Detection of Feral Hog Impacts to Water Quality and Wildlife
Final Report

Submitted to:

The Louisiana Department of Wildlife and Fisheries

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Executive Summary

1) All sites on which water quality was evaluated exhibited some level of pathogens, including the control LDEQ long-term water quality monitoring site. Samples yielded pathogens that cause leptospirosis (34 of 40), yersinosis (34 of 40), *Klebsiella* pneumonia (40 of 40), and salmonellosis (9 of 40) in humans and wildlife. All sites tested positive for ≥ 1 of these pathogens. These pathogens can cause severe intestinal diseases, kidney damage, meningitis, liver failure, or, if untreated, mortality in humans. For wildlife, these pathogens may cause kidney damage, renal failure, spontaneous abortions, severe respiratory illnesses, severe gastrointestinal illness, and mortality. Based on one or more criteria, every site evaluated was potentially unsafe for human-water and wildlife-water contact.

2) At 22 of 40 sites, DNA fingerprints positively matched water bourn *Escherichia coli* with fecal samples from Feral Hogs (water DNA fingerprints matched with 47 different Feral Hog DNA fingerprints from 12 of 39 sampled Feral Hogs), strongly associating feral hogs with high levels of *E. coli* and the pathogen that leads to salmonellosis in humans and wildlife. Non-significant associations were also noted between Feral Hog presence and heterotrophic bacteria counts, and microbes that may cause leptospirosis, yersinosis, and *Klebsiella* pneumonia.

3) Feral Hog matches suggested some patterns in associations among Feral Hogs (e.g., sounders or matrilineal-related groupings) and patterns, based on unequal visits to certain sites. Moreover, the spatial configuration of sites where Feral Hog matches were detected suggested a strong role in proximity to forested cover in the distribution of DNA fingerprint matches.

4) DNA fingerprinting also indicated that feral hog family groups (from the matrilineal line) were moving long distances (~ 45 km) and were genetically related to feral hogs specimens sampled this summer, as well as those sampled south of Ft. Polk Wildlife Management Area during 2005.

Project Scope and Background

The Feral Hog population has expanded to the point where impacts are widespread on both privately and publicly owned land. Feral Hogs are known carriers of over thirty zoonotic bacterial and viral diseases, including

several pathogens that may be spread through contact with water. This pilot project assessed these potential threats and health hazards to wildlife and humans from pathogens associated with Feral Hogs by determining whether Feral Hog impacts to water quality were occurring in recreationally, culturally, and ecologically important water resources on private lands adjacent Kisatchie National Forest between Natchitoches and Alexandria, Louisiana. Previous research in the Vernon District of the Kisatchie National Forest clearly indicated that feral hogs were a source of foreign *Escherichia coli* in streams (Kaller et al. 2007), and research in public lands near the Kisatchie National Forest indicated that feral hogs were associated with the presence of harmful pathogens, including *Pseudomonas* spp. (Kaller and Kelso 2003). However, these studies were localized and occurred prior to a rapid expansion of feral hog range and population density.

This project sampled 40 water bodies, including some locations suspected of contamination by feral hogs and other locations that were unlikely to have contamination. In these samples we identified *Leptospira* spp., *Escherichia coli*, *Shigella* spp., *Yersinia* spp., *Klebsiella* spp., and *Salmonella* spp., and conducted DNA fingerprinting to associate Feral Hogs with these pathogens.

Objectives

1. Determine the amount or presence of pathogenic bacteria and spirochetes in Louisiana water, including *Leptospira*, *Escherichia coli, Shigella*, *Yersinia*, *Klebsiella*, and *Salmonella*.
2. Assess through DNA fingerprinting the potential contribution of Feral Hogs to the presence of these pathogens.

Background

Recent evidence suggests Feral Hogs are present in all of Louisiana's parishes, which represents a tremendous increase in spatial coverage in the last 10 years. Concomitant with the increase in spatial coverage and, presumably, population density, have been reports of the negative impacts of Feral Hogs to other Louisiana wildlife, forest and crop resources (Tanger et al. 2015), and to property (Kaller et al. 2007; Kaller and Reed 2010). Feral Hogs can impact habitats and associated wildlife through foraging (rooting), wallowing, competition for space and food resources, and outright predation (Wood and Barrett 1979; Singer et al. 1984; Focardi et al. 2000). Reports of habitat and wildlife impacts due to establishment of Feral Hogs are extensive (see citations in Mayer

and Shedrow 2007 and Barros-Garcia and Ballari 2012). Vegetation regeneration and soil productivity decrease with Feral Hog activity (Bratton 1975; Howe et al. 1981; Lipscomb 1989; Ford and Grace 1998; Ickes et al. 2003; Siemann et al. 2009), and soil loss through erosion often increases (Belden and Pelton 1975, 1976). Through competition and opportunistic predation, increasing densities of Feral Hog populations have been associated with negative impacts to densities of White-tailed Deer (*Odocoileus virginianus*; Yarrow and Kroll 1989), Wild Turkey (*Meleagris gallopavo*; e.g., Dreibelbis et al. 2008), and American Alligator (*Alligator mississippiensis*; Elsey et al. 2012).

Other wildlife may be impacted by diseases transmitted and spread by Feral Hogs (Table 1). Of specific concern, Feral Hogs can carry *Leptospira* spp., which has been reported to cause kidney damage and loss of renal function in squirrels (*Sciurus* spp.; e.g., Dirsmith et al. 2013), Northern Raccoon (*Procyon lotor*; e.g., Junge et al. 2007), and White-tailed Deer (e.g., Davidson et al. 1985). Moreover, leptospirosis has caused abortions in experimentally infected White-tailed Deer (Trainer et al. 1961). *Leptospira* spp. may be transmitted through water, in addition to bodily fluids, and may lead to abortions and death in domestic animals and livestock, and leptospirosis in humans (Diesch et al. 1967; Jackson et al. 1993). Feral Hogs also carry *Salmonella* spp. that can result in salmonellosis in wild birds, including Wild Turkey, resulting in liver damage, severe diarrhea, and death (Hubálek 2004; United States Geological Survey Wildlife Health Bulletin 2009-01; Benskin et al. 2009). Transmission of salmonellosis can also occur through contaminated water. *Klebsiella* spp. has been associated with Feral Hogs and can cause respiratory problems (e.g., sinusitis and pneumonia) in wild birds and Wild Turkeys (Morishita et al. 1997; Fudge et al. 2001; Benskin et al. 2009). *Yersinia* spp. has been associated with Feral Hogs and can cause gastroenteritis in Black Bear (*Urus americanus*), White-tailed Deer and Northern Raccoons (Shayegani et al. 1986) and severe overwinter mortality in wild migratory birds (Hubálek 2004). *E. coli* inhabit the digestive tracts of birds and mammals, but encounters with unfamiliar *E. coli* from contaminated drinking water can cause mild to severe gastroenteritis. Although *Klebsiella* spp., *Yersinia* spp., and *E. coli* may not cause outright mortality in wildlife, increased stress from disease may reduce foraging, decrease condition, and reduce fitness.

A recent (2014) survey of 30 water bodies with known or suspected Feral Hog presence in the Kisatchie National Forest found many of these pathogens to be widespread. Colony counts of *E. coli* exceeded the limit for

safe human contact (200 fecal coliform colonies per 100mL) at 17 of 31 sites. Published guidelines for heterotrophic bacteria are not available, however, five sites had over 100,000 colonies per mL, which would be considered high and potentially harmful to humans and wildlife (Payment et al. 2003). Importantly, potentially harmful *Leptospira* spp. (19 of 31) and *Klebsiella* spp. (19 of 31) were found at a majority of sampling locations, whereas *Salmonella* spp. (8 of 31), *Yersinia* spp. (6 of 31), and *Shigella* spp. (1 of 31) were less frequently encountered. In humans, leptospirosis can lead to kidney damage, meningitis, liver failure, and death (when untreated), whereas the other pathogens will lead to highly problematic gastroenteritis. Therefore, contamination from Feral Hogs threatens wildlife and humans who may recreationally pursue wildlife.

Table 1. North American recreationally important and socio-culturally prominent wildlife species exposed to environmental pathogens resulting in sickness or death of individuals (summarized from Rausch 1947[1]; Mitchell and Ridgwell 1971[2]; Mair 1972[3]; Standridge et al. 1979[4]; Davidson et al. 1985[5]; Shayegani et al. 1986[6]; Bangert et al. 1988[7];Brand et al. 1988[8]; Cizek et al. 1995[9]; Dho-Moulin and Fairbrother 1999[10]; Thogmartin et al. 1999[11];Hudson et al. 2000[12]; Hubalek 2004[13]; Sayah et al. 2005[14]; Renter et al. 2006[15];Junge et al. 2007[16]; Benskin et al. 2009[17], Dirsmith et al. 2013[18]).

Species	Pathogen
Canada Goose (*Branta canadensis*), Mallard (*Anas platyrhynchos*), White-tailed Deer (*Odocoileus virginianus*), Wild turkey (*Meleagris gallopavo*)	*E. coli* [4,11,13,14, 17]
Fox Squirrels (*Sciurus niger*), Northern Raccoon (*Procyon lotor*), White-tailed Deer	*Leptospira* [5,16,18]
Owls (Strigiformes), Raptors (Falconiformes), Wild Turkey	*Klebsiella* [7,10]
American Black Duck (*A. rubripes*), American Coot (*Fulica americana*), Black-headed Gull (*Larus ridibundus*), Green-winged Teal (*A. crecca*), Blue-winged Teal (*A. discors*), Gadwall (*A. strepera*), Mallard, Northern Bobwhite Quail (*Colinus virginianus*), Ring-billed Gull (*L. delawarensis*), Wild Turkey, White-tailed Deer	Salmonellae [1,2,7,8,11,12,15]
Black Bear (*Ursus americanus*), Canada Goose, Canvasback (*Aythya valisineria*), Common Merganser (*Mergus merganser*), Eastern Cottontail (*Sylvilagous floridanus*), Gray squirrel (*Sciurus carolinensis*), Mallard, Snapping Turtle (*Chelydra serpentina*), White-tailed Deer, Wood Duck (*Aix sponsa*)	*Yersinia* [3,6]

Additionally, a recent survey of famers in Louisiana indicated Feral Hogs were causing production losses (estimated at $53 million) and increased costs of operations (estimated at $21 million; Tanger et al. 2015). Data indicated although only 30% of respondents reported Feral Hogs on their property, over 50% believed that Feral Hogs were a threat to their livelihoods, local wildlife, and personal health and safety (Tanger et al., in review). Landowners were surprisingly concerned and informed about threats to water quality and disease transfer to livestock and other wildlife. The authors concluded that the reputation of Feral Hogs preceded their arrival and that among farmers, the alarm has been raised, suggesting a call to action (Tanger et al., in review). Therefore, assessing the extent of pathogenic contamination associated with Feral Hogs will contribute to addressing stakeholder concerns.

Methodology

Water samples were collected between 18 June and 1 September 2015 on private lands between Natchitoches and Alexandria, Louisiana. One control site was selected at the Cane River Lake boat launch, which is a long-term water quality monitoring site for the Louisiana Department of Environmental Quality. At each site, 1 L of water was sampled by extendable pole from the shoreline, wherever possible, with every effort made to exclude benthic substrate. The sample was split in half and preserved on ice until return to the School of Renewable Natural Resources, Louisiana State University.

Within 12 hours of collection, 500 mL of the water samples was filtered and samples were split by media and replicate. Biochemical tests determined presence of *Leptospira* spp. (APHA Standard Method 9260 I), *Shigella* spp. (APHA Standard Method 9260 E), *Yersinia* spp. (APHA Standard Method 9260 K), *Klebsiella* spp. (APHA Standard Method 9225 A through E), and *Salmonella* spp. (APHA Standard Method 9260 B). Confirmation of *Leptospira* spp. required a 45-day incubation period and examination of live organisms through an inverted microscope. Additionally, 4-6 randomly-selected colonies from each site were identified to genus or species with the Enteropluri-Test (BD Diagnostic).

The other 500 mL was reserved for enumeration of total coliforms and *E.coli* and for DNA fingerprinting. Total coliforms and *E. coli* numbers were determined with Colilert medium in the Quanti-Tray/2000 system (IDEXX

Laboratories, Inc.). This most probable numbers approach uses *o*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolysis to enumerate total coliforms at 35°C and hydrolysis of the fluorogenic substrate, 4-methylumbelliferyl-β-D-glucuronide (MUG), to determine the numbers of *E. coli* and *Shigella* spp. Wells that were ONPG positive and MUG positive were scored as containing *E. coli*. For DNA fingerprinting analysis, *E. coli* strains were isolated from the Quanti-Tray2000 system by streak plate technique with Levine's EMB agar incubated at 37°C. Colonies presenting a green metallic sheen typical of *E. coli* were transferred to EMB agar, and selected colonies were confirmed as *E. coli* with a PCR assay based on primers directed against *gadAB* genes (Perry 2001). Presence of *gadAB* genes was shown to be strongly predictive of *E. coli* and *Shigella* (McDaniels et al. 1996), and because *Shigella* strains are excluded from testing by the initial bacteriology (i.e., *Shigella* does not ferment lactose), in this assay, the primers are specific for *E. coli*.

One hundred randomly selected *E. coli* underwent DNA fingerprinting. DNA fingerprinting of *E. coli* strains was by random amplification of polymorphic DNA (RAPD) based on a modification of methods in Pacheco et al. (1997). Polymerase chain reaction reagents were supplied as puRe Taq Ready-to-go beads (GE Healthcare Bio-Sciences Corp.). DNA banding patterns generated by RAPD analysis were analyzed with the Gel ComparII software (Applied Maths, Inc.) With this software, the densitometric curve-based Pearson product-moment correlation was used as the similarity coefficient and the dendrogram was generated based on the unweighted pair group method with arithmetric averages as the clustering method. DNA fingerprint patterns exhibiting similarity coefficients within 95% of each other were considered a match. The sample library of *E. coli* DNA fingerprints included fecal coliform colonies isolated from over 900 known wildlife and domestic sources, including North American Beaver (*Castor canadensis*), varieties of beef cattle (*Bos taurus*), Coyote (*Canis latrans*), White-tailed Deer (*Odocoileus virginianus*), domestic pigs (*Sus scrofa*), domestic and feral goats (*Capra aegagrus hircus*), hunter-harvested pigs, domestic and feral horses (*Equus caballus*), domestic and wild rabbits (*Sylvilagus* spp.), and Raccoons (*Procyon lotor*).

Feral Hog feces samples were obtained from trapping activities within the Bob R. Jones Idlewild Research Station, Kisatchie National Forest (one sample from 2005 and six samples from 2015), and on private lands between Natchitoches and Alexandria, Louisiana (25 samples from 2015-2016). Additionally, a sample of hunter

harvested hog collected prior to 2005 from the Leesville/DeRidder area was included. Samples from the Bob R. Jones Idlewild Research Station were from Feral Hogs originating from east of the Mississippi and served as genetic out-group for a control. Fecal samples were either extracted directly from the rectum on site and frozen, or the entire rectum was excised and frozen for later extraction. After delivery to the School of Renewable Natural Resources, DNA fingerprinting as outlined above was followed on *E. coli* isolated from the fecal samples.

Comparison between *E. coli* from the water and from Feral Hogs occurred in two steps. Initially, Feral Hog and water samples were randomly assigned to gels. If a match was detected, *E. coli* isolates were placed on the same gel for a second comparison. This procedure was performed for both primers. A match was considered confirmed if the same gel comparison resulted in a match for both primers. Previously sampling indicated a strong association between the presence of Feral Hogs and pathogens. Therefore, we analyzed whether Feral Hog presence and number of strains present were associated with *E. Coli*, heterotrophic plate counts, *Klebsiella* spp., *Leptospira* spp., *Salmonella* spp., or *Yersinia* spp. by generalized linear models with either a log link transformation and quasiPoisson error distribution (*E. Coli* and heterotrophic plate counts) or logit link transformation and binomial error distribution (*Klebsiella* spp., *Leptospira* spp., *Salmonella* spp., or *Yersinia* spp.).

Results

Pathogens were detected throughout the study sites sampled during summer 2015 (Table 2). Site 10 at the Cane River Lake boat launch is a long-term Louisiana Department of Environmental Quality monitoring site with a history of good water quality. Values for this site suggested natural, background microbial composition and counts and provided a benchmark for interpretation. All 40 sites tested positive for *Klebsiella* spp. Thirty-four (85%) sites tested positive for *Leptospira* spp., including a qualitative observation that site 7 exceeded the most *Leptospira* spp. ever observed in a water sample by the research team. Twenty-nine (73%) sites tested positive for *Yersinia* spp. At 21 of 40 sites (53%), heterotrophic bacteria colony forming units exceeded 100,000 units per mL, widely considered to be dangerous for humans and wildlife (Payment et al. 2003). Nine (23%) sites exceeded human health standards (200 colony forming units per 100 mL) for fecal coliforms (a measure of *E. coli*). Nine (23%) sites also tested positive for *Salmonella* spp., including *Salmonella cholera-suis* (swine cholera). Combining

these data with 30 water bodies with known Feral Hog presence in the adjacent Kisatchie National Forest sampled during summer 2014, many of these pathogens appeared to be widespread. In total, 53 of 70 sites (76%) tested positive for *Leptospira* spp, and 53 (76%) sites exceeded human health standards for fecal coliforms. Sixteen (23%) sites tested positive for *Salmonella* spp., including *Salmonella cholera-suis* (swine cholera). Fifty-nine (84%) sites tested positive for *Klebsiella* spp, and 46 (66%) sites tested positive for *Yersinia* spp.

Fingerprinting determined positive DNA matches of water sample *E. coli* with 47 Feral Hog *E. coli* strains at 22 of 40 sites (55%; Tables 2 and 3; Figures 1-4). Matches may represent individuals, given that *E. coli* are viable for 2-4 weeks in sediments (Garzio-Hadzik et al. 2010), but may also represent family groups related through common females. Five Feral Hogs sampled on the Kisatchie National Forest during 2015 yielded 16 E. coli strains that matched water samples at eight sites. Interestingly, four of the five Feral Hogs were matched to multiple sites (up to six sites), and sites 9, 18, and 26 were matched with multiple Feral Hogs. Five Feral Hogs trapped by a private contractor during 2015-2016 and delivered though LDWF were matched with six sites. Only one hog was matched with two sites, and sites 21 and 26 were the only sites matched with both recently-sampled sets of Feral Hogs. Intriguingly, two pre-2015 Feral Hog sources also matched sites sampled during 2015. A Feral Hog harvested by hunters in the DeRidder/Leesville area prior to 2005 matched four sites, and one Feral Hog sampled in 2005 on the Ft. Polk Wildlife Management Area matched 10 sites. Four sites (18, 21, 26, and 27) matched Feral Hogs from both historic and current samples. As sampling was lethal, these matches represent a familial (matrilineal) connection with sounder(s) sampled more than 10 years ago.

Although Feral Hogs were detected by DNA fingerprinting at 22 sites, the number of Feral Hogs uniquely identified by *E. coli* strains differed among sites (Figure 1). These patterns suggest that certain sites are favored over other sites, and potentially, based on co-occurrences of DNA fingerprints, that more than one sounder of Feral Hogs of different lineages was present. For example, site 26 matched *E. coli* DNA fingerprints with five different Feral Hogs. Sites 6, 18, and 21 all had DNA fingerprints from three different Feral Hogs, and sites 9, 23, and 27 had DNA fingerprints from two different Feral Hogs. The greater number of matches could be interpreted to indicate more Feral Hogs use these sites. Additionally, some geographic patterns were evident that may indicate different sounders were separating territorially. Feral Hogs associated with Kisatchie National Forest were

detected nearer to forest boundaries (Figure 2), which were similar sites to where Feral Hogs trapped by LDWF were detected (Figure 3). However, DNA fingerprints matched with the older Feral Hog samples were detected at a larger number of sites and in a different spatial configuration than the other two sources (Figure 4).

These results may suggest that more than one sounder or matrilineal-related groups with differing site selection are active in the area. The first interpretation based sounders or groupings primarily on sites used by few hogs. One sounder or grouping related to Feral Hogs sampled in the Kisatchie National Forest and by LDWF in 2015 exhibited similar site detections (Figures 2 and 3). DNA fingerprints matched with older Feral Hogs were found at other sites not visited by the other Feral Hog grouping (Figure 4). Potentially, there could be territorial differences or variation in the phenology of site visits between the two groupings as suggested by the unique sites attributable to each group. The second interpretation focuses on sites frequently used by many hogs (sites 6, 9, 18, 21, 23, 26, and 27; Table 3). These hogs co-occurred in the same window of time (0-4 weeks) suggesting that these hogs may be in the same sounder. For example, Feral Hogs 4, 9, 29, and one or more hogs related to the 2005 Feral Hog were all detected at site 26. However, it is important to note that seven of 12 Feral Hog samples were collected within or near the Kisatchie National Forest, including the older samples, and the observed differences in site use may be simply sampling artifacts representing widely dispersed hogs with a range including the Kisatchie National Forest.

Unlike previous studies, heterotrophic plate counts, *Klebsiella* spp., *Leptospira* spp., and *Yersinia* spp. were not significantly associated with Feral Hog presence. In contrast, both the amount of *E. coli* ($Z_{1,39} = 2.94$, $P = 0.003$) and the presence of Salmonella spp. ($Z_{1,38} = 2.72$, $P = 0.02$) were associated with the number of Feral Hog strains present in water samples.

Table 2. Pathogens detected and DNA fingerprinting results from summer 2015 sampling. Shading indicates *Escherichia coli* standard exceeded. MSW = Managed shallow water impoundment. TMTC = Too many too count.

Site	Date	Latitude	Longitude	Description	DNA Match	*Escherichia coli* per 100 mL	Heterotrophic plate count per 1 mL	*Leptospira*	*Klebsiella*	*Salmonella*	*Yersinia*
1	6 June	31.84891	93.06133	Bayou	No	3270.00	152,000		X	X	X
2	6 June	31.85113	93.06287	MSW	Yes	4100.00	122,000	X	X	X	
3	6 June	31.85881	93.06214	Bayou	Yes	3320.00	215,000		X	X	X
4	6 June	31.69607	92.98641	MSW	Yes	127.00	71,000	X	X	X	X
5	6 June	31.69685	92.98569	Drain	Yes	26000.00	TMTC		X	X	X
6	23 June	31.66730	93.02766	MSW	Yes	10.40	29,100		X	X	
7	23 June	31.66641	93.02466	Drain	Yes	99.20	298,000	X	X	X	X
8	23 June	31.66483	92.97887	Slough	No	10.30	127,000	X	X	X	X
9	23 June	31.67223	92.97183	MSW	Yes	74.60	6,400	X	X	X	X
10	23 June	31.66298	93.00125	Boat Launch	No	9.20	4,900		X	X	X
11	7 July	31.58439	92.82194	MSW	No	26.40	20,100		X	X	
12	7 July	31.54695	92.83104	MSW	No	71.80	60,000		X	X	X
13	7 July	31.56352	92.83463	MSW	No	8.20	7,000		X	X	
14	7 July	31.54449	92.84002	Bayou	Yes	197.00	19,300		X	X	X
15	7 July	31.50096	92.83685	MSW	No	23.80	13,500		X	X	X
16	14 July	31.59623	93.05573	Drain	Yes	116.00	96,000	X	X	X	
17	14 July	31.59824	93.07330	Wetland	No	5.20	10,800		X	X	X
18	14 July	31.59484	93.03457	Pond	Yes	100.00	4,200			X	X
19	14 July	31.58573	93.02541	Pond	Yes	115.00	580,000	X	X	X	X
20	14 July	31.58351	93.02227	Pond	No	0.00	35,000			X	X
21	22 July	31.54500	92.98000	Drain	Yes	10500.00	1,190,000		X	X	X
22	22 July	31.54900	92.97500	MSW	No	1370.00	95,000			X	X
23	22 July	31.55200	92.98100	Drain	Yes	16.60	203,000		X	X	X
24	22 July	31.55615	92.99081	MSW	No	60.20	1,200,000			X	
25	22 July	31.55106	92.99687	Drain	No	399.00	420,000		X	X	X
26	28 July	31.54534	92.78515	Drain	Yes	63.60	1,180,000	X	X	X	X
27	28 July	31.55678	92.79182	MSW	Yes	75.80	76,000			X	X
28	28 July	31.55312	92.77097	MSW	Yes	1160.00	195,000	X	X	X	X
29	28 July	31.54862	92.76864	Red Bayou	No	4.10	1,880,000		X	X	X

Table 2. Continued. MSW = Managed shallow water impoundment. TMTC = Too many to count.

Site	Date	Latitude	Longitude	Description	DNA Match	*Escherichia coli* per 100 mL	Heterotrophic plate count per 1 mL	*Leptospira*	*Klebsiella*	*Salmonella*	*Yersinia*
30	28 July	31.54424	92.76624	MSW	Yes	14200.00	11,900			X	X
31	25 August	31.53035	92.90174	Pond	No	94.80	133,000		X	X	X
32	25 August	31.53448	92.89967	Pierson Lake	No	2090.00	2,880,000		X	X	X
33	25 August	31.53473	92.90960	Pond	No	0.00	70,000			X	X
34	25 August	31.56612	92.90862	Bayou Charette	Yes	28.80	16,400		X	X	X
35	25 August	31.57132	92.91232	MSW	Yes	12.20	520,000		X	X	X
36	9 September	31.78850	93.25787	MSW	Yes	37.80	130,000			X	
37	9 September	31.79008	93.25307	MSW	No	19.20	113,000			X	X
38	9 September	31.79631	93.24343	MSW	No	2.00	28,400		X	X	X
39	9 September	31.84407	93.14941	MSW	Yes	8.20	TMTC			X	X
40	9 September	31.85569	93.14440	MSW	Yes	10.40	67,000			X	X

Table 3. Feral hog strains present at sampling sites.

Source of Feral Hog *E. coli*	Number of *E. coli* Strains Uniquely Attributable to Specific Hog Source that Matched to Water Samples	Water Sampling Sites
Feral Hog FH (2005 Ft. Polk WMA)	9	3, 6, 18, 21, 23, 26, 27, 28, 35, 36
Feral Hog WH (DeRidder/Leesville)	3	21, 23, 34, 40
Feral Hog 1 (2015 Kisatchie National Forest)	3	9, 27
Feral Hog 4 (2015 Kisatchie National Forest)	3	7, 26
Feral Hog 5 (2015 Kisatchie National Forest)	1	26
Feral Hog 7 (2015 Kisatchie National Forest)	5	2, 16, 18, 19, 26, 39
Feral Hog 9 (2015 Kisatchie National Forest)	4	6, 9, 18
Feral Hog 12 (2015 LDWF Sampling)	2	5, 21
Feral Hog 15 (2015 LDWF Sampling)	3	14
Feral Hog 16 (2015 LDWF Sampling)	12	4
Feral Hog 29 (2016 LDWF Sampling)	1	26
Feral Hog 36 (2016 LDWF Sampling)	1	30

Figure 1. Water sampling locations and results of DNA fingerprinting. Kisatchie National Forest (KNF) trapping locations = white dots. Water sampling sites without any *E. coli* matches = yellow dots. Water sampling sites with *E.coli* matches from multiple Feral Hog sources = black dots. Water sampling sites matched with only KNF trapped Feral Hogs samples= green dots. Water sampling sites matched with only LDWF-provided contractor trapped Feral Hogs = red dots. Water sampling sites matched with older (2005 and earlier) Ft. Polk/Leesville Feral Hog samples = blue dots.

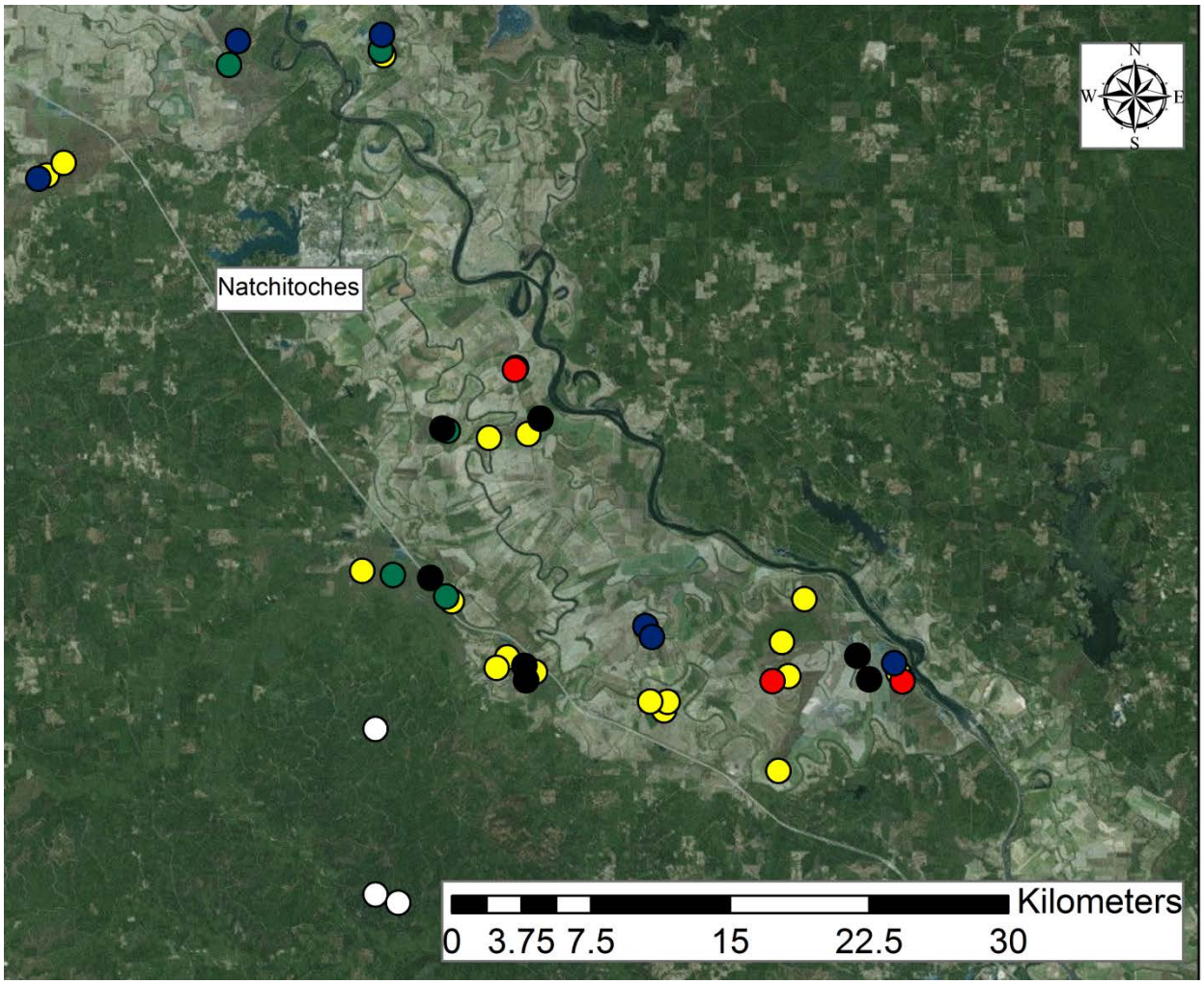


Figure 2. Water sampling locations and results of DNA fingerprinting. Kisatchie National Forest (KNF) trapping locations = white dots. Water sampling sites without KNF trapped Feral Hog *E. coli* matches = yellow dots. Water sampling sites matched with only KNF trapped Feral Hogs samples= green dots.

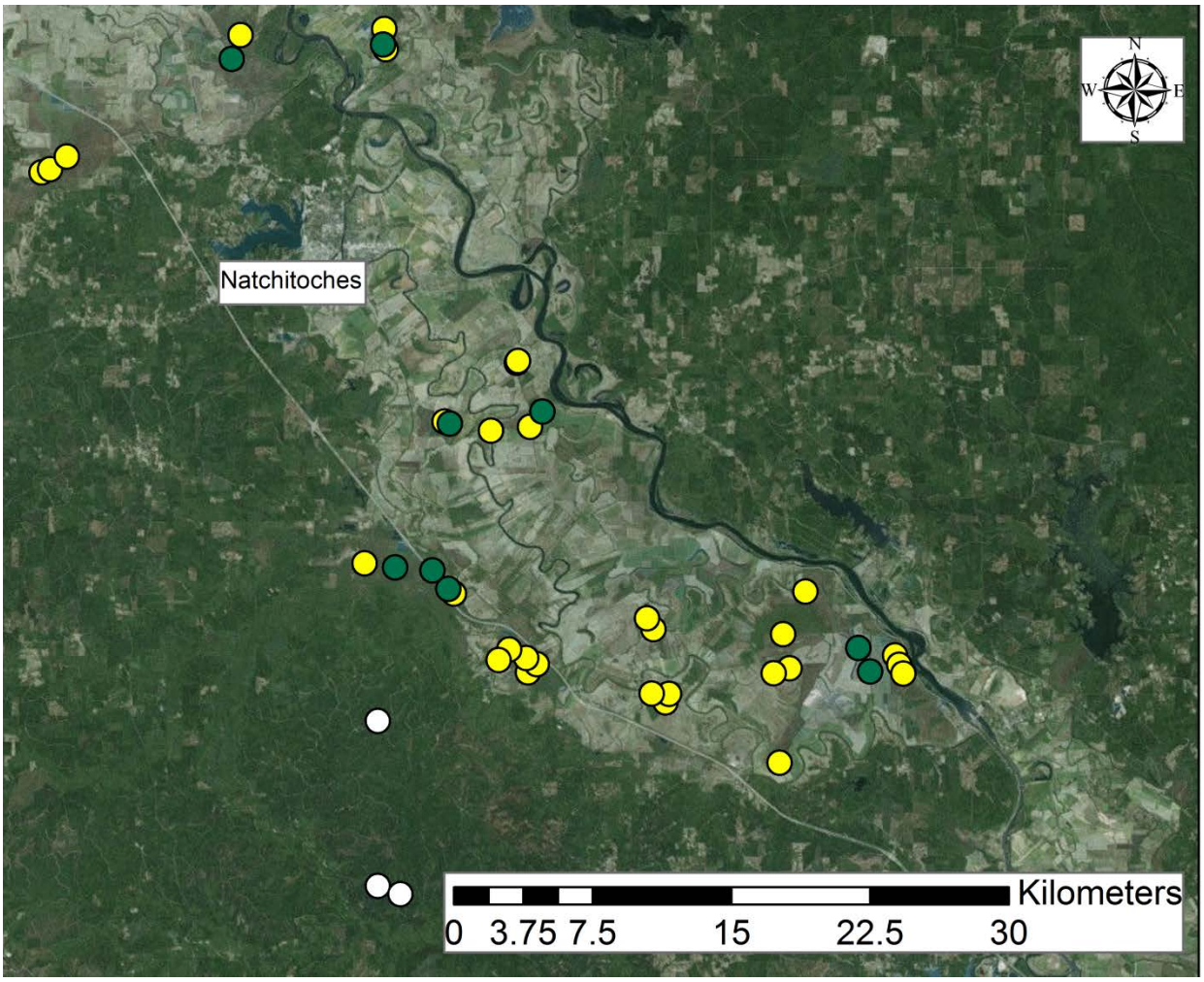


Figure 3. Water sampling locations and results of DNA fingerprinting. Water sampling sites without only LDWF-provided contractor trapped Feral Hogs *E. coli* matches = yellow dots. Water sampling sites matched with only LDWF-provided contractor trapped Feral Hogs = red dots.

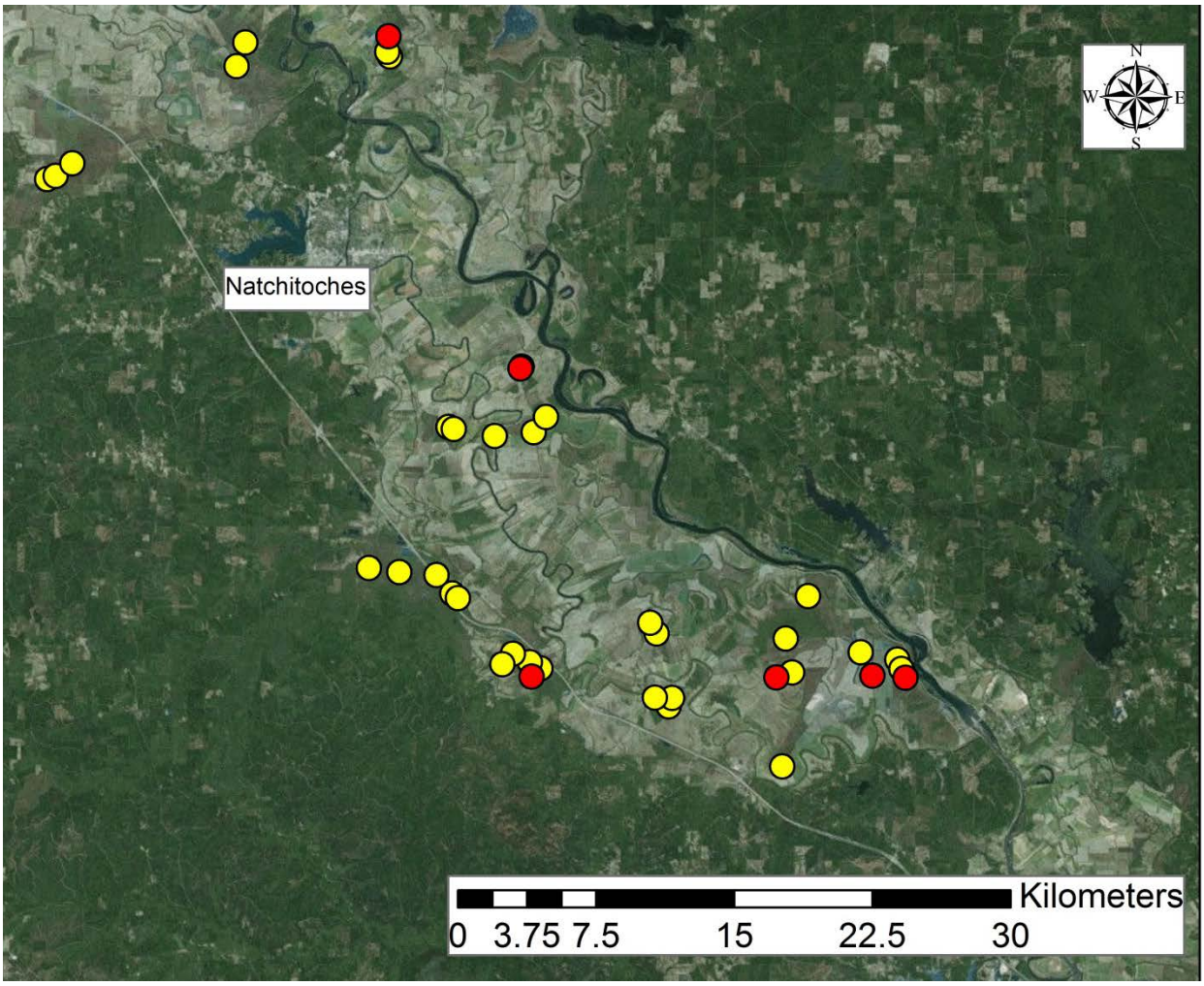
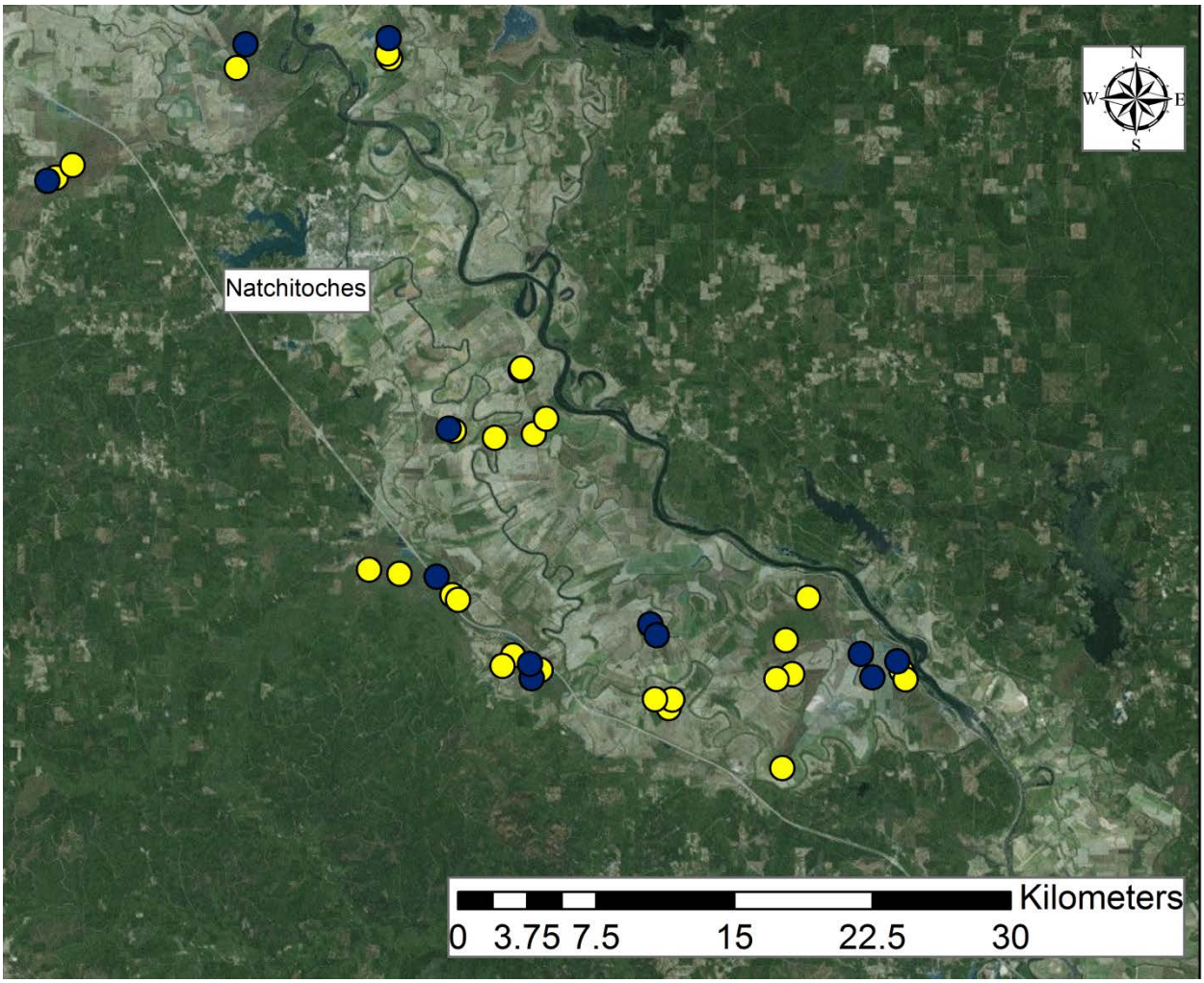


Figure 4. Water sampling locations and results of DNA fingerprinting. Water sampling sites without older (2005 and earlier) Ft. Polk/Leesville Feral Hog *E. coli* matches = yellow dots. Water sampling sites matched with older (2005 and earlier) Ft. Polk/Leesville Feral Hog samples = blue dots.



Discussion and Managemnt Implications

Pathogens were widespread in water bodies sampled on private lands and were associated with positive matches with feral hogs by DNA fingerprinting. These findings are very similar to strong associations between pathogens with feral hogs reported by Kaller and Kelso (2003) and very similar to DNA fingerprinting results demonstrating strong relationships between feral hogs with pathogens reported by Kaller et al. (2007). The results indicated that all sites, including the control LDEQ long-term water quality monitoring site, exhibited some level of pathogens, including pathogens that cause leptospirosis, yersinosis, *Klebsiella* pneumonia, and salmonellosis in

humans and wildlife. Based on one or more criteria, every site sampled was potentially unsafe for human-water and wildlife-water contact. At 22of 40 sites, DNA fingerprinting positively matched water bourn *E. coli* with fecal samples from 12 sampled Feral Hogs, and analyses strongly associated Feral Hogs with high levels of *E. coli* and the pathogen that causes salmonellosis in humans and wildlife. Moreover, spatial patterns suggested specific locations where numerous Feral Hog DNA fingerprints were detected, possibly indicating frequent use of these sites and, based on patterns of unique site visits and co-occurrences, potential sounders or matrilineal-related groupings among the hogs.

One consistent pattern in DNA fingerprint matches appeared to emerge in this study. Although only representing approximately 50% of sampled Feral Hogs, Feral Hogs sampled within Kisatchie National Forest were responsible for 28 of 47 (60%) matches with water samples. Site overlap with these hogs indicates two additional Feral Hogs (Feral Hog 12 and 29) may have been associated with these hogs. Therefore, a disproportionate number of strains detected were attributable to one potentially related group. An association with the Kisatchie National Forest is logical in that the forest offers cover, thermal refuge, and other food resources to the hogs. Additionally, the forest offers a means for dispersal suggesting Feral Hog management in this landscape would benefit from coordination between local landowners with the US Forest Service. It is important to note that this technique demonstrates matrilineal connection, however, the number of generations is not known (e.g., one cannot differentiate between siblings with a common mother or members of a sounder with a common great-grandmother). Therefore, this result could have arisen from displacement of sounders by natural demographic processes over time (i.e., dispersal to new habitats from crowding or resource exploitation) or anthropogenic transport. However, both explanations imply that the Kisatchie National Forest may be a source for feral hogs to expand onto adjacent private lands. In combination, these results strongly suggest that feral hogs are an active agent in the spread of pathogens and the introduction of substantial risk to humans and other wildlife.

Specifically, humans engaged in recreational activities, including swimming, kayaking, waterfowl hunting, fishing, or ORV riding, may come into direct contact with pathogens. Although not fatal, pathogenic *E. coli*, *Leptospira* spp., and *Klebsiella* spp. are of particular concern. Water-borne *E. coli* can lead to human health risks, including severe intestinal distress. During 2009-2010, which is the most recent data available, the Centers for

Disease Control (2014) reported 31 cases of *E. coli* O157:H7 directly associated with recreational contact of natural waters. Although human leptospirosis is not very common, some species of *Leptospira* spp. may be transmitted to humans during swimming or other contact with water (Diesch et al. 1967; Jackson et al. 1993) and may lead to kidney damage, meningitis, liver failure, or death, if left untreated. In humans, *Klebsiella* spp. may become problematic if contaminated water contacts mucus membranes leading to respiratory illness (Podschun et al. 2001), however, such infections are generally opportunistic (Allen et al. 2004). Despite being less frequently detected, *Salmonella* spp. and *Yersinia* spp. are potential threats. Although better known in relation to food contamination, *Salmonella* spp. also may be transmitted through water leading to infection (Threlfall 2002). Gastro-intestinal illnesses from *Yersinia* spp. are an increasing human health concern, possibly as a result of greater global trade and movement of people and animals (Sharma et al. 2003). In combination, these pathogens could cause mild to serious illness to humans engaged in recreation. Many of these pathogens are treatable, if recognized early by health care providers. However, because these pathogens are infrequently encountered in the clinical setting and their symptoms are similar to common illnesses, initial misdiagnosis and delays in treatment could lead to more serious harm and death (e.g., Simoes and Justino 2013). Therefore, in addition to limiting contact, education of risks to landowners and recreational users is warranted.

These results also suggest that wildlife may be at risk from disease. Specifically, White-Tailed Deer could suffer from illness ranging from gastroenteritis (Yersinia spp. and E. coli; Shayegani et al. 1986) to kidney damage and renal failure (Leptospira spp.; Davidson et al. 1985) to abortion (Leptospira spp.; Trainer et al. 1961). Wild Turkey and waterfowl could experience liver damage (Salmonella spp.; Hubálek 2004; United States Geological Survey Wildlife Health Bulletin 2009-01; Benskin et al. 2009) and respiratory illnesses (Klebsiella spp.; Morishita et al. 1997; Fudge et al. 2001; Benskin et al. 2009). Given the socio-cultural and economic importance of these species (Southwick Associates 2007), loss of individuals, reduced condition, and reduced fitness could have serious implications for human recreation and local economies. Other wildlife, including squirrels, raccoons, and other birds, also could contract similar illnesses resulting in decreased ecological status in the landscape.

In summary, our results indicate that all sampled water sources on private lands in this region have evidence of widespread pathogens. DNA fingerprinting implicated feral hogs as one active vector of

contamination. Unresolved by this study are the larger scale prevalence, persistence, frequency of contamination, and seasonality of contamination. Future work should address these unresolved factors.

Literature Cited

Allen, M.J., S.C. Edberg, and D.J. Reasoner. 2004. Heterotrophic plate count bacteria – what is their significance in drinking water? International Journal of Food Microbiology 92: 265-274

Bangert, R.L., A.C.S. Ward, E.H. Stauber, B.R. Cho, and P.R. Widders. 1988. A survey of aerobic bacteria in the feces of captive raptors. Avian Disease 32: 53-62

Barrett, R.H. 1982. Habitat preferences of Feral Hogs, deer, and cattle on a Sierra foothill range. Journal of Range Management 35:342-346

Barrios-Garcia, M.N. and S.A. Ballari. 2012. Impact of wild boar (*Sus scrofa*) in its introduced and native range: A review. Biological Invasions 14: 2283-2300

Benskin, C.M., K. Wilson, K. Jones, K., and I.R. Hartley. 2009. Bacterial pathogens in wild birds: a review of the frequency and effects of infection. Biological Reviews 84: 349-373

Brand, C.J., R.M. Windingstad, L.M. Siegfried, R.M. Duncan, and R.M. Cook. 1988. Avian morbidity and mortality from Botulism, Aspergillosis, and Salmonellosis at Jamaica Bay Wildlife Refuge, New York, USA. Colonial Waterbirds 11:284-292

Brittingham, M.C., S.A. Temple, and R.M. Duncan. 1988. A survey of the prevalence of selected bacteria in wild birds. Journal of Wildlife Diseases 24: 299-307

Centers for Disease Control and Prevention. 2014. 2009-2010 Recreational Water-associated Outbreak & Disease Surveillance Data. Morbidity and Mortality Weekly Report 63: 6-10.

Cizek, A., I. Literak, K. Hejlicek, F. Treml, and J. Smola. 1995. Salmonella contamination of the environment and its incidence in wild birds. Zentrabl Veterinarmed [B] 42: 128

Davidson, W.R., J.M. Crum, J.L. Blue, D.W. Sharp, and J.H. Phillips. 1985. Parasites, diseases, and health status of sympatric populations of fallow deer and white-tailed deer in Kentucky. Journal of Wildlife Diseases 21: 153-159

Dho-Moulin, M. and J.M. Fairbrother. 1999. Avian pathogenic Esherichia coli (APEC). Veterinary Research 30: 299-316

Diesch, S.L, R.P. Crawford, W.F. McCulloch, and F.H. Top. 1967. Human leptospirosis acquired from squirrels. New England Journal of Medicine 276: 838-842

Dirsmith, K., K. VanDalen, T. Fry, B. Charles, K. VerCauteren, and C. Duncan. 2013. Leptospirosis in Fox Squirrels (*Sciurus niger*) of Larimer County, Colorado, USA. Journal of Wildlife Diseases 49: 641-645

Fudge, A.M. 2001. Diagnosis and treatment of avian bacterial diseases. Seminars in Avian and Exotic Pet Medicine 10: 3-11.

Garzio-Hadzik, A., D.R. Shelton, R.L. Hill, Y.A. Pachepsky, A.K. Gruber, and R. Rowland. 2010. Survival of manure-borne E. coli in streambed sediment: Effects of temperature and sediment properties. Water Research 44: 2753-2762

Hubálek, Z. 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. Journal of Wildlife Diseases 40: 639-659

Hudson, C.R., C. Quist, M.D. Lee, K. Keyes, S.V. Dodson, C. Morales, S. Sanchez, D.G. White, and J.J. Maurer. 2000. Genetic relatedness of *Salmonella* isolates from nondomestic birds in the southeastern United States. Journal of Clinical Microbiology 38: 1860-1865

Jackson, L., A.F. Kaufmann, W.G. Adams, M.B. Phelps, C. Andreasen, C.W. Langkop, and J.D. Wenger. 1993. Outbreak of leptospirosis associated with swimming. The Pediatric Infectious Disease Journal 12: 48-53.

Junge, R.E., K. Bauman, M. King, and M.E. Gompper. 2007. A serologic assessment of exposure to viral pathogens and *Leptospira* in an urban raccoon (*Procyon lotor*) population inhabiting a large zoological park. Journal of Zoo and Wildlife Medicine 38: 18-26

Kaller, M.D., J.D. Hudson III, E.A. Achberger, and W.E. Kelso. 2007. Feral pig research in western Louisiana: Expanding populations and unforseen consequences. Human-Wildlife Conflicts 1: 168-177.

Kaller, M. D. and W. E. Kelso. 2003. Effects of feral swine on water quality in a coastal bottomland stream. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 57: 291-298.

Mair, N.S. 1972. Yersinosis in wildlife and its public health implications. Journal of Wildlife Diseases 9: 64-71

McDaniels, A., E. Rice, A.Reyes, C. Johnson, R. Haugland, and G. Stelma, Jr. 1996. Confirmational identification of Escherichia coli, a comparison of genotypeic and phenotypic assays for glutamate decarboxylase and beta-glucuronidase. Applied Environmental Microbiology 62: 3350-3354.

Mitchell, T.R. and T. Ridgwell. 1971. The frequency of salmonellae in wild ducks. Journal of Medical Microbiology 4: 359-361

Morishita, T.Y., P.P. Aye, and D.L. Brooks. 1997. A survey of diseases of raptorial birds. Journal of Avian Medicine and Surgery 11: 77-92

Payment, P., D.P. Sartory, and D.J. Reasoner. 2013. The history and use of HPC in drinking-water quality management. Pages 20-48 in Bartram, J., J. Cotruvo, M. Exner, C. Fricker, and A. Glasmacher (Editors). Heterotrophic Plate Counts and Drinking-Water Safety: The Significance of HPCs for Water Quality and Human Health. World Health Organization.

Pacheco, A.B.F., B.E.C. Guth, K.C.C. Soars, D.F. de Almeida, and L.C.S. Ferreira. 1997. Clonal relationships among *Escherichia coli* serogroup O6 based on RAPD. FEMS Microbiology Letters 148:255-260.

Perry, Q. L. 2001. The Evaluation of PCR-based Techniques for the Detection of Fecal Indicator Organisms in Environmental Samples. M.S. Thesis, Louisiana State University, LSU ETD Collection etd-1113101-162847

Podschun, R., S. Pietsch, C. Holler, and U. Ullman. 2001. Incidence of Klebsiella species in surface waters and their expression of virulence factors. Applied and Environmental Microbiology 67: 3325-3327

Rausch, R. 1947. Pullorum disease in the coot. Journal of Wildlife Management 11: 189

Renter, D.G., D.P Gnad, J.M. Sargent, and S.E. Hygnstrom. 2006. Prevalence and serovars of Salmonella in the feces of free-ranging White-tailed deer (*Odocoileus virginianus*) in Nebraska. Journal of Wildlife Diseases 43: 699-703

Sayah, R.S., J.B. Kaneene, Y. Johnson, and R.Miller. 2005. Patterns of antimicrobial resistance observed in *Esherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. Applied and Environmental Microbiology 71: 1394-1404

Shayegani, M.E.H.D.I., W.B. Stone, I. DeForge, T. Root, M.L. Parsons, and P. Maupin. 1986. *Yersinia enterocolitica* and related species isolated from wildlife in New York State. Applied and Environmental Microbiology 52: 420-424

Simoes, E. M. and J. D. Justino. 2013. Brucellosis infection in a feral swine hunter. Nurse Practitioner 38: 49-53.

Tanger, S.M., K.D. Guidry, and H. Niu. 2015. Monetary estimates of Feral hog damage to agricultural producers in Louisiana. Journal of the National Association of County Agricultural Agents 8 (2) ONLINE http://www.nacaa.com/journal/index.php?jid=553

Tanger, S.M., R.P. Vlosky, and M.D. Kaller, in review. Psychological and social impacts of Feral Hogs on farmers in Louisiana. Human-Wildlife Interactions

Threlfall, J.E. 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne illnesses. FEMS Microbiology Reviews 26: 141-148.

Trainer, D.O., L. Karstad, and R.P. Hanson. 1961. Experimental leptospirosis in white-tailed deer. Journal of Infectious Disease 108: 278.