Low Prevalence of Avian Influenza Virus in Shorebirds on the Pacific Coast of North America

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Abstract.—The emergence of highly pathogenic avian influenza (HPAI) H5N1 has elevated concerns about wild birds as virus hosts; however, little is known about the ecological and epidemiological factors of transmission by shorebirds. Here we summarize results for 2,773 shorebirds that were live-trapped on the Pacific coast of the United States during 2006-2007 and tested for avian influenza virus using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and virus isolation. As was the case throughout North America, HPAI H5N1 was not detected in shorebirds during this interval. Contrary to other wild bird groups, most notably waterfowl, the prevalence of even low pathogenicity virus among shorebirds in our study areas in California, Washington, and Alaska was extremely low (0.5%). Virus was detected by RT-PCR from four different species, including, Dunlin (*Calidris alpina*; N = 3), Western Sandpiper (*C. mauri*; N = 8), Long-billed Dowitcher (*Limnodromus scolopaceus*; N = 1), and American Avocet (*Recurvirostra americana*; N = 1), with the detections in the latter three constituting the first published records for these birds. Based on studies in the eastern United States, we expected, but did not detect (H_1 = 1.6, P = 0.21) elevated avian influenza prevalence among shorebirds during spring migration. Diagnostic tests, which were designed to evaluate testing and sampling methods, indicated poor functioning of traditional virus isolation methods and no improvement in detection likelihood by collecting oropharyngeal swabs in addition to cloacal swab samples for low pathogenicity viruses (Z_1 = 0.7, P = 0.48). *Received 18 December 2007, accepted 29 April 2008.*

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The widespread occurrence of avian influenza in waterbirds, particularly members of the orders Anseriformes (ducks, geese, and swans) and Charadriiformes (shorebirds and gulls), has led to their recognition as a natural reservoir of the virus in the wild (Webster *et al.* 1992; Horimoto and Kawaoka 2001; Olsen *et al.* 2006). From an epidemiological perspective, however, our understanding of taxonomic, geographic, and seasonal differences in avian influenza prevalence among waterbird hosts remains limited (Muzaffar *et al.* 2006). Such data are crucially important to address questions about the role of wild birds in the perpetuation, geographic spread, and cross-species

transmission of avian influenza viruses, including highly pathogenic avian influenza (HPAI) H5N1 (Yasue *et al.* 2006).

Shorebirds are known to harbor a broad spectrum of influenza *A* virus subtypes (Webster *et al.* 1992; Widjaja *et al.* 2004) and are adapted to the same marine and freshwater habitats that facilitate fecal-oral transmission of virus between hosts in the wild (Webster *et al.* 1992). Moreover, the majority of shorebirds migrate (62%, Warnock *et al.* 2001) and are capable of moving thousands of km in a matter of d (Driscoll and Ueta 2002; Gill *et al.* 2005), making them potential vectors for avian influenza dispersal over long-distances. The Pacific coast of the United States and nearby interior valleys are a major migratory corridor and winter destination for shorebirds that breed in the arctic and temperate-zones of eastern Siberia, Alaska, and Canada, as well as a year-round home to numerous non-migratory species (Page *et al.* 1999). Large populations of shorebirds are found in the region's wetlands intermixed with hunting areas, urban areas, and agriculture areas, including sizeable poultry and egg production industries.

In the mid-Atlantic region of the United States, studies spanning almost 30 y have investigated the relationships between shorebirds and avian influenza (Kawaoka *et al.* 1988; Makarova *et al.* 1999; Krauss *et al.* 2004; Widjaja *et al.* 2004). These studies have resulted in the isolation of viruses from a relatively small number of shorebird species; however, virus diversity has been high and included H5 and H7 hemagglutinin subtypes, which are commonly associated with HPAI in poultry (Webster *et al.* 1992; Widjaja *et al.* 2004). Viral sequences isolated from poultry in Pennsylvania and Florida are known to share ancestry with shorebirds [Ruddy Turnstone (*Arenaria interpres*)] and experimental infections have indicated that chickens are susceptible to shorebird viruses (Saito *et al.* 1994). It has also been suggested that shorebirds in the Delaware Bay region in the Atlantic Flyway exhibit seasonal variation in influenza *A* prevalence, with higher rates observed during spring compared to the autumn migration period (Kawaoka *et al.* 1988; Krauss *et al.* 2004), which is contrary to the pattern observed in ducks and has led to the hypothesis that shorebirds are involved in the over-winter persistence of viruses in the wild (Krauss *et al.* 2004).

Unfortunately, the generality of conclusions drawn about shorebirds and avian influenza from studies in the eastern United States is unclear (Munster *et al.* 2007). Until recently, only sporadic reports on prevalence and genetic subtypes have been reported from other locations (e.g., Honda *et al.* 1981; Mackenzie *et al.* 1984; Süss *et al.* 1994; Ito *et al.* 1995; Fouchier *et al.* 2003; Hurt *et al.* 2006; Gaidet *et al.* 2007; Munster *et al.* 2007; Winker *et al.* 2007), and we are aware of none that comprise birds from the Pacific coast of North America. Thus, our objective was to contribute knowledge about the taxonomic, geographic, and temporal variation in avian influenza prevalence among shorebirds on the Pacific coast of the United States. In addition, recognizing that current research methodology is optimized for poultry, and to a lesser extent for wild waterfowl, we collected supplementary data to improve diagnostic capabilities in shorebirds by evaluating testing and sampling procedures, including use of viral isolation in addition to real-time reverse transcriptase-polymerase chain reaction (RT-PCR) as a first pass screening methodology and the collection of oropharyngeal swab samples in addition to cloacal swabs to test for influenza *A* in live-trapped shorebirds.

METHODS

Study Species and Field Methods

In total, samples from 2,773 shorebirds were tested from seven locations along the Pacific coast of North America (Fig. 1). The samples were taken from 14 different species, representing three families (Charadriidae, Recurvirostridae, and Scolopacidae). The primary emphasis was on three abundant Scolopacidae species that were identified as priority candidates for United States HPAI H5N1 national surveillance efforts [(hereafter national surveillance; Long-billed Dowitcher (*Limnodromus scolopaceus*), Western Sandpiper (*Calidris mauri*), and Dunlin (*C. alpina*); Pacific Flyway Council 2006)]. Testing was also completed for samples from two non-migratory Recurvirostridae [American Avocet (*Recurvirostra americana*) and Black-necked Stilt (*Himantopus mexicanus*)], as well as smaller numbers of co-min-

Figure 1. Map depicting locations where shorebirds were sampled for avian influenza in the Pacific Flyway.

gling shorebird species that were captured opportunistically during our field efforts [Scolopacidae: Least Sandpiper (*Calidris minutilla*), Sanderling (*C. alba*), Semipalmated Sandpiper (*C. pusilla*), Red Knot (*C. canutus*), Red-necked Phalarope (*Phalaropus lobatus*), Short-billed Dowitcher (*Limnodromus griseus*), and Ruddy Turnstone; Charadriidae: Killdeer (*Charadrius vociferus*) and Semipalmated Plover (*C. semipalmatus*)].

Sampling and testing details, including the laboratory used for analysis, sample type, season and year, location, and species tested are described in Table 1. Briefly, birds were captured using mist nets, hand held net launchers, and rocket nets appropriate to the species and study areas under investigation. Capture locations were primarily coastal habitats (beaches, mudflats, and salt marshes), with the exception of one inland California site in the Central Valley, which was characterized by shallow freshwater marsh habitat. A majority of samples were collected during autumn 2006 (14 Jul-19 Dec; N = 1,414), when national surveillance efforts were most concerted. Samples also were collected during spring 2006 (16 Mar-13 May; N = 510), spring 2007 (8 Jan-9 May; N = 606), and autumn 2007 (8 Aug-9 Sep; N = 243).

The samples were collected using rayon tipped swabs (MicroPur™, PurFybr Inc., Munster, IN). They were preserved in 1.5 or 3.0 cc cryovial tubes containing viral transport media (U. S. Interagency HPAI Working Group 2006) and kept at 4°C until transfer (< six h) to a -20°C freezer (spring 2006) or -70°C liquid nitrogen vapor shipper (all samples thereafter; MVE Bio-Medical Division, Chart Industries, Inc., Burnsville, MN). They were then shipped in batches on dry ice to the U.S. Geological Survey National Wildlife Heath Center Diagnostic Virology Laboratory (USGS-NWHC) for inclusion in national surveillance efforts or the University of California, Davis - Veterinary Medicine Extension Laboratory (UCD) for follow-on analyses.

A single cloacal swab was collected from each bird unless otherwise noted. During autumn 2006, two cloacal and one oropharyngeal swabs were collected from birds in California to evaluate potential differences in detection probability when using paired cloacal and oropharyngeal samples (Table 1). The oropharyngeal samples were collected by rubbing the swab head across the top surface of the pharyngeal cavity and targeting the soft tissue of pharynx distal to choanal cleft. For these samples, one cloacal swab was submitted to USGS-NWHC laboratory for inclusion in national surveillance, and the other cloacal swab, as well as the oropharyngeal swab, to UCD for sample type comparison, thus controlling for potential differences between laboratories. The laboratory which received the first cloacal sample was alternated to control for sequential sampling of the cloaca and the UCD bound samples were held until after USGS-NWHC had confirmed they were not highly pathogenic. During spring 2007, the comparison trials between cloacal and oropharyngeal samples were continued with one sample of each type taken from birds in California and Alaska. During autumn 2007, samples collected in Washington followed revised national guidelines and both cloacal and oropharyngeal swab samples were collected from each bird and stored in a single cryovial tube (Table 1).

Laboratory Analysis

Samples from 993 shorebirds were tested exclusively at USGS-NWHC, samples from 897 exclusively at UCD, and samples from 883 at both laboratories (Table 1). All were analyzed as in accordance with U. S. Interagency Strategic Plan guidelines for early detection of HPAI H5N1 in wild birds (Spackman *et al.* 2002; U. S. Interagency HPAI Working Group 2006). Briefly, viral RNA was extracted from a 50 µl aliquot of sample using the Ambion MagMax Viral RNA extraction kit (Ambion Inc., Austin, Texas). From the aliquot, 50 µl (15 µl at UCD) of RNA was eluted and eight µl (2.5 µl at UC Davis) was tested using matrix influenza *A* gene assays. RT-PCR was performed using the Stratagene Mx3005P Real-Time PCR System (Stratagene Corp., La Jolla, California) at USGS-NWHC and the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, California) at UCD. A maximum Ct threshold of 35.0 was established to determine positive or negative status for samples included in national surveillance efforts (Ct values are inversely proportional to target concentrations in a sample). Recognizing that samples from small wild birds like shorebirds often have very low virus concentration, Ct values for follow-on analyses at UCD are reported without an assignment of positive or negative status.

Samples in which influenza *A* was detected were further tested using H5 and H7 RT-PCR assays. If positive for either, viral isolation was conducted by inoculation into the allantoic cavity of embryonated chicken eggs (Spackman *et al.* 2002; U. S. Interagency HPAI Working Group 2006), which allowed antigenic typing using inhibition assays to determine if the gene sequence was a highly pathogenic type. At UCD, all samples with detectable amounts of influenza *A* by RT-PCR were submitted for viral isolation irrespective of H5 and H7 assay results. A subset of 387 cloacal swab samples that were collected during spring 2007 were also submitted directly to both RT-PCR and viral isolation to determine whether detection rates could be improved by using isolation as a first pass screening methodology (Table 1). For these samples, virus was isolated in nine to eleven d old SPF embryonating chicken eggs (Charles River Laboratories, Wilmington, Massachusetts; Swayne *et al.* 1998).

Table 1. Sampling details for shorebirds tested for influenza *A* from the Pacific coast of the United States, including: laboratory, swab sample type, location, season and year and species tested. Number of samples for each category is given in parentheses.

Laboratory[1]	Swab sample type	Season and year	Location[2]	Species[3]
UCD (897)	Cloacal (510)	Spring 2006 (510)	SSFB (262); PM (80); CRD (66); GH (53); WB (49)	WESA (335); LBDO (44); AMAV (63); SBDO (32); BNST (18); SEPL (10); DUNL (7); RNPH (1)
	Paired cloacal and oropharyngeal (387)	Spring 2007 (387)	SSFB (263); CRD (80); SPB (44)	WESA (245); LBDO (50); DUNL (36); SBDO (29); LESA (24); SEPL (2); KILL (1)
USGS-NWHC (993)	Cloacal (750)	Autumn 2006 (531)	GH (398); WB (90); SSFB (33); SPB (10)	WESA (316); DUNL (114); LESA (53); SAND (11); SBDO (10); SEPL (12); REKN (2); RUTU (1)
		Spring 2007 (219)	GH (213); WB (6)	DUNL (214); WESA (4); SAND (1)
	Pooled cloacal and oropharyngeal (243)	Autumn 2007 (243)	GH (243)	WESA (207); LESA (31); SEPL (3); SESA (2)
USGS-NWHC and UCD (883)	Cloacal and paired cloacal and oropharyngeal (883)	Autumn 2006 (883)	CV (365); SPB (284), SSFB (234)	WESA (346); LBDO (243); DUNL (198); LESA (73); SEPL (20); SBDO (3)

[1]USGS-NWHC: U.S. Geological Survey National Wildlife Heath Center Diagnostic Virology Laboratory, Madison, Wisconsin; UCD: University of California, Davis - Veterinary Medicine Extension, Davis, California.

[2]CRD: Copper River Delta, Alaska; CV: Central Valley, California; GH: Grays Harbor, Washington; SPB: San Pablo Bay, California; SSFB: South San Francisco Bay, California; WB: Willapa Bay, Washington.

[3]AMAV: American Avocet; BNST: Black-necked Stilt; DUNL: Dunlin; KILL: Killdeer; LBDO: Long-billed Dowitcher; LESA: Least Sandpiper; REKN: Red Knot; RNPH: Red-necked Phalarope; RUTU: Ruddy Turnstone; SAND: Sanderling; SBDO: Short-billed Dowitcher; SEPL: Semipalmated Plover; SESA: Semipalmated Sandpiper; WESA: Western Sandpiper.

Statistical Analysis

Prevalence was summarized by species, season, location, and year. When appropriate, statistical variation was evaluated using single variable non-parametric Kruskal-Wallis ANOVAs (StatSoft 1995). Presence of influenza *A* virus was coded as a binary response variable and group effects were evaluated as categorical predictor variables. Low prevalence and methodological differences precluded robust multivariable analyses and caution was exercised when statistical differences were tested to reduce the possibility of Type I errors associated with inferences from poorly sampled variables. When evaluating diagnostic capabilities, sign tests were used to determine whether screening using multiple sampling procedures (i.e., use of viral isolation in addition to RT-PCR) or collection of multiple sample types (i.e., use of oropharyngeal swab samples in addition to cloacal) improved detection probability (MacDonald 2007). Statistical significance was judged at the $\alpha = 0.05$ level and mean prevalence estimates are presented ± standard error (SE).

RESULTS

Overall and Species-specific Influenza *A* Prevalence

Influenza *A* virus was detected from 13 of 2,773 (0.005 ± 0.001 SE) shorebirds. None were H5 or H7 subtypes and all were diagnosed using RT-PCR. Detections occurred in four of the 14 species that were studied and prevalence was uniformly low across species (range: 0-1.6%; Table 2). The host species included one American Avocet (spring 2006: San Francisco Bay, California); eight Western Sandpipers (autumn 2006: Grays Harbor, Washington [N = 1], San Pablo Bay, California [N = 2], San Francisco Bay, California [N = 4]; spring 2007: Copper River Delta, Alaska [N = 1]; one Long-billed Dowitcher (autumn 2006: San Francisco Bay); and three Dunlin (autumn 2006: Willapa Bay, Washington [N = 2]; spring 2007: Grays Harbor, Washington [N =1]).

Prevalence estimates were similar between laboratories, with some minor differences related to Ct thresholds. Four of 1,876 (0.2%) samples tested at USGS-NWHC were influenza *A* positive (Ct ≤35.0). Included among these were samples from 883 birds that were double swabbed, none of which were positive at USGS-NWHC, whereas seven (0.8%) had detectable virus at UCD (Ct range: 37-43). In all, influenza *A* was detected from nine of 1,780 (0.5%) shorebirds tested at UCD.

Temporal and Spatial Variation in Influenza *A* Prevalence

Seasonal variation was not evident in the single variable model ($H_1 = 1.6$, $P = 0.21$; $N = 2{,}273$). Three of 1,116 (0.2%) spring samples had detectable amounts of virus compared to nine of 1,657 (0.6%) autumn samples. Annual prevalence also was similar across years ($H_1 = 1.4$, $P = 0.23$; $N = 2{,}773$), with detections in eleven of 1,924 (0.6%) birds in 2006 and two of 849 (0.2%) in 2007. With respect to geographic location, prevalence estimates ranged from 0-1.4% and all locations except Point Mugu and Central Valley, California had at least one sample with detectable virus (Table 3).

Table 2. Prevalence of influenza *A* among shorebird species tested using RT-PCR on the Pacific coast of the United States during 2006-2007.

Species	Number sampled	Positive samples	Prevalence ± SE
Western Sandpiper	1,453	8	0.006 ± 0.002
Dunlin	569	3	0.005 ± 0.003
Long-billed Dowitcher	349	1	0.003 ± 0.004
Least Sandpiper	181	0	0.000 ± 0.005
Short-billed Dowitcher	74	0	0.000 ± 0.008
American Avocet	63	1	0.016 ± 0.009
Semipalmated Plover	47	0	0.000 ± 0.010
Other (N < 20 per species): Black-necked Stilt, Sanderling, Semipalmated Sandpiper, Red Knot, Ruddy Turnstone, Red-necked Phalarope, Killdeer	37	0	0.000 ± 0.012
All	2,773	13	0.005 ± 0.002

Table 3. Prevalence of avian influenza by sampling area for shorebirds tested on the Pacific coast of the United States during 2006-2007.

Sampling location	Number sampled	Positive samples	Prevalence ± SE
Point Mugu, California	80	0	0.000 ± 0.008
South San Francisco Bay, California	792	6	0.008 ± 0.002
San Pablo Bay, California	338	2	0.006 ± 0.004
Central Valley, California	365	0	0.000 ± 0.004
Willapa Bay, Washington	145	2	0.013 ± 0.006
Grays Harbor, Washington	907	2	0.002 ± 0.002
Copper River Delta, Alaska	146	1	0.007 ± 0.006

Influenza *A* Sampling Diagnostics

There were 1,270 shorebirds from which paired cloacal and oropharyngeal swabs were tested using RT-PCR. Among these, eight had influenza *A* virus, six of which were from cloacal swabs, two were oropharyngeal swabs, and none using both sample types. Although the detection rate was slightly higher when using both sample types opposed to cloacal samples alone, there was no statistical improvement in detection probability when both methods were used ($Z_1 = 0.7$, $P = 0.48$). There were no test-positive samples among the 243 from Washington in which cloacal and oropharyngeal swabs were pooled in a single cryovial tube.

The evaluation of viral isolation as a first pass screening methodology was equivocal. Influenza *A* was not detected by viral isolation in any of the 387 paired samples used for comparison, and only in one sample by RT-PCR. It was noted that virus could not be isolated from any of the eight other original samples in which influenza *A* was detected using RT-PCR at UCD before submission to viral isolation, indicating poor performance of traditional virus isolation techniques at low Ct values for live-trapped shorebirds.

Finally, there was no evidence that virus concentration decreases below detectable levels for RT-PCR analysis when sequential cloacal swabs are collected from the same individual. Of the seven duplicate samples in which detectable levels of influenza *A* were found at UCD, three were from first swabs and four were from second swabs.

DISCUSSION

Our testing of shorebirds on the Pacific coast of United States during 2006-2007 failed to detect gene sequences typically associated with high pathogenicity avian influenza virus in poultry, mirroring results for wild birds throughout North America. Notable were our low detection rates of any types of influenza *A*. Less than 0.5% of shorebirds we sampled had detectable virus, with prevalence being consistently low across species, locations, years, and seasons. These results stand in contrast to those reported in shorebird populations in the mid-Atlantic region of the United States, where prevalence estimates have been higher (Kawaoka *et al.* 1988; Makarova *et al.* 1999; Krauss *et al.* 2004; Widjaja *et al.* 2004), particularly because research conducted in the Delmarva Peninsula area has informed avian influenza surveillance priorities in wild birds around the world.

While opportunistic sampling has occurred in other areas using similar PCR-based testing methodologies, comprehensive studies involving shorebirds are few. For example, in the only other long-term surveillance study published to date in eastern Germany (1977-1989), a prevalence of 1.7% was estimated for "other waterbirds," a category that included Ruddy Turnstone, Red Knot, and Common Redshank (*Tringa tetanus*; Süss *et al.* 1994). Also in northern Europe, shorter-term studies have detected avian influenza viruses in <0.5% of 2,175 Charadriiformes, with no isolates from shorebirds (Fouchier *et al.* 2003), and Munster *et al.* (2007) detected no influenza virus from >3,000 European samples, concluding that

shorebirds do not play a significant role in the epidemiology of avian influenza in Europe as has been suggested in the Americas. Sampling in twelve African nations detected avian influenza virus in only three of 13 species [Ruff (*Philomachus pugnax*), Curlew Sandpiper (*Calidris ferruginea*), and Spotted Redshank (*Tringa erythropus*)] and an overall prevalence of 1.5% (Gaidet *et al.* 2007). In North America, recent sampling in Alaska has found very low prevalence among breeding shorebirds (Winker *et al.* 2007, Hon Ip, U.S. Geological Survey, *manuscript in review*). In contrast to our results and the findings discussed above, relatively high prevalence and highly pathogenic strains have occasionally been reported outside the eastern United States. Most notably, an Australian study found that >15% of 173 cloacal swab samples collected from six species of migratory shorebirds with links to east Asia were positive for influenza *A* (Hurt *et al.* 2006) and a preliminary report from Russia has documented the only known HPAI H5N1 infection in a shorebird to date, detected in a salvaged Green Sandpiper (*Tringa ochropus*; Brown *et al.* 2005).

We expected, but did not detect, elevated avian influenza prevalence among shorebirds during spring migration on the Pacific coast. In fact, infection rates were slightly higher during autumn for the species and study areas that we sampled most intensively. However, our target species differed from those studied in the eastern United States and most of our captures occurred in coastal, estuarine, mudflat habitats rather than beaches and agricultural lands described for the Delmarva Peninsula studies. Our results, combined with the findings described above, suggest that putative seasonal differences in prevalence among shorebirds are more likely to apply narrowly to certain species, habitats, and areas. Similarly, recent research including shorebirds in the high arctic indicates that sweeping hypotheses concerning the risk of intercontinental virus transfer via northern areas may be overstated (Peterson *et al.* 2007; Winker *et al.* 2007).

Our study confirmed influenza *A* virus in three previously undocumented species: American Avocet, Western Sandpiper, and Long-billed Dowitcher. The fourth species in which we detected virus, Dunlin, had previously been confirmed as a host (Honda *et al.* 1981; Ito *et al.* 1995), although, as was the case in our study, prevalence has been very low. Interestingly, influenza *A* virus was detected in four of the six species in our study for which >50 samples were collected and all of the species for which >300 were collected, thus indicating support for the generalization that most shorebirds are susceptible, if infrequent host organisms for influenza *A* viruses.

At present, our ability identify the full range of influenza *A* subtypes is limited because most diagnostic reagents and procedures are optimized for identifying viruses in poultry, not wild birds. Virus isolation is the only available method that allows complete characterization and genetic sequencing, but unfortunately might not work with some wild-bird viruses (Boyce 2007). A key area of avian influenza research has been determining the host-species range and phylogenetic relationships of various subtypes circulating in wild and domestic birds (Makarova *et al.* 1999; Saito *et al.* 1994; Widjaja *et al.* 2004). We were unable to determine how the Pacific coast viruses detected in our study relate to other lineages because we were unable to antigenically subtype them. Further complicating interpretation of results are a myriad of potentially interacting ecological factors and differences in sampling procedure. For example, the primary method of sample collection for the Delmarva Peninsula studies has been collection of fecal samples from the beach (Krauss *et al.* 2004) rather than cloacal swabbing of live-trapped birds, as has been the case with most recent efforts, including our own. Why fecal samples should yield higher detection rates than cloacal swab samples from live birds is unknown and it is not clear whether the observed differences are related to primarily to sampling methodology, for example difficulty obtaining sufficient sample from small birds, or ecological factors such as species, location, and the timing of sample collection.

In our study, it was recognized that research methodologies are not fully optimized for live-trapped shorebirds. We changed our field storage procedure after spring 2006 and began using a -70°C liquid nitrogen vapor shipper instead of a standard -20°C freezer. However, influenza *A* prevalence did not increase after the switch. With respect to sample type, recent evidence suggests that HPAI H5N1 attacks the respiratory system in wild waterfowl and detection may be higher using oropharyngeal rather than cloacal swabs for HPAI (Brown *et al.* 2006). Canadian researchers found that low pathogenicity virus detection rates were 10-20% higher in ducks when both cloacal and oropharyngeal samples were used compared to cloacal swabs alone (Canadian Cooperative Wildlife Health Centre 2006). Our data for shorebirds sampled in California and Alaska indicate that virus can be detected using either sample type. However, a majority of our influenza *A* detections were obtained using cloacal swab samples and the collection of oropharyngeal swabs did not significantly improve detection capabilities. Thus, we would recommend collection of oropharyngeal swab samples when testing for HPAI, but the method is not advisable for all studies.

Although a wide variety of viral subtypes have been isolated from shorebirds and basic phylogenetic relationships have been investigated, remarkably little is known about host-virus relationships for the vast majority of shorebird species. The research interest generated by HPAI H5N1 has fostered a dramatic increase in sampling efforts worldwide. However, low prevalence of virus makes it difficult for study teams working in isolation to draw wider inference about the predictors and correlates of influenza infection in wild birds. We believe that our findings contribute to the limited knowledge that is currently available regarding ecological correlates to avian influenza infections in wild birds and highlight the need for results to be amalgamated from multiple species and locations in order to more fully understand relationships between wild birds and avian influenza.

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