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IS THE OCCURRENCE OF AVIAN INFLUENZA VIRUS IN CHARADRIIFORMES SPECIES AND LOCATION DEPENDENT?

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ABSTRACT: Birds in the order Charadriiformes were sampled at multiple sites in the eastern half of the continental USA, as well as at Argentina, Chile, and Bermuda, during 1999–2005, and tested for avian influenza virus (AIV). Of more than 9,400 birds sampled, AIV virus was isolated from 290 birds. Although Ruddy Turnstones (*Arenaria interpres*) comprised just 25% of birds sampled, they accounted for 87% of isolates. Only eight AIV isolations were made from birds at four locations outside of the Delaware Bay, USA, region; six of these were from gulls (Laridae). At Delaware Bay, AIV isolations were predominated by hemagglutinin (HA) subtype H10, but subtype diversity varied each year. These results suggest that AIV infection among shorebirds (Scolopacidae) may be localized, species specific, and highly variable in relation to AIV subtype diversity.

Key words: Avian influenza virus, Charadriiformes, Delaware Bay, Ruddy Turnstone, shorebird.

INTRODUCTION

Before the detection of highly pathogenic avian influenza (HPAI) H5N1 virus in wild birds during 2002 (Ellis et al., 2004), isolations of HPAI from free-living wild bird populations were restricted to a single event in 1961, when an HPAI virus (A/tern/South Africa/61 [H5N3]) caused the deaths of approximately 1,300 Common Terns (*Sterna hirundo*) in South Africa (Becker, 1966). The South African event provided the first evidence of a wild bird reservoir for avian influenza viruses (AIV) and resulted in significant wild bird surveillance during the next 45 yr. Results from these field studies clearly identified species in the order Anseriformes as important AIV reservoirs (Stallknecht and Shane, 1988). The role of charadriiform species as reservoirs for AIV is less understood. Although a significant number of AIV isolations have been reported from species within Laridae and Scolopacidae, AIV prevalence, especially in the latter group, is generally low (Olsen et al., 2005). Several subtypes (H13 and H16) appear to be associated with charadriiform species, especially gulls (Hinshaw et al., 1982; Fouchier et al., 2005), and these may represent a unique gene pool. With other subtypes, phylogenetic studies currently do not support the existence of separate AIV gene pools associated with anseriform and charadriiform species (Widjaja et al., 2004; Spackman et al., 2005).

To date, Delaware Bay, USA, represents the only site worldwide where AIV isolations from shorebirds (Scolopacidae) have consistently been reported (Kawaoka et al., 1988; Krauss et al., 2004). Kawaoka et al. (1998) found that the Ruddy Turnstone (*Arenaria interpres*) accounted for 11.2% of charadriiform birds sampled, but 40% of all AIV isolates were made from this species. The most prevalent AIV hemagglutinin (HA) subtypes reported from this study were the H9 and H13 (26.1% and 17.9%, respectively); subtype diversity, however, varied yearly. In a more recent report from this site that included 16 yr of surveillance data, the H3 and H11 subtypes predominated in shorebirds; but again, subtype diversity varied between years (Krauss et al., 2004).

Delaware Bay, USA, represents a major migratory stopover for more than one million shorebirds every spring, and this may directly relate to the annual presence of AIV at this site. Worldwide, there are relatively few published reports of AIV isolations from shorebirds, and there are many reports of negative isolation results (Stallknecht, 1998). There is also a lack of data related to the probability of AIV isolations from individual species and the temporal and spatial factors that influence those probabilities. In a recent review of the AIV literature, Olsen et al. (2006) reported that the global cumulative sample sizes derived from all AIV studies in which avian species were identified were 2,637 for wading birds (10 species all included in Scolopacidae; 0.8% prevalence), 2,521 for terns (nine species; 0.9% prevalence), and 14,505 for gulls (nine species; 1.4% prevalence). Considering the global distribution and species diversity within this order and the temporal and spatial variations in AIV prevalence that have been reported in Anseriformes (Hinshaw and Webster, 1980), such sample sizes do not provide an adequate base to understand the potential role of charadriiform species in AIV epidemiology.

In this study, we conducted a long-term survey of shorebirds at Delaware Bay during spring migration to obtain species specific AIV prevalence estimates and information on AIV subtype diversity within these populations. We also tested a variety of charadriiform species outside of Delaware Bay to gain information on their potential to serve as AIV reservoirs. The objective of this study was to increase our understanding of AIV epidemiology during spring migration in the Delaware Bay area and to determine whether AIV infections in Charadriiformes, especially within shorebirds (Scolopacidae), are species and location dependent.

MATERIALS AND METHODS

During September 1999–November 2005, shorebirds, gulls, and terns were captured via cannon netting, mist netting, or trapping by multiple state, federal, and private organizations in the USA (Arkansas, Delaware, Florida, Georgia, Kansas, Louisiana, Massachusetts, Missouri, New Jersey, New York, South Carolina, and Texas), Argentina, Bermuda, and Chile. A cloacal swab was collected from each bird using sterile applicators with metal shafts and very small cotton tips (Puritan®, Hardwood Products Company, Guilford, Minnesota, USA) and individually placed in sterile polypropylene tubes (Corning Inc., Corning, New York, USA) containing 2–3 ml of brain-heart infusion broth (Becton Dickinson, Sparks, Maryland, USA) supplemented with penicillin G (10,000 u/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). Most samples were stored on ice in the field (<8 hr) and then placed in liquid nitrogen or on dry ice. All samples were then stored at −70 C until processed.

Samples were thawed, vortexed, and centrifuged at 1,500 × G for 15 min, and supernatant was inoculated (0.25 ml/egg) via the allantoic route into four 9-day-old specific-pathogen–free embryonated chicken eggs (Poultry Diagnostic Research Center, The University of Georgia, Athens, Georgia, USA; and Southeast Poultry Research Laboratory, Agricultural Research Service, US Department of Agriculture [USDA-ARS], Athens, Georgia, USA). Eggs were incubated at 34 C for 72 hr, and harvested amnio-allantoic fluid was tested by hemagglutination as previously described (Stallknecht et al., 1990b). For samples collected during 1999–2002, a second passage was attempted on HA-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 subtyped using hemagglutinating inhibition and neuraminidase (NA) inhibition tests at the National Veterinary Services Laboratories, Veterinary Services (Animal Plant Health Inspection Service, USDA, Ames, Iowa, USA). Differences in prevalence estimates among species, location, and season were tested using chi-square analysis (Sokal and Rohlf, 1981). The relationship between AIV prevalence and

TABLE 1. Species tested for avian influenza virus at all sites, 1999–2005.

Family	Species	Sample size[a]
Haematopodidae	American Oystercatcher (*Haematopus palliatus*)	84
Recurvirostridae	Black-necked Stilt (*Himantopus mexicanus*)	6
Charadriidae	Black-bellied Plover (*Pluvialis squatarola*)	7
	Wilson's Plover (*Charadrius wilsonia*)	5
	Semipalmated Plover (*Charadrius semipalmatus*)	21
	Killdeer (*Charadrius vociferous*)	12
Scolopacidae	Wilson's Snipe (*Gallinago delicata*)	4
	Short-billed Dowitcher (*Limnodromus griseus*)	63 (157)
	Long-billed Dowitcher (*Limnodromus scolopaceus*)	58
	Hudsonian Godwit (*Limosa haemastica*)	12
	Marbled Godwit (*Limosa fedoa*)	11
	Lesser Yellowlegs (*Tringa flavipes*)	17
	Spotted Sandpiper (*Actitis macularius*)	5
	Willet (*Tringa semipalmatus*)	10 (2)
	Ruddy Turnstone (*Arenaria interpres*)	21 (2,368)
	Red Knot (*Calidris canutus*)	938 (1,993)
	Sanderling (*Calidris alba*)	13 (745)
	Semipalmated Sandpiper (*Calidris pusilla*)	309 (439)
	Western Sandpiper (*Calidris mauri*)	78
	Least Sandpiper (*Calidris minutilla*)	155 (127)
	White-rumped Sandpiper (*Calidris fuscicollis*)	116
	Pectoral Sandpiper (*Calidris melanotos*)	42
	Dunlin (*Calidris alpina*)	138 (377)
	Stilt Sandpiper (*Calidris himantopus*)	77
	Wilson's Phalarope (*Phalaropus tricolor*)	3
Laridae	Ring-billed Gull (*Larus delawarensis*)	193
	Great Black-backed Gull (*Larus marinus*)	7
	Herring Gull (*Larus argentatus*)	35 (5)
	Laughing Gull (*Larus atricilla*)	529 (58)
	Sandwich Tern (*Thalasseus sandvicensis*)	4
	Royal Tern (*Thalasseus maxima*)	2
	Common Tern (*Sterna hirundo*)	105
	Forster's Tern (*Sterna forsteri*)	1
	Black Skimmer (*Rynchops niger*)	1 (49)

[a] Sample size outside of Delaware Bay (sample size at Delaware Bay, USA).

weight (as an indirect measure for time spent at Delaware Bay) in the Ruddy Turnstone population at Delaware Bay was investigated in 2002 and was also tested using chi-square analysis.

RESULTS

Samples from 9,402 birds, representing 34 species of Charadriiformes (Table 1), were tested for AIV. Five families were represented, including Haematopodidae (one species, $n=84$), Recurvirostridae (one species, $n=6$), Charadriidae (four species, $n=45$), Scolopacidae (19 species, $n=8{,}278$); and Laridae (nine species, $n=989$). The temporal and spatial distribution of samples and isolations for Delaware Bay and all other sites are shown in Tables 2 and 3, respectively. Avian influenza viruses were isolated only from species within Scolopacidae and Laridae; prevalence was 3.3% and 0.6%, respectively. Only eight AIVs were isolated from charadriiform birds outside the Delaware Bay (Table 3) compared with 282 AIV isolations from the Delaware Bay sites (Delaware and New Jersey, USA). Prevalence of AIV was higher in birds migrating through Delaware Bay (282/6,340; 4.4%) than at all other sites (8/3,076; 0.3%; $\chi^2=120.29$, $P<0.0001$).

TABLE 2. Avian influenza virus isolation for shorebirds and gulls at Delaware Bay, USA, from 2000 to 2005.

Species	Year (no. of isolates/no. of birds sampled [% positive])						Total isolates
	2000	2001	2002	2003	2004	2005	
Ruddy Turnstone	27/296 (9%)	58/394 (15%)	79/735 (11%)	46/441 (10%)	20/257 (8%)	32/245 (13%)	262/2,368 (11.1%)
Red Knot	7/857 (1%)	4/316 (1%)	2/364 (1%)	2/114 (2%)	0/130	0/212	15/1,993 (0.8%)
Sanderling	0/134	2/79 (3%)	3/247 (1%)	0/89	1/79 (1%)	2/117 (2%)	8/745 (1.1%)
Dunlin	1/85	0/83	0/74	0/13	0/62	0/60	1/377 (0.3%)
Semipalmated Sandpiper	3/226 (1%)	1/71 (1%)	0/138	0/4			4/439 (1%)
Short-billed Dowitcher	0/12	1/60 (2%)	0/80		0/5		1/157 (0.6%)
Least Sandpiper	0/127						0/127
Willet	0/1				0/1		0/2
Laughing Gull						1/58 (2%)	1/78 (1%)
Herring Gull						0/5	0/5
Black Skimmer						0/49	0/49
Total isolates	38	66	84	48	21	35	292

Prevalence of AIV infection in Ruddy Turnstones at Delaware Bay (253/2,368; 10.6%) was significantly higher than the AIV prevalence observed from the combined results of all other species of Charadriiformes (37/6,895; 0.5%; χ^2=595.14, $P<0.0001$), all other species within the Scolopacidae (30/5,889; 0.5%; χ^2=525.16, $P<0.0001$), and all other species within the Scolopacidae that were sampled at Delaware Bay (28/3,840; 0.7%; χ^2=333.59, $P<0.0001$). Ruddy Turnstones accounted for 25% of all birds sampled but 87% of all AIV isolates.

At Delaware Bay, subtypes varied between years (Table 4), and viruses representing all North American HA subtypes (H1–H13) were isolated, except H8 and H13. With the exception of the H1, H3, and H4 AIVs, all of these subtypes were represented in more than one year. All of the nine known NA subtypes were also represented at this site. Only AIVs that were completely subtyped were used in the analyses; 13 additional AIVs were isolated, but the subtype remains undetermined. All of the subtypes detected at Delaware Bay were represented within the 253 AIVs recovered from Ruddy Turnstones, and all subtypes recovered outside of Delaware Bay (Georgia and New York; H2N3, H6N1, H6N4, H7N3, H10N7, H11N6; Table 3), except an H3N8 isolated from a Least Sandpiper in Texas in 2000, were represented in the subtypes recovered at Delaware Bay within 12 mo of detection. The prevalence of AIV in Ruddy Turnstones during 2002 was dependent on weight class (Fig. 1; χ^2=17.79, $P<0.0001$).

DISCUSSION

Overall, shorebirds migrating through the Delaware Bay area during May had the highest prevalence of AIV compared with all other charadriiform populations sampled at other locations and times. The low prevalence or failure to detect AIV in these birds at sites other than the Delaware Bay is consistent with other studies (Slepuskin et al., 1972; Zakstelskaya et al., 1974; Bahl et al., 1977; Boudreault et al., 1980; Thorsen et al., 1980; Honda et al., 1981; Tsubokura et al., 1981; Abenes et al., 1982; Sinnecker et al., 1983; Mackenzie et al., 1984; Nettles et al., 1985; Slemons et al., 1991; Astorga et al., 1994; Okazaki et al., 2000; Munster et al., 2007). Although the reasons for this spatiotemporal cluster are not clear, one possibility is the high concentration of shorebirds that use this site during May. This includes up to 1.5 million shorebirds that concentrate at high-quality feeding sites in densities as high as 210 birds/m^2 (Gillings, 2007). Given that AIV is primarily transmitted via a fecal-oral route (Webster et al., 1992), it is likely that these crowded conditions facilitate virus transmission. Support for this theory appears when comparing the prevalence of AIV in Ruddy Turnstones to their body weight (Fig. 1). Ruddy Turnstones of a lower weight (those that have just arrived) have a significantly lower prevalence of AIV than Ruddy Turnstones of a greater weight. For year 2002, it was estimated that Ruddy Turnstones arrived at a weight of 98.0±2.7 g and gained approximately 5 g per day (Robinson et al., 2003). Of the 705 Ruddy Turnstones weighed and sampled for AIV that year, 10.5% were infected. However, just 2.8% of the birds at or near arrival weight were infected, whereas birds exceeding the arrival weight had a 13% AIV prevalence. Although it would be impossible to confirm that all Ruddy Turnstones become infected while in the Delaware Bay, this relationship suggests short-term on-site AIV amplification within the Ruddy Turnstone population at this specific site.

Like a previous study involving multiple shorebird species (Kawaoka et al., 1988), the Ruddy Turnstone had the highest AIV prevalence of birds sampled in the family Scolopacidae. The reasons for this are unknown, but species-related variation also has been suggested for ducks within the family Anatidae, that is, ducks of the tribe Anatini appear to have a higher prevalence of AIV than other duck tribes (Stallknecht, 1998). The potential for differences in closely related species within an individual avian family is not well documented but may have important implications in understanding AIV transmission risks. Such differences could be directly related to differences in species susceptibility or indirectly related to behavior. Most of the shorebirds sampled belong to the tribe Calidridini; however, the Ruddy Turnstone is the only member of the tribe Arenariini sampled in this study. Experimental infections would be needed to determine whether there are unique physiologic characteristics that make this species more susceptible to infection with AIV.

Further study also is needed to fully understand the ecology of AIV in shorebird populations of the Delaware Bay and to what extent shorebird behavior influences transmission. There is circumstantial evidence that many of the shorebirds separate by species while roosting at night. For example, Red Knots (*Calidris canutus*) appear to prefer sandy coastal habitats, whereas the Ruddy Turnstone will use any coastal maritime area, including wetlands and exposed mudflats (Sitters, pers. comm.). Given that AIV is transmitted primarily by a fecal-oral route in birds and that the virus can persist for weeks in water under favorable conditions (Stallknecht et al., 1990a), it seems plausible that some habitats could be more conducive to virus transmission. For example, wetlands have characteristics such as shallow pools of water that may increase the transmission of AIV versus habitats such as sandbars that usually lack stagnant water and are washed over by high tides twice a day. Another potential factor involves differences in feeding behavior. Unlike most other sandpipers, Ruddy Turnstones will consume carrion and human food wastes (Nettleship, 2000), and they are adept at digging and moving objects to reach their prey. They also

TABLE 3. Avian influenza isolation results from shore birds and gulls outside of Delaware Bay, USA, 1999–2005.

Location	Month	Year	Species (sample size; subtype [no. of isolations])[a]
Argentina	Nov/Dec	2000	Red Knot (240), White-rumped Sandpiper (113), Hudsonian Godwit (6), Sanderling (9)
	Nov	2001	Red knot (217), Hudsonian Godwit (6)
Bermuda	Dec	2002	Ruddy Turnstone (16)
Chile	Jan/Feb	2001	Red Knot (100)
USA, Arkansas	Aug/Sept	2001	Least Sandpiper (51), Pectoral Sandpiper (12), Semipalmated Sandpiper (26), Common Snipe (1), Killdeer (2)
USA, Florida	May	2001	Black-bellied Plover (7), Black-necked Stilt (2), Killdeer (5), Least Sandpiper (6), Marbled Godwit (5), Red Knot (6), Short-billed Dowitcher (10), Spotted Sandpiper (5), Willet (5), Wilson's Plover (5), Dunlin (3), Herring Gull (1), Laughing Gull (6), Lesser Yellowlegs (1), Ring-billed Gull (2), Royal Tern (2), Ruddy Turnstone (3), Sanderling (4), Sandwich Tern (4), Semipalmated Plover (3), Semipalmated Sandpiper (2), Western Sandpiper (1), Forster's Tern (1)
USA, Georgia	Sept	2000	Red Knot (66)
	Dec	2000	**Ring-billed Gull (48; H11N6 [1])**, Laughing Gull (2), Herring Gull (1)
	Jan	2001	American Oystercatcher (16), Marbled Godwit (3), Willet (4), Herring Gull (7), Ring-billed Gull (48)
	Feb	2001	American Oystercatcher (1)
	March	2001	**Ring-billed Gull (37; H6N4 [1])**, Laughing Gull (13)
	May	2001	Herring Gull (1), Laughing Gull (48), Ring-billed Gull (1)
	July	2001	Laughing Gull (35)
	Sept	2001	**Red Knot (91; H10N7 [1])**, American Oystercatcher (2), Ruddy Turnstone (2)
	Oct	2001	Laughing Gull (53)
	Nov	2001	American Oystercatcher (1)
	Dec	2001	American Oystercatcher (9), Ring-billed Gull (48), Laughing Gull (3), Herring Gull (8)
	Sept	2002	American Oystercatcher (17)
	Feb	2003	American Oystercatcher (16), Marbled Godwit (3), Willet (1)
	Feb	2005	Ring-billed Gull (7)
	March	2005	Killdeer (1), Ring-billed Gull (1)
	Aug	2005	Laughing Gull (58)
	March	2005	Laughing Gull (78)
USA, Kansas	April	2003	Common Snipe (1), Least Sandpiper (13), Long-billed Dowitcher (2), Semipalmated Sandpiper (46), Western Sandpiper (2), White-rumped Sandpiper (1), Wilson's Phalarope (3)
USA, Louisiana	April	2001	Least Sandpiper (1), Stilt Sandpiper (3)
USA, Missouri	April/May	2002	Killdeer (1), Least Sandpiper (17), Pectoral Sandpiper (10), Semipalmated Plover (1), Semipalmated Sandpiper (18), Short-billed Dowitcher (2), White-rumped Sandpiper (1)
	May	2003	Killdeer (1), Least Sandpiper (3), Pectoral Sandpiper (12), Semipalmated Plover (4), Semipalmated Sandpiper (1)
USA, Massachusetts	May	2000	Common Rern (105)
USA, New York	June	2000	**Laughing Gull (233; H6N1 [1], H6N4 [1], H7N3 [1]), Herring Gull (17; H2N3 [1])**, Great black-Backed Gull (7), Ring-billed Gull (1)
USA, South Carolina	Sept	1999	Red Knot (16)
	April	2000	Red Knot (202), Short-billed Dowitcher (24)
	May	2001	Dunlin (24), Least Sandpiper (16), Pectoral Sandpiper (1), Semipalmated Plover (9), Semipalmated Sandpiper (80), Short-billed Dowitcher (22), White-rumped Sandpiper (1)
	Nov	2005	American Oystercatcher (22), Short-billed Dowitcher (4)

Table 3. Continued.

Location	Month	Year	Species (sample size; subtype [no. of isolations])[a]
USA, Texas	April	2000	**Least Sandpiper (38; H3N8 [1])**, Black-necked Stilt (4), Dunlin (10), Lesser Yellowlegs (9), Long-billed Dowitcher (42), Semipalmated Sandpiper (122), Stilt Sandpiper (67), Western Sandpiper (13), Black Skimmer (1), Killdeer (2), Pectoral Sandpiper (3), Semipalmated Plover (3), Short-billed Dowitcher (1)
	April	2001	Least Sandpiper (10), Dunlin (101), Lesser Yellowlegs (7), Long-billed Dowitcher (14), Semipalmated Sandpiper (14), Stilt Sandpiper (7), Western Sandpiper (62), Pectoral Sandpiper (4), Semipalmated Plover (1), Common Snipe (2)

[a] Positive species are shown in bold.

search for food in unnatural areas (e.g., parking lots). Perhaps their feeding strategy exposes them to pathogens not normally encountered on the beach or enhances transmissibility of AIV within this population.

An indirect effect of behavior that may influence AIV prevalence or species distri-

Table 4. Avian influenza virus subtype diversity observed in shorebirds at Delaware Bay, USA, 2000–05.

	Year						
Hemagglutinin	2000	2001	2002	2003	2004	2005	Total isolates
H1		H1N9 (1)[a]					1
H2		H2N3 (1) H2N4 (9) H2N9 (25) H2N? (1)[b]	H2N6 (1) H2N9 (6)				43
H3					H3N6 (16) H3N8 (11) H3N4,8 (1)[b] H3N? (2)		30
H4	H4N6 (2)	H4N? (1)				H4N6 (1)	4
H5	H5N2 (1) H5N7 (3) H5N8 (1)		H5N2 (3) H5N9 (2)	H5N7 (1)	H5N3 (1)		12
H6	H6N2 (1) H6N5 (1)	H6N4 (4) H6N8 (1)	H6N8 (2)	H6N8 (6) H6N4,8 (1)		H6N1 (2) H6N4 (1)	19
H7		H7N3 (1)				H7N9 (2)	3
H9		H9N4 (2) H9N5 (2) H9N9 (8)	H9N2 (6) H9N5 (10) H9N7 (1) H9N8 (6) H9N9 (1) H9N1,4 (5)				41
H10	H10N7 (54)	H10N7 (1)		H10N7 (13)		H10N7 (12)	80
H11	H11N2 (4) H11N7 (1)	H11N2 (4) H11N3 (1) H11N4 (10) H11N9 (11)			H11N8 (3) H11N6 (2)	H11N6 (5)	41
H12	H12N7 (2)	H12N5 (1)	H12N4 (1) H12N5 (2) H12N9 (1)			H12N4 (4) H12N5 (11)	22
Total isolates	70	84	47	21	35	39	296

[a] Number of isolates in parentheses.

[b] N? = Neuramidase subtype not determined; N9,8 = Positive reaction for both neuraminidase subtypes.

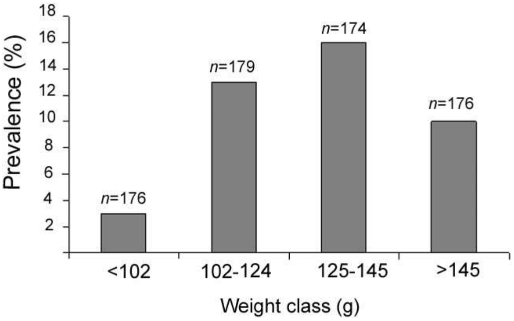

FIGURE 1. Prevalence of avian influenza viruses in Ruddy Turnstones (*n*) as a function of weight-class at Delaware Bay, USA, during 2002. Birds weighing <102 g are presumed to have recently arrived at Delaware Bay.

bution relates to age structure and the possible effects of prior AIV exposure and acquired immunity. In ducks, AIV prevalence is highest in juvenile ducks (Hinshaw and Webster, 1980), but how age affects AIV prevalence in charadriiforms is unknown. Most Ruddy Turnstones remain on or near their wintering grounds for more than 1 yr following their first fall migration (Nettleship, 2000). Thus, the vast majority of Ruddy Turnstones captured during spring migration in the Delaware Bay are at least 22 mo of age. In fact, of the 25 Ruddy Turnstones with recorded ages that tested positive for AIV in 2000, all 25 were classified as adults at least 2 yr of age. In 2002, 72 of the 76 Ruddy Turnstones for which age was determined and that tested positive for AIV were at least 2 yr of age. The species differences observed at Delaware Bay could be explained if species other than the Ruddy Turnstone acquired immunity before their spring arrival at Delaware Bay. However, there currently is no published information to suggest that this occurs.

Based on isolation results from this study, the H10 subtype predominated at Delaware Bay from 2000 to 2005. However, such results are misleading. In the initial study of AIV at this site, the H9, H11, and H13 subtypes were most prevalent in charadriiform species and the H4, H9, and H11 were most prevalent in Ruddy Turnstones during 1985–87 (Kawaoka et al., 1988). During a longer-term study (16 yr) at this site, the H3 and H11 subtypes predominated, but H1–H13 AIV were detected (Krauss et al., 2004). Such differences are not surprising as temporal shifts in predominant HA subtype have been observed in waterfowl (Sharp et al., 1993). Overall, no clear patterns of AIV subtype distribution are apparent at Delaware Bay, and represented subtypes can change dramatically between years. This almost-random pattern of subtype diversity may represent the product of annual introductions (and subsequent amplification) of AIV in the Ruddy Turnstone population at this site; such introductions may originate from ducks, gulls, or both. We do not think the Ruddy Turnstone or other members of the Scolopacidae are effective reservoirs for these viruses; rather, that AIV isolated from this and other species in this family represent viruses that spill over from both the duck and gull AIV reservoirs. This idea is supported by the observations that all of the AIV subtypes that were isolated from gulls (at Delaware Bay and other study areas) were represented at Delaware Bay (in Ruddy Turnstones) within 12 mo of detection and that predominant waterfowl HA subtypes (H3, H4, H6) were also present. It is further supported by the extremely low prevalence of AIV that has been consistently observed in Scolopacidae species (including Ruddy Turnstones at other sites) sampled outside of Delaware Bay. Consistent with our data from non-Delaware Bay sites, Munster et al. (2007) reported the detection of AIV (based on polymerase chain reaction results) from only two of 3,159 birds representing 47 species of Scolopacidae. One of these positive birds was a Red Knot sampled at Delaware Bay.

Because Delaware Bay is the last major stop before reaching the breeding grounds, it is critical that shorebirds maximize their time feeding and resting. It is unknown what effect, if any, an infection with AIV has on their ability to prepare for the flight to the breeding grounds. However, limited banding data derived from this study provides some evidence that shorebirds can survive infection with AIV. For example, during 2000–04, AIV was isolated from four Ruddy Turnstones that were recaptured 10 days to 2 yr later. One of these AIV-positive birds was captured on 16 May 2002 and recaptured (but not sampled for AIV) on 3 June 2002; it had gained 45 g during that time and weighed 9 g more than the average of the 30 Ruddy Turnstones captured that same day. Although this is just one example out of hundreds of thousands of birds in the Delaware Bay, it provides some evidence that AIV does not necessarily have detrimental effects on an individual bird's ability to gain weight in preparation for migration. With many shorebird populations believed to be in decline (Morrison et al., 1994), it is important to understand all the factors contributing to this decline, including the possible role of disease.

It is evident that our understanding of AIV within wild bird populations is far from complete (Hanson et al., 2005; Spackman et al., 2005). Although results from this and other studies to date support the idea that AIV infection in shorebirds outside of Delaware Bay is very low, it is important to note that the localized, short-term, and species-specific relationship that have been observed with AIV and Ruddy Turnstones at Delaware Bay may occur at other localized sites worldwide. Most North American Arctic-breeding shorebirds, for example, spend a majority of their lives in South America. Although AIV has recently been reported from a single wild duck from that continent (Spackman et al., 2007), there are few, if any, published reports of shorebird AIV surveillance in South America. Why Ruddy Turnstones appear to have a consistently higher AIV prevalence rate than other shorebirds at Delaware Bay is unknown. Given the current confusion surrounding incursions of HPAI H5N1 viruses into wild bird populations and recent evidence of AIV transmission directly to humans, it is increasingly important to gain a comprehensive understanding of the epidemiology and natural history of AIV within wild bird populations. Without such information, a realistic understanding of wildlife, domestic animal, or human health risks associated with existing or new (HPAI H5N1 viruses) AIV cannot be achieved.

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