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Potential of Double-crested Cormorants (*Phalacrocorax auritus*), American White Pelicans (*Pelecanus erythrorhynchos*), and Wood Storks (*Mycteria americana)* to Transmit a Hypervirulent Strain of *Aeromonas hydrophila* between Channel Catfish Culture Ponds

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ABSTRACT: *Aeromonas hydrophila* is a Gram-negative bacterium ubiquitous to freshwater and brackish aquatic environments that can cause disease in fish, humans, reptiles, and birds. Recent severe outbreaks of disease in commercial channel catfish (*Ictalurus punctatus*) aquaculture ponds have been associated with a hypervirulent *Aeromonas hydrophila* strain (VAH) that is genetically distinct from less virulent strains. The epidemiology of this disease has not been determined. Given that research has shown that Great Egrets (*Ardea alba*) can shed viable hypervirulent *A. hydrophila* after consuming diseased fish, we hypothesized that Double-crested Cormorants (*Phalacrocorax auritus*), American White Pelicans (*Pelecanus erythrorhynchos*), and Wood Storks (*Mycteria americana*) could also serve as a reservoir for VAH and spread the pathogen during predation of fish in uninfected catfish ponds. All three species, when fed VAH-infected catfish, shed viable VAH in their feces, demonstrating their potential to spread VAH.

Key words: American White Pelicans, aquaculture, catfish, Double-crested Cormorants, epidemiology, hypervirulent *Aeromonas hydrophila*, *Ictalurus punctatus*, Wood Storks.

In 2009, a hypervirulent strain of *Aeromonas hydrophila* (VAH) caused multiple disease outbreaks with high mortality in western Alabama channel catfish (*Ictalurus punctatus*) operations. Between June and October of 2009, VAH caused an estimated loss of more than 1,360 metric tons of market-sized catfish (Pridgeon and Klesius 2011b). The western Alabama isolates (VAH strain) required much lower doses to kill 50% of the test population value compared to a 1998 isolate (non-VAH strain), which suggested a higher virulence for the VAH strains (Pridgeon and Klesius 2011a). In a comprehensive comparison of VAH to non-VAH isolates, the genomes of five isolates cultured from the 2009 outbreak were sequenced and compared to six non-VAH isolates (Hossain et al. 2013). They found that the VAH strains were very similar to each other but differed from the non-VAH strains, having 313 unique genes and distinct biochemical pathways. Biochemically, VAH is unusual compared to the more common *Aeromonas* isolates of North America. They have the characteristic ability to ferment many complex sugars, are resistant to vibriostat 0/129, and produce indole, but unlike other *A. hydrophila* isolates they can metabolize inositol (Hossain et al. 2013; Hanson et al. 2014; Rasmussen-Ivey et al. 2016).

The epidemiology of this disease has not been determined, including how VAH is transported between catfish operations. Species of fish-eating birds such as Double-crested Cormorants (*Phalacrocorax auratus*; DCCO), Great Blue Herons (*Ardea herodias*), Great Egrets (*Ardea alba*; GREG), Wood Storks (*Mycteria americana*; WOST), and American White Pelicans (*Pelecanus erythrorhynchos*; AWPE) are frequently found on commercial catfish facilities (Glahn and King 2004) and could serve as vectors for bacterial pathogens such as VAH. Previous work demonstrated that GREG are capable of shedding viable VAH in their feces after consuming fish infected with VAH (Jubirt et al. 2015).

The objective of this study was to evaluate the potential for three species of fish-eating birds found on catfish ponds to act as

mechanical vectors of a hypervirulent strain of *A. hydrophila*. We evaluated the ability of DCCO, AWPE, and WOST to shed viable VAH when fed infected fish and the potential for VAH to colonize these birds.

All work was registered and conducted under the supervision of the US Department of Agriculture, Wildlife Services, National Wildlife Research Center Institutional Animal Care and Use Committee using approved protocols (QA 1969, 2040, 2105) to ensure humane handling and use. Ten DCCO, 10 AWPE, and three WOST were captured at commercial channel catfish fingerling ponds (Federal Scientific Collection Permit MB019065-0) in the Mississippi Delta using soft-catch leg-hold traps or a rocket net as previously described (King et al. 1998) and transported to the US Department of Agriculture, National Wildlife Research Center Mississippi Field Station, Mississippi State, Mississippi, avian test facility. A separate study was conducted for each bird species. All birds were weighed, marked with a unique leg band, and tested by fecal cultures prior to starting the trial to confirm they were negative for VAH (Jubirt et al. 2015). Birds were individually housed in 3.3×3.3×2 m cages containing shallow plastic feeding tanks filled with fresh water that was changed daily. Birds were fed live channel catfish ad libitum throughout the 10 d quarantine period and the 7 d study period except for WOST, which had a 10 d study period. Body weight was obtained by placing birds in a preweighed burlap sack and weighing them on a digital scale.

In each trial experimental birds (DCCO, n=6; AWPE, n=5; WOST, n=2) were fed VAH-injected channel catfish, and control birds (DCCO, n=4; AWPE, n=5; WOST, n=1) were fed noninjected catfish for three consecutive days. Injected catfish were produced by anesthetizing them with tricaine methanesulfonate (MS222, Western Chemical, Ferndale, Washington, USA) buffered to pH 7.0–7.5, at a rate of 100 mg/L of water followed by intraperitoneal injections with 0.5 mL of an overnight bacterial culture in brain heart infusion broth containing about 2×10^8 colony-forming units per mL of *A. hydrophila* isolate AL09 no. 2, previously confirmed as a VAH (Jubirt et al. 2015). Daily care and feeding of birds are detailed in Jubirt et al. (2015).

At the conclusion of DCCO trial (day 7), all birds were euthanatized using carbon dioxide in accordance with the guidelines published by the American Veterinary Medical Association (2013). The DCCO were then necropsied, and bacterial swabs were collected. Swabs were obtained from oral and nasal mucosa, upper and lower intestine, kidney, eye, esophagus, and lungs. The AWPE and WOST were donated to zoos, and only oral swabs were taken at the conclusion of their respective studies. Due to WOST shedding at day 10 they were held and tested until negative for shedding VAH (day 17) when they were released to the zoo.

Throughout each trial, feces were collected in the morning prior to feeding. Approximately 1 g feces were scraped from the concrete floor of each pen and placed in a sterile plastic bag (Nasco, Whirl-Pak, Fort Atkinson, Wisconsin, USA) and transported to the laboratory for analysis within 1 h. All microbial samples were cultured on CRITERION™ Ampicillin Dextrin Agar Base (Hardy Diagnostics, Santa Maria, California, USA). Swabbed plates were incubated overnight at 37 C, and yellow convex translucent colonies were tested for cytochrome oxidase C using filter paper and oxidase reagent (Becton Dickinson, Sparks, Maryland, USA). Microbiology methods were described in Jubirt et al. (2015).

Colonies from culture plates were harvested for DNA extraction by suspending bacteria in 1.5 mL of sterile saline using a plate spreader, and 100 µL was taken and pelleted in a centrifuge at 20,000 × G for 1 min. Saline was removed, and DNA was extracted from the pelleted bacteria using the Gentra Puregene DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's recommendation for Gram-negative bacteria. The concentration and purity of DNA was determined for each sample using the NanoDrop 8000 (Thermo Fisher Scientific, Wal-

tham, Massachusetts, USA). Any samples that had 260/280 nm optical density values of less than 1.8 were re-extracted. All samples were diluted to 3 ng/μL for quantitative PCR (qPCR). Standards for qPCR were made with 10-fold serial dilutions of hypervirulent strain *A. hydrophila* DNA, with the most concentrated standard being 5 ng/μL. A total of 10 μL of each sample or standard was used for qPCR that confirmed the presence or absence of *A. hydrophila*. Published protocols were followed to quantify the VAH strain (Griffin et al. 2013) and all other strains of *A. hydrophila* (Wang et al. 2009) in the DNA extracts of the samples using a final reaction volume of 25 μL as previously described (Jubirt et al. 2015).

Colonies obtained by bacterial culture of feces collected from the three avian species during the quarantine period were uniformly negative for *Aeromonas*-like colonies on Ampicillin Dextrin plates. The DNA extracted from the least diluted culture plate from each bird tested negative for VAH using qPCR as well. During the trials all birds who consumed fish injected with VAH shed VAH at some point (Table 1), whereas none of the control birds shed VAH. All DCCO shed VAH in their feces for multiple days. Two DCCO shed viable VAH for 6 days with one of the two DCCO continuing to shed VAH until the end of the study on day 7 (Table 1). All five of the AWPE fed injected fish shed viable VAH for 2 days with one shedding for 3 days (Table 1). Both of the treated WOST shed viable VAH sporadically for up to 10 d (Table 1).

Given the high densities of fish eating birds actively preying and scavenging at catfish ponds with VAH, we suspect that natural transmission routes for pond to pond spread of VAH involve these birds. Birds are known to be susceptible to motile aeromonads (Shane et al. 1984). A majority of the information reported on the relationship between *Aeromona*s and birds is limited to diagnostic submissions of birds that have been killed by the disease, but the history, prevalence, and other factors have not been extensively researched. While investigating routine avian diagnostic submissions over 25 mo, Shane et al. (1984) isolated *A. hydrophila* from 2% (20/1,000) cases. Their results suggest that *A. hydrophila* is an opportunistic pathogen of birds. Glünder and Siegmann (1989) concluded that the primary isolation site of *A. hydrophila* was the intestines with the lungs as a secondary site and that the recovery rate of *A. hydrophila* was highest from carnivorous aquatic birds compared to terrestrial avian species. We expected the aquatic birds tested in our study to function as vectors because we recently demonstrated that bacterium survived through the gut of Great Egrets (Jubirt et al. 2015).

This study broadened our earlier investigation (Jubirt et al. 2015) by including two very common predators (DCCO and AWPE) and WOST. Evaluating WOST was especially notable because it is an important scavenger that is commonly found at ponds containing channel catfish experiencing mortality caused by VAH. The WOST study was limited in numbers because it is a threatened species. *Aeromonas hydrophila* is known to infect birds, and fish-eating birds may serve as a reservoir for VAH and spread the pathogen via deposition of infected feces after flying to uninfected ponds.

All treatment birds that were fed VAH-infected catfish (day 0–2) shed VAH in their feces, but there was no consistent trend between the species of birds and the number of days they shed VAH. In Jubirt et al. (2015), 66% (4/6) of GREG in the treatment group shed VAH for multiple days. Interestingly, all GREG continued to shed VAH after they were no longer being fed VAH-infected fish. However, there were substantial variations between birds in the number of viable VAH in feces. In these studies, both WOST and DCCO shed VAH for multiple days after they were no longer fed VAH-infected fish. In WOST, VAH was detected in feces up to day 10 and in DCCO up to day 7 (end of study). In contrast, AWPE shed VAH only during the time when they were being fed and for 1 d (day 3) after feeding VAH-infected fish. The shedding of VAH by all three species after consuming VAH-infected fish could explain how VAH is transmitted from one commercial catfish pond to another seemingly without any

TABLE 1. Shedding of virulent *Aeromonas hydrophila* (VAH) in feces from Double-crested Cormorants (*Phalacrocorax auritus*; DCCO), American White Pelicans (*Pelecanus erythrorhynchos*; AWPE), and Wood Storks (*Mycteria americana*; WOST), by day for 7 d (DCCO and AWPE) and 10 d (WOST) after consuming VAH-injected fish on days 0, 1, and 2. Four DCCO, five AWPE, and one WOST control birds were negative for shedding VAH on all days.[a]

		Day									
Species	ID	0	1	2	3	4	5	6	7	8	9	10
DCCO	41	−	+	+	+	−	−	+	−	NA	NA	NA
DCCO	51	−	+	+	+	+	−	−	−	NA	NA	NA
DCCO	52	−	+	−	+	−	−	−	−	NA	NA	NA
DCCO	53	−	+	−	+	−	−	−	−	NA	NA	NA
DCCO	60	−	+	+	+	−	−	−	−	NA	NA	NA
DCCO	94	−	+	+	−	−	−	+	+	NA	NA	NA
AWPE	10	−	+	+	−	−	−	−	−	NA	NA	NA
AWPE	17	−	+	+	−	−	−	−	−	NA	NA	NA
AWPE	20	−		+	−	−	−	−	−	NA	NA	NA
AWPE	23	−	+	+	+	−	−	−	−	NA	NA	NA
AWPE	25	−	+	+	+	−	−	−	−	NA	NA	NA
WOST	40	−	−	−	+	+	−	−	−	−	−	+
WOST	41	−	−	−	+	+	−	+	−	−	−	+

[a] ID = bird identification; − = negative for VAH shedding in feces; + = positive for VAH shedding in feces; NA = not applicable.

connections between the ponds or farms. The relatively long-term shedding by WOST was especially intriguing because these birds are more common as scavengers in ponds containing channel catfish experiencing mortality caused by VAH. Furthermore, these birds may be more common in regions of the catfish industry where VAH outbreaks are more prevalent (west Alabama and east Mississippi) than in the Mississippi Delta. A more controlled analysis would be needed to determine the mechanisms that influence the concentrations of bacteria shed.

All three species of predatory birds that we tested showed strong potential to act as carriers for the transmission of VAH among catfish ponds. This study, along with our previous study with GREG (Jubirt et al. 2015), demonstrate that fish-eating birds serving as natural vectors may be important in VAH epidemiology. We were able to elucidate the transmission potential of VAH through our assay. We conclude that fish-eating birds may serve as a reservoir for VAH and can spread the pathogen by flying to uninfected ponds. These findings emphasize the need to reduce predation and scavenging on commercial catfish operations experiencing VAH outbreaks, which may help reduce losses to the industry caused by VAH.

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LITERATURE CITED

American Veterinary Medical Association. 2013. *AVMA guidelines for the euthanasia of animals*. 2013 Ed. https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx. Accessed August 2017.

Glahn JF, King DT. 2004. Bird depredation. In: *Biology and culture of channel catfish*, Tucker CS, Hargreaves JA, editors. *Developments in aquaculture and fisheries science*, Vol. 34. Elsevier, San Diego, California, pp. 503–529.

Glünder G, Siegmann O. 1989. Occurrence of *Aeromonas hydrophila* in wild birds. *Avian Pathol* 18:685–695.

Griffin MJ, Goodwin AE, Merry GE, Williams MA, Liles MR, Waldbieser GC. 2013. Rapid quantitative detection of *Aeromonas hydrophila* strains associated

with disease outbreaks in catfish aquaculture. *J Vet Diagn Invest* 25:473–481.

Hanson LA, Liles MR, Hossain MJ, Griffin MJ, Hemstreet WG. 2014. Motile aeromonas septicemia. In: AFS-FHS (American Fisheries Society–Fish Health Section), *FHS blue book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. Motile Aermonas Septicemia 1.2.9*. American Fisheries Society–Fish Health Section, Bethesda, Maryland, 11 pp.

Hossain MJ, Waldbieser GC, Sun D, Capps NK, Hemstreet WB, Carlisle K, Griffin MJ, Khoo L, Goodwin AE, Sonstegard TS, et al. 2013. Implication of lateral genetic transfer in the emergence of *Aeromonas hydrophila* isolates of epidemic outbreaks in channel catfish. *PLoS One* 8:e80943.

Jubirt MM, Hanson LA, Hanson-Dorr KC, Ford L, Lemmons S, Fioranelli P, Cunningham FL. 2015. Potential for Great Egrets (*Ardea alba*) to transmit a virulent strain of *Aeromonas hydrophila* among channel catfish (*Ictalurus punctatus*) culture ponds. *J Wildl Dis* 51:634–639.

King DT, Paulson JD, LeBlanc DJ, Bruce K. 1998. Two capture techniques for American White Pelican and Great Blue Herons. *Colon Waterbirds* 21:258–260.

Pridgeon JW, Klesius PH. 2011a. Molecular identification and virulence of three *Aeromonas hydrophila* isolates cultured from infected channel catfish during a disease outbreak in west Alabama (USA) in 2009. *Dis Aquat Organ* 94:249–253.

Pridgeon JW, Klesius PH. 2011b. Virulence of *Aeromonas hydrophila* to channel catfish *Ictalurus punctatus* fingerlings in the presence and absence of bacterial extracellular products. *Dis Aquat Organ* 95:209–215.

Rasmussen-Ivey CR, Hossain MJ, Odom SE, Terhune JS, Hemstreet WG, Shoemaker CA, Zhang D, Xu D-H, Griffin MJ, Liu Y-J, et al. 2016. Classification of a hypervirulent *Aeromonas hydrophila* pathotype responsible for epidemic outbreaks in warm-water fishes. *Front Microbiol* 7:1615.

Shane SM, Harrington KS, Montrose MS, Roebuck RG. 1984. The occurrence of *Aeromonas hydrophila* in avian diagnostic submissions. *Avian Dis* 28:804–807.

Wang HB, Wang DC, Bi ZQ, Kan B. 2009. Development of TaqMan real-time PCR in detection of *Aeromonas hydrophila*. *Zhonghua Yu Fang Xue Za Zhi* 43:611–614.

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