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Abstract: The objective of this study was to evaluate the mottled duck (*Anas fulvigula*), a nonmigratory dabbling duck, as a sentinel species for avian influenza virus (AIV) surveillance. A total of 235 cloacal swabs from 147 live-captured and 88 hunter-harvested mottled ducks during summer (June–August 2007) and winter (November 2007 to January 2008), respectively, were collected along the upper Texas coast. Samples were screened for AIV using real-time reverse transcription polymerase chain reaction (rRT-PCR); all rRT-PCR–positive samples were processed for virus isolation. Three samples were positive for AIV by AIV-matrix rRT-PCR. One of these samples also was positive for H5 by rRT-PCR, and a low pathogenic H5N2 AIV was isolated. Although isolation of AIVs from mottled ducks during the winter has been reported previously, to the authors' knowledge, this is the first H5 isolate from mottled ducks. Interestingly, this isolation was made during the same season that other H5N2 viruses were obtained from migratory waterfowl on the Texas coast, which suggests AIV transmission among waterfowl on the wintering grounds and the potential role of mottled ducks as a naturally occurring sentinel species for AIV surveillance.

Key words: *Anas fulvigula,* mottled duck, avian influenza virus, surveillance, Texas, waterfowl, sentinel.

BRIEF COMMUNICATION

Wild waterbirds, particularly Charadriiformes and Anseriformes, are considered the reservoir for all type A avian influenza viruses (AIV).[12] The migratory nature of waterfowl combined with virus persistence in these populations provides a potential mechanism for dispersal of AIVs among regions; exactly how specific subtypes are maintained from year to year and within waterbird populations remains unclear.[6,8] The mallard (*Anas platyrhynchos*) has emerged as a species of primary interest for AIV surveillance due to its abundance, wide distribution (Eurasia and North America), ease of capture, and high AIV prevalence.[5,8] Because mallards are highly adaptable to a variety of environmental conditions, they are easily domesticated and are useful sentinels for monitoring AIVs.[3] The mottled duck (*Anas fulvigula*) is closely related to the mallard, and these two species commonly interbreed and produce reproductively competent offspring.[7]

The mottled duck is a nonmigratory dabbling duck that inhabits a restricted range of coastal and subcoastal marshland along the Gulf Coast of the United States. Two distinct populations are recognized: one inhabits the Gulf Coast of Texas, Louisiana, and Mississippi; the other inhabits peninsular Florida.[7] The nonmigratory nature of the mottled duck coupled with its close relationship to the mallard, a high AIV prevalence species, and previous reports on the isolation of AIV from resident mottled ducks provides a unique opportunity for monitoring transmission of AIVs within waterfowl wintering areas of the central, Mississippi, and Atlantic flyways. The objective of this study was to evaluate the mottled duck as a sentinel species for AIV surveillance.[1,2,11]

Cloacal swabs were collected from live-captured mottled ducks during annual banding activities (June–August 2007) and from hunter-harvested mottled ducks (November 2007 to January 2008) at the J. D. Murphree Wildlife Management Area in Jefferson County (29°49′N, 94°02′W) and the McFaddin National Wildlife Refuge in Jefferson and Chambers counties (29°42′N, 94°05′W), located along the upper-Texas Gulf Coast. Data from both locations were combined for analysis.

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Table 1. Summary of avian influenza virus (AIV) testing from cloacal swabs collected from mottled ducks (*A. fulvigula*) June–August 2007 and November 2007 to January 2008, upper-Texas Gulf Coast, USA.

Mo	Site	Capture method	No.[a]	Age[b]		Sex			AIV rRT-PCR (prevalence)			Isolate (subtype)[c]
				Juvenile	Adult	M	F	Unk	Matrix	H5	H7	
Jun	Murphree	LC	49	48	1	26	23		0	0	0	
Jul	Murphree	LC	51	37	14	22	28	1	0	0	0	
Aug	Murphree-McFaddin	LC	47	39	8	21	26		0	0	0	
Nov	Murphree	HH	49	2	47	21	28		2 (4.1%)	1 (2.0%)	0	1 (H5N2)
Dec	Murphree	HH	36	0	36	15	21		1 (2.8%)	0	0	
Jan	Murphree	HH	3	0	3	1	2		0	0	0	
Total			235	126	109	106	128	1	3 (1.3%)	1 (0.4%)	0	1

AIV, avian influenza virus; rRT-PCR, real-time reverse transcription polymerase chain reaction; Murphree, J. D. Murphree Wildlife Management Area, Jefferson County, Texas; LC, live capture; Unk, sex undetermined; McFaddin, McFaddin National Wildlife Refuge, Chambers and Jefferson counties, Texas; HH, hunter harvested.

[a] Number of mottled ducks collected.

[b] Juvenile, hatch-year; Adult, after-hatch-year.

[c] Isolate obtained by virus isolation in embryonated chicken eggs.

All samples were collected, processed, and tested as previously described, with the single exception being that samples were transported from the field on ice (<10 hr collection and transport time) and stored at –80°C until processed, or collected and stored on location at –20°C (<5 days) until transported to the laboratory, where they were stored at –80°C until processed.[1,2] All the samples were screened for AIV by AIV-matrix real-time reverse transcription polymerase chain reaction (rRT-PCR), and virus isolation was performed on all rRT-PCR–positive samples. In addition, all rRT-PCR positive samples were screened for H5 and H7 subtype viruses by rRT-PCR. Primers and probes for the M, H5, and H7 genes were those previously described.[9,10] Any AIV isolates obtained were submitted to the National Veterinary Services Laboratory (Ames, Iowa, USA) for subtyping by hemagglutination and neuraminidase inhibition tests and screened for the presence of the N1 gene by rRT-PCR. In addition, any H5 and H7 isolates were pathotyped at the National Veterinary Services Laboratory by molecular analysis of the amino acid sequence at the hemagglutinin protein cleavage site.

A total of 235 cloacal swab samples were collected from mottled ducks during June–August 2007 and November 2007 to January 2008 (Table 1). During the summer (June–August 2007), most samples were collected from immature birds (124/147 [84.4%]), whereas, during the hunting season (November 2007 to January 2008), most samples were collected from adult birds (86/88 [97.7%]). Males made up 45.3% (106/234) of the sample, whereas females accounted for 57.1% (128/234); sex was undetermined for one individual. Three samples tested positive for AIV by matrix rRT-PCR (3/235 [1.3%]), of which, one sample tested positive for H5 (1/235 [0.4%]); the subtype of the other two samples was not determined (Table 1). A low pathogenic H5N2 virus was isolated from the H5 rRT-PCR–positive sample; no isolates were obtained from the other two matrix rRT-PCR–positive samples. Two of the AIV matrix rRT-PCR–positive samples were from adult males collected in November, whereas the third was collected from an adult female in December. The H5N2 virus was isolated from an adult male sampled in November.

AIV has previously been isolated from mottled ducks collected along the Gulf Coast during the winter but not during the summer, which suggests AIV transmission from migratory waterfowl to nonmigratory mottled ducks on wintering grounds.[1,2,4,11] Transmission from migratory waterfowl to the resident mottled duck was first detected in 1987 when two H6N2 AIVs were isolated from 75 hunter-harvested mottled ducks sampled in Cameron Parish, Louisiana.[11] Subsequently, two AIVs (H1N4, H6N5) were isolated from 103 hunter-harvested mottled ducks sampled between 2005 and 2009 in Brazoria County, Texas.[1,2] In addition, one AIV isolate (H6N8) was obtained from a hunter-harvested nonmigratory mallard × mottled duck cross.[1] The identification of an H5 AIV in mottled ducks reported herein also supports the concept of AIV transmission among waterfowl on their wintering grounds because H5 viruses were isolated from migratory waterfowl during the winter months (November–January) of the same hunting season (2007–

2008) but not during the previous two seasons.[1] Molecular characterization of these H5N2 viruses should help clarify the relationship between the viruses isolated from migratory waterfowl and nonmigratory mottled ducks.

This study reports the first isolation of a low pathogenic H5N2 AIV from mottled ducks during the winter on the Texas Gulf Coast. Interestingly, this isolation was made during the same season that other H5N2 viruses were obtained from migratory waterfowl on the Texas coast, which suggests AIV transmission among waterfowl on the wintering grounds.[1] Due to regulated harvest restrictions on mottled ducks, limited samples from hunters are available for evaluation of AIV transmission. To fully investigate the mottled duck as a sentinel species for AIV studies, blood samples for seroprevalence and cloacal swabs for virus identification should be collected any time that mottled ducks are captured, such as during banding projects. Such studies would provide further knowledge regarding the role of mottled duck populations in the ecology of AIV on waterfowl wintering grounds and would better allow an assessment of whether sampling mottled ducks along the Gulf Coast is a useful addition to current AIV monitoring programs.

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