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## Avian Influenza Viruses and Paramyxoviruses in Wintering and Resident Ducks in Texas

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**ABSTRACT:** Cloacal swabs were collected from teal (*Anas crecca*, *Anas cyanoptera*, *Anas discors*), mottled duck (*Anas fulvigula*) and northern pintail (*Anas acuta*) in Brazoria County, Texas, USA, during February 2001, mottled ducks during August 2001, and blue-winged teal (*A. discors*) during February 2002. Prevalence of avian influenza virus (AIV) infections during each sampling period were 11, 0, and 15%, respectively. The hemagglutinin (H) subtypes H2 and H7 were detected in both years, while the H8 subtype was detected in 2001 and the H1 subtype was detected in 2002. Avian paramyxovirus type 1 (APMV-1) was isolated from 13% of mottled ducks sampled in August 2001 and 30.7% of teal in February 2002. The season of isolation of both viruses and the majority of the AIV subtypes detected in this study are not typical based on previous reports of these viruses from North American ducks.

**Key words:** Avian influenza virus, avian paramyxovirus, mottled duck, pintail, teal, Texas.

Wild birds represent the natural reservoir for avian influenza viruses (AIVs) and avian paramyxoviruses (APMV), both of which are potential health threats to domestic poultry worldwide (Alexander, 2000). Although there has been significant progress in understanding the epidemiology of AIV and APMV in wild-bird populations, questions remain relating to the maintenance of these viruses in nature. In previous surveys of North American ducks, the prevalence of AIV infections have been consistently highest during the late summer and fall seasons. This has been attributed to the concentration of immunologically naive juvenile ducks during pre-migration staging and, in general, few AIVs have been isolated from ducks on

their wintering grounds or during other seasons (Webster et al., 1992). Although 15 of the 16 known hemagglutinin (H) subtypes have been represented in AIVs isolated from wild waterfowl (Sharp et al., 1997; L'vov et al., 2001), the predominate subtypes reported from North American ducks are the H3, H4, and H6 subtypes (Sharp et al., 1993).

Wild ducks also are considered the natural hosts of the APMV-1 (*Newcastle disease virus*), APMV-4, APMV-6, APMV-8, and APMV-9 serotypes (Alexander, 2000). Prevalence of APMV infections in wild ducks may vary due to multiple factors, including location, species, sex, age, and season of sampling (Stallknecht et al., 1991). However, the epidemiology of APMV is poorly understood and, unlike AIV, clear seasonal peaks in the transmission of APMV have not been detected.

Most studies investigating the prevalence of AIV and APMV infections in wild ducks have focused on mallards (*Anas platyrhynchos*) captured during the fall season in Canada and the northern half of the United States. However, because isolation of both AIVs and APMVs has been documented in ducks wintering on the Gulf Coast of the United States (Stallknecht et al., 1990b, 1991), additional information on the epidemiology of these viruses in wintering waterfowl is warranted. The objective of this study was to determine if AIVs and APMVs were present in migratory and resident duck species in the Gulf Coast region of Texas, with an emphasis on winter-time sampling.

Ducks were captured at prebaited sites using rocket nets over a freshwater marsh that is artificially and seasonally inundated at Peach Point Wildlife Management Area in Brazoria County, Texas (28°56'55"N, 095°26'17"W). Trapping sites were baited for approximately 1 wk prior to sampling. Cloacal swabs were collected by Texas Parks and Wildlife Department personnel using sterile cotton-tipped applicators (Puritan®, Hardwood Products Company, Guilford, Minnesota, USA) and placed in 3 ml of Brain Heart Infusion media (Becton Dickinson and Co., Sparks, Maryland, USA) supplemented with penicillin G (10,000 units/ml), streptomycin (2 mg/ml), kanamycin (0.6 mg/ml), gentamicin (1 mg/ml), and amphotericin B (0.02 mg/ml) (Sigma Chemical Company, St. Louis, Missouri, USA). Samples were stored on ice in the field, shipped overnight (approximately 24–72 hr on ice total), and frozen at –70 C until processed.

For virus isolation, samples were thawed, vortexed, and centrifuged at 1,500 × G for 15 min. The supernatant was inoculated (0.25 ml/egg) via the allantoic route into four 9-day-old specific-pathogen-free (SPF) embryonated chicken eggs. Eggs were incubated at 37 C for 72 hr and hemagglutination testing was completed as previously described (Stallknecht et al., 1990b). For negative samples, amnio-allantoic fluid was pooled by sample, diluted 1:10 in sterile phosphate-buffered saline, and repassaged into two additional eggs. All isolates were typed using hemagglutinin inhibition and neuraminidase inhibition tests at the National Veterinary Services Laboratory, Veterinary Services Laboratories, Ames, Iowa, USA.

During 2001 and 2002, 258 ducks were sampled and 57 hemagglutinating agents were detected. Fifty (87.7%) of the isolates were recovered on the first egg passage. Prevalence by year, month, and species is presented in Table 1. The sex and age of the birds were not consistently recorded and were not included in the analysis. Species diversity reflects the Texas

Parks and Wildlife Department's trap success and personnel available to collect swabs on sampling days.

Based on previous reports of the low prevalence of AIV infections in Gulf Coast wintering ducks (Stallknecht et al., 1990b), the >10% isolation rate during both winters was unexpected. In addition, the subtypes isolated in this study have not been frequently detected in ducks. For example, of more than 3,100 isolates from North American ducks during 1974–90, the H2, H7, and H8 each represented <1% of the total isolates (Webster et al., 1976; Boudreault et al., 1980; Hinshaw et al., 1980, 1985, 1986; Kocan et al., 1980; Smitka and Maassab, 1981; Deibel et al., 1985; Nettles et al., 1985; Kawaoka et al., 1988; Stallknecht et al., 1990b; Slemons et al., 1991; Alfonso et al., 1995). The more commonly reported H subtypes from ducks, including the H3, H4, and H6, were not detected in this study.

The distinctly different AIV-subtype assemblage in ducks tested in this study suggest that ducks in wintering areas, such as the coastal region of Texas, may play a unique role in the maintenance of many AIVs. This may be especially true for the less common AIV H subtypes. Although our sample size was small given the considerable population of ducks wintering in the management area (approx. 20,000; T. Merendino, pers. comm.), it is interesting that none of the AIV subtypes typically associated with ducks were detected, and AIV infection prevalence greater than 10% was detected during February in both years. This suggests that previously uncommon AIV subtypes may be transmitted among these wintering ducks. This is further supported by the isolation of H10N7 AIVs from a blue-winged teal and a northern shoveler (*Anas clypeata*) sampled during April 2000 approximately 100 miles south of Brazoria County (unpublished data).

Our results may represent the combined effects of multiple factors, including prior exposure and acquired immunity, host mi-

TABLE 1. Occurrence of avian influenza virus (AIV) and avian paramyxovirus (APMV) isolated from ducks, Brazoria County, Texas, USA.

Year	Month	Species	No.	AIV	APMV	No. serotype isolates
2001	February	Blue-winged teal ( <i>Anas discors</i> )	32	7 (0.22±0.14) <sup>a</sup>	0	H2N4 ( <i>n</i> =3) H7N3 ( <i>n</i> =3) H7N4 ( <i>n</i> =1)
	February	Cinnamon teal ( <i>Anas cyanoptera</i> )	2	1 (0.5±0.7)	0	H8N4 ( <i>n</i> =1)
	February	Green-winged teal ( <i>Anas crecca</i> )	29	2 (0.07±0.09)	0	H7N3 ( <i>n</i> =1) H8N4 ( <i>n</i> =1)
	February	Mottled duck <sup>b</sup> ( <i>Anas fulvigula</i> )	3	0	0	
	February	Northern pintail ( <i>Anas acuta</i> )	30	1 (0.03±0.06)	0	H8N4 ( <i>n</i> =1)
	August	Mottled duck	87	0	11 (0.13±0.07)	APMV-1 ( <i>n</i> =11)
2002	February	Blue-winged teal	75	11 (0.15±0.08)	24 (0.32±0.11)	H1N4 ( <i>n</i> =2) H1N3 ( <i>n</i> =2) H2N4 ( <i>n</i> =1) H2N9 ( <i>n</i> =1) H7N3 ( <i>n</i> =3) H7N4 ( <i>n</i> =2) APMV-1 ( <i>n</i> =23) APMV-7 ( <i>n</i> =1)

<sup>a</sup> No. (prevalence ± SD).<sup>b</sup> Resident species; all others, wintering species.

gratory patterns, or environmental influences on the virus.

Based on numerous published reports of the prevalence of H3, H4, and H6 AIV subtypes from ducks sampled during late summer and fall, population immunity to these specific subtypes should be high. Prior exposure and resulting immunity has been suggested as an explanation of the low prevalence of AIV infections in adult ducks versus juvenile ducks (Hinshaw et al., 1985; Webster et al., 1992) and is consistent with our failure to isolate AIVs representing the H3, H4, or H6 subtypes in this study. As evidence suggests that natural infections of AIV do not afford cross-protection between AIV subtypes (Suss et al., 1994), it is possible that wintering ducks would remain susceptible to those subtypes that do not predominate during the premigration and migration periods. The species composition of ducks sampled

in this study also may have influenced our results because it is plausible that the infected birds detected in Texas represent birds with little or no previous exposure to AIVs. Blue-winged teal, from which most of the AIVs were isolated, generally depart the breeding grounds earlier than most species, often arriving on the wintering grounds by September (Bellrose, 1980). This suggests that these early migrants may have minimal or no previous AIV exposure and thus represent a susceptible population on wintering areas. Avian influenza viruses have been reported from this species cohabiting with other ducks species during migration (Hinshaw et al., 1980;) as well as upon arrival to wintering areas in Louisiana (Stallknecht et al., 1990b). Although the prevalence of AIV infection was low in Louisiana, the more common H3, H4, and H6 subtypes pre-

dominated in birds sampled during the fall.

Although information is limited, persistence of AIV in water is influenced by the temperature, salinity, and pH and variation in environmental persistence between individual AIV isolates has been detected under experimental conditions (Stallknecht et al., 1990a). Although speculative, it may be possible that environmental conditions as well as variation in waterfowl populations influence local subtype diversity.

Both APMV-1 and APMV-7 have been previously reported from wild ducks (Stallknecht et al., 1991), although APMV-7 is generally associated with pigeons and doves (Alexander, 2000). The 30.7% APMV-1 infection rate detected in this study is the highest prevalence reported from wintering ducks. However, based on negative results from the first year of sampling, APMV-1 infections in these ducks may be a sporadic event.

Given the strong bias toward sampling ducks, especially mallards, during the fall season, it is not surprising that the ecology of both AIVs or APMVs are not fully understood. From these results, it appears that some of the assumptions regarding the seasonality of transmission and the virus subtype host associations of AIVs are not as clear as previously thought and that our understanding of APMV in wild ducks is incomplete. The transmission and maintenance of both of these viruses in free-living duck populations involves multiple interactions between many host species, many different subtypes of viruses, and occurs within many different environments. For these reasons, care must be taken in generalizations regarding the epidemiology of both AIVs and APMVs.

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