas with high probability for occurrence. The spatial scan statistic (SaTScan) was utilized to investigate clusters of infections. A follow up study was conducted in 2012 through 2013 to assess continuing prevalence of *T. gondii* and *Trichinella spiralis*, and to attempt isolation and genotyping of muscle larvae from tongues collected from feral hogs to determine the species of *Trichinella* spp. circulating in these animals.

2. Material and methods

2.1. Animals and sampled areas

Samples were collected from feral swine trapped or hunted in 32 states according to guidelines developed by USDA's Animal and Plant Health Inspection Service, Wildlife Services (Wildlife Services, 2008, USDA-APHIS-WS, NWDP, Comprehensive Feral Swine Disease Surveillance Procedure Manual) during 2006–2010. The main disease that drives feral pig surveillance is classical swine fever (CSF), and feral pig sampling targeted high risk areas based on potential entry pathways for CSF, such as international borders, and in areas near domestic pig production facilities, landfills, and high risk pig producers (small producers of non-contract animals for personal use or limited distribution). The 32 states sampled for feral pigs were divided into West, Midwest, South, or Northeast regions based on designations applied by the USA Census Bureau (www.census.gov). In the program, the number of pigs targeted for collection was determined from the number of samples needed per year for point prevalence estimates for CSF based on the estimated population of feral pigs within each state. The location of collected animals was determined using GPS units standardized to World Geodetic System (WGS-84) datum, collected in decimal degrees (Fig. 1). The longitude and latitude coordinates of the collection location, gender, and age class of the animals based on lower jaw tooth eruption criteria (incisor #2 absent = juveniles, less than two months old; incisor #2 erupted, deciduous canine = sub-adults, between two months and one year old; permanent canine = adults, over one year old; Matschke, 1967) were recorded for each collected sample.

Whole blood was collected directly from the heart, clavicle well, or orbital sinus into serum separator tubes. Tubes were labeled with a unique subject ID to link the samples and corresponding results back to the individual pig. Blood was allowed to clot for 5–10 min at ambient temperature before being placed in a cooler. Blood was centrifuged