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Waterfowl Ecology and Avian Influenza in California: Do Host Traits Inform Us About Viral Occurrence?

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SUMMARY. We examined whether host traits influenced the occurrence of avian influenza virus (AIV) in Anatidae (ducks, geese, swans) at wintering sites in California's Central Valley. In total, 3487 individuals were sampled at Sacramento National Wildlife Refuge and Conaway Ranch Duck Club during the hunting season of 2007–08. Of the 19 Anatidae species sampled, prevalence was highest in the northern shoveler (5.09%), followed by the ring-necked duck (2.63%), American wigeon (2.57%), bufflehead (2.50%), greater white-fronted goose (2.44%), and cinnamon teal (1.72%). Among host traits, density of lamellae (filtering plates) of dabbling ducks was significantly associated with AIV prevalence and the number of subtypes shed by the host, suggesting that feeding methods may influence exposure to viral particles.

RESUMEN. Ecología de las aves acuáticas y la influenza aviar en California: ¿Pueden las características de los hospederos informarnos acerca de la presentación del virus?

En este estudio, se estudió si las características de los hospedadores influyeron sobre la presentación del virus de la influenza aviar en miembros de la familia Anatidae (patos, gansos y cisnes) en los sitios de estancia invernal en el Valle Central de California. En total, se examinaron 3487 muestras individuales recolectadas en el Refugio Nacional de Vida Silvestre de Sacramento y en el Club Rancho de Patos Conaway durante la temporada de caza 2007–08. De los 19 ejemplares analizados de la familia Anatidae, la prevalencia más alta se registró en el pato cuchara del Norte (5.09%), seguido por el porrón acollarado (2.63%), el pato silbón americano (2.57%), el porrón albeola (2.50%), el ganso careto mayor (2.44%), y la cerceta colorada (1.72%). Entre las características de los huéspedes, la densidad de las lamelas (placas orales de filtrado) de los patos chapoteadores estuvo asociada significativamente con la prevalencia del virus de influenza aviar y el número de subtipos diseminados por el huésped, sugiriendo que los métodos de alimentación pueden influir sobre la exposición a partículas virales.

Key words: Anatidae, host ecology, hunter harvested, ecomorphology, lamellae, low pathogenic avian influenza, Pacific Flyway

Abbreviations: AF = allantoic fluid; AIV = avian influenza virus; ECE = embryonating chicken eggs; HA = hemagglutinin; HPAI = highly pathogenic avian influenza; LPAI = low pathogenic avian influenza; NA = neuraminadase; NWR = National Wildlife Refuge; rRT-PCR = real-time reverse transcriptase–polymerase chain reaction

Waterfowl in the family Anatidae (ducks, swans, and geese) are known reservoirs for avian influenza viruses (AIVs) in nature (17,46). This avian family exhibits a range of ecologic traits that promote the transmission of AIV. A probable key trait is their preference for freshwater habitat, which favors environmental persistence of the virus compared to saltwater (39) and allows efficient dispersal of viral particles (46). In addition, migration, ubiquitous in Anatidae, facilitates the long-distance spread of AIV between breeding sites at high latitudes and wintering sites at lower latitudes (17,18). Some studies have suggested that contact with surface waters, typical amongst dabbling ducks that forage in shallow wetlands, may increase fecal-oral transmission, thereby helping to complete the infection cycle of the virus (12,24). However, beyond these broad ecologic features of habitat preference, migratory movement, and foraging guild, our understanding of the host traits of Anatidae that promote the spread and circulation of AIV remains relatively limited.

A growing criticism of AIV studies is the lack of detailed ecologic information collected during sampling of birds, including demographics of the host population, the composition of bird species, and accurate habitat descriptions (26,47). Such data would provide valuable insights for developing more focused surveillance efforts in waterbirds, the reservoir for AIVs. Consequences of neglecting to document host ecology are demonstrated by the uncertainty that still surrounds the source (i.e., waterfowl of wild or domestic origin) and conditions (i.e., population density and physical state of birds) that prompted highly pathogenic avian influenza (HPAI) H5N1 outbreaks in waterfowl at Qinghai Lake in China (5). Reporting ecologic traits of hosts in combination with AIV subtypes, prevalence rates, and clinical effects may improve surveillance efforts by shedding light on the bird species that serve as vectors and the habitat settings that are conducive to an outbreak. Ecologic information on Anatidae hosts is necessary to inform surveillance efforts both in Asia, where HPAI H5N1 is endemic (6), and in North America, where AIV outbreaks in poultry have been reported with steady occurrence (40).

California's Central Valley attracts 60% of the migratory waterfowl in the Pacific Flyway during winter, approximately 20% of all waterfowl in North America (14). In California, wetlands designated for waterfowl are intermixed with urban centers that support nearly 38 million people, and they are in close contact with one of North America's highest yield rice-growing regions (45). California also hosts the largest free-ranging chicken industry in the United States. This situation may be conducive to the spillover of

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AIV from waterfowl into sympatric populations of humans and domestic animals that occupy areas of mixed land use. Consequently, identifying the host traits that promote AIV infection in waterfowl may better inform surveillance efforts in this region. Our study examined whether attributes of Anatidae, including morphology (body mass, lamellar density, and bill length), foraging ecology (diet, guild, foraging microhabitat, and feeding method), and abundance (density in rice fields and midwinter abundance), influenced AIV prevalence and occurrence of subtypes. Samples were collected from birds harvested during the 2007–08 hunting season in California's Central Valley, coinciding with the congregation of a range of Anatidae species during the winter.

MATERIALS AND METHODS

Study sites. Study sites included the Sacramento National Wildlife Refuge (NWR) in Glenn County (39°24′31″N, 122°9′54″W) and Conaway Ranch Duck Club in Yolo County (38°38′52″N, 121°40′1″W), a public and private hunting ground, respectively. These two sites are situated 95 km apart and represent geographically different basins in the Sacramento Valley of California. Sacramento NWR consists of 4378 ha of seasonal marsh and permanent wetlands in the Butte Basin managed by the U.S. Fish and Wildlife Service. In contrast, Conaway Ranch is a 6978-ha privately-owned property consisting mainly of rice fields in a flooded bypass area of the Sacramento River. Both sites are managed to provide a range of microhabitats for overwintering waterfowl. The dominant land use surrounding both sites is rice fields that are flooded postharvest to speed straw decomposition, a practice that provides forage such as waste grain and benthic invertebrates for waterfowl (23). The mixture of rice agriculture and protected refuge sites within the Central Valley attracts up to 6 million waterfowl during winter (35).

The study period coincided with a drought year in the state of California. The average minimum and maximum temperatures at Sacramento NWR (recorded at the nearest weather station, Willows 049699) during the study period were 2.94 C and 25.34 C, respectively, and the average monthly rainfall was 79.95 mm (CalClim: www.calclim.dri.edu). At Conaway Ranch, the average minimum and maximum temperatures (recorded at Woodland 049781) during the study period were 4.08 C and 23.55 C, respectively, and the average monthly rainfall was 103.83 mm.

Sample collection. Waterfowl were collected for sampling from hunters when they exited the check stations at Sacramento NWR and Conaway Ranch. Sampling occurred up to three times per week, targeting days when hunting activity was highest (Wednesday, Saturday, and Sunday) between 20 October 2007 (start of the hunting season) and 27 January 2008 (close of the hunting season). This period coincided with the congregation of resident and migratory waterfowl during the winter. Sampling efforts were similar between Sacramento NWR (28 days) and Conaway Ranch (24 days). The species and sex of all birds were determined, and morphometric measurements were collected, including exposed culmen length, short tarsus, and flat wing length. Where possible, age was determined as either hatch year (HY) or after hatch year (AHY) on the basis of plumage. To collect AIV samples, a rayon-tipped swab (MicroPur^{TM}, PurFybr Inc., Munster, IN) was inserted into the cloaca of the bird. Oropharyngeal samples were collected by rolling the head of a swab across the top surface of the pharyngeal cavity. In both cases, the tip of the swab was removed and preserved in cryovial tubes (Remel Inc., Lenexa, KS) containing viral transport media. Samples were kept on ice for up to 8 hr before storage in a −80 C freezer prior to laboratory analysis.

Laboratory analysis. Samples were screened for AIV by virus isolation in embryonating chicken eggs (ECE) followed by real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) of the allantoic fluids using previously published methods (38,41). In brief, each sample was inoculated into the allantoic cavity of 9- to 11-day-old ECEs and incubated at 37.5 C for 6 days or until embryo death, as detected by daily candling. Allantoic fluid (AF) from eggs with live embryos was tested for hemagglutinating activity with chicken erythrocytes following standard methods (41). RNA was extracted from AF harvested from all dead embryos and the hemagglutinating AF from live embryos using the MagMAX-96 Viral Isolation Kit (Ambion Inc. Austin, TX). RNA was screened for AIV with rRT-PCR targeting the matrix gene (38). PCR assays were run on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Genetic subtyping was performed by characterizing the hemagglutinin (HA) and neuraminidase (NA) gene using rRT-PCR, with universal primers (19,30). Amplicons were purified with clean-up columns (Millipore, Bedford, MA) and submitted for sequencing (Davis Sequencing, Inc. Davis, CA). Sequences were aligned (Invitrogen VectorNTI) and then compared with previously described isolates in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) to determine subtype.

Ecologic traits. Information on guild, diet, foraging microhabitat, and feeding method in the winter was verified from the compilation of references available in the Birds of North America database (http://bna.birds.cornell.edu/bna). For species of dabbling duck, the length of the bill and density of lamellae (or filtering plates) on the bill were based on Nearctic specimens reported in Nudds *et al.* (27). Body mass for each species was based on data reported for adult males in Bellrose (1), because this sex class accounted for the largest proportion of harvested birds. The density of birds in rice fields was obtained from waterfowl surveys performed in California during 1993–95 by Elphick and Oring (11). Density in rice fields could not be used as an index of habitat selection because it may partially reflect abundance. Estimates of abundance for each bird species were obtained from the Californian midwinter waterfowl survey conducted each January by the U.S. Fish and Wildlife Service, and an average was calculated for 5 yr between 2005 and 2009 (44). The age and sex ratios of waterfowl species harvested from the Pacific Flyway were taken from U.S. Fish and Wildlife Service estimates and averaged over 5 yr between 2003 and 2007 (36). All ecologic traits of Anatidae were continuous variables placed on a gradient from low to high.

Statistic analysis. Prevalence was defined as the number of infected individuals expressed as a percentage of the population of interest (4). To determine whether AIV prevalence in the entire population (n = 3487) was affected by demographic factors (age and sex), Pearson chi-square tests were performed. To analyze the effects of population host traits on AIV, we aggregated the data by species and applied the following criterion: the sample size from each species must be large enough to account for the low prevalence of AIV, using a random sample of 200 required to detect AIV at a prevalence of 1.5% in the study population (42). Only six species met this criterion, all of which were dabbling ducks (*Anas* spp.). In addition, the small number of species (n = 6) precluded models of multiple host traits (3). Consequently, separate linear regressions were performed to assess whether lamellar density, bill length, midwinter abundance, body mass, and density in rice fields corresponded to AIV prevalence or the number of subtypes. The response variable, AIV prevalence, was arcsine-square-root transformed to adjust for the non-normally distributed percentage data (48). A P-value of $\leq$0.05 indicated a significant difference. All statistic analyses were conducted using SPSS version 16 software for Macintosh (SPSS Inc., Chicago, IL).

RESULTS

AIV prevalence. We sampled 3487 individuals at Sacramento NWR and Conaway Ranch during the hunting season of 2007–08 (Table 1). This represented 19 species: eight species of dabbling ducks, six species of diving ducks, and five species of geese. In total, 54 birds were positive for AIV, equating to an overall prevalence of 1.55%. The species in which AIV prevalence was highest was the northern shoveler (5.09%), a consistent trend at both Sacramento NWR (5.08%) and Conaway Ranch (5.26%). The ring-necked

Table 1. AIV occurrence and sampling effort for 19 Anatidae species wintering at Sacramento National Wildlife Refuge and Conaway Ranch Duck Club in 2007–08. Biases in the age and sex ratio of harvested birds from the Pacific Flyway (based on a 5-yr average) are also shown.

Species	AIV prevalence (%)	AIV positive	Sampled	No. of subtypes	Harvested bird age bias (juvenile/adult)	Harvested bird sex bias (male/female)[B]
Northern shoveler (*Anas clypeata*)[A]	5.09	28	550	12	1.43	1.67
Ring-necked duck (*Aythya collaris*)	2.63	3	114	1	1.58	1.66
American wigeon (*Anas americana*)[A]	2.57	9	350	3	1.42	1.59
Bufflehead (*Bucephala albeola*)	2.50	1	40	1	1.02	1.58
Greater white-fronted goose (*Anser albifrons*)	2.44	2	82	2	0.79	–
Cinnamon teal (*Anas cyanoptera*)	1.72	1	58	1	1.32	1.38
Northern pintail (*Anas acuta*)[A]	0.84	3	356	2	1.01	2.78
American green-winged teal (*Anas crecca*)[A]	0.64	3	468	3	1.37	1.62
Gadwall (*Anas strepera*)[A]	0.51	2	390	1	1.21	1.67
Mallard (*Anas platyrhynchos*)[A]	0.23	2	867	1	1.59	2.33
Snow goose (*Chen caerulescens*)	0.00	0	55	0	0.81	–
Canvasback (*Aythya valisineria*)	0.00	0	44	0	1.44	1.19
Lesser scaup (*Aythya affinis*)	0.00	0	36	0	1.71	1.55
Ruddy duck (*Oxyura jamaicensis*)	0.00	0	30	0	1.26	–
Ross's goose (*Chen rossii*)	0.00	0	14	0	0.70	–
Cackling goose (*Branta hutchinsii*)	0.00	0	11	0	0.54	–
Wood duck (*Aix sponsa*)	0.00	0	9	0	1.91	1.56
Canada goose (*Branta canadensis*)	0.00	0	7	0	0.54	–
Common goldeneye (*Bucephala clangula*)	0.00	0	6	0	0.81	1.59
Total	–	54	3487	–	–	

[A]Species for which AIV detection is accurate based on an estimated prevalence of 1.5% (i.e., >200 samples collected).
[B]Ratio is not shown if based on a sample of less than 20.

duck (2.63%), American wigeon (2.57%), bufflehead (2.50%), greater white-fronted goose (2.44%), and cinnamon teal (1.72%) also had prevalence rates higher than the overall mean (Table 1).

A significant age bias was detected in the prevalence of AIV ($\chi^2 = 16.12$, $n = 2083$, $P < 0.001$). Of the subset of birds for which age could be determined by plumage ($n = 2083$), hatch-year birds had a higher rate of infection (3.93%) compared to after-hatch-year birds (1.17%). No significant sex bias in AIV prevalence was observed ($\chi^2 = 0.29$, $n = 3487$, $P = 0.87$).

The abundance of dabbling duck species in California ($R^2 = 0.08$, $n = 6$, $P = 0.58$), their density in rice fields ($R^2 = 0.01$, $n = 6$, $P = 0.84$), and their body mass ($R^2 = 0.29$, $n = 6$, $P = 0.55$) were not significantly related to AIV prevalence. Their bill length was not significantly related to AIV prevalence ($R^2 = 0.54$, $n = 6$, $P = 0.16$); however, the lamellar density showed a significant positive relationship with AIV prevalence ($R^2 = 0.79$, $n = 6$, $P = 0.03$). The northern shoveler, the dabbling species with the most densely spaced lamellae, had the highest prevalence (5.09%), compared to mallards (0.23%), which had coarsely spaced lamellae.

AIV subtypes. Nine of the 16 known HA subtypes were detected during the study (H2, H3, H4, H5, H6, H7, H8, H10, H11). In addition, eight of the nine known NA subtypes were detected (N1, N2, N3, N4, N5, N7, N8, N9). The most common subtype, H6N1, was shed by 29.6% of infected birds, while the next most common subtype, H10N7, was shed by 9.3% of infected birds (Fig. 1). HPAI H5N1 was not detected, and all subtypes were characterized as low pathogenic.

Similar to results for AIV prevalence, the abundance of dabbling duck species in California ($R^2 = 0.06$, $n = 6$, $P = 0.63$), their density in rice fields ($R^2 = 0.49$, $n = 6$, $P = 0.68$), and their body mass ($R^2 = 0.16$, $n = 6$, $P = 0.43$) were not significantly related to the number of subtypes shed. Bill length was not significantly related to the number of subtypes shed ($R^2 = 0.20$, $n = 6$, $P < 0.21$); however, the lamellar density showed a significant positive relationship with the number of subtypes ($R^2 = 0.89$, $n = 6$, $P < 0.01$). The dabbling species with the most densely spaced lamellae, the northern shoveler, shed the highest number of subtypes (12 of 19) compared to the mallard (1 of 19), which had coarsely spaced lamellae (Fig. 1).

DISCUSSION

Our findings provide preliminary information on the relationship between the occurrence of AIV and ecologic traits of Anatidae. For a small subset of dabbling ducks, AIV prevalence and the number of subtypes shed were positively associated with the density of their lamellae. Lamellae are plate-like structures on the side of the bill that are important in retaining food particles during filtration. In our study, the northern shoveler, the dabbling species with the most densely spaced lamellae, demonstrated the highest rate of AIV infection (5.09%) and shed the largest number of subtypes (12 of 19), compared to the low infection rate (0.23%) and number of subtypes (1 of 19) of the mallard, which has coarsely spaced lamellae (Table 2). Studies have shown that finely spaced lamellae enhance water filtration rates (22) and intake of small invertebrates (16) among dabbling ducks. Superior efficiency at filtering surface water may therefore increase the exposure of the northern shoveler to AIV particles during foraging. In addition, as filtration is the preferred foraging method of the northern shoveler, it spends a longer time with the bill submerged than other dabbling ducks that rely on pecking at surface waters to acquire food (10). Long bouts with the bill submerged in surface waters may inadvertently maximize opportunities for contact with AIV. Although based on a small number of dabbling species ($n = 6$ species) sampled over a single winter season ($n = 4$ mo), our findings provide preliminary evidence that ducks equipped for efficient filtration may be at higher risk of ingesting AIV particles.

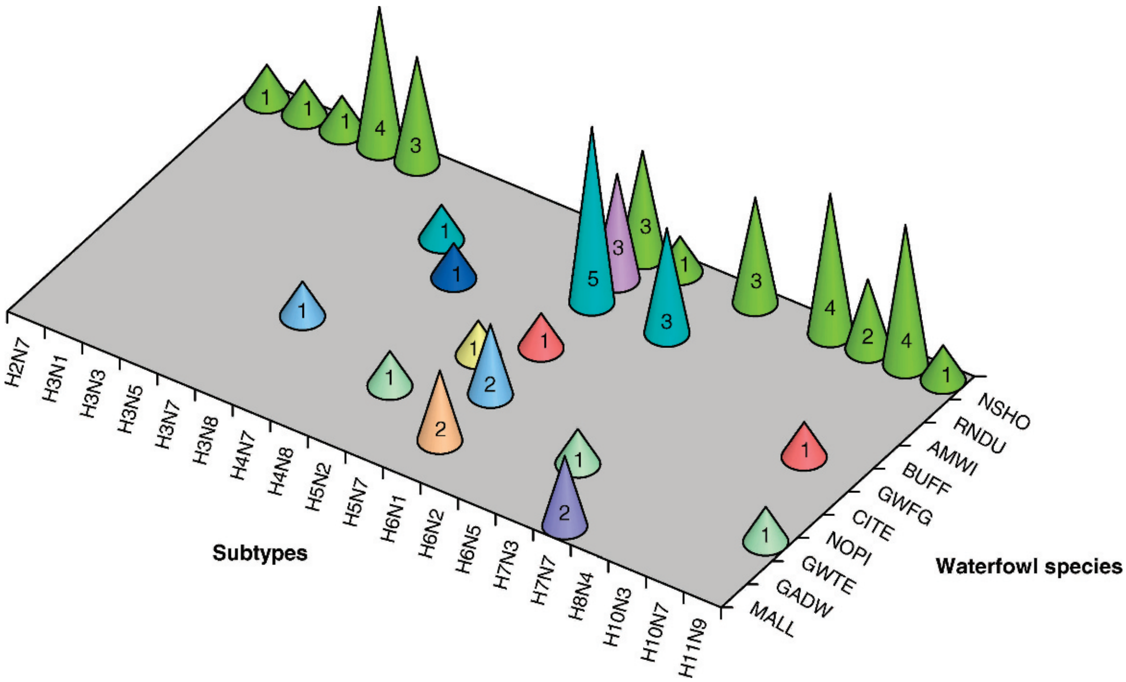

Fig. 1. Distribution of the 54 AIV-positive samples according to subtype and Anatidae species. Species are organized in descending order of prevalence from right to left, including, northern shoveler (NOSH), ring-necked duck (RNDU), American wigeon (AMWI), bufflehead (BUFF), greater white-fronted goose (GWFG), cinnamon teal (CITE), northern pintail (NOPI), green-winged teal (GWTE), gadwall (GADW), and mallard (MALL).

The emergence of the northern shoveler as an important host in this study may reflect the large number of samples collected for this species ($n = 550$) compared to earlier studies that may have had sample sizes too small to accurately detect AIV. This species is rarely a focus of AIV surveillance, in contrast to the northern pintail and mallard, for which sampling effort is considerably higher in the Pacific Flyway (29,37). Our findings highlight that uneven sampling effort across species may mask the contribution of different waterfowl hosts to the epidemiology of AIV. A sufficient sample size is especially pertinent at wintering grounds where AIV infection rates reach low levels in waterfowl populations, compared to the elevated prevalence rates observed at breeding sites (15). Our study population proved large enough to detect the influence of age on the prevalence of AIV, which showed a higher number of hatch-year birds infected compared to after-hatch-year birds. Consistent with previous studies, our findings imply that young birds lacked the immunity needed to limit infection, while prior exposure may have allowed adults infected with the virus to shed for shorter durations and at lower titers (20).

Among the 19 subtypes detected, H6N1 demonstrated the broadest host range, infecting six of the ten waterfowl species that were positive for AIV. This indiscriminate infection of species suggests that a wide variety of waterfowl in the Central Valley may be involved in the perpetuation of this AIV subtype. Previous studies have identified interspecific transmission of AIV as most likely to occur at common feeding or breeding grounds, due to the congregation of a diversity of species (7,17,18). In the Central Valley during the winter, flooded rice fields provide forage such as waste grain and benthic invertebrates (23), attracting over 57 species of waterbirds, including geese, ducks, herons, ibises, rails, shorebirds, grebes, and pelicans (9). We therefore speculate that the concentration of waterbird species in postharvest flooded rice fields may facilitate the spread of AIV subtypes, including H6N1. In view of the importance of rice paddies in the epidemiology of HPAI H5N1 in Asia (13,25), the potential for rice fields to promote AIV infection of waterfowl in California warrants further study.

As with most bird sampling methods, hunter harvesting is associated with numerous biases that interfere with the ability to collect samples representative of the underlying population. Biases are implicit with hunting methods because juveniles are more vulnerable to being shot (8,28), and species that make better "table birds" for consumption (i.e., northern pintail and mallard) are preferentially harvested. In addition, hunting regulations place limits on the number of females that can be shot, resulting in a sex ratio biased toward males, particularly for mallard and northern pintail (36). However, biases associated with sampling harvested birds are, we believe, outweighed by the benefits. Hunting is an efficient method to collect samples due to the large number of birds yielded during a popular weekend of the hunting season. For example, 431 birds were shot by 120 hunters at Sacramento NWR on a single Saturday during December 2007 (43). Sampling harvested birds also eliminates the expense of capture, which can be a labor-intensive exercise and requires materials for trapping and baiting. Furthermore, hunting has assisted with the detection of H5N1 in wild birds from Eurasian countries where the practice is commonplace, including Germany (34), Greece (33), Italy (32), and Russia (31). Consequently, although care should be taken to recognize bias associated with this method, hunter-harvested waterfowl provide a low-cost, high-yield source of samples from wild birds.

Finally, other factors unrelated to ecologic traits may have been responsible for the observed difference in prevalence and number of subtypes hosted by each species. For example, pathologic response to AIV may differ between hosts such that infection causes longer bouts of shedding and higher viral titer in the northern shoveler compared to the mallard. This may have influenced the detection of virus from samples and consequently the prevalence and number of subtypes reported. Experimental studies have demonstrated that the mallard can remain asymptomatic upon HPAI H5N1 infection, excreting

Table 2. AIV prevalence and ecologic traits of six dabbling duck species sampled in 2007–08.

Species	AIV prevalence (%)	No. of subtypes	Guild	Diet	Foraging microhabitat	Lamellar density (no./cm)[A,B]	Bill length (cm)	Feeding method	Density in rice fields (no./km^2)	Mass of adult males (g)	Midwinter abundance in California
Northern shoveler	5.09	12	Dabbler	Omnivore: invertebrates, seeds	Offshore with no emergent vegetation	21.5	4.8	Filtering, churning up sediments, and social foraging	160	680	628,400
American wigeon	2.57	3	Dabbler	Herbivore: land and aquatic plant parts	On land, marshes, shallow wetlands	11.0	2.6	Grazing on land	58	821	536,500
Northern pintail	0.84	2	Dabbler	Omnivore: aquatic plant seeds, invertebrates	Shallow wetlands	10.1	3.8	Filtering/pecking at surface	252	1025	1,259,300
American green-winged teal	0.64	3	Dabbler	Omnivore: seeds, aquatic vegetation, invertebrates	Shallow wetlands	13.3	2.8	Filtering/pecking at surface	184	322	471,600
Gadwall	0.51	1	Dabbler	Omnivore: seeds, aquatic vegetation, invertebrates	Shallow to deep wetlands with submerged vegetation	12.2	3.4	Filtering/pecking at surface	13	966	171,300
Mallard	0.23	1	Dabbler	Omnivore: seeds, acorns, aquatic vegetation, invertebrates	Shallow wetlands with tall, dense vegetation	8.0	4.1	Filtering/pecking at surface	119	1247	357,500

[A]Indicates an ecologic trait significantly related to AIV prevalence in Anatidae ($P < 0.05$).
[B]Indicates an ecologic trait significantly related to the number of subtypes shed by Anatidae ($P < 0.05$).

virus for shorter durations and at lower viral load compared to other dabbling ducks (2,21). Similar host challenge trials have not been performed for the northern shoveler but may provide insights into the role that host pathobiology plays in the detection of AIV during surveillance studies. Further studies are also needed to account for the spatial and temporal variation in waterfowl infection patterns, a hallmark of AIV (17) that influences differences in circulating subtypes, prevalence rates, and species infected observed between years and sampling locations. Continued sampling of waterfowl populations at both the wintering and breeding grounds within the Pacific Flyway will help to ascertain whether ecologic host traits may prove useful for the purpose of delimiting species for surveillance.

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