DOI: 10.7589/2011-02-052

*Journal of Wildlife Diseases*, 48(4), 2012, pp. 910–917


# AVIAN INFLUENZA INFECTIONS IN NONMIGRANT LAND BIRDS IN ANDEAN PERU

**Richard A. J. Williams,[1,2,6] Karen Segovia-Hinostroza,[3] Bruno M. Ghersi,[3,4] Victor Gonzaga,[4] A. Townsend Peterson,[1] and Joel M. Montgomery[4,5]**

[1] Biodiversity Institute, 1345 Jayhawk Blvd., University of Kansas, Lawrence, Kansas 66045, USA
[2] Departamento de Zoología y Antropología Física, Universidad Complutense de Madrid, C/José Antonio Novais, 2 Ciudad Universitaria, 28040 Madrid, Spain
[3] Facultad de Mecicina Veterinaria de Universidad Nacional Mayor de San Marcos, Av. Circunvalación Cdra. 28 San Borja, Lima, Peru
[4] United States Naval Medical Research Center Detachment, Unit 6, Av. Venezuela Cdra. 36, Callao 2, Lima, Peru
[5] Current address: International Emerging Infections Program, GDDER-KenyaCDC-KenyaMbagathi Rd, Off Mbagathi Way, PO Box 606, Village Market 00621, Nairobi, Kenya
[6] Corresponding author (email: ricw.ku@gmail.com)

ABSTRACT: As part of ongoing surveillance for avian influenza viruses (AIV) in Peruvian birds, in June 2008, we sampled 600 land birds of 177 species, using real-time reverse-transcription PCR. We addressed the assumption that AIV prevalence is low or nil among land birds, a hypothesis that was not supported by the results—rather, we found AIV infections at relatively high prevalences in birds of the orders Apodiformes (hummingbirds) and Passeriformes (songbirds). Surveillance programs for monitoring spread and identification of AIV should thus not focus solely on water birds.

*Key words:* Avian influenza viruses, ecology, hummingbirds, land birds, Peru.

## INTRODUCTION

The emergence and hemispheric spread of highly pathogenic avian influenza (HPAI) H5N1 over the past 13 yr led to renewed surveillance for avian influenza viruses (AIV) and study of influenza ecology. Wild birds, particularly of the aquatic bird orders, Anseriformes (ducks and geese) and Charadriiformes (shorebirds, gulls, and terns), are widely considered to be AIV reservoirs, particularly for low-pathogenic strains (Swayne and Suarez, 2000). As a result, surveillance for AIV (and particularly for HPAI H5N1) has emphasized surveillance in water birds (DeLiberto et al., 2009; Hesterberg et al., 2009). Despite the apparent association with water birds, however, AIV (including HPAI H5N1) have been detected more broadly across class Aves (Peterson et al., 2008; Hesterberg et al., 2009; Cumming et al., 2012; Thinh et al., 2012), although some studies have shown 0% prevalence in land birds (Munster et al., 2007). Nonetheless, known HPAI H5N1 hosts are diverse, and land birds may play an important transmission role that is poorly characterized (Kou et al., 2009).

Given the limited surveillance of South American wild birds, information on AIV host distribution and genotypes there is sparse (Spackman et al., 2007). To date, AIV has been detected in wild birds in Argentina (Pereda et al., 2008; Alvarez et al., 2010), Bolivia (Spackman et al., 2007), Brazil (Senne, 2007), Chile (Suarez et al., 2004), Peru (Ghersi et al., 2009), and the Caribbean (Douglas et al., 2007). Surveys in North America have detected AIV in birds that migrate between North and South America (Hanson et al., 2008; Dusek et al., 2009). Highly pathogenic influenza has been detected once in South America: H7N3 in poultry at Los Lagos, Chile (Suarez et al., 2004). Highly pathogenic avian influenza H5N1 has not been detected in the Western Hemisphere.

We conducted broad-scale influenza surveillance among land birds to assess the distribution of AIV strains among land birds in Peru. Our goal was to produce a baseline understanding of AIV prevalence before the annual influx of boreal migrant species and any AIV that they might introduce into the environment. Ours is the first broad-scale analysis of AIV distribution in South American land birds; to our knowledge, we document the first detection of influenza in hummingbirds (family

Trochilidae), and indeed in all avian host species found positive in this study.

## METHODS

### Field methods

In June 2008, we sampled birds from two, mostly forested, Andean sites in Ayacucho Department, Peru, within 3 km of the towns of Ccano (12.785°S, 73.995°W) and Tutumbaro (12.733°S, 73.956°W), at elevations of 2,800–3,100 m and 1,800–2,000 m, respectively (Fig. 1). Ccano is best characterized as covered by anthropogenically modified, high-elevation cloud forest near treeline, whereas Tutumbaro presents mid-elevation cloud forest along a rushing Andean river. The two sites were separated by approximately 10 km of horizontal distance (considerably more by road). Sampling was conducted by mist netting and selective harvesting with shotguns; all individuals were apparently healthy when sampled.

All birds sampled were euthanized before sampling, following protocols approved by the University of Kansas Institutional Animal Care and Use Committee. Voucher specimens were prepared following standard procedures for the identification of each sample and to document host sex, age, and condition. Voucher specimens were deposited at the University of Kansas Natural History Museum, Lawrence, Kansas, USA and Centro de Ornitologia y Biodiversidad (CORBIDI), Lima, Peru. Ages of host individuals were determined by plumage characteristics, skull ossification, and dissection to determine presence or absence of a bursa of Fabricius.

All tissue sampling for virus testing was done in field camps, to minimize capture-to-processing time intervals; live-captured birds were maintained in cloth bags under cool conditions, and all were euthanized within 1 hr of capture. Oral pharyngeal and cloacal swabs were collected by inserting a sterile cotton swab into the body orifice and moving it across all surfaces inside. In general, the entire lung was collected,

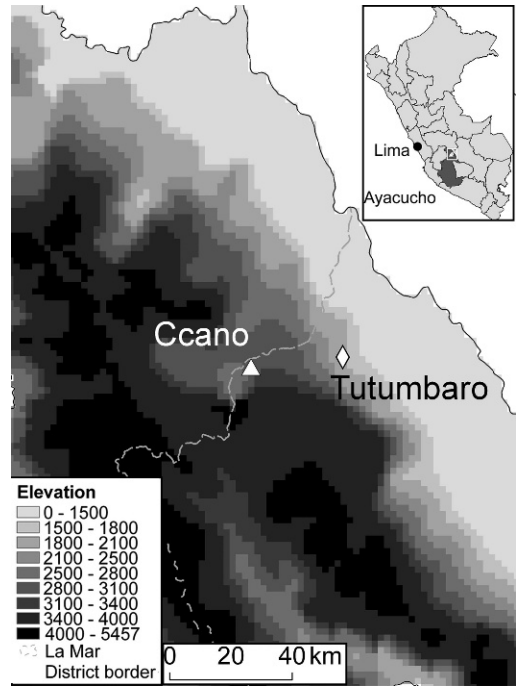


FIGURE 1. Northern Ayacucho, Peru, showing the two Andean sites from which land birds were collected and tested for influenza A virus infections. Prevalences were 1.5% ($n$=265) in Ccano (triangle): and 1.2% ($n$=335) in Tutumbaro (diamond). Elevation is indicated by shading; low elevation (light gray) through to high elevation (dark gray)—see legend for detail. Ayacucho Department is highlighted in dark gray in the map of Peru (inset), and the Northern Ayacucho study site is marked (square).

although for larger birds, only a single lobe was preserved; for most birds, the entire intestine was sampled, although for larger birds, only the anterior portion was preserved. Swabs and tissue samples were collected immediately after euthanasia, frozen immediately in liquid $N_2$ in cryotubes without any buffers, and maintained at −80 C until testing; individuals collected by shotgun were prepared as soon as feasible postmortem, and kept as cool as feasible in the interim.

### RNA extraction and viral characterization

Each specimen type (oral pharyngeal and cloacal swabs, intestine and lung tissue samples) was pooled by species (maximum six individuals/pool). Viral RNA from intestine and lung samples was extracted on

dry ice, or directly from swabs using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, California, USA). Intestine and lung tissue were homogenized using a high-speed mechanical homogenizer (Mixer Mill MM300; Qiagen) for 1 min at 6,000 × G, and viral RNA was extracted from the supernatant using the RNEasy Mini Kit (Qiagen), with the minor modification that 10 μL of β-mercaptoethanol was added to 1 mL of buffer RLT before use.

All samples were tested for influenza Matrix gene by real-time reverse-transcription (rRT)-PCR using the US Centers for Disease Control and Prevention (CDC) protocol released on 6 October 2009 (revision 2) (www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html). Invitrogen SuperScript™III Platinum® One-Step Quantitative Kit (Invitrogen Inc., Carlsbad, California, USA) was used for nucleic acid amplification, with a 20-μL reaction mixture containing: 5.5 μL of nuclease-free water, 0.5 μL of each primer (20 nmol), probe, and kit-supplied RT-PCR superscript enzyme, 12.5 μL of kit-supplied RT-PCR master mix, and 5 μL of extracted viral RNA. The primers/probe for reverse transcription of viral RNA gene were: GAC CRA TCC TGT CAC CTC TGA C (forward); AGG GCA TTY TGG ACA AAK CGT CTA (reverse); FAM-TGC AGT CCT CGC TCA CTG GGC ACG-BHQ-1 (probe). The RRT-PCR was carried out in an ABI Prism 7500 machine (Applied Biosystems™, Foster City, California, USA), with the following conditions for the RT step (50 C for 30 min and 95 C for 2 min) and for the PCR amplification (95 C for 15 sec and 55 C for 30 sec for 45 cycles), collecting fluorescence data during the 55 C incubation step. Results were read before 40 cycles were completed. Pools with Ct (threshold cycles) values of $<40$ were considered positive, following the CDC protocol referenced above. Individual samples from positive pools were tested further to establish the identity of the positive individual using the same protocols as described above. Two positive controls were used for all rRT-PCR testing, one human positive control, supplied in the CDC kit, and the other obtained from a wild bird sample (Ghersi et al., 2009). Primers for internal control were unavailable.

Samples determined positive by rRT-PCR were processed for conventional PCR to amplify the matrix (M) and neuraminidase (NA) genes. The gene segments of AIV were amplified by RT-PCR (Hoffmann et al., 2001), using the primers described therein. Briefly, 4 μL of RNA were transcribed into complementary (c)DNA using avian myeloblastosis virus reverse transcriptase (Promega, Madison, Wisconsin, USA) following the manufacturer's protocol, and 500 ng of Uni12 primer in a 30-μL reaction mix. The RT reaction was performed at 42 C for 60 min; 1 μL of the RT reaction was used for each PCR reaction. The cDNA was amplified using the Expand High-Fidelity PCR system (Roche Diagnostics, Mannheim, Germany) following manufacturer's protocols. The final concentration of $Mg^{2+}$ ions was 1.5 mM; primer concentrations were 1.5 μM. The first cycle consisted of 4 min at 94 C, followed by 30 cycles with the following conditions: 94 C for 20 sec, 58 C for 30 sec, and 72 C for 7 min, and a final extension of 7 min at 72 C.

After amplification, 10 μL of each reaction mixture was analyzed by electrophoresis with a 2% agarose gel containing 0.5 μg/mL ethidium bromide in tris-borate-ethylenediaminetetra-acetic acid buffer gels. Products were visualized under UV light. A 1-kb DNA ladder was included on each gel. Bands from this amplification were purified using a QIAquick PCR Purification Kit (Qiagen) and directly sequenced with the BigDye 3.1 terminator kit (Applied Biosystems™) on an ABI 3730 (Applied Biosystems™).

We attempted viral isolation to obtain reference material, and to confirm the findings of rRT-PCR. Positive pools and all individual samples from positive pools (stored before RNA extraction) were

TABLE 1. *K* (number of positive individuals) of *N* (total number of individuals), for families and orders in which avian influenza virus (AIV) infections were detected. Prevalences are shown as percentages in parentheses. Overall prevalences are shown at bottom of the table. Eight individuals (from five families and two orders) were AIV positive from 600 individuals sampled. The detected AIV prevalence was zero in the remaining 31 families/12 orders of birds.

| Positive orders | Positive families | % Positive Ccano | % Positive Tutumbaro | % Positive total |
|---|---|---|---|---|
| Apodiformes | Trochilidae | 0/38 (0.00) | 3/37 (8.11) | 3/75 (4.00) |
| Passeriformes | All | 4/202 (1.98) | 1/257 (0.39) | 5/459 (1.09) |
| | Furnariidae | 1/20 (5.00) | 0/29 (0.00) | 1/49 (2.04) |
| | Parulidae | 1/10 (10.00) | 1/27 (3.70) | 2/37 (5.41) |
| | Thraupidae | 1/51 (1.96) | 0/52 (0.00) | 1/103 (0.97) |
| | Troglodytidae | 1/16 (6.25) | 0/10 (0.00) | 1/26 (3.85) |
| All: 14 orders | 36 families | 4/263 (1.52) | 4/337 (2.37) | 8/600 (1.33) |

centrifuged and filtered (Millex 0.45 μm) before inoculation of 0.2 mL into the allantoic cavity of four specific-pathogen-free, 9-day-old chicken embryos. Eggs were incubated for 6 days; survival was checked daily. Allantoic fluid of each egg was tested for hemagglutinating agents by a direct hemagglutination assay (Swayne et al., 1998). First-passage negative pools were repassaged twice for a total of three passages. The influenza sequence that was obtained (GenBank HQ420901) was compared with published sequences using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) to identify closely related strains; sequence alignments were carried out with ClustalX (Larkin et al., 2007) using default parameters.

### Statistical testing

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to measure associations between numbers of positive versus negative influenza cases in individuals and species of different avian orders and families (e.g., Apodiformes versus Passeriformes; taxonomy and general ecological classification follow Schulenberg et al., 2007). Relationships were tested using Pearson's chi-square tests or Fisher's exact tests when sample sizes were $<100$. Associations were considered statistically significant on the basis of an $\alpha<0.05$. For the purposes of statistical analysis, we made the conservative assumption that only one individual was positive in the two pools for which we were unable to determine the infected individual's identity. We developed analyses across all individuals and species so as to avoid dominant effects caused by large samples of single species in broader analyses. All statistical analyses were carried out using SigmaPlot 11.0 (Systat Software Inc., Chicago, Illinois, USA).

## RESULTS

Samples were collected from 600 individuals of 177 species (Table 1, Supplementary Table 1). Eight of 196 pools (4.1%) of all sampling types were positive by rRT-PCR (Table 1). The identity of six of the eight individuals that were PCR-positive for AIV was established; the identity of the positive individual within two pools could not be established (Table 2). Positive species were all from the orders Apodiformes (3 of 19 species; 15.8%; all Trochilidae) and Passeriformes (5 of 127 species; 3.9%); these orders constituted 10.7% and 76.3% of the species in the overall sample, respectively. Among Passeriformes, two positive pools were detected in the New World warbler family Parulidae (two of six species; 33.3%). Four positive pools were detected from each site. Five of 194 oral pharyngeal, one of 192 cloacal swabs, three of 194 lung, and zero of 191 intestine pools were positive. Only one sample from the Peruvian Wren (*Cinnycerthia peruana*) was

TABLE 2. Summary of avian influenza virus (AIV) reverse transcriptase (RT)-PCR-positive pool samples, showing type of sample; positive number of individuals per pool; if PCR positive, for M (matrix) or NA (neuraminidase, N1, N2, N4, N5, N8), and catalog number, if established. One 922-nucleotide M gene sequence (GenBank accession number HQ420901) was obtained from a woodcreeper (*Campylorhamphus pucherani*).

| Species | Sample type [a] | No. samples in RT-PCR positive pool | PCR-positive gene | KUNHM[b]/CORBIDI[c] catalog number |
|---|---|---|---|---|
| Apodiformes | | | | |
| *Chaetocercus mulsant* | C | 1 | | CORBIDI uncatalogued[d] |
| *Chlorostilbon mellisugus* | O | 1 | | CORBIDI uncatalogued[d] |
| *Coeligena coeligena* | O | 4 | | Not established |
| Passeriformes | | | | |
| *Anisognathus lacrymosus* | L | 1 | NA | KU 112795 |
| *Basileuterus coronatus* | O | 5 | M | CORBIDI uncatalogued[d] |
| *Basileuterus luteoviridis* | O | 6 | | Not established |
| *Campylorhamphus pucherani* | L | 1 | M, NA | KU 112898 |
| *Cinnycerthia peruana* | O, L | 4 | M | CORBIDI uncatalogued[d] |

[a] C = cloacal swab; L = lung; O = oropharyngeal swab; no intestine samples were positive.

[b] Kansas University Natural History Museum.

[c] Centro de Ornitología y Biodiversidad.

[d] The identity of the individual was established, and the specimen is awaiting incorporation into the CORBIDI collection.

positive for more than one sample type (oral pharyngeal swab and lung). All sample types (i.e., lung, intestine, swabs) were tested from all positive pools, with the exception of the Blue-tailed Emerald, a hummingbird (*Chlorostilbon mellisugus*), for which insufficient lung was available for testing.

We found a significant association (Supplementary Table 2) of AIV prevalence and host order, with Apodiformes (hummingbirds; 3 of 19 species and 75 individuals) more prone to AIV infection than all other individuals (5 of 158 species and 525 individuals; OR 5.7; 95% CI 1.4–24.1; $P=0.012$; OR 4.3; 95% CI 1.1–16.8; $P=0.031$; for species and individuals, respectively). Families Parulidae (New World warblers) and Trochilidae (hummingbirds) showed prevalences statistically significantly higher than expected as compared with other families.

No positive species belonged to bird groups particularly associated with water (e.g., ducks, kingfishers, or shorebirds); five species sampled that are associated with water tested negative for influenza among samples available. Prevalence among nectarivorous species was significantly higher than in nonnectarivores (OR 3.4; 95% CI 0.9–13.7; $P=0.045$). We did not detect significant differences in influenza prevalences between open-country (1.7%) and forest (1.2%) species (cumulative binomial probability, $P=0.312$). We detected no differences in AIV prevalence among sites (cumulative binomial probability, $P=0.718$).

Three individuals from rRT-PCR-positive pools were positive for the AIV matrix gene (using primer pair Bm-M-1/Bm-M-1027R; Table 2), and one 922-nucleotide sequence (GenBank HQ420901) was obtained, from the Greater Scythebill (*Campylorhamphus pucherani*; Passeriformes; Dendrocolaptidae). Two individuals from rRT-positive pools were positive for NA (using primer pair Bm-NA-1/Bm-NA-1413R, which is designed to detect NA strains 1, 2, 4, 5, and 8). We found no evidence for HPAI H5N1 presence, though we were unable to fully characterize strains. No samples were positive by the hemagglutination assay, and none showed obvious cytopathic effects in egg culture.

The BLAST searches revealed that our influenza strain shared >97% identity with the 100 most similar influenza sequences. Maximum identity was found to M sequences from H10N7 serotypes; however, at least 22 influenza serotypes were found among the 100 most similar. The 100 most closely related sequences were detected in at least three bird orders, but all were from North America.

## DISCUSSION

This is the first broad survey for influenza distributions in land birds in South America. We are not aware of previous detection of AIV in any of the influenza-positive species we identified. We also describe the first AIV detection in hummingbirds (Apodiformes: Trochilidae) to the best of our knowledge; all hummingbirds are at least in large part nectarivorous. It is noteworthy that prevalence was higher among hummingbird taxa than in other bird groups. Nectarivory is a plausible transmission route for respiratory diseases. However, as other nectarivores were few in the communities sampled, the high prevalence among hummingbirds may be explained by factors unrelated to feeding type. We should interpret these results tentatively given small sample sizes.

Consideration of natural history characteristics suggests that influenza infections are broadly distributed with regard to ecological characteristics of host species. For instance, influenza infection prevalence was higher in Parulidae and Trochilidae than in other families. Parulidae are highly social, insectivorous species that generally travel in mixed flocks; in our sample, all forest birds. By contrast, Trochilidae are solitary, aggressively territorial, largely nectarivorous, and (in our sample) mixed in use of forest and more open habitats. We did not detect significant differences between influenza prevalences in open-country versus forest species, though we note that the information on habitat use by species reported (Schulenberg et al., 2007) is an average for that species and may introduce uncertainty.

Overall prevalence in Ayacucho (1.3%) was more than triple that documented recently in Peruvian water birds (0.4%) tested in the Lima region of Peru (Ghersi et al., 2009). Higher detection rates may result from differences in field collection protocols (swabs and tissue versus fecal samples), laboratory detection methods (viral isolation versus PCR protocols), or epidemiologic aspects (e.g., temporal variation in prevalence is usually reported in infected ducks), rather than reflecting higher prevalences in land birds than in water birds. Ayacucho prevalence was nonetheless lower than the 2.3% found in southern Chinese birds, and somewhat lower than the prevalence in nonmigratory birds in that study (1.8%; Peterson et al., 2008). Our prevalence in Passeriformes (1.1%) is lower than the prevalence found in Anseriformes (3.0%), somewhat higher than that in Charadriiformes (0.8%), and 10 times higher than that in Passeriformes (0.1%) in a recent European study (Hesterberg et al., 2009), and considerably lower than prevalences found in a water-bird-dominated surveillance in North America (9.7% and 11% in 2007 and 2008, respectively; DeLiberto et al., 2009). However, our findings and others suggest that land birds may play some role in hosting AIV and merits further study (Peterson et al., 2008; Hesterberg et al., 2009).

We detected AIV presence more frequently in respiratory samples—oral pharyngeal swabs ($n=5$) and lung ($n=3$) than in gastrointestinal samples—cloacal swab ($n=1$) and intestine ($n=0$). Only one sample (from *Cinnycerthia peruana*) was positive in more than one sample type (oral pharyngeal swab and lung). The reasons for these differential detections may be related to presence of inhibitors, particularly in the fecal material in cloacal and intestinal samples (Suarez et al. 2007), or because infection was localized to single tissue. In the latter case, oral pharyngeal sampling (note the higher detection rates in tissue

samples related to respiratory function) for AIV may result in higher detection rates, although tissue specificity and localization may vary among AIV types (Mo et al. 1997; Gu et al. 2007; van Riel et al. 2007). Regardless of the cause, this result indicates the need for broader sampling of more diverse tissues from birds for testing for AIV.

## ACKNOWLEDGMENTS

We thank ornithologists Michael Andersen, Roger Boyd, Adolfo Navarro-Sigüenza, Luis Sánchez-González, and Mark Robbins; the staff of CORBIDI, Lima, Peru, for extensive assistance with logistics associated with field sampling of birds; M. Papeş for assistance with maps; and A. E. González for extending facilities and hospitality at the School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos. The study was funded by the National Institutes of Health–Fogarty International Center, US Department of Defense AFHSC-GEIS grant GF0121_07_LI, and R.A.J.W. was supported by grant CGL2010-15734/BOS, Ministerio de Ciencia e Innovación, Spain, and a Tinker Field Research grant.

## LITERATURE CITED

Alvarez, P., R. Mattiello, P. Rivailler, A. Pereda, C. T. Davis, L. Boado, E. D'ambrosio, S. Aguirre, C. Espinosa, J. La Torre, R. Donis, and N. Mattion. 2010. First isolation of an H1N1 avian influenza virus from wild terrestrial non-migratory birds in Argentina. Virology 396: 76–84.

Birdlife International. Datazone: species accounts 2009. http://www.birdlife.org. Accessed October 2009.

Cumming, G. S., A. Caron, C. Abolnik, G. Cattoli, L. W. Bruinzeel, C. E. Burger, K. Cecchettin, N. Chiweshe, B. Mochotlhoane, G. L. Mutumi, and M. Ndlovu. 2011. The ecology of Influenza A viruses in wild birds in southern Africa. Ecohealth 8: 4–13.

DeLiberto, T. J., S. R. Swafford, D. L. Nolte, K. Pedersen, M. W. Lutman, B. B. Schmit, J. A. Baroch, D. J. Kohler, and A. Franklin. 2009. Surveillance for highly pathogenic avian influenza in wild birds in the USA. Integrative Zoology 4: 426–439.

Douglas, K. O., M. C. Lavoie, L. M. Kim, C. L. Afonso, and D. L. Suarez. 2007. Isolation and genetic characterization of avian influenza viruses and a Newcastle Disease virus from wild birds in Barbados: 2003–2004. Avian Diseases 51: 781–787.

Dusek, R. J., J. B. Bortner, T. J. DeLiberto, J. Hoskins, J. C. Franson, B. D. Bales, D. Yparraguirre, S. R. Swafford, and H. S. Ip. 2009. Surveillance for high-pathogenicity avian influenza virus in wild birds in the Pacific Flyway of the United States, 2006–2007. Avian Diseases 53: 222–230.

Ghersi, B. M., D. L. Blazes, E. Icochea, R. I. Gonzalez, T. Kochel, Y. Tinoco, M. M. Sovero, S. Lindstrom, B. Shu, A. Klimov, A. E. Gonzalez, and J. M. Montgomery. 2009. Avian influenza in wild birds, central coast of Peru. Emerging Infectious Diseases 15: 935–938.

Gu, J., Z. G. Xie, Z. C. Gao, J. H. Liu, C. Kortweg, J. X. Ye, L. T. Lau, J. Lu, Z. F. Gao, B. Zhang, M. A. Mcnutt, M. Lu, V. M. Anderson, E. C. Gong, A. C. H. Yu, and W. I. Lipkin. 2007. H5N1 infection of the respiratory tract and beyond: A molecular pathology study. Lancet 370: 1137–1145.

Hanson, B. A., M. P. Luttrell, V. H. Goekjian, L. Niles, D. E. Swayne, D. A. Senne, and D. E. Stallknecht. 2008. Is the occurrence of avian influenza virus in Charadriiformes species location dependent? Journal of Wildlife Diseases 44: 351–361.

Hesterberg, U., K. Harris, D. Stroud, V. Guberti, L. Busani, M. Pittman, V. Piazza, A. Cook, and I. Brown. 2009. Avian influenza surveillance in wild birds in the European Union in 2006. Influenza and Other Respiratory Viruses 3: 1–14.

Hoffmann, E., J. Stech, Y. Guan, R. G. Webster, and D. R. Perez. 2001. Universal primer set for the full-length amplification of all influenza A viruses. Archives of Virology 146: 2275–2289.

Holder, M., and P. O. Lewis. 2003. Phylogeny estimation: Traditional and Bayesian approaches. Nature Reviews Genetics 4: 275–284.

Kou, Z., Y. D. Li, Z. H. Yin, S. Guo, M. L. Wang, X. B. Gao, P. Li, L. J. Tang, P. Jiang, Z. Luo, Z. Xin, C. Ding, Y. B. He, Z. Y. Ren, P. Cui, H. F. Zhao, Z. Zhang, S. A. Tang, B. P. Yan, F. M. Lei, and T. X. Li. 2009. The survey of H5N1 flu virus in wild birds in 14 provinces of China from 2004 to 2007. Plos One 4: e-6926.

Larkin, M. A., G. Blackfields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. C. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.

Mo, I. P., M. Brugh, O. J. Fletcher, G. N. Rowland, and D. E. Swayne. 1997. Comparative pathology of chickens experimentally inoculated with avian influenza viruses of low and high pathogenicity. Avian Diseases 41: 125–136.

Munster, V. J., C. Baas, P. Lexmond, J. Waldenström, A. Wallensten, T. Fransson, G. F. Rimmelzwaan, W. E. Beyer, M. Schutten, B. Olsen, A. D. Osterhaus, and R. A. Fouchier.

2007. Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. PLoS Pathogens 3: e61.

Pereda, A. J., M. Uhart, A. A. Perez, M. E. Zaccagnini, L. La Sala, J. Decarre, A. Goijman, L. Solari, R. Suarez, M. I. Craig, A. Vagnozzi, A. Rimondi, G. Konig, M. V. Terrera, A. Kaloghlian, H. C. Song, E. M. Sorrell, and D. R. Perez. 2008. Avian influenza virus isolated in wild waterfowl in Argentina: Evidence of a potentially unique phylogenetic lineage in South America. Virology 378: 363–370.

Peterson, A., S. Bush, E. Spackman, D. Swayne, and H. Ip. 2008. Influenza A virus infections in land birds, People's Republic of China. Emerging Infectious Diseases 4: 1644–1646.

Schulenberg, T. S., D. F. Douglas, F. Stotz, D. F. Daniel, F. Lane, J. P. John, P. O'Neill, and T. P. Parker. 2007. Birds of Peru. Christopher Helm, London, UK, 656 pp.

Senne, D. A. 2007. Avian influenza in North and South America, 2002–2005. Avian Diseases 51: 167–173.

Spackman, E., K. G. McCracken, K. Winker, and D. E. Swayne. 2007. An avian influenza virus from waterfowl in South America contains genes from North American avian and equine lineages. Avian Diseases 51: 273–274.

Suarez, D. L., D. A. Senne, J. Banks, I. H. Brown, S. C. Essen, C. W. Lee, R. J. Manvell, C. Mathieu-Benson, V. Moreno, J. C. Pedersen, B. Panigrahy, H. Rojas, E. Spackman, and D. J. Alexander. 2004. Recombination resulting in virulence shift in avian influenza outbreak, Chile. Emerging Infectious Diseases 10: 693–699.

———, A. Das, and E. Ellis. 2007. Review of rapid molecular diagnostic tools for avian influenza virus. Avian Diseases 51S: 201–208.

Swayne, D., D. Senne, and C. Beard. 1998. Avian influenza. *In* A laboratory manual for the isolation and identification of avian pathogens, D. Swayne, J. Glisson and M. Jackwood (eds.). International Book Distributing Co., Kennett Square, Pennsylvania, pp. 150–155.

Swayne, D. E., and D. L. Suarez. 2000. Highly pathogenic avian influenza. Revue Scientifique et Technique de l'Office International des Epizooties 19: 463–482.

Thinh, T. V., M. Gilbert, N. Bunpapong, A. Amonsin, D. T. Nguyen, P. F. Doherty, and K. P. Huyvaert. 2012. Avian influenza viruses in wild land birds in northern Vietnam. Journal of Wildlife Diseases 48: 195–200.

van Riel, D., V. Munster, E. De Wit, G. Rimmelzwaan, R. Fouchier, A. Osterhaus, and T. Kuiken. 2007. Human and avian influenza viruses target different cells in the lower respiratory tract of humans and other mammals. American Journal of Pathology 171: 1215–1223.

*Submitted for publication 21 February 2011.*
*Accepted 13 June 2012.*