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# Detection of *Clostridium difficile* and *Salmonella* in Feral Swine Population in North Carolina

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ABSTRACT: We sampled 161 feral pigs in eastern North Carolina, USA, to determine the prevalence and antimicrobial resistance profile of *Clostridium difficile* and *Salmonella*. Seven (4.4%) and eight (5.0%) pigs tested positive for *C. difficile* and *Salmonella*, respectively, highlighting the importance of determining the epidemiology of these pathogens in feral pigs.

Feral pigs are widely distributed in the USA, with established populations in 37 states (SCWDS, 2011). Recent studies have clearly indicated the ability of feral pigs to act as reservoirs of important viral and bacterial pathogens, including the porcine circovirus 2, and *Escherichia coli* O157:H7 (Corn et al., 2009; Sandfoss, 2010). In addition, feral pigs have been shown to come in contact with domestic pigs and potentially transmit pathogens (Corn et al., 2009). *Clostridium difficile* and *Salmonella* cause gastrointestinal disorders in domesticated pigs (Songer and Anderson, 2006; Thakur et al., 2007, 2010). However, the status of these two pathogens in feral swine has not been studied in detail except for a few reports on *Salmonella* in the USA (Zygmont, 1981), Australia (Fenwick et al., 2004), and Spain (Vicente et al., 2002). Our objective was to determine the status of *C. difficile* and *Salmonella* in feral pigs in North Carolina, the second largest pork-producing state in the USA.

We sampled 161 feral pigs from 2007 to 2009 in four North Carolina counties: Bertie (36°01′28.3″N, 76°57′51.5″W), Bladen (34°35′17.7″N, 78°33′57.9″W), Columbus (34°15′17.5″N, 78°44′51.4″W), and Johnston (35°26′25.3″N, 78°23′03.2″W). In addition, samples were collected from Howell Woods (35°22′14.7″N, 78°18′23.4″W), an 11-km$^2$ private property, in Johnston County. Pigs were either captured in walk-in drop door traps (1.3×2×1-m box-style traps and 6×6×2-m corral type traps) baited with corn, shot with the aid of spotlights at night, or hunted from tree stands. Pigs were necropsied in the field, and approximately 10 g of fecal samples was stored in individual sterile whirl pack bags and transported on ice to the laboratory.

*Clostridium difficile* and *Salmonella* were isolated from fecal samples by using a standard bacteriologic method (Rodriguez-Palacios et al., 2007; Thakur et al., 2010). Presumptive *C. difficile* isolates were confirmed by PCR detection of the triose phosphate isomerase (*tpi*) housekeeping gene (Lemee et al., 2004). *Salmonella* serotyping was conducted using the Kauffman White scheme at the National Veterinary Services Laboratory (NVSL, Ames, Iowa, USA). The Epsilometric (E) and the Kirby-Bauer disk diffusion test were used to determine the antimicrobial susceptibility profile of *C. difficile* and *Salmonella* isolates to a panel of eight and 12 antimicrobials, respectively (Thakur et al., 2007, 2010). *Clostridium difficile* amplification of the toxins genes coding for Toxin A, Toxin B, and the binary toxin (CDT) was conducted by PCR (Stubbs et al., 2000; Lemee et al., 2004). Toxinotyping of the *C. difficile* isolates followed the methods of Rupnik et al. (1997).

*Clostridium difficile* and *Salmonella* were isolated from seven (4.4%) and eight (5.0%) feral pigs, respectively. We tested

multiple colonies of *C. difficile* (three/pig) and *Salmonella* (five/pig); therefore, 21 *C. difficile* and 40 *Salmonella* isolates in total were further characterized at the phenotypic level. The *Salmonella* serotypes detected included *Salmonella* Braenderup, Inverness, Berta, and Bareilly in addition to serotypes from subspecies III (antigenic formula: III_16:z10:e,n,x,z15 and III_48:g,z51:-) and IV (IV_40:z4, z32:-). All the *Salmonella* isolates were pansusceptible to the antimicrobial panel. Antimicrobial resistance was detected in *C. difficile* isolates for six of the eight antimicrobials tested. All isolates (100%) were resistant to ciprofloxacin (CIP) at the highest concentration (32 μg/ml). *Clostridium difficile* isolates exhibited high frequency of resistance to tetracycline (57%) and levofloxacin (30%). None of the isolates exhibited resistance to metronidazole and vancomycin. All *C. difficile* isolates were positive for the genes encoding Toxin A (*tcdA*), Toxin B (*tcdB*), and CDT except for a single isolate that was negative for the CDT gene. The predominant toxinotype (90.4%) we observed was toxinotype V ($A^+B^+$, $CDT^+$), and single isolates were identified as toxinotype 0 ($A^+B^+$, $CDT^-$) and XXIV ($A^+B^+$, $CDT^+$).

Although only a few samples were positive, our study is the first to describe the status of *C. difficile* in free-ranging feral pigs. The low frequency of *C. difficile* isolation may be because feral pigs are not regularly exposed to the pathogen. There has been an alarming pattern of fluoroquinolone resistance in human hospitals and the association with hypervirulent *C. difficile* strains that has resulted in high mortality and morbidity in humans (Razavi et al., 2007). We have reported similar high level of CIP resistance in *C. difficile* pigs from commercial farms in North Carolina (Thakur et al., 2010). Only eight feral pigs were *Salmonella* positive, comparable with the low prevalence reported from transitional antimicrobial-free hogs in North Carolina (Thakur et al., 2007). The *Salmonella* serotypes detected in this study, those belonging to subspecies III and IV, are rarely seen in pigs; the NVSL has observed these rare *Salmonella* strains in only 68 isolates from 2002 to 2009 from various sources, out of which 33 (49%) were from reptiles and amphibians (Erdman, pers. comm.). It is possible that feral pigs contacted with these unique *Salmonella* strains by coming in contact with reptiles.

All of our pig *C. difficile* isolates were positive for *tcd*A and *tcd*B genes that code for toxins responsible for the development of pseudomembranous colitis. Except for one isolate, the feral swine population was positive for the binary toxin genes. Toxinotype V was the predominant *C. difficile* toxinotype that we identified, and it has been reported in pig populations (Thakur et al., 2010). Another key result of our study was the identification of toxinotype 0 ($A^+B^+$, $CDT^-$) from feral swine, which has been reported from commercial based piglets (Pirs et al., 2008).

In conclusion, we report the prevalence of *Salmonella* and *C. difficile* isolated from free-ranging feral swine in areas with extensive commercial swine production. Our study highlights the potential role that feral pigs may play as a reservoir of these two pathogens. We recommend testing additional feral pigs to better understand the epidemiology of these pathogens in free-ranging feral pig populations.

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