

Avian Influenza

Table of Contents

Avian Influenza and other diseases	2
Avian Influenza 2016	2
Avian Influenza 2015	13
Avian Influenza 2014	38
Avian Influenza 2013	59
Avian Influenza 2012	64
Avian Influenza 2011	67
Avian Influenza 2010	69
Avian Influenza 2009 and earlier	71

Avian Influenza and other diseases

Avian Influenza 2016

Xu, Y., P. Gong, B. Wielstra, and Y. Si. 2016. Southward autumn migration of waterfowl facilitates crosscontinental transmission of the highly pathogenic avian influenza H5N1 virus. *Scientific Reports* **6**:30262.

(Abstract)

The highly pathogenic avian influenza subtype H5N1 (HPAI H5N1) is a worldwide zoonotic infectious disease, threatening humans, poultry and wild birds. The role of wild birds in the spread of HPAI H5N1 has previously been investigated by comparing disease spread patterns with bird migration routes. However, the different roles that the southward autumn and northward spring migration might play in virus transmission have hardly been explored. Using direction analysis, we analyze HPAI H5N1 transmission directions and angular concentration of currently circulating viral clades, and compare these with waterfowl seasonal migration directions along major waterfowl flyways. Out of 22 HPAI H5N1 transmission directions, 18 had both a southward direction and a relatively high concentration. Differences between disease transmission and waterfowl migration directions were significantly smaller for autumn than for spring migration. The four northward transmission directions were found along Asian flyways, where the initial epicenter of the virus was located. We suggest waterfowl first picked up the virus from East Asia, then brought it to the north via spring migration, and then spread it to other parts of world mainly by autumn migration. We emphasize waterfowl autumn migration plays a relatively important role in HPAI H5N1 transmission compared to spring migration.

Huang, S., H. Tian, X. Wu, S. Zhou, X. Li, T. Zhang, X. Zhao, Y. Wang, Y. Pei, and B. Xu. 2016. Risk analysis of H5N1 highly pathogenic avian influenza in poultry at the Poyang Lake area, China. *Environmental Earth Sciences* **75**:955.

(Abstract)

Migratory birds have been known to spread the highly pathogenic avian influenza virus (HPAIV) H5N1 over large distances. Moreover, previous studies have shown it to be widely circulated in live-bird markets. However, how live-bird markets in addition to wild birds affect the local persistence of avian influenza virus (AIV) remains uncertain. The abundance of fresh water and widely distributed wetlands make the Poyang lake area a suitable overwintering site for migratory birds. The intensive local poultry industry and poor biosecurity potentially increase the risk of interactions between the wild and domestic birds. These factors likely contributed to the spread and persistence of AIVs in the Poyang lake area in these years. Here, a cross-sectional survey together with sampling was conducted randomly in the Poyang lake area to investigate the local distribution of the H5N1 viruses. In total, 891 poultry isolate from 31 villages around the Poyang lake area were randomly sampled in poultry sectors and tested for the presence of avian influenza using RTPCR assays. Environmental factors

such as poultry density, road accessibility, and wild bird biodiversity together with breeding characteristics derived from these questionnaires were collected and utilized for risk analysis modelling. Our results revealed that distance to the nearest wetlands with a high number of birds and accessibility by road were the main risk factors for the occurrence HPAIV H5N1, suggesting that the high density of road networks may facilitate the transmission of this virus. Investigating the regional distribution characteristics of the virus is of great significance, therefore the general public would benefit from an early warning, prevention, and taking control measures in high-risk regions.

Bi, Y., H. Liu, C. Xiong, D. Liu, W. Shi, M. Li, S. Liu, J. Chen, G. Chen, Y. Li, G. Yang, Y. Lei, Y. Xiong, F. Lei, H. Wang, Q. Chen, J. Chen, and G. F. Gao. 2016. Novel avian influenza A (H5N6) viruses isolated in migratory waterfowl before the first human case reported in China, 2014. *Scientific Reports* **6**:29888.

(Abstract)

In May 2014, China formally confirmed the first human infection with the novel H5N6 avian influenza virus (AIV) in Sichuan Province. Before the first human case was reported, surveillance of AIVs in wild birds resulted in the detection of three H5N6 viruses in faecal samples from migratory waterfowl in Chenhu wetlands, Hubei Province, China. Genetic and phylogenetic analyses revealed that these three novel viruses were closely related to the H5N6 virus that has caused human infections in China since 2014. A Bayesian phylogenetic reconstruction of all eight segments suggests multiple reassortment events in the evolution of these viruses. The hemagglutinin (HA) and neuraminidase (NA) originated from the H5N2 and H6N6 AIVs, respectively, whereas all six internal genes were derived from avian H5N1 viruses. The reassortant may have occurred in eastern China during 2012–2013. A phylogeographic analysis of the HA and NA genes traced the viruses to southern China, from where they spread to other areas via eastern China. A receptor-binding test showed that H5N6 viruses from migratory waterfowl had human-type receptor-binding activity, suggesting a potential for transmission to humans. These data suggest that migratory waterfowl may play a role in the dissemination of novel H5N6 viruses.

Su, S., G. Xing, J. Wang, Z. Li, J. Gu, L. Yan, J. Lei, S. Ji, B. Hu, G. C. Gray, Y. Yan, and J. Zhou. 2016. Characterization of H7N2 Avian Influenza Virus in Wild Birds and Pikas in Qinghai-Tibet Plateau Area. *Scientific Reports* **6**:30974.

(Abstract)

Qinghai Lake is a major migrating bird breeding site that has experienced several recent highly pathogenic avian influenza virus (HPAIV) epizootics. From 2006 to 2009 we studied Qinghai's wild birds and pikas for evidence of AIV infections. We sampled 941 healthy wild animals and isolated seventeen H7N2 viruses (eight from pikas and nine from wild birds). The H7N2 viruses were phylogenetically closely related to each other and to viruses isolated in Hong Kong in the 1970s. We

determined the pathogenicity of the H7N2 viruses by infecting chickens and mice. Our results suggest that pikas might play an important role in the ecology of AIVs, acting as intermediate hosts in which viruses become more adapted to mammals. Our findings of AI infection in pikas are consistent with previous observations and raise the possibility that pikas might play a previously unrecognized role in the ecology of AIVs peridomestic aquatic environments.

Belkhiria, J., M. A. Alkhamis, and B. Martínez-López. 2016. Application of Species Distribution Modeling for Avian Influenza surveillance in the United States considering the North America Migratory Flyways. *Scientific Reports* **6**:33161.

(Abstract)

Highly Pathogenic Avian Influenza (HPAI) has recently (2014–2015) re-emerged in the United States (US) causing the largest outbreak in US history with 232 outbreaks and an estimated economic impact of \$950 million. This study proposes to use suitability maps for Low Pathogenic Avian Influenza (LPAI) to identify areas at high risk for HPAI outbreaks. LPAI suitability maps were based on wild bird demographics, LPAI surveillance, and poultry density in combination with environmental, climatic, and socio-economic risk factors. Species distribution modeling was used to produce high-resolution (cell size: 500m x 500m) maps for Avian Influenza (AI) suitability in each of the four North American migratory flyways (NAMF). Results reveal that AI suitability is heterogeneously distributed throughout the US with higher suitability in specific zones of the Midwest and coastal areas. The resultant suitability maps adequately predicted most of the HPAI outbreak areas during the 2014–2015 epidemic in the US (i.e. 89% of HPAI outbreaks were located in areas identified as highly suitable for LPAI). Results are potentially useful for poultry producers and stakeholders in designing risk-based surveillance, outreach and intervention strategies to better prevent and control future HPAI outbreaks in the US.

Mundava, J., A. Caron, M. De Garine-Wichatitsky, C. Abolnik, P. Mundy, and N. Gaidet. 2016. The role of breeding phenology and aggregation of waterfowl on avian influenza dynamics in southern Africa. *Ibis* **158**:762–775.

(Abstract)

Recent outbreaks of highly pathogenic avian influenza virus (AIV) in birds, humans and other mammalian species calls for a better understanding of virus dynamics in wild bird species and populations that act as maintenance hosts. Host ecology influences the transmission of pathogens and can be used to explore and infer pathogen dynamics. Most of the ecological processes proposed to explain AIV transmission in wild birds have been derived from studies conducted in the temperate and boreal regions of the northern hemisphere. We evaluate the role of two key drivers of AIV dynamics in a waterfowl community in Zimbabwe (southern Africa): (1) the recruitment of young birds and (2) the seasonal aggregation of birds. We analyse the seasonal variation of AIV prevalence in

waterfowl and overlay these data with the phenology of reproduction and the seasonal variation in the local abundance of these species. We find that the breeding period of southern Afrotropical waterfowl species is more extended and somewhat less synchronized among species in the community than is the case in temperate and boreal waterfowl communities. Young birds are recorded at most times of the year, and these immunologically naive individuals can therefore act as new hosts for AIV throughout the year within the wild bird population. Although host aggregation peaks in the cold-dry to hot-dry season, birds still aggregate throughout the year and this potentially spreads the opportunities for first infection of juveniles and other naive birds temporally. We did not find a relationship between season, AIV prevalence in waterfowl, the influx of juveniles or the gradual aggregation of birds during the dry season. Therefore, the main drivers of AIV dynamics (juvenile influx and host abundance/aggregation), although present in Afrotropical regions, could not explain the AIV seasonal patterns in our study in contrast to results reported from temperate and boreal regions. These differences imply variation in the risk of AIV circulation in waterfowl and in the risk of spread to poultry, other animals or humans.

Li, X., J. Yang, S. Huang, T. Zhang, J. Chang, and B. Xu. 2016. Environmental factors contributing to avian influenza risk in Poyang Lake region, China. *Environmental Earth Sciences* **75**:1292.

(Abstract)

The avian influenza (AI) virus isolated from and adapted to an avian host could cause high morbidity, mortality and enormous economic losses in poultry farming. Epidemiological field investigation was performed to study environmental conditions for infected poultry, which possibly spread avian influenza viruses in the Poyang Lake region. A total of 1695 cloacal and oropharyngeal samples were collected in 54 sites in September and December of 2009. Among which, 16 sites with positive samples were confirmed by viral typing and sequencing. The results showed that the highest viral isolation rate was found in live poultry markets. Subsequently, a cross-sectional study was performed to identify environmental risk factors contributing to infection using logistic regression analysis. Based on the predictive model, a risk map was developed to improve our understanding on risk distribution and likelihood of local circulation of avian influenza viruses. In conclusion, (1) the local live poultry market serves as major source of risk for avian influenza; (2) the closer to the administrative centers of counties and the Poyang Lake, the higher is the probability of infection in birds; (3) the human settlements in western, southwestern and southeastern parts of the Poyang Lake were exposed to high risks. Based on the risk factors, combined measures should be taken to control and prevent the avian influenza transmission.

Krauss, S., D. E. Stallknecht, R. D. Slemons, A. S. Bowman, R. L. Poulson, J. M. Nolting, J. P. Knowles, and R. G. Webster. 2016. The enigma of the apparent disappearance of Eurasian highly pathogenic H5 clade 2.3.4.4 influenza A viruses in North American waterfowl. *PNAS* **113**:9033–9038.

(Abstract)

One of the major unresolved questions in influenza A virus (IAV) ecology is exemplified by the apparent disappearance of highly pathogenic (HP) H5N1, H5N2, and H5N8 (H5Nx) viruses containing the Eurasian hemagglutinin 2.3.4.4 clade from wild bird populations in North America. The introduction of Eurasian lineage HP H5 clade 2.3.4.4 H5N8 IAV and subsequent reassortment with lowpathogenic H7N2 and H7N1 North American wild bird-origin IAVs in late 2014 resulted in widespread HP H5Nx IAV infections and outbreaks in poultry and wild birds across two-thirds of North America starting in November 2014 and continuing through June 2015. Although the stamping out strategies adopted by the poultry industry and animal health authorities in Canada and the United States—which included culling, quarantining, increased biosecurity, and abstention from vaccine use—were successful in eradicating the HP H5Nx viruses from poultry, these activities do not explain the apparent disappearance of these viruses from migratory waterfowl. Here we examine current and historical aquatic bird IAV surveillance and outbreaks of HP H5Nx in poultry in the United States and Canada, providing additional evidence of unresolved mechanisms that restrict the emergence and perpetuation of HP avian influenza viruses in these natural reservoirs.

Kim, Y.-I., S.-W. Kim, Y.-J. Si, H.-I. Kwon, S.-J. Park, E.-H. Kim, S. m. Kim, I.-W. Lee, M.-S. Song, and Y.-K. Choi. 2016. Genetic diversity and pathogenic potential of low pathogenic H7 avian influenza viruses isolated from wild migratory birds in Korea. *Infection, Genetics and Evolution* **45**:268–284.

(Abstract)

To detect the circulation of H7 avian influenza viruses, we characterized H7 viruses found in migratory birds and live poultry markets of South Korea from 2005 to 2014. Phylogenetic analysis revealed that while all viruses clustered into the Eurasian-lineage of H7 avian viruses, at least 12 distinct genotypes were represented. Most H7 viruses contained at least one gene segment from the highly-pathogenic A/Sck/Hong Kong/YU100/02(H5N1)-like avian virus, and they could be separated into at least two antigenic groups. Although we did not detect genetically identical strains, HI assay demonstrated close cross-reactivity of some isolates with the H7N9 viruses from China. Animal studies revealed that most of the genotypes could replicate in the lungs of mice and chickens without prior adaptation and some, particularly H7N4 and H7N7 subtypes, induced mortality in mice. These results reinforce growing pandemic concerns regarding recent H7 viruses and emphasize the importance of continued surveillance of avian influenza viruses in the wild.

Hill, N. J., and J. A. Runstadler. 2016. A Bird's Eye View of Influenza A Virus Transmission: Challenges with Characterizing Both Sides of a Co-Evolutionary Dynamic. *Integrative and Comparative Biology* **56**:304-316.

(Abstract)

In nature, wild birds and influenza A viruses (IAV) are continually co-evolving, locked into a back-and-forth of resistance and conquest that has approached a stable equilibrium over time. This co-

evolutionary relationship between bird host and IAV may appear stable at the organismal level, but is highly dynamic at the molecular level manifesting in a constant trade-off between transmissibility and virulence of the virus. Characterizing both sides of the host-virus dynamic has presented a challenge for ecologists and virologists alike, despite the potential for this approach to provide insights into which conditions destabilize the equilibrium state resulting in outbreaks or mortality of hosts in extreme cases. The use of different methods that are either host-centric or virus-centric has made it difficult to reconcile the disparate fields of host ecology and virology for investigating and ultimately predicting wild bird-mediated transmission of IAV. This review distills some of the key lessons learned from virological and ecological studies and explores the promises and pitfalls of both approaches. Ultimately, reconciling ecological and virological approaches hinges on integrating scales for measuring host-virus interactions. We argue that prospects for finding common scales for measuring wild bird-influenza dynamics are improving due to advances in genomic sequencing, host-tracking technology and remote sensing data, with the unit of time (months, year, or seasons) providing a starting point for crossover.

Hoye, B. J., V. J. Munster, N. Huig, P. P. de Vries, K. Oosterbeek, W. Tijssen, M. Klaassen, R. A. M. Fouchier, and J. A. van Gils. 2016. Hampered Performance of Migratory Swans: Intra- and Inter-Seasonal Effects of Avian Influenza Virus. *Integrative and Comparative Biology* **56**:317–329.

(Abstract)

The extent to which animal migrations shape parasite transmission networks is critically dependent on a migrant's ability to tolerate infection and migrate successfully. Yet, sub-lethal effects of parasites can be intensified through periods of increased physiological stress. Long-distance migrants may, therefore, be especially susceptible to negative effects of parasitic infection. Although a handful of studies have investigated the short-term, transmission-relevant behaviors of wild birds infected with low-pathogenic avian influenza viruses (LPAIV), the ecological consequences of LPAIV for the hosts themselves remain largely unknown. Here, we assessed the potential effects of naturally-acquired LPAIV infections in Bewick's swans, a long-distance migratory species that experiences relatively low incidence of LPAIV infection during early winter. We monitored both foraging and movement behavior in the winter of infection, as well as subsequent breeding behavior and interannual resighting probability over 3 years. Incorporating data on infection history we hypothesized that any effects would be most apparent in naive individuals experiencing their first LPAIV infection. Indeed, significant effects of infection were only seen in birds that were infected but lacked antibodies indicative of prior infection. Swans that were infected but had survived a previous infection were indistinguishable from uninfected birds in each of the ecological performance metrics. Despite showing reduced foraging rates, individuals in the naive-infected category had similar accumulated body stores to re-infected and uninfected individuals prior to departure on spring migration, possibly as a result of having higher scaled mass at the time of infection. And yet individuals in the naive-infected category were unlikely to be resighted 1 year after infection, with 6 out of 7 individuals that never resighted again compared to 20 out of 63 uninfected individuals and 5 out of 12 individuals in the re-infected category. Collectively, our findings indicate that acute and superficially harmless infection with LPAIV may have indirect effects on individual performance and

recruitment in migratory Bewick's swans. Our results also highlight the potential for infection history to play an important role in shaping ecological constraints throughout the annual cycle.

Weiser, E. L., R. B. Lanctot, S. C. Brown, J. A. Alves, P. F. Battley, R. Bentzen, J. Bêty, M. A. Bishop, M. Boldenow, L. Bollache, B. Casler, M. Christie, J. T. Coleman, J. R. Conklin, W. B. English, R. Gates, O. Gilg, M.-A. Giroux, K. Gosbell, C. Hassell, J. Helmericks, A. Johnson, B. Katrínardóttir, K. Koivula, E. Kwon, J.-F. Lamarre, J. Lang, D. B. Lank, N. Lecomte, J. Liebezeit, V. Loverti, L. McKinnon, C. D. T. Minton, D. S. Mizrahi, E. Nol, V.-M. Pakanen, J. Perz, R. Porter, J. Rausch, J. Reneerkens, N. Rönkä, S. T. Saalfeld, N. R. Senner, B. Sittler, P. A. Smith, K. M. Sowl, A. Taylor, D. H. Ward, S. Yezerinac, and B. K. Sandercock. 2016. Effects of geolocators on hatching success, return rates, breeding movements, and change in body mass in 16 species of Arctic-breeding shorebirds. *Movement Ecology* 4:12.

(Abstract)

Background: Geolocators are useful for tracking movements of long-distance migrants, but potential negative effects on birds have not been well studied. We tested for effects of geolocators (0.8–2.0 g total, representing 0.1–3.9% of mean body mass) on 16 species of migratory shorebirds, including five species with 2–4 subspecies each for a total of 23 study taxa. Study species spanned a range of body sizes (26–1091 g) and eight genera, and were tagged at 23 breeding and eight nonbreeding sites. We compared breeding performance and return rates of birds with geolocators to control groups while controlling for potential confounding variables.

Results: We detected negative effects of tags for three small-bodied species. Geolocators reduced annual return rates for two of 23 taxa: by 63 % for semipalmated sandpipers and by 43% for the *arcticola* subspecies of dunlin. High resighting effort for geocator birds could have masked additional negative effects. Geolocators were more likely to negatively affect return rates if the total mass of geolocators and color markers was 2.5–5.8% of body mass than if tags were 0.3–2.3% of body mass. Carrying a geocator reduced nest success by 42% for semipalmated sandpipers and tripled the probability of partial clutch failure in semipalmated and western sandpipers. Geolocators mounted perpendicular to the leg on a flag had stronger negative effects on nest success than geolocators mounted parallel to the leg on a band. However, parallel-band geolocators were more likely to reduce return rates and cause injuries to the leg. No effects of geolocators were found on breeding movements or changes in body mass. Among-site variation in geocator effect size was high, suggesting that local factors were important.

Conclusions: Negative effects of geolocators occurred only for three of the smallest species in our dataset, but were substantial when present. Future studies could mitigate impacts of tags by reducing protruding parts and minimizing use of additional markers. Investigators could maximize recovery of tags by strategically deploying geolocators on males, previously marked individuals, and successful breeders, though targeting subsets of a population could bias the resulting migratory movement data in some species.

Zack, S., and J. Liebezeit. 2012. Arctic shorebirds: Conservation of a moving target in changing times. Pages 204-225. in J. F. Brodie, E. S. Post, and D. F. Doak, editors. *Wildlife Conservation in a Changing Climate*. University of Chicago Press, Chicago.

(No abstract available)

Bahl, J., T. T. Pham, N. J. Hill, I. T. M. Hussein, E. J. Ma, B. C. Easterday, R. A. Halpin, T. B. Stockwell, D. E. Wentworth, G. Kayali, S. Krauss, S. Schultz-Cherry, R. G. Webster, R. J. Webby, M. D. Swartz, G. J. D. Smith, and J. A. Runstadler. 2016. Ecosystem Interactions Underlie the Spread of Avian Influenza A Viruses with Pandemic Potential. *PLoS Pathogens* **12**:e1005620.

(Abstract)

Despite evidence for avian influenza A virus (AIV) transmission between wild and domestic ecosystems, the roles of bird migration and poultry trade in the spread of viruses remain enigmatic. In this study, we integrate ecosystem interactions into a phylogeographic model to assess the contribution of wild and domestic hosts to AIV distribution and persistence. Analysis of globally sampled AIV datasets shows frequent two-way transmission between wild and domestic ecosystems. In general, viral flow from domestic to wild bird populations was restricted to within a geographic region. In contrast, spillover from wild to domestic populations occurred both within and between regions. Wild birds mediated long-distance dispersal at intercontinental scales whereas viral spread among poultry populations was a major driver of regional spread. Viral spread between poultry flocks frequently originated from persistent lineages circulating in regions of intensive poultry production. Our analysis of long-term surveillance data demonstrates that meaningful insights can be inferred from integrating ecosystem into phylogeographic reconstructions that may be consequential for pandemic preparedness and livestock protection.

Hu, J., and X. Liu. 2016. Endemicity of H9N2 and H5N1 avian influenza viruses in poultry in China poses a serious threat to poultry industry and public health. *Frontiers in Agricultural Science and Engineering* **3**:11–24.

(Abstract)

The H9N2 and H5N1 avian influenza viruses (AIVs) have been circulating in poultry in China and become endemic since 1998 and 2004, respectively. Currently, they are prevalent in poultry throughout China. This endemicity makes them actively involved in the emergence of the novel lineages of other subtypes of influenza viruses, such as the well-known viruses of the highly pathogenic avian influenza (HPAI) H5N2 and the 2013 novel H7N7, H7N9 and H10N8 subtypes, thereby threatening both the poultry industry and public health. Here, we will review briefly the prevalence and evolution, pathogenicity, transmission, and disease control of these two subtypes and also discuss the possibility of emergence of potentially virulent and highly transmissible AIVs to humans.

Wei, Y.-R., X.-Y. Yang, Y.-G. Li, J. Wei, W.-G. Ma, Z.-G. Ren, H.-L. Guo, T.-C. Wang, X.-Y. Mi, G. Adili, S.-K. Miao, A. Shaha, Y.-W. Gao, J. Huang, and X.-Z. Xia. 2016. Serological survey of avian influenza virus infection in non-avian wildlife in Xinjiang, China. *Archives of Virology* **161**:867–872.

(Abstract)

We conducted a serological survey to detect antibodies against avian influenza virus (AIV) in *Gazella subgutturosa*, *Canis lupus*, *Capreolus pygargus*, *Sus scrofa*, *Cervus elaphus*, *Capra ibex*, *Ovis ammon*, *Bos grunniens* and *Pseudois nayaur* in Xinjiang, China. Two hundred forty-six sera collected from 2009 to 2013 were assayed for antibodies against H5, H7 and H9 AIVs using hemagglutination inhibition (HI) tests and a pan-influenza competitive ELISA. Across all tested wildlife species, 4.47 % harbored anti-AIV antibodies that were detected by the HI assay. The seroprevalence for each AIV subtype across all species evaluated was 0 % for H5 AIV, 0.81 % for H7 AIV, and 3.66 % for H9 AIV. H7-reactive antibodies were found in *Canis lupus* (9.09 %) and *Ovis ammon* (4.55 %). H9-reactive antibodies were found in *Gazella subgutturosa* (4.55 %), *Canis lupus* (27.27 %), *Pseudois nayaur* (23.08 %), and *Ovis ammon* (4.55 %). The pan-influenza competitive ELISA results closely corresponded to the cumulative prevalence of AIV exposure as measured by subtype-specific HI assays, suggesting that H7 and H9 AIV subtypes predominate in the wildlife species evaluated. These data provide evidence of prior infection with H7 and H9 AIVs in non-avian wildlife in Xinjiang, China.

Ramey, A. M., A. B. Reeves, J. L. TeSlaa, S. W. Nashold, T. Donnelly, J. Bahl, and J. S. Hall. 2016. Evidence for common ancestry among viruses isolated from wild birds in Beringia and highly pathogenic intercontinental reassortant H5N1 and H5N2 influenza A viruses. *Infection, Genetics and Evolution* **40**:176–185.

(Abstract)

Highly pathogenic clade 2.3.4.4 H5N8, H5N2, and H5N1 influenza A viruses were first detected in wild, captive, and domestic birds in North America in November–December 2014. In this study, we used wild waterbird samples collected in Alaska prior to the initial detection of clade 2.3.4.4 H5 influenza A viruses in North America to assess the evidence for: (1) dispersal of highly pathogenic influenza A viruses from East Asia to North America by migratory birds via Alaska and (2) ancestral origins of clade 2.3.4.4 H5 reassortant viruses in Beringia. Although we did not detect highly pathogenic influenza A viruses in our sample collection from western Alaska, we did identify viruses that contained gene segments sharing recent common ancestry with intercontinental reassortant H5N2 and H5N1 viruses. Results of phylogenetic analyses and estimates for times of most recent common ancestry support migratory birds sampled in Beringia as maintaining viral diversity closely related to novel highly pathogenic influenza A virus genotypes detected in North America. Although our results do not elucidate the route by which highly pathogenic influenza A viruses were introduced into North America, genetic evidence is consistent with the hypothesized trans-Beringian route of introduction via migratory birds.

Prosser, D. J., E. C. Palm, J. Y. Takekawa, D. Zhao, X. Xiao, P. Li, Y. Liu, and S. H. Newman. 2016. Movement analysis of free-grazing domestic ducks in Poyang Lake, China: a disease connection. *International Journal of Geographical Information Science* **30**:869-880.

(Abstract)

Previous work suggests domestic poultry are important contributors to the emergence and transmission of highly pathogenic avian influenza throughout Asia. In Poyang Lake, China, domestic duck production cycles are synchronized with arrival and departure of thousands of migratory wild birds in the area. During these periods, high densities of juvenile domestic ducks are in close proximity to migratory wild ducks, increasing the potential for the virus to be transmitted and subsequently disseminated via migration. In this paper, we use GPS dataloggers and dynamic Brownian bridge models to describe movements and habitat use of free-grazing domestic ducks in the Poyang Lake basin and identify specific areas that may have the highest risk of H5N1 transmission between domestic and wild birds. Specifically, we determine relative use by free-grazing domestic ducks of natural wetlands, which are the most heavily used areas by migratory wild ducks, and of rice paddies, which provide habitat for resident wild ducks and lower densities of migratory wild ducks. To our knowledge, this is the first movement study on domestic ducks, and our data show potential for free-grazing domestic ducks from farms located near natural wetlands to come in contact with wild waterfowl, thereby increasing the risk for disease transmission. This study provides an example of the importance of movement ecology studies in understanding dynamics such as disease transmission on a complicated landscape.

Wang H, Zhang Z, Chen Z, Zhang Y, Lv Q, An X, Tong Y, Carr MJ, Sun S, Shi W. 2016. High genetic diversity and frequent genetic reassortment of avian influenza A(H9N2) viruses along the East Asian–Australian migratory flyway. *Infection, Genetics and Evolution* **39**:325–329.

(Abstract)

To understand the molecular epidemiology and evolution of avian influenza viruses (AIV) along the East Asian–Australian migration flyway, we collected faecal samples (n = 2859) between November 2014 and March 2015 from poultry, environmental sources and wild birds in Dongying, Shandong province and Yancheng, Jiangsu province in eastern China. The presence of AIV RNA was evaluated by real-time PCR and the positivity rate ranged from 0 to 29.3%. In both Dongying and Yancheng, samples collected from live poultry markets had the highest positivity rate for AIV RNA. AIV whole genomes were generated and phylogenetically analysed. Our results demonstrate that most of the viruses belonged to the H9N2 subtype, and could be classified into nine novel genotypes based on the phylogenetic analysis of the eight gene segments of the AIV genomes. This revealed a high genetic diversity of H9N2 in this region and suggested that they might have undergone frequent genetic reassortment. In addition, the internal genes (PB2, etc.) of two viruses from wild birds and several viruses from poultry belonged to the same gene constellation, suggesting a potential inter-

host transmission of AIV between wild birds and poultry in live markets along routes of migratory flyways. Our results highlight the high genetic diversity of AIV along the East Asian–Australian migration flyway and the need for more extensive AIV surveillance in eastern China.

Ferenczi M, Beckmann C, Warner S, Loyn RH, O’Riley K, Wang X, Klaassen M. 2016. Avian influenza infection dynamics under variable climatic conditions, viral prevalence is rainfall driven in waterfowl from temperate, south-east Australia. *Veterinary Research* **47**:23.

(Abstract)

Understanding Avian Influenza Virus (AIV) infection dynamics in wildlife is crucial because of possible virus spill over to livestock and humans. Studies from the northern hemisphere have suggested several ecological and environmental drivers of AIV prevalence in wild birds. To determine if the same drivers apply in the southern hemisphere, where more irregular environmental conditions prevail, we investigated AIV prevalence in ducks in relation to biotic and abiotic factors in south-eastern Australia. We sampled duck faeces for AIV and tested for an effect of bird numbers, rainfall anomaly, temperature anomaly and long-term ENSO (El-Niño Southern Oscillation) patterns on AIV prevalence. We demonstrate a positive long term effect of ENSO-related rainfall on AIV prevalence. We also found a more immediate response to rainfall where AIV prevalence was positively related to rainfall in the preceding 3–7 months. Additionally, for one duck species we found a positive relationship between their numbers and AIV prevalence, while prevalence was negatively or not affected by duck numbers in the remaining four species studied. In Australia largely non-seasonal rainfall patterns determine breeding opportunities and thereby influence bird numbers. Based on our findings we suggest that rainfall influences age structures within populations, producing an influx of immunologically naïve juveniles within the population, which may subsequently affect AIV infection dynamics. Our study suggests that drivers of AIV dynamics in the northern hemisphere do not have the same influence at our south-east Australian field site in the southern hemisphere due to more erratic climatological conditions.

Bengtsson D, Safi K, Avril A, Fiedler W, Wikelski M, Gunnarsson G, Elmberg J, Tolf C, Olsen B, Waldenström J. 2016. Does influenza A virus infection affect movement behaviour during stopover in its wild reservoir host? *Royal Society Open Science* **3**:150633.

(Abstract)

The last decade has seen a surge in research on avian influenza A viruses (IAVs), in part fuelled by the emergence, spread and potential zoonotic importance of highly pathogenic virus subtypes. The mallard (*Anas platyrhynchos*) is the most numerous and widespread dabbling duck in the world, and one of the most important natural hosts for studying IAV transmission dynamics. In order to predict the likelihood of IAV transmission between individual ducks and to other hosts, as well as between geographical regions, it is important to understand how IAV infection affects the host. In this study, we

analysed the movements of 40 mallards equipped with GPS transmitters and three-dimensional accelerometers, of which 20 were naturally infected with low pathogenic avian influenza virus (LPAIV), at a major stopover site in the Northwest European flyway. Movements differed substantially between day and night, as well as between mallards returning to the capture site and those feeding in natural habitats. However, movement patterns did not differ between LPAIV infected and uninfected birds. Hence, LPAIV infection probably does not affect mallard movements during stopover, with high possibility of virus spread along the migration route as a consequence.

Avian Influenza 2015

Steiof, K., J. Mooij, and P. Petermann. 2015. The “wild bird hypothesis” in the epidemiology of highly pathogenic avian influenza – recent situation and critical assessment of the position of the German Federal Research Institute for Animal Health (FLI) (state of June 2015). *Vogelwelt* **135**:131-145.

(Abstract)

In 2005-2007 the highly pathogenic Avian Influenza Virus “H5N1” spread through large parts of Europe. Since November 2014 the subtype H5N8, a recombinant descendant of H5N1 has made a similar appearance. This subtype has been known since 2013 in East Asia where it has caused devastating outbreaks among poultry in South Korea in 2014, also infecting wild birds. In Europe the virus caused outbreaks between November 2014 and February 2015 in eleven poultry holdings in Germany, the Netherlands, England, Italy and Hungary. It is most likely, that the virus came to Western Europe by poultry trade with South Korea, since no outbreaks are known in the space between these regions. Following the outbreaks in Western European poultry holdings, a few cases of infected wild birds were reported (some ducks, two Mute Swans and one gull), however, so far without scientific and transparent documentation. In January 2015 there were three more outbreaks in a zoo, a public park with domesticated animals and a small animal holding in cities of Mecklenburg-Vorpommern. As in 2006 and 2007, the Friedrich-Loeffler-Institute (FLI), the German Federal Institute for Animal Diseases, propagated the “wild bird hypothesis” as explanation for the introduction of highly pathogenic AIV in Europe. According to this hypothesis wild birds play a key role as reservoir and vector of HPAI-viruses. However, no evidence for this thesis has been found anywhere in the world. To the contrary, it seems rather unlikely: among 800,000 wild birds tested worldwide HPAIV was usually only detected, where the virus was already circulating in commercial poultry holdings. In poultry it frequently appears first in large factory farm holdings with high biosecurity. Wild birds are dead ends for HPAI viruses. If they die, the virus vanishes as well. Longer chains of infection are not known from the wild. Even after events of mass infections of wild birds the virus disappears from the environment in a few weeks, at most. Additionally, there has never been any evidence for wild birds carrying the virus into a poultry holding. Furthermore, the FLI still composes its Avian Influenza Risk Assessment on the assumption of the wild bird hypothesis. To support this the FLI authors cite several publications, some of which, however, have already been shown some years ago to be faulty or inadequate (Steiof 2005, Petermann 2006, Feare 2007). Therefore, the risk assessment and actions taken by veterinary authorities are badly focused. Indeed, the monitoring of avian influenza in wild birds and poultry has so far failed to detect highly pathogenic Avian Influenza in any one case before

outbreaks became obvious. Similarly the way of introduction of HPAIV could not be determined in any one case. It is an error to focus the monitoring effort on wild birds, free-range and backyard poultry. This should urgently be replaced by effective controls and actions in factory poultry farming. Without any real solution, some common values may be endangered: wild birds are threatened by release of perilous viruses from poultry holdings to the environment. Furthermore, rare poultry breeds and races are threatened by obligatory housing due to veterinary law, which makes their keeping impossible. Eco-farming as well as animal welfare is being challenged by the difficulties to keep free-range poultry in species-appropriate conditions, while factory poultry holdings in closed biosecurity premises are being privileged.

Flint, P. L., J. M. Pearce, C. Franson, and D. V. Derksen. 2015. Wild bird surveillance for highly pathogenic avian influenza H5 in North America. *Virology Journal* **12**:151.

(Abstract)

It is unknown how the current Asian origin highly pathogenic avian influenza H5 viruses arrived, but these viruses are now poised to become endemic in North America. Wild birds harbor these viruses and have dispersed them at regional scales. What is unclear is how the viruses may be moving from the wild bird reservoir into poultry holdings. Active surveillance of live wild birds is likely the best way to determine the true distribution of these viruses. We also suggest that sampling be focused on regions with the greatest risk for poultry losses and attempt to define the mechanisms of transfer to enhance biosecurity. Responding to the recent outbreaks of highly pathogenic avian influenza in North America requires an efficient plan with clear objectives and potential management outcomes.

Sequeira Trovao, N., M. A. Suchard, G. Baele, M. Gilbert, and P. Lemey. 2015. Bayesian Inference Reveals Host-Specific Contributions to the Epidemic Expansion of Influenza A H5N1. *Molecular Biology and Evolution* **32**:3264–3275.

(Abstract)

Since its first isolation in 1996 in Guangdong, China, the highly pathogenic avian influenza virus (HPAIV) H5N1 has circulated in avian hosts for almost two decades and spread to more than 60 countries worldwide. The role of different avian hosts and the domestic-wild bird interface has been critical in shaping the complex HPAIV H5N1 disease ecology, but remains difficult to ascertain. To shed light on the large-scale H5N1 transmission patterns and disentangle the contributions of different avian hosts on the tempo and mode of HPAIV H5N1 dispersal, we apply Bayesian evolutionary inference techniques to comprehensive sets of hemagglutinin and neuraminidase gene sequences sampled between 1996 and 2011 throughout Asia and Russia. Our analyses demonstrate that the large-scale H5N1 transmission dynamics are structured according to different avian flyways, and that the incursion of the Central Asian flyway specifically was driven by *Anatidae* hosts coinciding with rapid rate of spread and an epidemic wavefront acceleration. This also resulted in long-distance

dispersal that is likely to be explained by wild bird migration. We identify a significant degree of asymmetry in the large-scale transmission dynamics between *Anatidae* and *Phasianidae*, with the latter largely representing poultry as an evolutionary sink. A joint analysis of host dynamics and continuous spatial diffusion demonstrates that the rate of viral dispersal and host diffusivity is significantly higher for *Anatidae* compared with *Phasianidae*. These findings complement risk modeling studies and satellite tracking of wild birds in demonstrating a continental-scale structuring into areas of H5N1 persistence that are connected through migratory waterfowl.

Verhagen, J. H., H. P. van der Jeugd, B. A. Nolet, R. Slaterus, S. P. Kharitonov, P. P. de Vries, O. Vuong, F. Majoor, T. Kuiken, and R. A. M. Fouchier. 2015. Wild bird surveillance around outbreaks of highly pathogenic avian influenza A (H5N8) virus in the Netherlands, 2014, within the context of global flyways. *Euro Surveillance* **20**:21-32.

(Abstract)

Highly pathogenic avian influenza (HPAI) A(H5N8) viruses that emerged in poultry in east Asia since 2010 spread to Europe and North America by late 2014. Despite detections in migrating birds, the role of free-living wild birds in the global dispersal of H5N8 virus is unclear. Here, wild bird sampling activities in response to the H5N8 virus outbreaks in poultry in the Netherlands are summarised along with a review on ring recoveries. HPAI H5N8 virus was detected exclusively in two samples from ducks of the Eurasian wigeon species, among 4,018 birds sampled within a three months period from mid-November 2014. The H5N8 viruses isolated from wild birds in the Netherlands were genetically closely related to and had the same gene constellation as H5N8 viruses detected elsewhere in Europe, in Asia and in North America, suggesting a common origin. Ring recoveries of migratory duck species from which H5N8 viruses have been isolated overall provide evidence for indirect migratory connections between East Asia and Western Europe and between East Asia and North America. This study is useful for better understanding the role of wild birds in the global epidemiology of H5N8 viruses. The need for sampling large numbers of wild birds for the detection of H5N8 virus and H5N8-virus-specific antibodies in a variety of species globally is highlighted, with specific emphasis in north-eastern Europe, Russia and northern China.

Samuel, M. D., J. S. Hall, J. D. Brown, D. R. Goldberg, H. S. Ip, and V. V. Baranyuk. 2015. The dynamics of avian influenza in Lesser Snow Geese: implications for annual and migratory infection patterns. *Ecological Applications* **25**:1851–1859.

(Abstract)

Wild water birds are the natural reservoir for low-pathogenic avian influenza viruses (AIV). However, our ability to investigate the epizootiology of AIV in these migratory populations is challenging and, despite intensive worldwide surveillance, remains poorly understood. We conducted a cross-sectional, retrospective analysis in Pacific Flyway Lesser Snow Geese, *Chen caerulescens*, to investigate AIV

serology and infection patterns. We collected nearly 3000 sera samples from Snow Geese at two breeding colonies in Russia and Canada during 1993–1996 and swab samples from >4000 birds at wintering and migration areas in the United States during 2006–2011. We found seroprevalence and annual seroconversion varied considerably among years. Seroconversion and infection rates also differed between Snow Goose breeding colonies and wintering areas, suggesting that AIV exposure in this gregarious waterfowl species is likely occurring during several phases (migration, wintering, and potentially breeding areas) of the annual cycle. We estimated AIV antibody persistence was longer (14 months) in female geese compared to males (6 months). This relatively long period of AIV antibody persistence suggests that subtype-specific serology may be an effective tool for detection of exposure to subtypes associated with highly pathogenic AIV. Our study provides further evidence of high seroprevalence in Arctic goose populations, and estimates of annual AIV seroconversion and antibody persistence for North American waterfowl. We suggest future AIV studies include serology to help elucidate the epizootiological dynamics of AIV in wild bird populations.

Arnal, A., M. Vittecoq, J. Pearce-Duvet, M. Gauthier-Clerc, T. Boulinier, and E. Jourdain. 2015. Laridae: A neglected reservoir that could play a major role in avian influenza virus epidemiological dynamics. *Critical Reviews in Microbiology* **41**:508-519.

(Abstract)

Avian influenza viruses (AIVs) are of great concern worldwide due to their economic impact and the threat they represent to human health. As wild birds are the natural reservoirs of AIVs, understanding AIV dynamics in different avian taxa is essential for deciphering the epidemiological links between wildlife, poultry and humans. To date, only the *Anatidae* (ducks, geese and swans) have been widely studied. Here, we aim to shed light on the current state of knowledge on AIVs in *Laridae* (gulls, terns and kittiwakes) versus that in *Anatidae* by setting forth four fundamental questions: how, when, where and to which host species are AIVs transmitted? First, we describe ecological differences between *Laridae* and *Anatidae* and discuss how they may explain observed contrasts in preferential transmission routes and the evolution of specific AIV subtypes. Second, we highlight the dissimilarities in the temporal patterns of AIV shedding between *Laridae* and *Anatidae* and address the role that immunity likely plays in shaping these patterns. Third, we underscore that *Laridae* may be key in promoting intercontinental exchanges of AIVs. Finally, we emphasize the crucial epidemiological position that *Laridae* occupy between wildlife, domestic birds and humans.

Miller, R. S., S. J. Sweeney, J. E. Akkina, and E. K. Saito. 2015. Potential Intercontinental Movement of Influenza A(H7N9) Virus into North America by Wild Birds: Application of a Rapid Assessment Framework. *Transboundary and Emerging Diseases* **62**:650–668.

(Abstract)

A critical question surrounding emergence of novel strains of avian influenza viruses (AIV) is the ability for wild migratory birds to translocate a complete (unreassorted whole genome) AIV intercontinentally. Virus translocation via migratory birds is suspected in outbreaks of highly pathogenic strain A(H5N1) in Asia, Africa and Europe. As a result, the potential intercontinental translocation of newly emerging AIV such as A(H7N9) from Eurasia to North America via migratory movements of birds remains a concern. An estimated 2.91 million aquatic birds move annually between Eurasia and North America with an estimated AIV prevalence as high as 32.2%. Here, we present a rapid assessment to address the likelihood of whole (unreassorted)-genome translocation of Eurasian strain AIV into North America. The scope of this assessment was limited specifically to assess the weight of evidence to support the movement of an unreassorted AIV intercontinentally by migratory aquatic birds. We developed a rapid assessment framework to assess the potential for intercontinental movement of avian influenzas by aquatic birds. This framework was iteratively reviewed by a multidisciplinary panel of scientific experts until a consensus was established. Our assessment framework identified four factors that may contribute to the potential for introduction of any AIV intercontinentally into North America by wild aquatic birds. These factors, in aggregate, provide a framework for evaluating the likelihood of new forms of AIV from Eurasia to be introduced by aquatic birds into North America. Based on our assessment, we determined that the potential for introduction of A(H7N9) into North America through aquatic migratory birds is possible, but the likelihood ranges from extremely low to low.

Okuya, K., T. Kawabata, K. Nagano, K. Tsukiyama-Kohara, I. Kusumoto, K. Takase, and M. Ozawa. 2015. Isolation and characterization of influenza A viruses from environmental water at an overwintering site of migratory birds in Japan. *Archives of Virology* **160**:3037–3052.

(Abstract)

The Izumi plain in Kagoshima prefecture, Japan, is an overwintering site of more than 10,000 cranes. The wet paddy areas are artificially created to provide roosting sites for the cranes every winter. Since wild ducks, known to be a natural reservoir of influenza A viruses, also overwinter in this area, the cranes' roost water likely serves as a source of influenza A virus infection. To assess this potential risk, we collected 126 water samples from the cranes' roost in the 2012/2013 winter season for virus isolation. We isolated six influenza viruses of three subtypes (H3N8, H4N6, and H4N8) from the water samples collected in the months of November and December. Genetic analysis of our isolates indicated that these viruses were genetically similar to the low-pathogenic avian influenza viruses circulating among Eurasian waterfowl. These findings suggest the possibility of the cranes becoming infected with the avian influenza viruses that are present in their roost water.

Grillo, V. L., K. E. Arzey, P. M. Hansbro, A. C. Hurt, S. Warner, J. Bergfeld, G. W. Burgess, B. Cookson, C. J. Dickason, M. Ferenczi, T. Hollingsworth, M. A. Hoque, R. B. Jackson, M. Klaassen, P. D. Kirkland, N. Y. Kung, S. Lisovski, M. A. O'Dea, K. O'Riley, D. A. Roshier, L. F. Skerratt, J. P. Tracey, X. Wang,

R. Woods, and L. Post. 2015 Avian influenza in Australia: a summary of 5 years of wild bird surveillance. *Australian Veterinary Journal* **93**:387-393.

(Abstract)

Avian influenza viruses (AIVs) are found worldwide in numerous bird species, causing significant disease in gallinaceous poultry and occasionally other species. Surveillance of wild bird reservoirs provides an opportunity to add to the understanding of the epidemiology of AIVs.

Methods: This study examined key findings from the National Avian Influenza Wild Bird Surveillance Program over a 5-year period (July 2007–June 2012), the main source of information on AIVs circulating in Australia.

Results: The overall proportion of birds that tested positive for influenza A via PCR was $1.9 \pm 0.1\%$, with evidence of widespread exposure of Australian wild birds to most low pathogenic avian influenza (LPAI) subtypes (H1–13, H16). LPAI H5 subtypes were found to be dominant and widespread during this 5-year period.

Conclusion: Given Australia's isolation, both geographically and ecologically, it is important for Australia not to assume that the epidemiology of AIV from other geographic regions applies here. Despite all previous highly pathogenic avian influenza outbreaks in Australian poultry being attributed to H7 subtypes, widespread detection of H5 subtypes in wild birds may represent an ongoing risk to the Australian poultry industry.

Lee, D.-H., M. K. Torchetti, K. Winker, H. S. Ip, C.-S. Song, and D. E. Swayne. 2015. Intercontinental Spread of Asian-origin H5N8 to North America through Beringia by Migratory Birds. *Journal of Virology* **89**:6521-6524.

(Abstract)

Phylogenetic network analysis and understanding of waterfowl migration patterns suggest the Eurasian H5N8 clade 2.3.4.4 avian influenza virus emerged in late 2013 in China, spread in early 2014 to South Korea and Japan, and reached Siberia and Beringia by summer 2014 via migratory birds. Three genetically distinct subgroups emerged and subsequently spread along different flyways during fall 2014 into Europe, North America, and East Asia, respectively. All three subgroups reappeared in Japan, a wintering site for waterfowl from Eurasia and parts of North America.

Bodenstein, B., K. Beckmen, G. Sheffield, K. J. Kuletz, C. Van Hemert, B. Berlowski, and V. Shearn-Bochsler. 2015. Avian Cholera Causes Marine Bird Mortality in the Bering Sea of Alaska. *Journal of Wildlife Diseases* **51**:934-937.

(Abstract)

The first known avian cholera outbreak among wild birds in Alaska occurred during November 2013. Liver, intestinal, and splenic necrosis consistent with avian cholera was noted, and *Pasteurella*

multocida serotype 1 was isolated from liver and lung or spleen in Crested Auklets (*Aethia cristatella*), Thick-billed Murres (*Uria lomvia*), Common Eider (*Somateria mollissima*), Northern Fulmars (*Fulmarus glacialis*), and gulls (*Larus* spp.).

Tian, H., and B. Xu. 2015. Anthropogenic factors and societal response to challenges in the transmission of highly pathogenic avian influenza A (H5N1). *Annals of GIS* **21**:149–156.

(Abstract)

Research suggested that human infection with the novel avian influenza virus might be derived from live poultry markets. The sale of freshly slaughtered poultry, live poultry transportation, and mixed trading of different domestic animals in the live poultry markets all provide environments conducive to genome segment reassortment, gene mutation, and interspecies transmission of avian influenza virus. Production methods, human lifestyle, and the earth environment have been dramatically altered owing to rapid expansion of cities, industrialization, transportation, and accelerated population growth, in contrast to preserving traditional culture. People are exposed to the double burden of continued increasing incidence and mortality of the disease. In response to the huge challenge in the transmission of the disease, especially to prevent the spread of future avian influenza outbreaks, changing lifestyle and production habit should partly control the spread of the infections such as temporally closing live poultry markets, together with efforts to control the movement of illegal poultry products and wild birds. It is anticipated that by constantly seeking better solutions new models of living and development throughout society will gradually come into being.

Ramey, A. M., A. B. Reeves, R. L. Poulson, J. Wasley, D. Esler, and D. E. Stallknecht. 2015. Sampling of Sea Ducks for Influenza A viruses in Alaska during winter provides lack of evidence for epidemiologic peak of infection. *Journal of Wildlife Diseases* **51**:938-941.

(Abstract)

Sampling of sea ducks for influenza A viruses in Alaska during winter provided no evidence for an epidemiologic peak of infection. Isolates were recovered, however, that provide information on viral diversity and dispersal that may not be realized through sampling efforts focused on other avian taxa.

van Dijk, J. G. B., E. Kleyheeg, M. B. Soons, B. A. Nolet, R. A. M. Fouchier, and M. Klaassen. 2015. Weak negative associations between avian influenza virus infection and movement behaviour in a key host species, the mallard *Anas platyrhynchos*. *Oikos* **124**:1293–1303.

(Abstract)

Animal movements may contribute to the spread of pathogens. In the case of avian influenza virus, [migratory] birds have been suggested to play a role in the spread of some highly pathogenic strains (e.g. H5N1, H5N8), as well as their low pathogenic precursors which circulate naturally in wild birds. For a better understanding of the emergence and spread of both highly pathogenic (HPAIV) and low pathogenic avian influenza virus (LPAIV), the potential effects of LPAIVs on bird movement need to be evaluated. In a key host species, the mallard *Anas platyrhynchos*, we tested whether LPAIV infection status affected daily local (<100 m) and regional (>100 m) movements by comparing movement behaviour 1) within individuals (captured and sampled at two time points) and 2) between individuals (captured and sampled at one time point). We fitted free-living adult males with GPS loggers throughout the autumn LPAIV infection peak, and sampled them for LPAIV infection at logger deployment and at logger removal on recapture. Within individuals, we found no association between LPAIV infection and daily local and regional movements. Among individuals, daily regional movements of LPAIV infected mallards in the last days of tracking were lower than those of non-infected birds. Moreover, these regional movements of LPAIV infected birds were additionally reduced by poor weather conditions (i.e. increased wind and/or precipitation and lower temperatures). Local movements of LPAIV infected birds in the first days of tracking were higher when temperature decreased. Our study thus demonstrates that bird-assisted dispersal rate of LPAIV may be lower on a regional scale than expected on the basis of the movement behaviour of non-infected birds. Our study underlines the importance of understanding the impact of pathogen infection on host movement in order to assess its potential role in the emergence and spread of infectious diseases.

Kurscheid, J., J. Millar, M. Abdurrahman, I. G. Agung, A. Ambarawati, W. Suadnya, R. P. Yusuf, S. Fenwick, and J.-A. L. M. Toribio. 2015. Knowledge and Perceptions of Highly Pathogenic Avian Influenza (HPAI) among Poultry Traders in Live Bird Markets in Bali and Lombok, Indonesia. *PLoS ONE* **10**:e0139917.

(Abstract)

Highly Pathogenic Avian Influenza (HPAI) has been prevalent in Indonesia since 2003 causing major losses to poultry production and human deaths. Live bird markets are considered high risk areas due to the density of large numbers of mixed poultry species of unknown disease status. Understanding trader knowledge and perceptions of HPAI and biosecurity is critical to reducing transmission risk and controlling the disease. An interview-administered survey was conducted at 17 live bird markets on the islands of Bali and Lombok in 2008 and 2009. A total of 413 live poultry traders were interviewed. Respondents were mostly male (89%) with a mean age of 45 years (range: 19–81). The main source of AI information was TV (78%), although personal communication was also identified to be an important source, particularly among female traders (60%) and respondents from Bali (43%). More than half (58%) of live poultry traders interviewed knew that infected birds can transmit HPAI viruses but were generally unaware that viruses can be introduced to markets by fomites. Cleaning cages and disposing of sick and dead birds were recognized as the most important steps to prevent the spread of disease by respondents. Two thirds (n = 277) of respondents were unwilling to report sudden or suspicious bird deaths to authorities. Bali vendors perceive biosecurity to be of higher importance than Lombok vendors and are more willing to improve biosecurity within markets than

traders in Lombok. Collectors and traders selling large numbers (>214) of poultry, or selling both chickens and ducks, have better knowledge of HPAI transmission and prevention than vendors or traders selling smaller quantities or only one species of poultry. Education was strongly associated with better knowledge but did not influence positive reporting behavior. Our study reveals that most live poultry traders have limited knowledge of HPAI transmission and prevention and are generally reluctant to report bird deaths. Greater efforts are needed to engage local government, market managers and traders in education and awareness programs, regulatory measures and incentive mechanisms. Understanding and evaluating the social responses to such an integrated approach could lead to more effective HPAI prevention and control.

Guillemain, M., J. Champagnon, M.-L. Gourlay-Larour, F. Cavallo, A.-L. Brochet, J. Hars, G. Massez, T. George, P.-Y. Perroi, V. Jestin, and A. Caizergues. 2015. Blood and cloacal swab sampling for avian influenza monitoring has no effect on survival rates of free-ranging ducks. *Ibis* **157**:743–753.

(Abstract)

Concerns about the spread of avian influenza viruses (AIVs) have led to cloacal swab sampling of hundreds of thousands of birds worldwide as part of AIV surveillance schemes, but the effects of cloacal swabbing have not been adequately evaluated. We tested for differences between swabbed, swabbed and bled, and non-sampled wild ducks in terms of live re-encounter and dead recoveries for Common Pochard *Aythya ferina* and Tufted Duck *Aythya fuligula*, and also determined re-encounter and recovery rates for Mallard *Anas platyrhynchos* and Common Teal *Anas crecca*. No effects of sampling methods were detected, except in Teal. Re-encounter rates were lower in sampled Teal than in controls, with annual re-encounter probabilities being 25% and 35% lower in males and females, respectively. Teal possibly left or avoided sampling sites, or sought sites where they were less detectable after sampling. In general, no deleterious effects were found, suggesting that cloacal swabbing and blood sampling are suitable methods for conducting AIV surveillance in ducks.

Nguyen, T. H., V. T. Than, H. D. Thanh, V. Q. Nguyen, K. H. Nguyen, D. T. Nguyen, J.-H. Park, I. S. Chung, D. G. Jeong, K.-T. Chang, T. K. Oh, and W. Kim. 2015. The evolutionary dynamics of highly pathogenic avian influenza H5N1 in south-central Vietnam reveals multiple clades evolving from Chinese and Cambodian viruses. *Comparative Immunology, Microbiology and Infectious Diseases* **42**:21–30.

(Abstract)

In Vietnam, highly pathogenic avian influenza (HPAI), such as that caused by H5N1 viruses, is the most highly contagious infectious disease that has been affecting domestic poultry in recent years. Vietnam might be an evolutionary hotspot and a potential source of globally pandemic strains. However, few studies have reported viruses circulating in the south-central region of Vietnam. In the present study, 47H5N1-positive samples were collected from both vaccinated and unvaccinated

poultry farms in the South Central Coast region of Vietnam during 2013–2014, and their genetic diversity was analyzed. A common sequence motif for HPAI virus was identified at HA-cleavage sites in all samples: either RERRRKR/G (clades 2.3.2.1c and 2.3.2.1a) or REGRRKCR/G (clade 1.1.2). Phylogenetic analysis of HA genes identified three clades of HPAI H5N1: 1.1.2 (n = 1), 2.3.2.1a (n = 1), and 2.3.2.1c (n = 45). The phylogenetic analysis indicated that these Vietnamese clades may have evolved from Chinese and Cambodian virus clades isolated in 2012–2013 but are less closely related to the clades detected from the Tyva Republic, Bulgaria, Mongolia, Japan, and Korea in 2009–2011. Detection of the coexistence of virus clades 2.3.2.1 and the very virulent 1.1.2 in the south-central regions suggests their local importance and highlights concerns regarding their spread, both northwards and southwards, as well as the potential for reassortment. The obtained data highlight the importance of regular identification of viral evolution and the development and use of region-specific vaccines.

Bi, Y., Z. Zhang, W. Liu, Y. Yin, J. Hong, X. Li, H. Wang, G. Wong, J. Chen, Y. Li, W. Ru, R. Gao, D. Liu, Y. Liu, B. Zhou, G. F. Gao, W. Shi, and F. Lei. 2015. Highly Pathogenic Avian Influenza A(H5N1) Virus Struck Migratory Birds in China in 2015. *Scientific Reports* 5:12986.

(Abstract)

Approximately 100 migratory birds, including whooper swans and pochards, were found dead in the Sanmenxia Reservoir Area of China during January 2015. The causative agent behind this outbreak was identified as H5N1 highly pathogenic avian influenza virus (HPAIV). Genetic and phylogenetic analyses revealed that this Sanmenxia H5N1 virus was a novel reassortant, possessing a Clade 2.3.2.1c HA gene and a H9N2-derived PB2 gene. Sanmenxia Clade 2.3.2.1c-like H5N1 viruses possess the closest genetic identity to A/Alberta/01/2014 (H5N1), which recently caused a fatal respiratory infection in Canada with signs of meningoencephalitis, a highly unusual symptom with influenza infections in humans. Furthermore, this virus was shown to be highly pathogenic to both birds and mammals, and demonstrate tropism for the nervous system. Due to the geographical location of Sanmenxia, these novel H5N1 viruses also have the potential to be imported to other regions through the migration of wild birds, similar to the H5N1 outbreak amongst migratory birds in Qinghai Lake during 2005. Therefore, further investigation and monitoring is required to prevent this novel reassortant virus from becoming a new threat to public health.

Hiono, T., A. Ohkawara, K. Ogasawara, M. Okamatsu, T. Tamura, D.-H. Chu, M. Suzuki, S. Kuribayashi, S. Shichinohe, A. Takada, H. Ogawa, R. Yoshida, H. Miyamoto, N. Nao, W. Furuyama, J. Maruyama, N. Eguchi, G. Ulziibat, B. Enkhbold, M. Shatar, T. Jargalsaikhan, S. Byambadorj, B. Damdinjav, Y. Sakoda, and H. Kida. 2015. Genetic and antigenic characterization of H5 and H7 influenza viruses isolated from migratory water birds in Hokkaido, Japan and Mongolia from 2010 to 2014. *Virus Genes* 51:57–68.

(Abstract)

Migratory water birds are the natural reservoir of influenza A viruses. H5 and H7 influenza viruses are isolated over the world and also circulate among poultry in Asia. In 2010, two H5N1 highly pathogenic avian influenza viruses (HPAIVs) were isolated from fecal samples of water birds on the flyway of migration from Siberia, Russia to the south in Hokkaido, Japan. H7N9 viruses are sporadically isolated from humans and circulate in poultry in China. To monitor whether these viruses have spread in the wild bird population, we conducted virological surveillance of avian influenza in migratory water birds in autumn from 2010 to 2014. A total of 8103 fecal samples from migratory water birds were collected in Japan and Mongolia, and 350 influenza viruses including 13 H5 and 19 H7 influenza viruses were isolated. A phylogenetic analysis revealed that all isolates are genetically closely related to viruses circulating among wild water birds. The results of the antigenic analysis indicated that the antigenicity of viruses in wild water birds is highly stable despite their nucleotide sequence diversity but is distinct from that of HPAIVs recently isolated in Asia. The present results suggest that HPAIVs and Chinese H7N9 viruses were not predominantly circulating in migratory water birds; however, continued monitoring of H5 and H7 influenza viruses both in domestic and wild birds is recommended for the control of avian influenza.

Koçer, Z. A., R. Carter, G. Wu, J. Zhang, and R. G. Webster. 2015. The Genomic Contributions of Avian H1N1 Influenza A Viruses to the Evolution of Mammalian Strains. *PLoS ONE* **10**:e0133795.

(Abstract)

Among the influenza A viruses (IAVs) in wild aquatic birds, only H1, H2, and H3 subtypes have caused epidemics in humans. H1N1 viruses of avian origin have also caused 3 of 5 pandemics. To understand the reappearance of H1N1 in the context of pandemic emergence, we investigated whether avian H1N1 IAVs have contributed to the evolution of human, swine, and 2009 pandemic H1N1 IAVs. On the basis of phylogenetic analysis, we concluded that the polymerase gene segments (especially PB2 and PA) circulating in North American avian H1N1 IAVs have been reintroduced to swine multiple times, resulting in different lineages that led to the emergence of the 2009 pandemic H1N1 IAVs. Moreover, the similar topologies of hemagglutinin and nucleoprotein and neuraminidase and matrix gene segments suggest that each surface glycoprotein coevolved with an internal gene segment within the H1N1 subtype. The genotype of avian H1N1 IAVs of Charadriiformes origin isolated in 2009 differs from that of avian H1N1 IAVs of Anseriformes origin. When the antigenic sites in the hemagglutinin of all 31 North American avian H1N1 IAVs were considered, 60%-80% of the amino acids at the antigenic sites were identical to those in 1918 and/or 2009 pandemic H1N1 viruses. Thus, although the pathogenicity of avian H1N1 IAVs could not be inferred from the phylogeny due to the small dataset, the evolutionary process within the H1N1 IAV subtype suggests that the circulation of H1N1 IAVs in wild birds poses a continuous threat for future influenza pandemics in humans.

Rees, J. D., J. K. Webb, M. S. Crowther, and M. Letnic. 2015. Carrion subsidies provided by fishermen increase predation of beach-nesting bird nests by facultative scavengers. *Animal Conservation* **18**:44–49.

(Abstract)

Many predators are also scavengers that feed on carrion and human refuse. Therefore, the availability of carrion can elevate the abundance or activity of facultative scavengers, amplifying predation pressure on prey. On Australian beaches, fishermen often discard fish carcasses that could attract facultative scavengers, both native, such as Australian ravens *Corvus coronoides*, and invasive, such as European red foxes *Vulpes vulpes*, and result in elevated rates of predation on wildlife. We tested whether the presence of fish carcasses increased the risk of depredation for nearby nests of beach-nesting birds by deploying artificial nests in 12 subsidized and 12 control patches, spaced 1 km apart, on a beach. We placed a fish carcass in each subsidized patch, but not at control patches. In each patch, we placed two artificial nests, which resembled redcapped plover *Charadrius ruficapillus* nests, 80 m apart and 40 m from carcasses at subsidized patches. Nest predators were identified from tracks and predator activity near subsidized and control nests was measured by counting tracks crossing a straight transect (220 m). The activity of a native predator, the Australian raven, was 17 times higher near (<80 m) nests with fish carcasses than nests without carcasses. After 72 h, 96% of nests near carcasses were depredated compared with 30% of nests without carcasses. Ravens were identified as the culprit for 80% of depredated nests. Although other predators were present in the study area, they did not depredate artificial nests in this experiment. Previous studies have highlighted the effects of permanent and/or large-scale food resources on scavenger abundance and impact. A key management implication of our study is that even small, sparsely distributed, temporally irregular food subsidies, provided by humans, can elevate the activity and predatory impacts of facultative scavengers.

Read, A. F., S. J. Baigent, C. Powers, L. B. Kgosana, L. Blackwell, L. P. Smith, D. A. Kennedy, S. W. Walkden-Brown, and V. K. Nair. 2015. Imperfect Vaccination Can Enhance the Transmission of Highly Virulent Pathogens. *PLoS Biol* **13**:e1002198.

(Abstract)

Could some vaccines drive the evolution of more virulent pathogens? Conventional wisdom is that natural selection will remove highly lethal pathogens if host death greatly reduces transmission. Vaccines that keep hosts alive but still allow transmission could thus allow very virulent strains to circulate in a population. Here we show experimentally that immunization of chickens against Marek's disease virus enhances the fitness of more virulent strains, making it possible for hyperpathogenic strains to transmit. Immunity elicited by direct vaccination or by maternal vaccination prolongs host survival but does not prevent infection, viral replication or transmission, thus extending the infectious periods of strains otherwise too lethal to persist. Our data show that anti-disease vaccines that do not prevent transmission can create conditions that promote the emergence of pathogen strains that cause more severe disease in unvaccinated hosts.

Author Summary: There is a theoretical expectation that some types of vaccines could prompt the evolution of more virulent ("hotter") pathogens. This idea follows from the notion that natural selection

removes pathogen strains that are so “hot” that they kill their hosts and, therefore, themselves. Vaccines that let the hosts survive but do not prevent the spread of the pathogen relax this selection, allowing the evolution of hotter pathogens to occur. This type of vaccine is often called a leaky vaccine. When vaccines prevent transmission, as is the case for nearly all vaccines used in humans, this type of evolution towards increased virulence is blocked. But when vaccines leak, allowing at least some pathogen transmission, they could create the ecological conditions that would allow hot strains to emerge and persist. This theory proved highly controversial when it was first proposed over a decade ago, but here we report experiments with Marek’s disease virus in poultry that show that modern commercial leaky vaccines can have precisely this effect: they allow the onward transmission of strains otherwise too lethal to persist. Thus, the use of leaky vaccines can facilitate the evolution of pathogen strains that put unvaccinated hosts at greater risk of severe disease. The future challenge is to identify whether there are other types of vaccines used in animals and humans that might also generate these evolutionary risks.

Hill, S. C., Y.-J. Lee, B.-M. Song, H.-M. Kang, E.-K. Lee, A. Hanna, M. Gilbert, I. H. Brown, and O. G. Pybus. 2015. Wild waterfowl migration and domestic duck density shape the epidemiology of highly pathogenic H5N8 influenza in the Republic of Korea. *Infection, Genetics and Evolution* **34**:267–277.

(Abstract)

Highly pathogenic avian influenza (HPAI) viruses threaten human and animal health yet their emergence is poorly understood, partly because sampling of the HPAI Asian-origin H5N1 lineage immediately after its identification in 1996 was comparatively sparse. The discovery of a novel H5N8 virus in 2013 provides a new opportunity to investigate HPAI emergence in greater detail. Here we investigate the origin and transmission of H5N8 in the Republic of Korea, the second country to report the new strain. We reconstruct viral spread using phylogeographic methods and interpret the results in the context of ecological data on poultry density, overwintering wild bird numbers, and bird migration patterns. Our results indicate that wild waterfowl migration and domestic duck density were important to H5N8 epidemiology. Specifically, we infer that H5N8 entered the Republic of Korea via Jeonbuk province, then spread rapidly among western provinces where densities of overwintering waterfowl and domestic ducks are higher, yet rarely persisted in eastern regions. The common ancestor of H5N8 in the Republic of Korea was estimated to have arrived during the peak of inward migration of overwintering birds. Recent virus isolations likely represent re-introductions via bird migration from an as-yet unsampled reservoir. Based on the limited data from outside the Republic of Korea, our data suggest that H5N8 may have entered Europe at least twice, and Asia at least three times from this reservoir, most likely carried by wild migrating birds.

Backer, J. A., H. J. W. van Roermund, E. A. J. Fischer, M. A. P. M. van Asseldonk, and R. H. M. Bergevoet. 2015. Controlling highly pathogenic avian influenza outbreaks: An epidemiological and economic model analysis. *Preventive Veterinary Medicine* **121**:142–150.

(Abstract)

Outbreaks of highly pathogenic avian influenza (HPAI) can cause large losses for the poultry sector and for animal disease controlling authorities, as well as risks for animal and human welfare. In the current simulation approach epidemiological and economic models are combined to compare different strategies to control highly pathogenic avian influenza in Dutch poultry flocks. Evaluated control strategies are the minimum EU strategy (i.e., culling of infected flocks, transport regulations, tracing and screening of contact flocks, establishment of protection and surveillance zones), and additional control strategies comprising pre-emptive culling of all susceptible poultry flocks in an area around infected flocks (1 km, 3 km and 10 km) and emergency vaccination of all flocks except broilers around infected flocks (3 km). Simulation results indicate that the EU strategy is not sufficient to eradicate an epidemic in high density poultry areas. From an epidemiological point of view, this strategy is the least effective, while pre-emptive culling in 10km radius is the most effective of the studied strategies. But these two strategies incur the highest costs due to long duration (EU strategy) and large-scale culling (pre-emptive culling in 10km radius). Other analysed pre-emptive culling strategies (i.e., in 1 km and 3 km radius) are more effective than the analysed emergency vaccination strategy (in 3 km radius) in terms of duration and size of the epidemics, despite the assumed optimistic vaccination capacity of 20 farms per day. However, the total costs of these strategies differ only marginally. Extending the capacity for culling substantially reduces the duration, size and costs of the epidemic. This study demonstrates the strength of combining epidemiological and economic model analysis to gain insight in a range of consequences and thus to serve as a decision support tool in the control of HPAI epidemics.

Sims, L. D., J. Domenech, C. Benigno, S. Kahn, A. Kamata, J. Lubroth, V. Martin, and P. Roeder. 2005. Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. *Veterinary Record* **157**:159-164.

(Abstract)

Outbreaks of highly pathogenic avian influenza caused by H5N1 viruses were reported almost simultaneously in eight neighbouring Asian countries between December 2003 and January 2004, with a ninth reporting in August 2004, suggesting that the viruses had spread recently and rapidly. However, they had been detected widely in the region in domestic waterfowl and terrestrial poultry for several years before this, and the absence of widespread disease in the region before 2003, apart from localised outbreaks in the Hong Kong Special Autonomous Region (SAR), is perplexing. Possible explanations include limited virus excretion by domestic waterfowl infected with H5N1, the confusion of avian influenza with other serious endemic diseases, the unsanctioned use of vaccines, and the under-reporting of disease as a result of limited surveillance. There is some evidence that the excretion of the viruses by domestic ducks had increased by early 2004, and there is circumstantial evidence that they can be transmitted by wild birds. The migratory birds from which viruses have been isolated were usually sick or dead, suggesting that they would have had limited potential for carrying the viruses over long distances unless subclinical infections were prevalent. However, there is strong circumstantial evidence that wild birds can become infected from domestic poultry and potentially can exchange viruses when they share the same environment. Nevertheless, there is little reason to believe that wild birds have played a more significant role in spreading disease than trade through

live bird markets and movement of domestic waterfowl. Asian H5N1 viruses were first detected in domestic geese in southern China in 1996. By 2000, their host range had extended to domestic ducks, which played a key role in the genesis of the 2003/04 outbreaks. The epidemic was not due to the introduction and spread of a single virus but was caused by multiple viruses which were genotypically linked to the Goose/GD/96 lineage via the haemagglutinin gene. The H5N1 viruses isolated from China, including the Hong Kong SAR, between 1999 and 2004 had a range of genotypes and considerable variability within genotypes. The rising incidence and widespread reporting of disease in 2003/04 can probably be attributed to the increasing spread of the viruses from existing reservoirs of infection in domestic waterfowl and live bird markets leading to greater environmental contamination. When countries in the region started to report disease in December 2003, others were alerted to the risk and disease surveillance and reporting improved. The H5N1 viruses have reportedly been eliminated from three of the nine countries that reported disease in 2003/04, but they could be extremely difficult to eradicate from the remaining countries, owing to the existence of populations and, possibly, production and marketing sectors, in which apparently normal birds harbour the viruses.

Shin, J.-H., C. Woo, S.-J. Wang, J. Jeong, I.-J. An, J.-K. Hwang, S.-D. Jo, S. D. Yu, K. Choi, H.-M. Chung, J.-H. Suh, and S.-H. Kim. 2015. Prevalence of avian influenza virus in wild birds before and after the HPAI H5N8 outbreak in 2014 in South Korea. *Journal of Microbiology* **53**:475-480.

(Abstract)

Since 2003, highly pathogenic avian influenza (HPAI) virus outbreaks have occurred five times in Korea, with four HPAI H5N1 outbreaks and one HPAI H5N8 outbreak. Migratory birds have been suggested to be the first source of HPAI in Korea. Here, we surveyed migratory wild birds for the presence of AI and compared regional AI prevalence in wild birds from September 2012 to April 2014 for birds having migratory pathways in South Korea. Finally, we investigated the prevalence of AI in migratory birds before and after HPAI H5N8 outbreaks. Overall, we captured 1617 migratory wild birds, while 18,817 feces samples and 74 dead birds were collected from major wild bird habitats. A total of 21 HPAI viruses were isolated from dead birds, and 86 low pathogenic AI (LPAI) viruses were isolated from captured birds and from feces samples. Spatiotemporal distribution analysis revealed that AI viruses were spread southward until December, but tended to shift north after January, consistent with the movement of migratory birds in South Korea. Furthermore, we found that LPAI virus prevalence within wild birds were notably higher in 2013–2014 than the previous prevalence during the northward migration season. The data from our study demonstrate the importance of the surveillance of AI in wild birds. Future studies including in-depth genetic analysis in combination with evaluation of the movement and ecology of migratory birds might help us to bridge the gaps in our knowledge and better explain, predict, and ultimately prevent future HPAI outbreaks.

Kocer, Z. A., S. Krauss, M. Zanin, A. Danner, S. Gulati, J. C. Jones, K. Friedman, A. Graham, H. Forrest, J. Seiler, G. M. Air, and R. G. Webster. 2015. Possible basis for the emergence of H1N1 viruses with pandemic potential from avian hosts. *Emerging Microbes and Infections* 4:e40.

(Abstract)

Influenza A viruses of the H1N1 subtype have emerged from the avian influenza gene pool in aquatic birds and caused human pandemics at least twice during the past century. Despite this fact, surprisingly little is known about the H1N1 gene pool in the aquatic bird reservoir. A preliminary study showed that an H1N1 virus from a shorebird of the Charadriiformes order was transmitted between animals through the airborne route of infection, whereas an H1N1 virus from a bird of the Anseriformes order was not. Here we show that two of the three H1N1 viruses isolated from Charadriiformes species in 2009 were transmitted between animals through the airborne route of infection, and five H1N1 isolates from Anseriformes species were not. The one H1N1 virus from a Charadriiformes species that failed to transmit through the airborne route was a reassortant possessing multiple internal gene segments from Anseriformes species. The molecular differences between the airborne-transmissible and non-airborne-transmissible H1N1 viruses were multigenic, involving the selection of virus with human-like receptor-binding specificity (α 2-6 sialic acid) and multiple differences in the polymerase complex, mainly in the PB2, PB1-F2, and nonstructural genes.

Nallar, R., Z. Papp, T. Epp, F. A. Leighton, S. R. Swafford, T. J. DeLiberto, R. J. Dusek, H. S. Ip, J. S. Hall, Y. Berhane, S. E. Gibbs, and C. Soos. 2015. Demographic and Spatiotemporal Patterns of Avian Influenza Infection at the Continental Scale, and in Relation to Annual Life Cycle of a Migratory Host. *PLoS ONE* 10:e0130662.

(Abstract)

Since the spread of highly pathogenic avian influenza (HPAI) H5N1 in the eastern hemisphere, numerous surveillance programs and studies have been undertaken to detect the occurrence, distribution, or spread of avian influenza viruses (AIV) in wild bird populations worldwide. To identify demographic determinants and spatiotemporal patterns of AIV infection in long distance migratory waterfowl in North America, we fitted generalized linear models with binomial distribution to analyze results from 13,574 blue-winged teal (*Anas discors*, BWTE) sampled in 2007 to 2010 year round during AIV surveillance programs in Canada and the United States. Our analyses revealed that during late summer staging (July-August) and fall migration (September-October), hatch year (HY) birds were more likely to be infected than after hatch year (AHY) birds, however there was no difference between age categories for the remainder of the year (winter, spring migration, and breeding period), likely due to maturing immune systems and newly acquired immunity of HY birds. Probability of infection increased non-linearly with latitude, and was highest in late summer prior to fall migration when densities of birds and the proportion of susceptible HY birds in the population are highest. Birds in the Central and Mississippi flyways were more likely to be infected compared to those in the Atlantic flyway. Seasonal cycles and spatial variation of AIV infection were largely driven by the dynamics of AIV infection in HY birds, which had more prominent cycles and spatial variation in infection compared to AHY birds. Our results demonstrate demographic as well as seasonal, latitudinal and flyway trends

across Canada and the US, while illustrating the importance of migratory host life cycle and age in driving cyclical patterns of prevalence.

Krauss, S., K. M. Stucker, S. A. Schobel, A. Danner, K. Friedman, J. P. Knowles, G. Kayali, L. J. Niles, A. D. Dey, G. Raven, P. Pryor, X. Lin, S. R. Das, T. B. Stockwell, D. E. Wentworth, and R. G. Webster. 2015. Long-term surveillance of H7 influenza viruses in American wild aquatic birds: are the H7N3 influenza viruses in wild birds the precursors of highly pathogenic strains in domestic poultry? *Emerging Microbes and Infections* 4:e35.

(Abstract)

The emergence of influenza A virus (IAV) in domestic avian species and associated transmissions to mammals is unpredictable. In the Americas, the H7 IAVs are of particular concern, and there have been four separate outbreaks of highly pathogenic (HP) H7N3 in domestic poultry in North and South America between 2002 and 2012, with occasional spillover into humans. Here, we use long-term IAV surveillance in North American shorebirds at Delaware Bay, USA, from 1985 to 2012 and in ducks in Alberta, Canada, from 1976 to 2012 to determine which hemagglutinin (HA)–neuraminidase (NA) combinations predominated in Anseriformes (ducks) and Charadriiformes (shorebirds) and whether there is concordance between peaks of H7 prevalence and transmission in wild aquatic birds and the emergence of H7 IAVs in poultry and humans. Whole-genome sequencing supported phylogenetic and genomic constellation analyses to determine whether HP IAVs emerge in the context of specific internal gene segment sequences. Phylogenetic analysis of whole-genome sequences of the H7N3 influenza viruses from wild birds and HP H7N3 outbreaks in the Americas indicate that each HP outbreak was an independent emergence event and that the low pathogenic (LP) avian influenza precursors were most likely from dabbling ducks. The different polybasic cleavage sites in the four HP outbreaks support independent origins. At the 95% nucleotide percent identity-level phylogenetic analysis showed that the wild duck HA, PB1, and M sequences clustered with the poultry and human outbreak sequences. The genomic constellation analysis strongly suggests that gene segments/virus flow from wild birds to domestic poultry.

Bridge, E. S., J. F. Kelly, X. Xiao, N. Batbayar, T. Natsagdorj, N. J. Hill, J. Y. Takekawa, L. A. Hawkes, C. M. Bishop, P. J. Butler, and S. H. Newman. 2015. Stable Isotopes Suggest Low Site Fidelity in Bar-headed Geese *Waterbirds* 38:123-132.

(Abstract)

Population connectivity is an important consideration in studies of disease transmission and biological conservation, especially with regard to migratory species. Determining how and when different subpopulations intermingle during different phases of the annual cycle can help identify important geographical regions or features as targets for conservation efforts and can help inform our understanding of continental-scale disease transmission. In this study, stable isotopes of hydrogen

and carbon in contour feathers were used to assess the degree of molt-site fidelity among Bar-headed Geese (*Anser indicus*) captured in north-central Mongolia. Samples were collected from actively molting Bar-headed Geese (n = 61), and some individual samples included both a newly grown feather (still in sheath) and an old, worn feather from the bird's previous molt (n = 21). Although there was no difference in mean hydrogen isotope ratios for the old and new feathers, the isotopic variance in old feathers was approximately three times higher than that of the new feathers, which suggests that these birds use different and geographically distant molting locations from year to year. To further test this conclusion, online data and modeling tools from the isoMAP website were used to generate probability landscapes for the origin of each feather. Likely molting locations were much more widespread for old feathers than for new feathers, which supports the prospect of low molt-site fidelity. This finding indicates that population connectivity would be greater than expected based on data from a single annual cycle, and that disease spread can be rapid even in areas like Mongolia where Bar-headed Geese generally breed in small isolated groups.

Cumming, G. S., C. Abolnik, A. Caron, N. Gaidet, J. Grewar, E. Hellard, D. A. W. Henry, and C. Reynolds. 2015. A social–ecological approach to landscape epidemiology: geographic variation and avian influenza. *Landscape Ecology* **30**:963–985.

(Abstract)

Context: Landscape structure influences host–parasite–pathogen dynamics at multiple scales in space and time. Landscape epidemiology, which connects disease ecology and landscape ecology, is still an emerging field.

Objective: We argue that landscape epidemiology must move beyond simply studying the influence of landscape configuration and composition on epidemiological processes and towards a more comparative, systems approach that better incorporates social–ecological complexity.

Methods: We illustrate our argument with a detailed review, based on a single conceptual systems model, of geographic variation in drivers of avian influenza in Western Europe, Southeast Asia, and Southern Africa.

Results: Our three study regions are similar in some ways but quite different in others. The same underlying mechanisms apply in all cases, but differences in the attributes of key components and linkages (most notably avian diversity, the abiotic environment, land use and land cover, and food production systems) create significant differences in avian influenza virus prevalence and human risk between regions.

Conclusions: Landscape approaches can connect local- and continental-scale elements of epidemiology. Adopting a landscape-focused systems perspective on the problem facilitates the identification of the most important commonalities and differences, guiding both science and policy, and helps to identify elements of the problem on which further research is needed. More generally, our review demonstrates the importance of social–ecological interactions and comparative approaches for landscape epidemiology.

Ozawa, M., A. Matsuu, K. Tokorozaki, M. Horie, T. Masatani, H. Nakagawa, K. Okuya, T. Kawabata, and S. Toda. 2015. Genetic diversity of highly pathogenic H5N8 avian influenza viruses at a single overwintering site of migratory birds in Japan, 2014/15. *Euro Surveillance* **20**:1-13.

(Abstract)

We isolated eight highly pathogenic H5N8 avian influenza viruses (H5N8 HPAIVs) in the 2014/15 winter season at an overwintering site of migratory birds in Japan. Genetic analyses revealed that these isolates were divided into three groups, indicating the co-circulation of three genetic groups of H5N8 HPAIV among these migratory birds. These results also imply the possibility of global redistribution of the H5N8 HPAIVs via the migration of these birds next winter.

Lebarbenchon, C., A. Jaeger, C. J. Feare, M. Bastien, M. Dietrich, C. S. Larose, E. Lagadec, G. Rocamora, N. Shah, H. Pascalis, T. Boulinier, M. Le Corre, D. E. Stallknecht, and K. Dellagi. 2015. Influenza A Virus on Oceanic Islands: Host and Viral Diversity in Seabirds in the Western Indian Ocean. *PLoS Pathogens* **11**:e1004925.

(Abstract)

Ducks and seabirds are natural hosts for influenza A viruses (IAV). On oceanic islands, the ecology of IAV could be affected by the relative diversity, abundance and density of seabirds and ducks. Seabirds are the most abundant and widespread avifauna in the Western Indian Ocean and, in this region, oceanic islands represent major breeding sites for a large diversity of potential IAV host species. Based on serological assays, we assessed the host range of IAV and the virus subtype diversity in terms of the islands of the Western Indian Ocean. We further investigated the spatial variation in virus transmission patterns between islands and identified the origin of circulating viruses using a molecular approach. Our findings indicate that terns represent a major host for IAV on oceanic islands, not only for seabird-related virus subtypes such as H16, but also for those commonly isolated in wild and domestic ducks (H3, H6, H9, H12 subtypes). We also identified strong species-associated variation in virus exposure that may be associated to differences in the ecology and behaviour of terns. We discuss the role of tern migrations in the spread of viruses to and between oceanic islands, in particular for the H2 and H9 IAV subtypes.

Author Summary: Avian influenza viruses circulate in wild birds, worldwide, in particular in ducks and seabirds from which a large diversity of viruses has been described. The continued emergence of influenza viruses in poultry and humans has stimulated both research activities and surveillance programs; however, there are still many gaps in our knowledge on virus ecology and epidemiology, in particular in the Southern Hemisphere. In this study we investigated influenza virus circulation in seabirds in the islands of the Western Indian Ocean. We demonstrate that terns act as a major host for influenza viruses on oceanic islands and that, in addition to being infected with virus subtypes usually associated to wild birds, they also could regularly be in contact with viruses that represent a significant threat to veterinary and human health. This study demonstrates that the spatial isolation of these oceanic islands does not limit connectivity with the global avian influenza virus epidemiology and that it may create opportunities for local viral maintenance in wild bird communities.

Dalby, A. R., and M. Iqbal. 2015. The European and Japanese outbreaks of H5N8 derive from a single source population providing evidence for the dispersal along the long distance bird migratory flyways. *PeerJ* **3**:e934.

(Abstract)

The origin of recent parallel outbreaks of the high pathogenicity H5N8 avian flu virus in Europe and in Japan can be traced to a single source population, which has most likely been spread by migratory birds. By using Bayesian coalescent methods to analyze the DNA sequences of the virus to find the times for divergence and combining this sequence data with bird migration data we can show the most likely locations and migratory pathways involved in the origin of the current outbreak. This population was most likely located in the Siberian summer breeding grounds of long-range migratory birds. These breeding grounds provide a connection between different migratory flyways and explain the current outbreaks in remote locations. By combining genetic methods and epidemiological data we can rapidly identify the sources and the dispersion pathways for novel avian influenza outbreaks.

Li, X.-L., K. Liu, H.-W. Yao, Y. Sun, W.-J. Chen, R.-X. Sun, S. J. de Vlas, L.-Q. Fang, and W.-C. Cao. 2015. Highly Pathogenic Avian Influenza H5N1 in Mainland China. *International Journal of Environmental Research and Public Health* **12**:5026-5045.

(Abstract)

Highly pathogenic avian influenza (HPAI) H5N1 has posed a significant threat to both humans and birds, and it has spanned large geographic areas and various ecological systems throughout Asia, Europe and Africa, but especially in mainland China. Great efforts in control and prevention of the disease, including universal vaccination campaigns in poultry and active serological and virological surveillance, have been undertaken in mainland China since the beginning of 2006. In this study, we aim to characterize the spatial and temporal patterns of HPAI H5N1, and identify influencing factors favoring the occurrence of HPAI H5N1 outbreaks in poultry in mainland China. Our study shows that HPAI H5N1 outbreaks took place sporadically after vaccination campaigns in poultry, and mostly occurred in the cold season. The positive tests in routine virological surveillance of HPAI H5N1 virus in chicken, duck, goose as well as environmental samples were mapped to display the potential risk distribution of the virus. Southern China had a higher positive rate than northern China, and positive samples were mostly detected from chickens in the north, while the majority were from duck in the south, and a negative correlation with monthly vaccination rates in domestic poultry was found ($R = -0.19$, p value = 0.005). Multivariate panel logistic regression identified vaccination rate, interaction between distance to the nearest city and national highway, interaction between distance to the nearest lake and wetland, and density of human population, as well as the autoregressive term in space and time as independent risk factors in the occurrence of HPAI H5N1 outbreaks, based on which a predicted risk map of the disease was derived. Our findings could provide new understanding of the distribution and transmission of HPAI H5N1 in mainland China and could be used to inform targeted

surveillance and control efforts in both human and poultry populations to reduce the risk of future infections.

Tian, H., S. Zhou, L. Dong, T. P. Van Boeckel, Y. Pei, Q. Wu, W. Yuan, Y. Guo, S. Huang, W. Chen, X. Lu, Z. Liu, Y. Bai, T. Yu, B. T. Grenfell, and B. Xu. 2015. Climate change suggests a shift of H5N1 risk in migratory birds. *Ecological Modelling* **306**:6–15.

(Abstract)

Migratory birds are considered to have played an important role in the spread of highly pathogenic avian influenza H5N1. However, how bird species are expected to modify their wintering sites in response to climate change, and in turn affect the geographic distribution of the risk associated with H5N1 outbreaks, is unknown. We explored the association between past climate variability and H5N1 outbreaks that were attributed to migratory birds from 2005 to 2009. We then predicted the distribution of risk associated with H5N1 outbreaks based on future climate change scenarios. Overlapping the probabilities of bird distribution and H5N1 outbreaks produced final emergence risk. Our results suggest that minimum daily temperature in the winter (-15 to -11 °C, 15 to 17 °C) and maximum daily temperature in the summer (12 to 15 °C, 30 to 35 °C) governed wild bird migratory routes, while high mean air pressure and low mean specific humidity in the winter impacted the outbreaks of H5N1 among migratory birds. By the end of 2030, Europe may be at higher risk for H5N1 outbreaks in January and February. Northern Africa and Southern and Western Asia will likely be a higher risk for H5N1 outbreaks from April to June. Our findings suggest a potential shift in H5N1 risk from Southeast Asia to the western part of the world due to climate change. The results of this study could be used to inform policy in the area of H5N1 outbreak detection and preparedness.

Kang, H.-M., E.-K. Lee, B.-M. Song, J. Jeong, J.-G. Choi, J. Jeong, O.-K. Moon, H. Yoon, Y. Cho, Y.-M. Kang, H.-S. Lee, and Y.-J. Lee. 2015. Novel Reassortant Influenza A(H5N8) Viruses among Inoculated Domestic and Wild Ducks, South Korea, 2014. *Emerging Infectious Diseases* **21**:298-304.

(Abstract)

An outbreak of highly pathogenic avian influenza, caused by a novel reassortant influenza A (H5N8) virus, occurred among poultry and wild birds in South Korea in 2014. The aim of this study was to evaluate the pathogenesis in and mode of transmission of this virus among domestic and wild ducks. Three of the viruses had similar pathogenicity among infected domestic ducks: the H5N8 viruses were moderately pathogenic (0%–20% mortality rate); in wild mallard ducks, the H5N8 and H5N1 viruses did not cause severe illness or death; viral replication and shedding were greater in H5N8-infected mallards than in H5N1-infected mallards. Identification of H5N8 viruses in birds exposed to infected domestic ducks and mallards indicated that the viruses could spread by contact. We propose active surveillance to support prevention of the spread of this virus among wild birds and poultry, especially domestic ducks.

Fearnley, L. 2015. Wild goose chase: The Displacement of Influenza Research in the Fields of Poyang Lake, China. *Cultural Anthropology* 30:12-35.

(Abstract)

This article follows transnational avian influenza scientists as they move their experimental systems and research objects into what they refer to as the “epicenter” of flu pandemics, southern China. Based on the hypothesis that contact between wild and domestic bird species could produce new pandemic flu viruses, scientists set up a research program into the wild–domestic interface at China’s Poyang Lake. As influenza comes to be understood in terms of multispecies relations and ecologies in addition to the virus proper, the scientific knowledge of influenza is increasingly dependent on research conducted at particular sites, such as Poyang Lake. What does this movement of influenza research from laboratory to field mean for anthropological concepts of scientific knowledge? A widely shared premise among anthropologists is that scientific knowledge is made in experimental practice, but this practice turn in science studies draws largely from fieldwork inside laboratories. In this article, drawing on fieldwork with both influenza scientists and poultry breeders, I show how scientific research objects can be displaced by the practices of poultry breeders rather than by experimental practice itself. For these poultry breeders, refusing to respect the distinction of wild and domestic, were breeding wild birds.

Tian, H., Y. Cui, L. Dong, S. Zhou, X. Li , S. Huang, R. Yang, and B. Xu. 2015. Spatial, temporal and genetic dynamics of highly pathogenic avian influenza A (H5N1) virus in China.

(Abstract)

Background: The spatial spread of H5N1 avian influenza, significant ongoing mutations, and long-term persistence of the virus in some geographic regions has had an enormous impact on the poultry industry and presents a serious threat to human health.

Methods: We applied phylogenetic analysis, geospatial techniques, and time series models to investigate the spatiotemporal pattern of H5N1 outbreaks in China and the effect of vaccination on virus evolution.

Results: Results showed obvious spatial and temporal clusters of H5N1 outbreaks on different scales, which may have been associated with poultry and wild-bird transmission modes of H5N1 viruses. Lead–lag relationships were found among poultry and wild-bird outbreaks and human cases. Human cases were preceded by poultry outbreaks, and wild-bird outbreaks were led by human cases. Each clade has gained its own unique spatiotemporal and genetic dominance. Genetic diversity of the H5N1 virus decreased significantly between 1996 and 2011; presumably under strong selective pressure of vaccination. Mean evolutionary rates of H5N1 virus increased after vaccination was adopted in China. A clear signature of positively selected sites in the clade 2.3.2 virus was discovered and this may have resulted in the emergence of clade 2.3.2.1.

Conclusions: Our study revealed two different transmission modes of H5N1 viruses in China, and indicated a significant role of poultry in virus dissemination. Furthermore, selective pressure posed by vaccination was found in virus evolution in the country.

Ling, F., E. Chen, Q. Liu, Z. Miao, and Z. Gong. 2015. Hypothesis On The Source, Transmission and Characteristics of Infection of Avian Influenza A (H7N9) Virus – Based On Analysis of Field Epidemiological Investigation and Gene Sequence Analysis. *BMC Infectious Diseases* **15**:54-68.

(Abstract)

- The virus was transmitted by migratory birds, which stop along the East Asian–Australasian Flyway, to the local wild waterfowl around Taihu Lake.
- At present, human susceptibility to the virus is not high; the virus may only infect close contacts of infected birds; however, it is possible that the H7N9 virus can adapt well to people who are immunocompromised.
- The main sources of human infection were the secondary wholesale markets or farmers markets where the poultry had been infected by migratory birds or from outside environmental pollution.

On 31 March 2013, the National Health and Family Planning Commission announced that human infections with influenza A (H7N9) virus had occurred in Shanghai and Anhui provinces, China. H7N9 cases were later detected in Jiangsu and Zhejiang provinces. It was estimated that the virus first spread northward along the route taken by migratory birds and then spread to neighbouring provinces with the sale of poultry. Epidemiological studies were carried out on samples from the external environment of infected cases, transmission routes, farmers markets and live poultry markets. Phylogenetic study of viral sequences from human and avian infections in Zhejiang showed that those from Shanghai and Jiangsu provinces along Taihu Lake were highly homologous with those from the external environment. This suggests that avian viruses carried by waterfowl combined with the virus carried by migratory birds, giving rise to avian influenza virus H7N9, which is highly pathogenic to humans. It is possible that the virus was transmitted by local wildfowl to domestic poultry and then to humans, or spread further by means of trading in wholesale poultry markets. As the weather has turned warm, and with measures adopted to terminate poultry trade and facilitate health communication, the epidemic in the first half of the year has been kept under control. However, the infection source in the triangular area around Taihu Lake still remains. The H7N9 epidemic will probably hit the area later in the year and next spring when the migratory birds return and may even spread to other areas. Great importance should therefore be attached to the wildfowl in Taihu Lake as the repository and disseminator of the virus: investigation and study of this population is essential.

Ni, X., F. He, M. Hu, X. Zhou, B. Wang, C. Feng, Y. Wu, Y. Li, J. Tu, H. Li, M. Liu, H. Chen, and S. Chen. 2015. Investigation of avian influenza virus in poultry and wild birds due to novel avian-origin influenza A(H10N8) in Nanchang City, China. *Microbes and Infection* **17**:48-53.

(Abstract)

Multiple reassortment events within poultry and wild birds had resulted in the establishment of another novel avian influenza A(H10N8) virus, and finally resulted in human death in Nanchang, China. However, there was a paucity of information on the prevalence of avian influenza virus in poultry and wild birds in Nanchang area. We investigated avian influenza virus in poultry and wild birds from live poultry markets, poultry courtyards, delivery vehicles, and wild-bird habitats in Nanchang. We analyzed 1036 samples from wild birds and domestic poultry collected from December 2013 to February 2014. Original biological samples were tested for the presence of avian influenza virus using specific primer and probe sets of H5, H7, H9, H10 and N8 subtypes by real-time RT-PCR. In our analysis, the majority (97.98%) of positive samples were from live poultry markets. Among the poultry samples from chickens and ducks, AIV prevalence was 26.05 and 30.81%, respectively. Mixed infection of different HA subtypes was very common. Additionally, H10 subtypes coexistence with N8 was the most prevalent agent during the emergence of H10N8. This event illustrated a long-term surveillance was so helpful for pandemic preparedness and response.

Wang, S., S. Wang, and P. Smith. 2015. Ecological impacts of windfarms on birds: Questions, hypotheses, and research needs. *Renewable and Sustainable Energy Reviews* **44**:599–607.

(Abstract)

Wind power is increasingly being used worldwide as an important contribution to renewable energy, due to its low greenhouse gas emissions compared to fossil fuels. However, it has been suggested that the development of wind power has caused an adverse impact on birds. We summarise current evidence of bird fatalities resulting from wind power, outline the reasons why and how birds are killed by wind power developments, and identify research needs to better inform researchers, decision-makers, developers and other stakeholders, to help mitigate any adverse impacts of wind power developments on birds.

Hoque, M. A., G. W. Burgess, A. L. Cheam, and L. F. Skerratt. 2015. Epidemiology of avian influenza in wild aquatic birds in a biosecurity hotspot, North Queensland, Australia. *Preventive Veterinary Medicine* **118**:169–181

(Abstract)

Migratory birds may introduce highly pathogenic H5N1 avian influenza from Southeast Asia into Australia via North Queensland, a key stopover along the East Asian-Australasian Flyway, with severe consequences for trade and human health. A 3-year repeated cross sectional study on the epidemiology of avian influenza in Australian nomadic wild aquatic birds was conducted in this potential biosecurity hotspot using molecular and serological techniques. Avian influenza virus subtypes H6 and H9 were commonly present in the studied population. It is likely that one of the H6 viruses was newly introduced through migratory birds confirming the perceived biosecurity risk. The matrix gene of another H6 virus was similar to the Australian H7 subtypes, which suggests the

reassortment of a previously introduced H6 and local viruses. Similarly, a H9 subtype had a matrix gene similar to that found in Asian H9 viruses suggesting reassortment of viruses originated from Australia and Asia. Whilst H5N1 was not found, the serological study demonstrated a constant circulation of the H5 subtype in the sampled birds. The odds of being reactive for avian influenza viral antibodies were 13.1 (95% CI: 5.9–28.9) for Pacific Black Ducks over Plumed Whistling Ducks, highlighting that some species of waterfowl pose a greater biosecurity risk. Antibody titres were slightly higher during warm wet compared with warm dry weather. Routine surveillance programmes should be established to monitor the introduction of avian influenza viruses from Asia and the interactions of the introduced viruses with resident viruses in order to better detect emerging pathogens in aquatic birds of North Queensland. Surveillance should be targeted towards highly susceptible species such as the Pacific Black Duck and carried out during favourable environmental conditions for viral transmission such as the wet season in northern Australia.

Tian, H., S. Zhou, L. Dong, T. P. Van Boeckel, Y. Cui, Y. Wu, B. Cazelles, S. Huang, R. Yang, B. T. Grenfell, and B. Xu. 2015. Avian influenza H5N1 viral and bird migration networks in Asia. *PNAS* **112**:172–177.

(Abstract)

The spatial spread of the highly pathogenic avian influenza virus H5N1 and its long-term persistence in Asia have resulted in avian influenza panzootics and enormous economic losses in the poultry sector. However, an understanding of the regional long-distance transmission and seasonal patterns of the virus is still lacking. In this study, we present a phylogeographic approach to reconstruct the viral migration network. We show that within each wild fowl migratory flyway, the timing of H5N1 outbreaks and viral migrations are closely associated, but little viral transmission was observed between the flyways. The bird migration network is shown to better reflect the observed viral gene sequence data than other networks and contributes to seasonal H5N1 epidemics in local regions and its large-scale transmission along flyways. These findings have potentially far-reaching consequences, improving our understanding of how bird migration drives the periodic reemergence of H5N1 in Asia.

Fan, Z., Y. Ci, L. Liu, Y. Ma, Y. Jia, D. Wang, Y. Guan, G. Tian, J. Ma, Y. Li, and H. Chen. 2015. Phylogenetic and pathogenic analyses of three H5N1 avian influenza viruses (clade 2.3.2.1) isolated from wild birds in Northeast China. *Infection, Genetics and Evolution* **29**:138–145.

(Abstract)

From April to September 2012, periodic surveillance of avian influenza H5N1 viruses from different wild bird species was conducted in Northeast China. Three highly pathogenic avian influenza (HPAI) H5N1 viruses were isolated from a yellow-browed warbler, common shoveler, and mallard. To trace the genetic lineage of the isolates, nucleotide sequences of all eight gene segments were determined and phylogenetically analyzed. The data indicated that three viruses belonged to the same antigenic

virus group: clade 2.3.2.1. To investigate the pathogenicity of these three viruses in different hosts, chickens, ducks, and mice were inoculated. The results showed that chickens were susceptible to each of the three HPAI H5N1 viruses, resulting in 100% mortality within 2–6 days after infection, whereas the three isolates exhibited distinctly different virulence in ducks and mice. The results of this study demonstrated that HPAI H5N1 viruses of clade 2.3.2.1 are still circulating in wild birds through overlapping migratory flyways. Therefore, continuous monitoring of H5N1 in both domestic and wild birds is necessary to prevent a potentially wider outbreak.

Avian Influenza 2014

Moskvitina, N. S., I. G. Korobitsyna, O. Y. Tyutenkova, S. I. Gashkova, Y. V. Kononova, S. S. Moskvitina, V. N. Romanenko, T. P. Mikryukova, E. V. Protopopova, M. Y. Kartashov, E. V. Chausov, S. N. Konovalova, N. L. Tupota, A. O. Sementsova, V. A. Ternovoy, and V. B. Loktev. 2014. The Potential Role of Migratory Birds in the Spread of Tick-borne Infections in Siberia and the Russian Far East. *Achievements in the Life Sciences* 8:118–120.

(Abstract)

From 2006 to 2011, in the Tomsk region (south of Western Siberia), eight species of pathogens were detected in birds and the ticks feeding on them: Tick-borne encephalitis virus (TBEV), West Nile virus (WNV), *Borrelia* spp., *Rickettsia* spp., *Bartonella* spp., *Anaplasma* spp., *Ehrlichia* spp., and *Babesia* spp. The identification of a number of strains of viruses and bacterial genovariants related geographically with the Russian Far East, Eastern Siberia, China and Japan and confirms the possibility of the role of birds in the spread of pathogens in the direction of Western Siberia and back. Most of the species that breed and migrate in Western Siberia are of Eastern origin and mostly fly for wintering to South-East Asia. Among these species in our samples, *Phylloscopus proregulus* was a carrier of both TBEV and *Bartonella* spp.; *Luscinia calliope* were infected with both TBEV and *Borrelia*, while *Tarsiger cyanurus* were infected with WNV.

Ku, K. B., E. H. Park, J. Yum, J. A. Kim, S. K. Oh, and S. H. Seo. 2014. Highly Pathogenic Avian Influenza A(H5N8) Virus from Waterfowl, South Korea, 2014. *Emerging Infectious Diseases* 20:1587-1588.

(Abstract)

Because all 8 genes of A/waterfowl/Korea/S005/2014 (H5N8) are closely related to those of the A/duck/Korea/Buan2/2014 (H5N8) isolate that was obtained from a duck farm, it is likely that A/waterfowl/Korea/S005/2014 (H5N8) originated from infected waterfowl that had visited poultry on

an infected farm. Our laboratory has studied the feces of wild birds in Chungnam Province since 2009, surveying >20,000 fecal samples from wild birds in this area each year, but we had not previously isolated avian influenza A(H5N8) virus from any samples. (...) Most genes of the virus we isolated are related to those of avian influenza viruses isolated in China, but the HA gene of A/waterfowl/Korea/S005/2014 (H5N8) showed only 97% homology to the closest HA gene in GenBank, which indicates that this gene may have been created in poultry in South Korea. To our knowledge, no outbreak of this virus in poultry farms in China has been reported, and we found no previous reports in the literature that migratory birds could carry the virus. Taken together, our data suggest that A/waterfowl/Korea/S005/2014 (H5N8) may have been reassorted in a duck farm in South Korea.

Verhagen, J. H., J. G. B. van Dijk, O. Vuong, T. Bestebroer, P. Lexmond, M. Klaassen, and R. A. M. Fouchier. 2014. Migratory Birds Reinforce Local Circulation of Avian Influenza Viruses. *PLoS ONE* 9:e112366.

(Abstract)

Migratory and resident hosts have been hypothesized to fulfil distinct roles in infectious disease dynamics. However, the contribution of resident and migratory hosts to wildlife infectious disease epidemiology, including that of low pathogenic avian influenza virus (LPAIV) in wild birds, has largely remained unstudied. During an autumn H3 LPAIV epizootic in free-living mallards (*Anas platyrhynchos*) — a partially migratory species — we identified resident and migratory host populations using stable hydrogen isotope analysis of flight feathers. We investigated the role of migratory and resident hosts separately in the introduction and maintenance of H3 LPAIV during the epizootic. To test this we analysed (i) H3 virus kinship, (ii) temporal patterns in H3 virus prevalence and shedding and (iii) H3-specific antibody prevalence in relation to host migratory strategy. We demonstrate that the H3 LPAIV strain causing the epizootic most likely originated from a single introduction, followed by local clonal expansion. The H3 LPAIV strain was genetically unrelated to H3 LPAIV detected both before and after the epizootic at the study site. During the LPAIV epizootic, migratory mallards were more often infected with H3 LPAIV than residents. Low titres of H3-specific antibodies were detected in only a few residents and migrants. Our results suggest that in this LPAIV epizootic, a single H3 virus was present in resident mallards prior to arrival of migratory mallards followed by a period of virus amplification, importantly associated with the influx of migratory mallards. Thus migrants are suggested to act as local amplifiers rather than the often suggested role as vectors importing novel strains from afar. Our study exemplifies that a multifaceted interdisciplinary approach offers promising opportunities to elucidate the role of migratory and resident hosts in infectious disease dynamics in wildlife.

Rao, D. M. 2014. Enhancing epidemiological analysis of intercontinental dispersion of H5N1 viral strains by migratory waterfowl using phylogeography. *BMC Proceedings* 8:S1-10.

(Abstract)

Background: Intercontinental migratory waterfowl are the primary vectors for dispersion of H5N1 viruses and have been implicated in several zoonotic epidemics and pandemics. Recent investigations have established that with a single mutation, the virus gains the ability to transmit between humans. Consequently, there is a heightened urgency to identify innovative approaches to proactively mitigate emergent epidemics. Accordingly, a novel methodology combining temporo-geospatial epidemiology and phylogeographic analysis of viral strains is proposed to identify critical epicenters and epidemic pathways along with high risk candidate regions for increased surveillance.

Results: Epidemiological analysis was used to identify 91,245 candidate global infection transmission pathways between 22 high risk waterfowl species. Dominant infection pathways (25,625 and 54,500 in summering and wintering zones) were identified through annotation using phylogeographical data computed from the phylogram of 2417 H5N1 HA isolates (from GISAID EpiFlu database). Annotation of infection pathways in turn delineated 23 influential clades out of 130 clades in the phylogram.

Conclusions: The phylogeographic analyses provides strong cross-validation of epidemic pathways and identifies the dominant pathways for use in other epidemiological and prophylactic studies. The temporo-geospatial characteristics of infection transmission provides corroborating, but novel evidence for rapid genesis of H5N1 lineages in S.E. Asia. The proposed method pinpoints several regions, particularly in the southern hemisphere, as candidates for increased surveillance.

Hsieh, Y.-H., J. Wu, J. Fang, Y. Yang, and J. Lou. 2014. Quantification of Bird-to-Bird and Bird-to-Human Infections during 2013 Novel H7N9 Avian Influenza Outbreak in China. *PLoS ONE* **9**:e111834.

(Abstract)

From February to May, 2013, 132 human avian influenza H7N9 cases were identified in China resulting in 37 deaths. We developed a novel, simple and effective compartmental modeling framework for transmissions among (wild and domestic) birds as well as from birds to human, to infer important epidemiological quantifiers, such as basic reproduction number for bird epidemic, bird-to-human infection rate and turning points of the epidemics, for the epidemic via human H7N9 case onset data and to acquire useful information regarding the bird-to-human transmission dynamics. Estimated basic reproduction number for infections among birds is 4.10 and the mean daily number of human infections per infected bird is 3.16×10^{25} [3.08×10^{25} , 3.23×10^{25}]. The turning point of 2013 H7N9 epidemic is pinpointed at April 16 for bird infections and at April 9 for bird-to-human transmissions. Our result reveals very low level of bird-to-human infections, thus indicating minimal risk of widespread bird-to-human infections of H7N9 virus during the outbreak. Moreover, the turning point of the human epidemic, pinpointed at shortly after the implementation of full-scale control and intervention measures initiated in early April, further highlights the impact of timely actions on ending the outbreak. This is the first study where both the bird and human components of an avian influenza epidemic can be quantified using only the human case data.

Fan, S., L. Zhou, D. Wu, X. Gao, E. Pei, T. Wang, Y. Gao, and X. Xia. 2014. A novel highly pathogenic H5N8 avian influenza virus isolated from a wild duck in China. *Influenza and Other Respiratory Viruses* 8:646-653.

(Abstract)

Migrating wild birds are considered natural reservoirs of influenza viruses and serve as a potential source of novel influenza strains in humans and livestock. During routine avian influenza surveillance conducted in eastern China, a novel H5N8 (SH-9) reassortant influenza virus was isolated from a mallard duck in China. BLAST analysis revealed that the HA, NA, PB1, PA, NP, and M segments of SH-9 were most closely related to the corresponding segments of A/duck/Jiangsu/k1203/2010 (H5N8). The SH-9 virus preferentially recognized avian-like influenza virus receptors and was highly pathogenic in mice. Our results suggest that wild birds could acquire the H5N8 virus from breeding ducks and spread the virus via migratory bird flyways.

Miller RS, Sweeney SJ, Akkina JE, Saito EK (in press) Potential Intercontinental Movement of Influenza A(H7N9) Virus into North America by Wild Birds: Application of a Rapid Assessment Framework. *Transboundary and Emerging Diseases*.

(Abstract)

A critical question surrounding emergence of novel strains of avian influenza viruses (AIV) is the ability for wild migratory birds to translocate a complete (unreassorted whole genome) AIV intercontinentally. Virus translocation via migratory birds is suspected in outbreaks of highly pathogenic strain A(H5N1) in Asia, Africa and Europe. As a result, the potential intercontinental translocation of newly emerging AIV such as A(H7N9) from Eurasia to North America via migratory movements of birds remains a concern. An estimated 2.91 million aquatic birds move annually between Eurasia and North America with an estimated AIV prevalence as high as 32.2%. Here, we present a rapid assessment to address the likelihood of whole (unreassorted)-genome translocation of Eurasian strain AIV into North America. The scope of this assessment was limited specifically to assess the weight of evidence to support the movement of an unreassorted AIV intercontinentally by migratory aquatic birds. We developed a rapid assessment framework to assess the potential for intercontinental movement of avian influenzas by aquatic birds. This framework was iteratively reviewed by a multidisciplinary panel of scientific experts until a consensus was established. Our assessment framework identified four factors that may contribute to the potential for introduction of any AIV intercontinentally into North America by wild aquatic birds. These factors, in aggregate, provide a framework for evaluating the likelihood of new forms of AIV from Eurasia to be introduced by aquatic birds into North America. Based on our assessment, we determined that the potential for introduction of A(H7N9) into North America through aquatic migratory birds is possible, but the likelihood ranges from extremely low to low.

Parvin, R., A. H. M. Kamal, M. E. Haque, E. H. Chowdhury, M. Giasuddin, M. R. Islam, and T. W. Vahlenkamp. 2014. Genetic characterization of highly pathogenic H5N1 avian influenza virus from live migratory birds in Bangladesh. *Virus Genes* **49**:438–448.

(Abstract)

Since the first outbreak of highly pathogenic avian influenza virus (HPAIV) subtype H5N1 in Bangladesh in 2007, the virus has been circulating among domestic poultry causing severe economic losses. To investigate the presence of HPAIV H5N1 in migratory birds and their potential role in virus spread, 205 pools of fecal samples from live migratory birds were analyzed. Here, the first virus isolation and genome characterization of two HPAIV H5N1 isolates from migratory birds (*A/migratory bird/Bangladesh/P18/2010* and *A/migratorybird/Bangladesh/P29/2010*) are described. Full-length amplification, sequencing, and a comprehensive phylogenetic analysis were performed for HA, NA, M, NS, NP, PA, PB1, and PB2 gene segments. The selected migratory bird isolates belong to clade 2.3.2.1 along with recent Bangladeshi isolates from chickens, ducks, and crows which grouped in the same cluster with contemporary South and South-East Asian isolates. The studied isolates were genetically similar to other H5N1 isolates from different species within the respective clade although some unique amino acid substitutions were observed among them. Migratory birds remain a real threat for spreading pathogenic avian influenza viruses across the continent and introduction of new strains into Bangladesh.

Jin, Y., D. Yu, H. Ren, Z. Yin, H. Zhisong, M. Hu, B. Li, W. Zhou, J. Yue, and L. Liang. 2014. Phylogeography of Avian influenza A H9N2 in China. *BMC Genomics* **15**:1110.

(Abstract)

Background: During the past two decades, avian influenza A H9N2 viruses have spread geographically and ecologically in China. Other than its current role in causing outbreaks in poultry and sporadic human infections by direct transmission, H9N2 virus could also serve as a progenitor for novel human avian influenza viruses including H5N1, H7N9 and H10N8. Hence, H9N2 virus is becoming a notable threat to public health. However, despite multiple lineages and genotypes that were detected by previous studies, the migration dynamics of the H9N2 virus in China is unclear. Increasing such knowledge would help us better prevent and control H9N2 as well as other future potentially threatening viruses from spreading across China. The objectives of this study were to determine the source, migration patterns, and the demography history of avian influenza A H9N2 virus that circulated in China.

Results: Using Bayesian phylogeography framework, we showed that the H9N2 virus in mainland China may have originated from the Hong Kong Special Administrative Region (SAR). Southern China, most likely the Guangdong province acts as the primary epicentre for multiple H9N2 strains spreading across the whole country, and eastern China, most likely the Jiangsu province, acts as an important secondary source to seed outbreaks. Our demography inference suggests that during the long-term migration process, H9N2 evolved into multiple diverse lineages and then experienced a selective sweep, which reduced its genetic diversity. Importantly, such a selective sweep may pose

a greater threat to public health because novel strains confer higher fitness advantages than strains being replaced and could generate new viruses through reassortment.

Conclusion: Our analyses indicate that migratory birds, poultry trade and transportation have all contributed to the spreading of the H9N2 virus in China. The ongoing migration and evolution of H9N2, which poses a constant threat to the human population, highlights the need for a more comprehensive surveillance of wild birds and for the enhancement of biosafety for China's poultry industry.

Cui, P., D. Zhou, Y. Wu, J. Wu, J. Lei, M. Shao, G. Liu, X. Wu, J. Wu, W. Ji, and F. Lei. 2014. The Ecological Risks of Avian Influenza Virus Spread via Migratory Birds: A Case Study at Poyang Lake, China. *Pakistan Journal of Zoology* **46**:1545-1555.

(Abstract)

The highly pathogenic avian influenza H5N1 virus (here after H5N1) still produces devastating effects in humans, poultries and wild birds. Migratory birds were thought to play a role in the long-distance spread of H5N1. This study identified 7 high-risk species and 18 potential high-risk species that may transmit H5N1 into Poyang Lake and determined four resident bird species as "bridge species" that may disperse H5N1 around the lake. The high-risk species were selected according to the following behavioral and ecological factors: migratory status, abundance, degree of mixing of species and gregariousness, migration from or stopovers at epidemic areas, and previous infection status. Among the 25 high-risk and potential high-risk species, 22 belonged to the orders Anseriformes (n = 11) and Charadriiformes (n = 11). The risk of intra-species transmission was high for Anseriformes (ducks and geese) and Charadriiformes birds, and was relatively low for Gruiformes and Ciconiiformes birds. This study can be informative and useful in the surveillance of avian influenza epidemics at breeding, stopover, and wintering sites other than Poyang Lake along the East Asian-Australian Flyway.

Gale P, Goddard A, Breed AC, Irvine RM, Kelly L, Snary EL (2014) Entry of H5N1 highly pathogenic avian influenza virus into Europe through migratory wild birds: a qualitative release assessment at the species level. *Journal of Applied Microbiology* **116**, 1405-1417.

(Abstract)

Aims: To estimate qualitatively the probabilities of release (or entry) of Eurasian lineage H5N1 highly pathogenic avian influenza (HPAI) virus into Great Britain (GB), the Netherlands and Italy through selected higher risk species of migratory water bird.

Methods and Results: The probabilities of one or more release events of H5N1 HPAI per year (Pre-release) were estimated qualitatively for 15 avian species, including swans, geese, ducks and gulls, by assessing the prevalence of H5N1 HPAI in different regions of the world (weighted to 2009) and estimates of the total numbers of birds migrating from each of those regions. The release

assessment accommodated the migration times for each species in relation to the probabilities of their surviving infection and shedding virus on arrival. Although the predicted probabilities of release of H5N1 per individual bird per year were low, very low or negligible, Prelease was high for a few species reflecting the high numbers of birds migrating from some regions. Values of Prelease were generally higher for the Netherlands than for GB, while ducks and gulls from Africa presented higher probabilities to Italy compared to the Netherlands and GB.

Conclusions: Bird species with high values of Prelease in GB, the Netherlands and Italy generally originate from within Europe based on data for global prevalence of H5N1 between 2003 and 2009 weighted to 2009. Potential long-distance transfer of H5N1 HPAI from North Asia and Eurasia to GB, the Netherlands and Italy is limited to a few species and does not occur from South-East Asia, an area where H5N1 is endemic.

Significance and Impact of the Study: The approach accommodates biogeographical conditions and variability in the estimated worldwide prevalence of the virus. The outputs of this release assessment can be used to inform surveillance activities through focusing on certain species and migratory pathways.

Sharshov, K., M. Sivay, D. Liu, M. Pantin-Jackwood, V. Marchenko, A. Durymanov, A. Alekseev, D. Damdindorj, G. F. Gao, D. E. Swayne, and A. Shestopalov. 2014. Molecular characterization and phylogenetics of a reassortant H13N8 influenza virus isolated from gulls in Mongolia. *Virus Genes* **49**, 237–249.

(Abstract)

Double reassortant H13N8 influenza A virus was isolated from gull in Mongolia. The basic virological characteristics were studied. Complete genome sequence analysis indicated the complicated evolutionary history. The PA gene belongs to classical Avian-like lineage and more likely originated from non-gull avian virus pool. Data confirm the state of extensive geographic mosaicism in AIV from gulls in the Northern Hemisphere.

Hall, J. S., G. T. Hallgrimsson, K. Suwannanarn, S. Sreevatsen, H. S. Ip, E. Magnusdottir, J. L. TeSlaa, S. W. Nashold, and R. J. Dusek. 2014. Avian influenza virus ecology in Iceland shorebirds: Intercontinental reassortment and movement. *Infection, Genetics and Evolution* **28**, 130–136.

(Abstract)

Shorebirds are a primary reservoir of avian influenza viruses (AIV). We conducted surveillance studies in Iceland shorebird populations for 3 years, documenting high serological evidence of AIV exposure in shorebirds, primarily in Ruddy Turnstones (*Arenaria interpres*; seroprevalence = 75%). However, little evidence of virus infection was found in these shorebird populations and only two turnstone AIVs (H2N7; H5N1) were able to be phylogenetically examined. These analyses showed that viruses from

Iceland shorebirds were primarily derived from Eurasian lineage viruses, yet the H2 hemagglutinin gene segment was from a North American lineage previously detected in a gull from Iceland the previous year. The H5N1 virus was determined to be low pathogenic, however the PB2 gene was closely related to the PB2 from highly pathogenic H5N1 isolates from China. Multiple lines of evidence suggest that the turnstones were infected with at least one of these AIV while in Iceland and confirm Iceland as an important location where AIV from different continents interact and reassort, creating new virus genomes. Mounting data warrant continued surveillance for AIV in wild birds in the North Atlantic, including Canada, Greenland, and the northeast USA to determine the risks of new AI viruses and their intercontinental movement in this region.

Ramis, A., G. van Amerongen, M. W. G. van de Bildt, L. Leijten, R. Vanderstichel, A. D. M. E. Osterhaus, and T. Kuiken. 2014. Experimental infection of highly pathogenic avian influenza virus H5N1 in black-headed gulls (*Chroicocephalus ridibundus*). *Veterinary Research* **45**, 84-94.

(Abstract)

Historically, highly pathogenic avian influenza viruses (HPAIV) rarely resulted in infection or clinical disease in wild birds. However, since 2002, disease and mortality from natural HPAIV H5N1 infection have been observed in wild birds including gulls. We performed an experimental HPAIV H5N1 infection of black-headed gulls (*Chroicocephalus ridibundus*) to determine their susceptibility to infection and disease from this virus, pattern of viral shedding, clinical signs, pathological changes and viral tissue distribution. We inoculated sixteen black-headed gulls with 1×10^4 median tissue culture infectious dose HPAIV H5N1 (A/turkey/Turkey/1/2005) intratracheally and intraesophageally. Birds were monitored daily until 12 days post inoculation (dpi). Oropharyngeal and cloacal swabs were collected daily to detect viral shedding. Necropsies from birds were performed at 2, 4, 5, 6, 7, and 12 dpi. Sampling from selected tissues was done for histopathology, immunohistochemical detection of viral antigen, PCR, and viral isolation. Our study shows that all inoculated birds were productively infected, developed systemic disease, and had a high morbidity and mortality rate. Virus was detected mainly in the respiratory tract on the first days after inoculation, and then concentrated more in pancreas and central nervous system from 4 dpi onwards. Birds shed infectious virus until 7 dpi from the pharynx and 6 dpi from the cloaca. We conclude that black-headed gulls are highly susceptible to disease with a high mortality rate and are thus more likely to act as sentinel species for the presence of the virus than as long-distance carriers of the virus to new geographical areas.

Repérant, L. A., M. W. G. van de Bildt, G. van Amerongen, D. M. Buehler, A. D. M. E. Osterhaus, S. Jenni-Eiermann, T. Piersma, and T. Kuiken. 2014. Highly Pathogenic Avian Influenza Virus H5N1 Infection in a Long-Distance Migrant Shorebird under Migratory and Non-Migratory States. *PLoS ONE* **6**, e27814.

(Abstract)

Corticosterone regulates physiological changes preparing wild birds for migration. It also modulates the immune system and may lead to increased susceptibility to infection, with implications for the spread of pathogens, including highly pathogenic avian influenza virus (HPAIV) H5N1. The red knot (*Calidris canutus islandica*) displays migratory changes in captivity and was used as a model to assess the effect of high plasma concentration of corticosterone on HPAIV H5N1 infection. We inoculated knots during pre-migration (N = 6), fueling (N = 5), migration (N = 9) and post-migration periods (N = 6). Knots from all groups shed similar viral titers for up to 5 days post-inoculation (dpi), peaking at 1 to 3 dpi. Lesions of acute encephalitis, associated with virus replication in neurons, were seen in 1 to 2 knots per group, leading to neurological disease and death at 5 to 11 dpi. Therefore, the risk of HPAIV H5N1 Infection in wild birds and of potential transmission between wild birds and poultry may be similar at different times of the year, irrespective of wild birds' migratory status. However, in knots inoculated during the migration period, viral shedding levels positively correlated with pre-inoculation plasma concentration of corticosterone. Of these, knots that did not become productively infected had lower plasma concentration of corticosterone. Conversely, elevated plasma concentration of corticosterone did not result in an increased probability to develop clinical disease. These results suggest that birds with elevated plasma concentration of corticosterone at the time of migration (ready to migrate) may be more susceptible to acquisition of infection and shed higher viral titers— before the onset of clinical disease— than birds with low concentration of corticosterone (not ready for take-off). Yet, they may not be more prone to the development of clinical disease. Therefore, assuming no effect of subclinical infection on the likelihood of migratory take-off, this may favor the spread of HPAIV H5N1 by migratory birds over long distances.

Hiley JR, Bradbury RB, Thomas CD. 2014. Introduced and natural colonists show contrasting patterns of protected area association in UK wetlands. *Diversity and Distributions* **20**, 943–951.

(Abstract)

Aim: Protected wildlife habitats provide valuable stepping stones for species that shift their distributions in response to climatic and other environmental changes, but they might also aid the spread of invasive alien species. Here, we quantify the use of protected areas (PAs) by both introduced and natural wetland colonists in the UK to analyse patterns of colonization and examine the propensity of invaders to use PAs.

Location: United Kingdom.

Methods: We calculate PA associations for six species of wetland birds deliberately introduced to the UK and compare these with eight others that have recently colonized the UK naturally. We assess PA associations at three different stages of establishment – first breeding in each county, early establishment of a population (4–6 years after initial breeding) and subsequent consolidation (14–16 after initial breeding) – and analyse changes in PA association over time.

Results: Introduced wetland bird species were less associated with PAs than natural colonists at each stage of establishment. During the later stages of colonization, the PA association of introduced species tended to increase. In contrast, natural colonists usually colonized PAs first, and their established populations subsequently spread into non-PA sites.

Main conclusions: The United Kingdom PA network did not facilitate the invasion of introduced species during the initial stages of their colonization, but was vulnerable to colonization as populations established. This is in contrast to natural colonists, which are more reliant on PAs during initial colonization but become less dependent as they establish. During a period of rapid environmental change, PAs have facilitated expansions of natural colonists, without acting as the prime sites for invasion by introduced species.

Zamani-Ahmadmahmoodi R, Alahverdi M, Mirzaei R. 2014. Mercury Concentrations in Common Tern *Sterna hirundo* and Slender-billed Gull *Larus genei* from the Shadegan Marshes of Iran, in North-western Corner of the Persian Gulf. *Biol Trace Elem Res* **159**, 161–166.

(Abstract)

We examined mercury levels in several tissues of Common Terns and Slender-billed Gulls collected from Shadegan Marshes of south-western Iran. In both species, total mercury content was highest in feathers followed by liver, kidney and muscle tissue. We found a significant correlation between mercury concentrations in kidney and breast feather ($r=0.83$, $p<0.05$), breast feather and liver ($r=0.81$, $p<0.05$) as well as liver and kidney ($r=0.83$, $p<0.05$). The contaminant levels in the feathers (11.53 and 15.32 $\mu\text{g/g}$ in breast feather and tail feather, respectively) of Common Terns from Shadegan Marshes are higher than those reported for other tern species from elsewhere in the world, but feather mercury of Slender-billed Gull (6.61 and 5.35 $\mu\text{g/g}$ in breast feather and tail feather, respectively) was similar to those reported for gull species worldwide. Mean values for mercury in the feather of two seabird species were higher than the levels known to cause adverse effects.

D'Amico VL, Gonzalez PM, Baker AJ, Buehler DM, Bertellotti M. 2014. Multi-year surveillance of selected avian pathogens in the migrant shorebird Red Knot (*Calidris canutus rufa*) at its main stopover site in Patagonia, Argentina. *Journal of Ornithology* **155**, 555-559.

(Abstract)

To investigate possible reasons for recent declines in Red Knot (*Calidris canutus rufa*) populations we surveyed for selected pathogens in Red Knots captured in San Antonio Bay, Argentina, on their northward migration during the period 2006–2011. Blood, cloacal swabs and faeces were analysed for bacteria [*Salmonella* sp., *Shigella* sp., enteropathogenic *Escherichia coli* (N = 42) and other coliforms (N = 35)], viral agents [responsible for avian influenza (N = 239), St. Louis encephalitis (N = 51) and Newcastle disease (N = 239)] and avian malaria parasites [*Plasmodium* sp. and *Haemoproteus* sp. (N = 284)]. All 698 samples taken from 303 individuals were negative, providing no evidence that Red Knots sampled at this stopover site were infected with these pathogens at the time of sampling.

Pardal S, Alves JA, Ze-Ze L, Osorino H, Rocha A, Lopes RJ, Potts PM, Amaro F, Santiago-Quesada F, Sanchez-Guzman JM, Masero JA, Alves MJ, Perez-Tris J, Ramos JA, Mendes L. 2014. Shorebird low spillover risk of mosquito-borne pathogens on Iberian wetlands. *Journal of Ornithology* **155**, 549–554.

(Abstract)

Migratory shorebirds are exposed to a wide range of pathogens along their migratory flyways, but their capacity to acquire or spread pathogens beyond endemic areas is poorly known. We focused on the spillover and acquisition of mosquito-borne pathogens such as haemosporidians and West Nile virus (WNV) on key-staging Iberian wetlands during different seasons. We screened seven shorebird species (447 individuals), and detected low haemosporidian prevalence (0.6 %). Furthermore, no WNV infections could be detected, though 6.2 % revealed antibodies against flaviviruses. Although Iberian wetlands congregate numerous shorebirds of different species and origins, the potential introduction of foreign pathogens is not a common event.

Ogden JC, Baldwin JD, Bass OL, Browder JA, Cook MI, Frederick PC, Frezza PE, Galvez RA, B. HA, Meyer KD, Oberhofer LD, Paul AF, Fletcher PJ, Davis SM, J. LJ. 2014. Waterbirds as indicators of ecosystem health in the coastal marine habitats of southern Florida: 1. Selection and justification for a suite of indicator species. *Ecological Indicators* **44**, 148–163.

(Abstract)

The coastal marine environment is currently under threat from many anthropogenic pressures that were identified by the MARES project. Indicators of ecosystem health are needed so that targets can be set to guide protection and restoration efforts. Species of birds that are dependent on coastal habitats are ubiquitous along the coasts of southern Florida. Generally referred to as waterbirds, these species, although not all taxonomically related, share a common dependency on the marine environment for food, nesting habitat, or both. A suite of waterbirds was selected based on their perceived sensitivity to pressures in multiple coastal habitat types. The list of species was refined on the basis of a review of life history for characteristics that might make the species particularly vulnerable. Each selected species was then evaluated for sensitivity to the identified pressures using a hierarchical assessment that took into account the sensitivity, severity, and the temporal and spatial scales of the indicator to the given pressures. The selected suite of indicators was collectively sensitive to all the pressures except one.

Ogden JC, Baldwin JD, Bass OL, Browder JA, Cook MI, Frederick PC, Frezza PE, Galvez RA, B. HA, Meyer KD, Oberhofer LD, Paul AF, Fletcher PJ, Davis SM, J. LJ. 2014. Waterbirds as indicators of

ecosystem health in the coastal marine habitats of southern Florida: 2. Conceptual ecological models. *Ecological Indicators*. **44**, 128–147.

(Abstract)

In our companion manuscript we identified 11 waterbirds as indicators of various pressures on the coastal marine ecosystems of southern Florida. Here, we identify the habitats on which these species depend and the ecological linkages that make them representative of those habitats. Through the use of conceptual ecological models (CEMs), we develop tools that can be used by managers/decision makers to evaluate the health of the various habitats in order to rectify myriad problems that are occurring or will possibly occur in the future such that the valuable ecosystem services provided by these habitats can be maximized. We also demonstrate the practical use of these tools by documenting data availability, benchmarks, and scientific needs for each species.

Cappelle J, Zhao D, Gilbert M, Nelson MI, Newman SH, Takekawa JY, Gaidet N, Prosser DJ, Liu Y, Li P, Shu Y, Xiao X. 2014. Risks of Avian Influenza Transmission in Areas of Intensive Free-Ranging Duck Production with Wild Waterfowl. *EcoHealth* **11**, 109–119.

(Abstract)

For decades, southern China has been considered to be an important source for emerging influenza viruses since key hosts live together in high densities in areas with intensive agriculture. However, the underlying conditions of emergence and spread of avian influenza viruses (AIV) have not been studied in detail, particularly the complex spatiotemporal interplay of viral transmission between wild and domestic ducks, two major actors of AIV epidemiology. In this synthesis, we examine the risks of avian influenza spread in Poyang Lake, an area of intensive free-ranging duck production and large numbers of wild waterfowl. Our synthesis shows that farming of free-grazing domestic ducks is intensive in this area and synchronized with wild duck migration. The presence of juvenile domestic ducks in harvested paddy fields prior to the arrival and departure of migrant ducks in the same fields may amplify the risk of AIV circulation and facilitate the transmission between wild and domestic populations. We provide evidence associating wild ducks migration with the spread of H5N1 in the spring of 2008 from southern China to South Korea, Russia, and Japan, supported by documented wild duck movements and phylogenetic analyses of highly pathogenic avian influenza H5N1 sequences. We suggest that prevention measures based on a modification of agricultural practices may be implemented in these areas to reduce the intensity of AIV transmission between wild and domestic ducks. This would require involving all local stakeholders to discuss feasible and acceptable solutions.

De Marco MA, Delogu M, Sivay M, Sharshov K, Yurlov A, Cotti C, Shestopalov A. 2014. Virological Evaluation of Avian Influenza Virus Persistence in Natural and Anthropogenic Ecosystems of Western Siberia (Novosibirsk Region, Summer 2012). *PLoS ONE* **9**, e100859.

(Abstract)

Background: Wild aquatic birds, reservoir of low-pathogenicity (LP) avian influenza viruses (AIVs), congregate in huge numbers in Western Siberia wetlands, where major intra- and inter-continental bird flyways overlap. In 2005 and 2006, highly pathogenic (HP) AIV H5N1 epizootics affected wild and domestic birds in the Novosibirsk Region. In 2012, we evaluated AIV persistence in Siberian natural and anthropic ecosystems.

Methodology/Principal Findings: In Novosibirsk Region, 166 wild birds ecologically linked to aquatic environments and 152 domestic waterfowl were examined for AIV isolation in embryonating chicken eggs. Biological samples were obtained by integrating the conventional cloacal swab collection with the harvesting of samples from birds' plumage. Haemagglutinating allantoic fluids were further characterized by serological and molecular methods. In August-September 2012, 17 AIVs, including three H3N8, eight H4N6, two H4N?, one H2N?, one H?N2, and two untyped LPAIVs, were isolated from 15 wild ducks. Whereas comparable proportions of wild Anseriformes (n.118) tested virus isolation (VI)-positive from cloaca and feathers (5.9% vs 8.5%) were detected, the overall prevalence of virus isolation, obtained from both sampling methods, was 2.4 times higher than that calculated on results from cloacal swab examination only (14.4% vs 5.9%). Unlike previously described in this area, the H4N6 antigenic subtype was found to be the prevalent one in 2012. Both cloacal and feather samples collected from domestic waterfowl tested VI-negative.

Conclusion/Significance: We found lack of evidence for the H5N1 HPAIV circulation, explainable by the poor environmental fitness of HPAIVs in natural ecosystems. Our LPAIV isolation data emphasise the importance of Siberia wetlands in influenza A virus ecology, providing evidence of changes in circulation dynamics of HN antigenic subtypes harboured in wild bird reservoirs. Further studies of isolates, based on bioinformatic approaches to virus molecular evolution and phylogenesis, will be needed to better elucidate mechanisms involved in AIV perpetuation in this area.

Barton HD, Rohani P, Stallknecht DE, Brown JD, Drake JM. 2014. Subtype diversity and reassortment potential for co-circulating avian influenza viruses at a diversity hot spot. *Journal of Animal Ecology* **83**, 566-575.

(Abstract)

1. Biological diversity has long been used to measure ecological health. While evidence exists from many ecosystems that declines in host biodiversity may lead to greater risk of disease emergence, the role of pathogen diversity in the emergence process remains poorly understood. Particularly, because a more diverse pool of pathogen types provides more ways in which evolutionary innovations may arise, we suggest that host-pathogen systems with high pathogen diversity are more prone to disease emergence than systems with relatively homogeneous pathogen communities. We call this prediction the diversity-emergence hypothesis.

2. To show how this hypothesis could be tested, we studied a system comprised of North American shorebirds and their associated low-pathogenicity avian influenza (LPAI) viruses. These viruses are important as a potential source of genetic innovations in influenza. A theoretical contribution of this study is an expression predicting the rate of viral subtype reassortment to be proportional to both prevalence and Simpson's Index, a formula that has been used traditionally to quantify biodiversity.

We then estimated prevalence and subtype diversity in host species at Delaware Bay, a North American AIV hotspot, and used our model to extrapolate from these data.

3. We estimated that 4 to 39 virus subtypes circulated at Delaware Bay each year between 2000 and 2008, and that surveillance coverage (percentage of co-circulating subtypes collected) at Delaware Bay is only about 63_0%. Simpson's Index in the same period varied more than fourfold from 0.22 to 0.93. These measurements together with the model provide an indirect, model-based estimate of the reassortment rate. A proper test of the diversity-emergence hypothesis would require these results to be joined to independent and reliable estimates of reassortment, perhaps obtained through molecular surveillance.

4. These results suggest both that subtype diversity (and therefore reassortment) varies from year to year and that several subtypes contributing to reassortment are going undetected. The similarity between these results and more detailed studies of one host, ruddy turnstone (*Arenaria interpres*), further suggests that this species may be the primary host for influenza reassortment at Delaware Bay.

5. Biological diversity has long been quantified using Simpson's Index. Our model links this formula to a mechanistic account of reassortment in multipathogen systems in the form of subtype diversity at Delaware Bay, USA. As a theory of how pathogen diversity may influence the evolution of novel pathogens, this work is a contribution to the larger project of understanding the connections between biodiversity and disease.

Zhang Z, Chen D, Chen Y, Wang B, Hu Y, Gao J, Sun L, Li R, Xiong C. 2014. Evaluating the Impact of Environmental Temperature on Global Highly Pathogenic Avian Influenza (HPAI) H5N1 Outbreaks in Domestic Poultry. *International Journal of Environmental Research and Public Health* **11**, 6388-6399.

(Abstract)

The emergence and spread of highly pathogenic avian influenza (HPAI) A virus subtype H5N1 in Asia, Europe and Africa has had an enormously socioeconomic impact and presents an important threat to human health because of its efficient animal-to-human transmission. Many factors contribute to the occurrence and transmission of HPAI H5N1 virus, but the role of environmental temperature remains poorly understood. Based on an approach of integrating a Bayesian Cox proportional hazards model and a Besag-York-Mollié (BYM) model, we examined the specific impact of environmental temperature on HPAI H5N1 outbreaks in domestic poultry around the globe during the period from 1 December 2003 to 31 December 2009. The results showed that higher environmental temperature was a significant risk factor for earlier occurrence of HPAI H5N1 outbreaks in domestic poultry, especially for a temperature of 25 °C. Its impact varied with epidemic waves (EWs), and the magnitude of the impact tended to increase over EWs.

Li R, Jiang Z, Xu B. 2014. Global spatiotemporal and genetic footprint of the H5N1 avian influenza virus. *International Journal of Health Geographics* **13**, 14.

(Abstract)

Background: Since 2005, the Qinghai-like lineage of the highly pathogenic avian influenza A virus H5N1 has rapidly spread westward to Europe, the Middle East and Africa, reaching a dominant level at a global scale in 2006.

Methods: Based on a combination of genetic sequence data and H5N1 outbreak information from 2005 to 2011, we use an interdisciplinary approach to improve our understanding of the transmission pattern of this particular clade 2.2, and present cartography of global spatiotemporal transmission footprints with genetic characteristics.

Results: Four major viral transmission routes were derived with three sources— Russia, Mongolia, and the Middle East (Kuwait and Saudi Arabia)—in the three consecutive years 2005, 2006 and 2007. With spatiotemporal transmission along each route, genetic distances to isolate A/goose/Guangdong/1996 are becoming significantly larger, leading to a more challenging situation in certain regions like Korea, India, France, Germany, Nigeria and Sudan. Europe and India have had at least two incursions along multiple routes, causing a mixed virus situation. In addition, spatiotemporal distribution along the routes showed that 2007/2008 was a temporal separation point for the infection of different host species; specifically, wild birds were the main host in 2005–2007/2008 and poultry was responsible for the genetic mutation in 2009–2011. “Global-to-local” and “high-to-low latitude” transmission footprints have been observed.

Conclusions: Our results suggest that both wild birds and poultry play important roles in the transmission of the H5N1 virus clade, but with different spatial, temporal, and genetic dominance. These characteristics necessitate that special attention be paid to countries along the transmission routes.

Bridge ES, Kelly JF, Xiao X, Takekawa JY, Hill NJ, Yamage M, UI Haque E, Islam MA, Mundkur T, Erciyas Yavuz K, Leader P, Leung CYH, Smith B, Spragens KA, Vandegrift KJ, Hosseini PR, Saif S, Mohsanin S, Mikolon A, Islam A, George A, Sivananthaperumal B, Daszak P, Newman SH. 2014. Bird migration and avian influenza: A comparison of hydrogen stable isotopes and satellite tracking methods. *Ecological Indicators* **45**, 266–273.

(Abstract)

Satellite-based tracking of migratory waterfowl is an important tool for understanding the potential role of wild birds in the long-distance transmission of highly pathogenic avian influenza. However, employing this technique on a continental scale is prohibitively expensive. This study explores the utility of stable isotope ratios in feathers in examining both the distances traveled by migratory birds and variation in migration behavior. We compared the satellite-derived movement data of 22 ducks from 8 species captured at wintering areas in Bangladesh, Turkey, and Hong Kong with deuterium ratios (δD) in the feathers of these and other individuals captured at the same locations. We derived likely molting locations from the satellite tracking data and generated expected isotope ratios based on an interpolated map of δD in rainwater. Although δD was correlated with the distance between wintering and molting locations, surprisingly, measured δD values were not correlated with either expected values or latitudes of molting sites. However, population-level parameters derived from the

satellite-tracking data, such as mean distance between wintering and molting locations and variation in migration distance, were reflected by means and variation of the stable isotope values. Our findings call into question the relevance of the rainfall isotope map for Asia for linking feather isotopes to molting locations, and underscore the need for extensive ground truthing in the form of feather-based isoscapes. Nevertheless, stable isotopes from feathers could inform disease models by characterizing the degree to which regional breeding populations interact at common wintering locations. Feather isotopes also could aid in surveying wintering locations to determine where high-resolution tracking techniques (e.g. satellite tracking) could most effectively be employed. Moreover, intrinsic markers such as stable isotopes offer the only means of inferring movement information from birds that have died as a result of infection. In the absence of feather based-isoscapes, we recommend a combination of isotope analysis and satellite-tracking as the best means of generating aggregate movement data for informing disease models.

Tosh C, Nagarajan S, Murugkar HV, Bhatia S, Kulkarni DD. 2014. Evolution and spread of avian influenza H5N1 viruses. *Advances in Animal and Veterinary Sciences* **2**, 33-41.

(Abstract)

The Asian lineage of H5N1 avian influenza virus emerged from geese (A/Goose/Guangdong/1/96) in China in 1996, and is continuing its spread infecting domestic poultry, other birds and sporadic infection to humans revealing its pandemic potential. Since late 2003, H5N1 virus has been reported over 60 countries in Asia, Europe and Africa killing millions of poultry and infected humans in 16 countries with a total of 650 confirmed cases including 386 fatal have been reported to WHO. Spread of H5N1 virus has been linked with bird migration, although trade of poultry and poultry products has also been attributed in the spread. During the last one and half decades of circulation in poultry, the H5N1 virus has undergone significant genetic diversification leading to emergence of 10 major virus clades (clades 0-9), with subsequent diversification into 2nd-, 3rd- and 4th- order clades. Vaccination is one of the strategies for control and prevention of H5N1 virus infection; however, some countries still face challenges to eliminate H5N1 virus in poultry. One of the major problems is antigenic drift in viruses that develops due to vaccine pressure, particularly when vaccination is not implemented properly. Two classes of anti-viral drugs (neuraminidase inhibitors and adamantanes) are available for treatment and prophylaxis of influenza; however, there is emergence of drug resistant variants in poultry and humans. Wide-spread circulation and ever changing nature of H5N1 virus make avian influenza control program more difficult. Therefore, educational programs to enhance awareness on avian influenza, animal husbandry practices and biosecurity measures are important in control of the disease.

Shi J, Gao L, Zhu Y, Chen T, Liu Y, Dong L, Liu F, Yang H, Cai Y, Yu M, Yao Y, Xu C, Xiao X, Shu Y. 2014. Investigation of Avian Influenza Infections in Wild Birds, Poultry and Humans in Eastern Dongting Lake, China. *PLoS ONE* **9**, e95685.

(Abstract)

We investigated avian influenza infections in wild birds, poultry, and humans at Eastern Dongting Lake, China. We analyzed 6,621 environmental samples, including fresh fecal and water samples, from wild birds and domestic ducks that were collected from the Eastern Dongting Lake area from November 2011 to April 2012. We also conducted two cross-sectional serological studies in November 2011 and April 2012, with 1,050 serum samples collected from people exposed to wild birds and/or domestic ducks. Environmental samples were tested for the presence of avian influenza virus (AIV) using quantitative PCR assays and virus isolation techniques. Hemagglutination inhibition assays were used to detect antibodies against AIV H5N1, and microneutralization assays were used to confirm these results. Among the environmental samples from wild birds and domestic ducks, AIV prevalence was 5.19 and 5.32%, respectively. We isolated 39 and 5 AIVs from the fecal samples of wild birds and domestic ducks, respectively. Our analysis indicated 12 subtypes of AIV were present, suggesting that wild birds in the Eastern Dongting Lake area carried a diverse array of AIVs with low pathogenicity. We were unable to detect any antibodies against AIV H5N1 in humans, suggesting that human infection with H5N1 was rare in this region.

Coman A, Maftai DN, Chereches RM, Zavrotchi E, Bria P, Dragnea C, McKenzie PP, Valentine MA, Gray GC. 2014. Avian Influenza Surveillance in the Danube Delta Using Sentinel Geese and Ducks. *Influenza Research and Treatment* **Article ID 965749**, 6.

(Abstract)

Highly pathogenic avian influenza (HPAI) H5N1 virus incursions from migrating birds have occurred multiple times in Romania since 2005. Beginning in September 2008 through April 2013, seasonal sentinel surveillance for avian influenza A viruses (AIVs) using domestic geese (*Anser cygnoides*) and ducks (*Anas platyrhynchos*) in the Danube Delta was established by placing 15 geese and 5 ducks at seven sites. Tracheal and cloacal swabs, and sera collections (starting in 2009) were taken monthly. We studied a total of 580 domestic birds and collected 5,520 cloacal and tracheal swabs from each and 2,760 sera samples. All swabs were studied with real-time reverse transcription polymerase chain reaction (rRT-PCR) for evidence of AIV. Serological samples were studied with hemagglutination inhibition assays against avian H5, H7, and H9 influenza viruses. From 2009 to 2013, 47 swab specimens from Cot Candura, Enisala, and Saon screened positive for AIV; further subtyping demonstrated that 14 ducks and 20 geese had cloacal evidence of H5N3 carriage. Correspondingly, 4 to 12 weeks after these molecular detections, sentinel bird sera revealed elevated HI titers against H5 virus antigens. We posit that domestic bird surveillance is an effective method to conduct AIV surveillance among migrating birds in delta areas.

Dusek RJ, Hallgrimsson GT, Ip HS, Jonsson JE, Sreevatsan S, Nashold SW, TeSlaa JL, Enomoto S, Halpin RA, Lin X, Fedorova N, Stockwell TB, Dugan VG, Wentworth DE, Hall JS. 2014. North Atlantic

Migratory Bird Flyways Provide Routes for Intercontinental Movement of Avian Influenza Viruses. *PLoS ONE* **9**, e92075.

(Abstract)

Avian influenza virus (AIV) in wild birds has been of increasing interest over the last decade due to the emergence of AIVs that cause significant disease and mortality in both poultry and humans. While research clearly demonstrates that AIVs can move across the Pacific or Atlantic Ocean, there has been no data to support the mechanism of how this occurs. In spring and autumn of 2010 and autumn of 2011 we obtained cloacal swab samples from 1078 waterfowl, gulls, and shorebirds of various species in southwest and west Iceland and tested them for AIV. From these, we isolated and fully sequenced the genomes of 29 AIVs from wild caught gulls (Charadriiformes) and waterfowl (Anseriformes) in Iceland. We detected viruses that were entirely (8 of 8 genomic segments) of American lineage, viruses that were entirely of Eurasian lineage, and viruses with mixed American-Eurasian lineage. Prior to this work only 2 AIVs had been reported from wild birds in Iceland and only the sequence from one segment was available in GenBank. This is the first report of finding AIVs of entirely American lineage and Eurasian lineage, as well as reassortant viruses, together in the same geographic location. Our study demonstrates the importance of the North Atlantic as a corridor for the movement of AIVs between Europe and North America.

Olson SH, Parmley J, Soos C, Gilbert M, Latorre-Margalef N, Hall JS, Hansbro PM, Leighton F, Munster VJ, Joly D. 2014. Sampling Strategies and Biodiversity of Influenza A Subtypes in Wild Birds. *PLoS ONE* **9**, e90826.

(Abstract)

Wild aquatic birds are recognized as the natural reservoir of avian influenza A viruses (AIV), but across high and low pathogenic AIV strains, scientists have yet to rigorously identify most competent hosts for the various subtypes. We examined 11,870 GenBank records to provide a baseline inventory and insight into patterns of global AIV subtype diversity and richness. Further, we conducted an extensive literature review and communicated directly with scientists to accumulate data from 50 non-overlapping studies and over 250,000 birds to assess the status of historic sampling effort. We then built virus subtype sample-based accumulation curves to better estimate sample size targets that capture a specific percentage of virus subtype richness at seven sampling locations. Our study identifies a sampling methodology that will detect an estimated 75% of circulating virus subtypes from a targeted bird population and outlines future surveillance and research priorities that are needed to explore the influence of host and virus biodiversity on emergence and transmission.

Kirunda H, Erima B, Tumushabe A, Kiconco J, Tugume T, Mulei S, Mimbe D, Mworozzi E, Bwogi J, Luswa L, Kibuuka H, Millard M, Byaruhanga A, Ducatez MF, Krauss S, Webby RJ, Webster RG,

Wurapa K, Byarugaba DK, Wabwire-Mangen F. 2014. Prevalence of influenza A virus in livestock and free-living waterfowl in Uganda. *BMC Veterinary Research* **10**, 50.

(Abstract)

Abstract(Background): Avian influenza viruses may cause severe disease in a variety of domestic animal species worldwide, with high mortality in chickens and turkeys. To reduce the information gap about prevalence of these viruses in animals in Uganda, this study was undertaken.

Results: Influenza A virus prevalence by RT-PCR was 1.1% (45/4,052) while seroprevalence by ELISA was 0.8% (24/2,970). Virus prevalence was highest in domestic ducks (2.7%, 17/629) and turkeys (2.6%, 2/76), followed by free-living waterfowl (1.3%, 12/929) and swine (1.4%, 7/511). A lower proportion of chicken samples (0.4%, 7/1,865) tested positive. No influenza A virus was isolated. A seasonal prevalence of these viruses in waterfowl was 0.7% (4/561) for the dry and 2.2% (8/368) for the wet season. In poultry, prevalence was 0.2% (2/863) for the dry and 1.4% (24/1,713) for the wet season, while that of swine was 0.0% (0/159) and 2.0% (7/352) in the two seasons, respectively. Of the 45 RT-PCR positive samples, 13 (28.9%) of them were H5 but none was H7. The 19 swine sera positive for influenza antibodies by ELISA were positive for H1 antibodies by HAI assay, but the subtype(s) of ELISA positive poultry sera could not be determined. Antibodies in the poultry sera could have been those against subtypes not included in the HAI test panel.

Conclusions: The study has demonstrated occurrence of influenza A viruses in animals in Uganda. The results suggest that increase in volumes of migratory waterfowl in the country could be associated with increased prevalence of these viruses in free-living waterfowl and poultry.

Brown VL, Drake JM, Barton HD, Stallknecht DE, Brown JD, Rohani P. 2014. Neutrality, Cross-Immunity and Subtype Dominance in Avian Influenza Viruses. *PLoS ONE* **9**, e88817.

(Abstract)

Avian influenza viruses (AIVs) are considered a threat for their potential to seed human influenza pandemics. Despite their acknowledged importance, there are significant unknowns regarding AIV transmission dynamics in their natural hosts, wild birds. Of particular interest is the difference in subtype dynamics between human and bird populations—in human populations, typically only two or three subtypes cocirculate, while avian populations are capable of simultaneously hosting a multitude of subtypes. One species in particular—ruddy turnstones (*Arenaria interpres*)—has been found to harbour a very wide range of AIV subtypes, which could make them a key player in the spread of new subtypes in wild bird populations. Very little is known about the mechanisms that drive subtype dynamics in this species, and here we address this gap in our knowledge. Taking advantage of two independent sources of data collected from ruddy turnstones in Delaware Bay, USA, we examine patterns of subtype diversity and dominance at this site. We compare these patterns to those produced by a stochastic, multi-strain transmission model to investigate possible mechanisms that are parsimonious with the observed subtype dynamics. We find, in agreement with earlier experimental work, that subtype differences are unnecessary to replicate the observed

dynamics, and that neutrality alone is sufficient. We also evaluate the role of subtype cross-immunity and find that it is not necessary to generate patterns consistent with observations. This work offers new insights into the mechanisms behind subtype diversity and dominance in a species that has the potential to be a key player in AIV dynamics in wild bird populations.

Sharshov K, Marchenko V, Yang F, Alekseev A, Cao J, Li Z, Shestopalov A, Li L. 2014. Avian Influenza H5N1 Surveillance in Geese of Qinghai Province, China (2012). *Advances in Infectious Diseases* **4**, 18-21.

(Abstract)

The aim of study was to detect H5N1 virus in wild geese in Qinghai Province in 2012. The work was provided according to WHO and OIE guidelines. In 2012, we collected 532 samples from wild geese of two species: Bar-headed Goose (*Anser indicus*) and Graylag Goose (*Anser anser*). We analyzed samples by chicken embryo inoculation and PCR. No avian influenza viruses were isolated. History of HPAI H5N1 shows obvious importance of Central Asian region in its spreading. The outbreaks of the H5N1 Highly Pathogenic Avian Influenza (HPAI H5N1) were reported in wild birds at the Qinghai Lake since 2005. This area seems to be key point for H5N1 avian influenza surveillance in wild birds. We did not find viruses although H5N1 cases in poultry were reported from 5 provinces of China in 2012. Annual surveillance is required for early AIV detection in this region.

Huang Y, Wille M, Dobbin A, Walzthoni NM, Robertson GJ, Ojkic D, Whitney H, Lang AS. 2014. Genetic Structure of Avian Influenza Viruses from Ducks of the Atlantic Flyway of North America. *PLoS ONE* **9**, e86999.

(Abstract)

Wild birds, including waterfowl such as ducks, are reservoir hosts of influenza A viruses. Despite the increased number of avian influenza virus (AIV) genome sequences available, our understanding of AIV genetic structure and transmission through space and time in waterfowl in North America is still limited. In particular, AIVs in ducks of the Atlantic flyway of North America have not been thoroughly investigated. To begin to address this gap, we analyzed 109 AIV genome sequences from ducks in the Atlantic flyway to determine their genetic structure and to document the extent of gene flow in the context of sequences from other locations and other avian and mammalian host groups. The analyses included 25 AIVs from ducks from Newfoundland, Canada, from 2008–2011 and 84 available reference duck AIVs from the Atlantic flyway from 2006–2011. A vast diversity of viral genes and genomes was identified in the 109 viruses. The genetic structure differed amongst the 8 viral segments with predominant single lineages found for the PB2, PB1 and M segments, increased diversity found for the PA, NP and NS segments (2, 3 and 3 lineages, respectively), and the highest diversity found for the HA and NA segments (12 and 9 lineages, respectively). Identification of inter-hemispheric transmissions was rare with only 2% of the genes of Eurasian origin. Virus transmission between ducks and other bird groups was investigated, with 57.3% of the genes having highly similar

(99% nucleotide identity) genes detected in birds other than ducks. Transmission between North American flyways has been frequent and 75.8% of the genes were highly similar to genes found in other North American flyways. However, the duck AIV genes did display spatial distribution bias, which was demonstrated by the different population sizes of specific viral genes in one or two neighbouring flyways compared to more distant flyways.

Marinova-Petkova A, Feeroz MM, Alam SMR, Hasan MK, Akhtar S, Jones-Engel L, Walker D, McClenaghan L, Rubrum A, Franks J, Seiler P, Jeevan T, McKenzie P, Krauss S, Webby RJ, Webster RG. 2014. Multiple introductions of highly pathogenic avian influenza H5N1 viruses into Bangladesh. *Emerging Microbes and Infections* **3**, e11.

(Abstract)

Highly pathogenic H5N1 and low pathogenic H9N2 influenza viruses are endemic to poultry markets in Bangladesh and have cocirculated since 2008. H9N2 influenza viruses circulated constantly in the poultry markets, whereas highly pathogenic H5N1 viruses occurred sporadically, with peaks of activity in cooler months. Thirty highly pathogenic H5N1 influenza viruses isolated from poultry were characterized by antigenic, molecular, and phylogenetic analyses. Highly pathogenic H5N1 influenza viruses from clades 2.2.2 and 2.3.2.1 were isolated from live bird markets only. Phylogenetic analysis of the 30 H5N1 isolates revealed multiple introductions of H5N1 influenza viruses in Bangladesh. There was no reassortment between the local H9N2 influenza viruses and H5N1 genotype, despite their prolonged cocirculation. However, we detected two reassortant H5N1 viruses, carrying the M gene from the Chinese H9N2 lineage, which briefly circulated in the Bangladesh poultry markets and then disappeared. On the other hand, interclade reassortment occurred within H5N1 lineages and played a role in the genesis of the currently dominant H5N1 viruses in Bangladesh. Few 'human-like' mutations in H5N1 may account for the limited number of human cases. Antigenically, clade 2.3.2.1 H5N1 viruses in Bangladesh have evolved since their introduction and are currently mainly homogenous, and show evidence of recent antigenic drift. Although reassortants containing H9N2 genes were detected in live poultry markets in Bangladesh, these reassortants failed to supplant the dominant H5N1 lineage.

Chang H, Dai F, Liu Z, Yuan F, Zhao S, Xiang X, Zou F, Zeng B, Fan Y, Duan G. 2014. Seroprevalence Survey of Avian Influenza A (H5) in wild migratory birds in Yunnan Province, Southwestern China. *Virology Journal* **11**, 18.

(Abstract)

Background: Highly pathogenic avian influenza virus (HPAIV) is a highly contagious disease which is a zoonotic pathogen of significant economic and public health concern. The outbreaks caused by HPAIV H5N1 of Asian origin have caused animal and human disease and mortality in several countries of Southeast Asia, such as Bangladesh, Cambodia, China, India, Indonesia, Laos, Myanmar,

Thailand and Viet Nam. For the first time since 1961, this HPAIV has also caused extensive mortality in wild birds and has sparked debate of the role wild birds have played in the spread of this virus. Other than confirmed mortality events, little is known of this virus in wild birds. There is no report on the seroprevalence of avian influenza H5 infection in wild migratory birds in Yunnan Province. In this study we examined live wild birds in Yunnan Province for H5 specific antibody to better understand the occurrence of this disease in free living birds.

Methods: Sera from 440 wild birds were collected from in Kunming and Northern Ailaoshan of Yunnan Province, Southwestern China, and assayed for H5 antibodies using the hemagglutination inhibition HI assays.

Results: The investigation revealed that the seroprevalence of avian influenza H5 was as following: Ciconiiformes 2.6%, Strigiformes 13.04%, Passeriformes 20%, Cuculiformes 21.74%, Gruiformes 0%, Columbiformes 0%, Charadriiformes 0% and Coraciiformes 0%. Statistical analyses showed that there was a significant difference of prevalence between the orders ($P < 0.01$). Specific avian influenza H5 antibodies were detected in 23 of 440 (5.23%) sera. Mean HI titer 23 positive sera against H5 were 5.4 \log_2

Conclusions: The results of the present survey indicated that the proportion of wild birds had previously infected AIV H5 at other times of the year. To our knowledge, this is the first seroprevalence report of avian influenza H5 infection in wild migratory birds in China's southwestern Yunnan Province. The results of the present survey have significant public health concerns.

Avian Influenza 2013

Deng, G., D. Tan, J. Shi, P. Cui, Y. Jiang, L. Liu, G. Tian, Y. Kawaoka, C. Li, and H. Chen. 2013. Complex Reassortment of Multiple Subtypes of Avian Influenza Viruses in Domestic Ducks at the Dongting Lake Region of China. *Journal of Virology* **87**:9452–9462.

(Abstract)

To gain insight into the ecology of avian influenza viruses (AIV), we conducted active influenza virus surveillance in domestic ducks on farms located on the flyway of migratory birds in the Dongting Lake region of Hunan Province, China, from winter 2011 until spring 2012. Specimens comprising 3,030 duck swab samples and 1,010 environmental samples were collected from 101 duck farms. We isolated AIV of various HA subtypes, including H3, H4, H5, H6, H9, H10, H11, and H12. We sequenced the entire coding sequences of the genomes of 28 representative isolates constituting 13 specific subtypes. When the phylogenetic relationships among these isolates were examined, we observed that extensive reassortment events had occurred. Among the 28 Dongting Lake viruses, 21 genotypes involving the six internal genes were identified. Furthermore, we identified viruses or viral genes introduced from other countries, viral gene segments of unknown origin, and a novel HA/NA combination. Our findings emphasize the importance of farmed domestic ducks in the Dongting Lake region to the genesis and evolution of AIV and highlight the need for continued surveillance of domestic ducks in this region.

Kang, H.-M., J.-G. Choi, K.-I. Kim, B.-S. Kim, D. Batchuluun, T.-O. Erdene-Ochir, M.-C. Kim, J.-H. Kwon, C.-K. Park, and Y.-J. Lee. 2013. Pathogenicity in domestic ducks and mice of highly pathogenic H5N1 clade 2.3.2.1 influenza viruses recently circulating in Eastern Asia. *Veterinary Microbiology* **167**:327–333.

(Abstract)

Influenza virus A (H5N1) clade 2.3.2.1 has recently caused widespread outbreaks of disease in domestic poultry and wild birds in Eastern Asia. In the current study, the antigenicity and pathogenicity of three clade 2.3.2.1 viruses (Ck/Kr/Gimje/08, Ws/Mongolia/1/09, and Ws/Mongolia/7/10) were investigated in domestic ducks and mice. The H5N1 influenza viruses in this study were antigenically similar to each other (r-values of 0.35–1.4). The three viruses replicated systemically in all tissues tested in domestic ducks, indicating high pathogenicity. However, the viruses produced different clinical signs and mortality rates: Ck/Kr/Gimje/08 and Ws/Mongolia/1/09 resulted in 100% mortality with severe neurological signs, whereas Ws/Mongolia/7/10 resulted in 50% mortality with relatively mild neurological signs. In mice, infection with Ck/Kr/Gimje/08 and Ws/Mongolia/7/10 resulted in weight loss that peaked at 4 days post-infection (22.3% and 20.8%, respectively), same MLD50 (2.2 Log₁₀ EID₅₀) and systemic replication. The three viruses had K deletion at the -2 position of the HA1-connecting peptide (PQRERRRK-R), which is associated with increased virulence in domestic ducks and harbored NA stalk deletion, NS1 deletion and mutation of P42S in NS1, and full length (90aa) in PB1-F2, which confer increased virulence in mice. Our study shows that clade 2.3.2.1 viruses from Korea and Mongolia are antigenically similar and highly pathogenic in both domestic ducks and mice. Moreover, we provide molecular determinants of the clade 2.3.2.1 viruses associated with the pathogenicity in domestic ducks and mice, respectively.

Zhao K, Gu M, Zhong L, Duan Z, Zhang Y, Zhu Y, Zhao G, Zhao M, Chen Z, Hua S, Liu W, Liu X, Peng D, Liu X. 2013. Characterization of three H5N5 and one H5N8 highly pathogenic avian influenza viruses in China. *Veterinary Microbiology* **163**, 351–357.

(Abstract)

One H5N8 and three H5N5 highly pathogenic avian influenza (HPAI) viruses which derived their HA genes from the Asian H5N1 lineage were isolated from poultry during 2009–2010 in mainland China. Pathogenicity studies showed that these viruses were all highly virulent to chickens, while they varied from moderate to high virulence in mice and from mild to intermediate virulence in mallards. Phylogenetic analyses showed that these viruses were reassortants bearing the H5N1 backbone while acquiring PB1, NP and NA genes from unidentified non-H5N1 viruses, and had developed into three distinct genotypes (B–D). Molecular characterization indicated that all these viruses might resist to antiviral agents. Our findings highlight the emergence and development of HPAI H5 viruses of other NA subtypes in H5N1 endemic areas and their potential threat to poultry industry and public health.

Kariwa H, Murata R, Totani M, Yoshii K, Takashima I. 2013. Increased Pathogenicity of West Nile Virus (WNV) by Glycosylation of Envelope Protein and Seroprevalence of WNV in Wild Birds in Far Eastern Russia. *International Journal of Environmental Research and Public Health* **10**, 7144-7164.

(Abstract)

In this review, we discuss the possibility that the glycosylation of West Nile (WN) virus E-protein may be associated with enhanced pathogenicity and higher replication of WN virus. The results indicate that E-protein glycosylation allows the virus to multiply in a heat-stable manner and therefore, has a critical role in enhanced viremic levels and virulence of WN virus in young-chick infection model. The effect of the glycosylation of the E protein on the pathogenicity of WN virus in young chicks was further investigated. The results indicate that glycosylation of the WN virus E protein is important for viral multiplication in peripheral organs and that it is associated with the strong pathogenicity of WN virus in birds. The micro-focus reduction neutralization test (FRNT) in which a large number of serum samples can be handled at once with a small volume (15 μ L) of serum was useful for differential diagnosis between Japanese encephalitis and WN virus infections in infected chicks. Serological investigation was performed among wild birds in the Far Eastern region of Russia using the FRNT. Antibodies specific to WN virus were detected in 21 samples of resident and migratory birds out of 145 wild bird samples in the region.

Zhang L, Guo ZW, Bridge ES, Li YM, Xiao X. 2013. Distribution and dynamics of risk factors associated with highly pathogenic avian influenza H5N1. *Epidemiology & Infection*.

(Abstract)

Within China's Poyang Lake region, close interactions between wild migratory birds and domestic poultry are common and provide an opportunity for the transmission and subsequent outbreaks of highly pathogenic avian influenza (HPAI) virus. We overlaid a series of ecological factors associated with HPAI to map the risk of HPAI in relation to natural and anthropogenic variables, and we identified two hotspots for potential HPAI outbreaks in the Poyang Lake region as well as three corridors connecting the two hotspot areas. In hotspot I, there is potential for migratory birds to bring new avian influenza (AI) strains that can reassort with existing strains to form new AI viruses. Hotspot II features high-density poultry production where outbreaks of endemic AI viruses are likely. The three communication corridors that link the two hotspots further promote HPAI H5N1 transmission and outbreaks and lead to the persistence of AI viruses in the Poyang Lake region. We speculate that the region's unevenly distributed poultry supply-and-demand system might be a key factor inducing HPAI H5N1 transmission and outbreaks in the Poyang Lake region.

Gerloff NA, Jones J, Simpson N, Balish A, ElBadry MA, Baghat V, Rusev I, de Mattos CC, de Mattos CA, Zonkle LEA, Kis Z, Davis CT, Yingst S, Cornelius C, Soliman A, Mohareb E, Klimov A, Donis RO. 2013. A High Diversity of Eurasian Lineage Low Pathogenicity Avian Influenza A Viruses Circulate among Wild Birds Sampled in Egypt. *PLoS ONE* **8**, e68522.

(Abstract)

Surveillance for influenza A viruses in wild birds has increased substantially as part of efforts to control the global movement of highly pathogenic avian influenza A (H5N1) virus. Studies conducted in Egypt from 2003 to 2007 to monitor birds for H5N1 identified multiple subtypes of low pathogenicity avian influenza A viruses isolated primarily from migratory waterfowl collected in the Nile Delta. Phylogenetic analysis of 28 viral genomes was performed to estimate their nearest ancestors and identify possible reassortants. Migratory flyway patterns were included in the analysis to assess gene flow between overlapping flyways. Overall, the viruses were most closely related to Eurasian, African and/or Central Asian lineage low pathogenicity viruses and belonged to 15 different subtypes. A subset of the internal genes seemed to originate from specific flyways (Black Sea-Mediterranean, East African-West Asian). The remaining genes were derived from a mixture of viruses broadly distributed across as many as 4 different flyways suggesting the importance of the Nile Delta for virus dispersal. Molecular clock date estimates suggested that the time to the nearest common ancestor of all viruses analyzed ranged from 5 to 10 years, indicating frequent genetic exchange with viruses sampled elsewhere. The intersection of multiple migratory bird flyways and the resulting diversity of influenza virus gene lineages in the Nile Delta create conditions favoring reassortment, as evident from the gene constellations identified by this study. In conclusion, we present for the first time a comprehensive phylogenetic analysis of full genome sequences from low pathogenic avian influenza viruses circulating in Egypt, underscoring the significance of the region for viral reassortment and the potential emergence of novel avian influenza A viruses, as well as representing a highly diverse influenza A virus gene pool that merits continued monitoring.

Pandit PS, Bunn DA, Pande SA, Aly SS. 2013. Modeling highly pathogenic avian influenza transmission in wild birds and poultry in West Bengal, India. *Scientific Reports* **3**, 1-8.

(Abstract)

Wild birds are suspected to have played a role in highly pathogenic avian influenza (HPAI) H5N1 outbreaks in West Bengal. Cluster analysis showed that H5N1 was introduced in West Bengal at least 3 times between 2008 and 2010. We simulated the introduction of H5N1 by wild birds and their contact with poultry through a stochastic continuous-time mathematical model. Results showed that reducing contact between wild birds and domestic poultry, and increasing the culling rate of infected domestic poultry communities will reduce the probability of outbreaks. Poultry communities that shared habitat with wild birds or those in districts with previous outbreaks were more likely to suffer an outbreak. These results indicate that wild birds can introduce HPAI to domestic poultry and that limiting their contact at shared habitats together with swift culling of infected domestic poultry can greatly reduce the likelihood of HPAI outbreaks.

Reeves AB, Pearce JM, Ramey AM, Ely CR, Schmutz JA, Flint PL, Derksen DV, Ip HS, Trust KA. 2013. Genomic analysis of avian influenza viruses from waterfowl in western Alaska, USA. *Journal of Wildlife Diseases* **49**, 600-610.

(Abstract)

The Yukon-Kuskokwim Delta (Y-K Delta) in western Alaska is an immense and important breeding ground for waterfowl. Migratory birds from the Pacific Americas, Central Pacific, and East Asian-Australasian flyways converge in this region, providing opportunities for intermixing of North American- and Eurasian-origin hosts and infectious agents, such as avian influenza virus (AIV). We characterized the genomes of 90 low pathogenic (LP) AIV isolates from 11 species of waterfowl sampled on the Y-K Delta between 2006 and 2009 as part of an interagency surveillance program for the detection of the H5N1 highly pathogenic (HP) strain of AIV. We found evidence for subtype and genetic differences between viruses from swans and geese, dabbling ducks, and sea ducks. At least one gene segment in 39% of all isolates was Eurasian in origin. Target species (those ranked as having a relatively high potential to introduce HP H5N1 AIV to North America) were no more likely than nontarget species to carry viruses with genes of Eurasian origin. These findings provide evidence that the frequency at which viral gene segments of Eurasian origin are detected does not result from a strong species effect, but rather we suspect it is linked to the geographic location of the Y-K Delta in western Alaska where flyways from different continents overlap. This study provides support for retaining the Y-K Delta as a high priority region for the surveillance of Asian avian pathogens such as HP H5N1 AIV.

USDA. 2013. 'Assessment of introduction pathway for novel avian influenza virus into North America by wild birds.' USDA: APHIS: VS: Center for Epidemiology and Animal Health. *Fort Collins, CO*.

(Abstract)

A critical question surrounding emergence of novel strains of avian influenza viruses (AIV) is the ability for wild migratory birds to translocate a complete (unreassorted whole genome) AIV intercontinentally. Virus translocation via migratory birds is suspected in outbreaks of highly pathogenic strain A (H5N1) in Asia, Africa and Europe. As a result, the potential intercontinental translocation of newly emerging AIV (e.g. A(H7N9) from Eurasia to North America via migratory movements of birds) remains a concern. An estimated 1.48 to 2.91 million aquatic birds, principally Anseriformes (ducks, geese, and swans) and Charadriiformes (gulls, terns and shorebirds) move annually between Eurasia and North America. AIV prevalence in Alaskan waterfowl populations shared between Eurasia and North America has been reported to range from <0.1 to 32.2 percent. Here we present an assessment to address the likelihood of whole (unreassorted) genome translocation of Eurasian strain AIV into North America. The scope of this assessment was limited specifically to assess the weight of evidence to support the movement of an unreassorted AIV intercontinentally by migratory aquatic birds. We reviewed high impact scientific publications to assess the evidence related to intercontinental

movement of avian influenzas by aquatic birds, specifically between Eurasia and North America. In addition, we reviewed the available information for bird populations and movements (migratory and other) between North America and all other continents. We then identified common factors described across the publications related to the pathway of introduction of a novel AIV intercontinentally into North America. In our assessment of the scientific published literature, there appear to be at least four factors that may contribute to the potential for introduction of a specific AIV intercontinentally into North America by wild birds. These factors, in aggregate, may provide a framework for evaluating the likelihood of new forms of AIV from Eurasia to be introduced through aquatic birds and become established in North America.

Avian Influenza 2012

Ahmed SSU, Ersbøll AK, Biswas PK, Christensen JP, Hannan ASMA, Toft N. 2012. Ecological Determinants of Highly Pathogenic Avian Influenza (H5N1) Outbreaks in Bangladesh. *PLoS ONE* 7, e33938.

(Abstract)

Abstract(Background): The agro-ecology and poultry husbandry of the south Asian and south-east Asian countries share common features, however, with noticeable differences. Hence, the ecological determinants associated with risk of highly pathogenic avian influenza (HPAI-H5N1) outbreaks are expected to differ between Bangladesh and e.g., Thailand and Vietnam. The primary aim of the current study was to establish ecological determinants associated with the risk of HPAI-H5N1 outbreaks at subdistrict level in Bangladesh. The secondary aim was to explore the performance of two different statistical modeling approaches for unmeasured spatially correlated variation.

Methodology/Principal Findings: An ecological study at subdistrict level in Bangladesh was performed with 138 subdistricts with HPAI-H5N1 outbreaks during 2007–2008, and 326 subdistricts with no outbreaks. The association between ecological determinants and HPAI-H5N1 outbreaks was examined using a generalized linear mixed model. Spatial clustering of the ecological data was modeled using 1) an intrinsic conditional autoregressive (ICAR) model at subdistrict level considering their first order neighbors, and 2) a multilevel (ML) model with subdistricts nested within districts. Ecological determinants significantly associated with risk of HPAI-H5N1 outbreaks at subdistrict level were migratory birds' staging areas, river network, household density, literacy rate, poultry density, live bird markets, and highway network. Predictive risk maps were derived based on the resulting models. The resulting models indicate that the ML model absorbed some of the covariate effect of the ICAR model because of the neighbor structure implied in the two different models.

Conclusions/Significance: The study identified a new set of ecological determinants related to river networks, migratory birds' staging areas and literacy rate in addition to already known risk factors, and clarified that the generalized concept of free grazing duck and duck-rice cultivation interacted

ecology are not significant determinants for Bangladesh. These findings will refine current understanding of the HPAI-H5N1 epidemiology in Bangladesh.

The emergence and spread of highly pathogenic avian influenza (HPAI) A virus subtype H5N1 in Asia, Europe and Africa has had an enormously socioeconomic impact and presents an important threat to human health because of its efficient animal-to-human transmission. Many factors contribute to the occurrence and transmission of HPAI H5N1 virus, but the role of environmental temperature remains poorly understood. Based on an approach of integrating a Bayesian Cox proportional hazards model and a Besag-York-Mollié (BYM) model, we examined the specific impact of environmental temperature on HPAI H5N1 outbreaks in domestic poultry around the globe during the period from 1 December 2003 to 31 December 2009. The results showed that higher environmental temperature was a significant risk factor for earlier occurrence of HPAI H5N1 outbreaks in domestic poultry, especially for a temperature of 25 °C. Its impact varied with epidemic waves (EWs), and the magnitude of the impact tended to increase over EWs.

Fuller TL, Bensch S, Muller I, Novembre J, Perez-Tris J, Ricklefs RE, Smith TB, Waldenstrom J. 2012. The Ecology of Emerging Infectious Diseases in Migratory Birds: An Assessment of the Role of Climate Change and Priorities for Future Research. *EcoHealth* **9**, 80-88.

(Abstract)

Pathogens that are maintained by wild birds occasionally jump to human hosts, causing considerable loss of life and disruption to global commerce. Preliminary evidence suggests that climate change and human movements and commerce may have played a role in recent range expansions of avian pathogens. Since the magnitude of climate change in the coming decades is predicted to exceed climatic changes in the recent past, there is an urgent need to determine the extent to which climate change may drive the spread of disease by avian migrants. In this review, we recommend actions intended to mitigate the impact of emergent pathogens of migratory birds on biodiversity and public health. Increased surveillance that builds upon existing bird banding networks is required to conclusively establish a link between climate and avian pathogens and to prevent pathogens with migratory bird reservoirs from spilling over to humans.

Newman SH, Hill NJ, Spragens KA, Janies D, Voronkin IO, Prosser DJ, Yan B, Lei F, Batbayar N, Natsagdorj T, Bishop CM, Butler PJ, Wikelski M, Balachandran S, Mundkur T, Douglas DC, Takekawa JY. 2012. Eco-Virological Approach for Assessing the Role of Wild Birds in the Spread of Avian Influenza H5N1 along the Central Asian Flyway. *PLoS ONE* **7**, e30636.

(Abstract)

A unique pattern of highly pathogenic avian influenza (HPAI) H5N1 outbreaks has emerged along the Central Asia Flyway, where infection of wild birds has been reported with steady frequency since 2005.

We assessed the potential for two hosts of HPAI H5N1, the bar-headed goose (*Anser indicus*) and ruddy shelduck (*Tadorna tadorna*), to act as agents for virus dispersal along this ‘thoroughfare’. We used an eco-virological approach to compare the migration of 141 birds marked with GPS satellite transmitters during 2005–2010 with: 1) the spatio-temporal patterns of poultry and wild bird outbreaks of HPAI H5N1, and 2) the trajectory of the virus in the outbreak region based on phylogeographic mapping. We found that biweekly utilization distributions (UDs) for 19.2% of bar-headed geese and 46.2% of ruddy shelduck were significantly associated with outbreaks. Ruddy shelduck showed highest correlation with poultry outbreaks owing to their wintering distribution in South Asia, where there is considerable opportunity for HPAI H5N1 spillover from poultry. Both species showed correlation with wild bird outbreaks during the spring migration, suggesting they may be involved in the northward movement of the virus. However, phylogeographic mapping of HPAI H5N1 clades 2.2 and 2.3 did not support dissemination of the virus in a northern direction along the migration corridor. In particular, two subclades (2.2.1 and 2.3.2) moved in a strictly southern direction in contrast to our spatio-temporal analysis of bird migration. Our attempt to reconcile the disciplines of wild bird ecology and HPAI H5N1 virology highlights prospects offered by both approaches as well as their limitations.

Gaidet N, Ould El Mamy AB, Cappelle J, Caron A, Cumming GS, Grosbois V, Gil P, Hammoumi S, Servan de Almeida R, Fereidouni SR, Cattoli G, Abolnik C, Mundava J, Fofana B, Ndlovu M, Diawara Y, Hurtado R, Newman SH, Dodman T, Balanca G. 2012. Investigating Avian Influenza Infection Hotspots in Old-World Shorebirds. *PLoS ONE* 7, e46049.

(Abstract)

Heterogeneity in the transmission rates of pathogens across hosts or environments may produce disease hotspots, which are defined as specific sites, times or species associations in which the infection rate is consistently elevated. Hotspots for avian influenza virus (AIV) in wild birds are largely unstudied and poorly understood. A striking feature is the existence of a unique but consistent AIV hotspot in shorebirds (Charadriiformes) associated with a single species at a specific location and time (ruddy turnstone *Arenaria interpres* at Delaware Bay, USA, in May). This unique case, though a valuable reference, limits our capacity to explore and understand the general properties of AIV hotspots in shorebirds. Unfortunately, relatively few shorebirds have been sampled outside Delaware Bay and they belong to only a few shorebird families; there also has been a lack of consistent oropharyngeal sampling as a complement to cloacal sampling. In this study we looked for AIV hotspots associated with other shorebird species and/or with some of the larger congregation sites of shorebirds in the old world. We assembled and analysed a regionally extensive dataset of AIV prevalence from 69 shorebird species sampled in 25 countries across Africa and Western Eurasia. Despite this diverse and extensive coverage we did not detect any new shorebird AIV hotspots. Neither large shorebird congregation sites nor the ruddy turnstone was consistently associated with AIV hotspots. We did, however, find a low but widespread circulation of AIV in shorebirds that contrast with the absence of AIV previously reported in shorebirds in Europe. A very high AIV antibody prevalence coupled to a low infection rate was found in both first-year and adult birds of two migratory sandpiper species, suggesting the potential existence of an AIV hotspot along their migratory flyway that is yet to be discovered.

Cappelle J, Servan de Almeida R, Fofana B, Dakouo M, Balanca G, Gil P, Albina E, Gaidet N. 2012. Circulation of avian influenza viruses in wild birds in Inner Niger Delta, Mali. *Influenza and Other Respiratory Viruses* **6**, 240-244.

(Abstract)

Background: Avian influenza viruses (AIV) have been detected in wild birds in West Africa during the northern winter, but no information is available on a potential year-round circulation of AIV in West Africa. Such year-round circulation would allow reassortment opportunities between strains circulating in Afro-tropical birds and strains imported by migratory birds wintering in West Africa.

Objective and Method: A 2-year longitudinal survey was conducted in the largest continental wetland of West Africa, the Inner Niger Delta in Mali, to determine the year-round circulation of AIV in wild birds.

Results and Conclusions: Avian influenza virus RNA was detected during all periods of the year. Very low prevalence was detected during the absence of the migratory wild birds. However, a year-round circulation of AIV seems possible in West Africa, as shown in other African regions. West Africa may hence be another potential site of reassortment between AIV strains originating from both Afro-tropical and Eurasian regions.

Avian Influenza 2011

Iverson, S. A., A. Gavrilov, T. E. Katzner, J. Y. Takekawa, T. A. Miller, W. Hagemeijer, T. Mundkur, B. Sivananthaperumal, C. C. Demattos, L. a. S. Ahmed, and S. H. Newman. 2011. Migratory movements of waterfowl in Central Asia and avian influenza emergence: sporadic transmission of H5N1 from east to west. *Ibis* **153**:279–292.

(Abstract)

Waterfowl in the genera *Anas* and *Tadorna* are suspected as vectors in the long-distance transmission of highly pathogenic avian influenza H5N1. The former Soviet Republics of Central Asia are situated at an important migratory crossroads for these and other species of birds that bridges regions where the disease is prevalent. However, waterfowl movements through Central Asia are poorly quantified. In this study, historical data derived from over 80 years of bird ringing are combined with recent satellite tracking data to delineate migration routes, movement chronology and habitat use patterns of waterfowl in relation to H5N1 outbreak locations. Results confirm migratory linkage between breeding and moulting areas in northern Kazakhstan and southern Siberia, with nonbreeding areas in the Caspian, Black and eastern Mediterranean Sea basins, as well as with South Asia. However, unlike the situation in neighbouring regions, most notably western China, H5N1 outbreaks have not been recurrent in Central Asia after they were first reported during summer 2005 and spring 2006. These findings have implications in relation to potential sampling biases, species-specific variation in migratory behaviour and continuing regional H5N1 transmission risks.

Sivay, M. V., N. Y. Silko, K. A. Sharshov, A. V. Prokudin, L. Li, M. Yang, S. Cao, and A. M. Shestopalov. 2011. The role of wild goose (*Anser*) populations of Russia and the Tibet Plateau in the spread of the avian influenza virus. *Chinese Birds* 2:140–146.

(Abstract)

Wild birds of the orders Anseriformes and Charadriiformes represent a natural reservoir of low pathogenic avian influenza (LPAI) viruses (family Orthomyxoviridae). Wild geese (order Anseriformes) relating to waterfowls undertake extensive migration flights reaching thousands of kilometers. Isolation of the avian influenza virus (AIV) from wild geese is quite low or absent. The aims of this study are to monitor the AIV in different wild goose species, nesting on Russian territory and the Tibet Plateau and to analyze the derived data for the purpose of determining the role of these wild bird species in spreading pathogens. In our study 3245 samples from nine wild goose species in nine regions of Russia and on the territory of the Tibet Plateau (the Xizang Autonomous Region) were tested and no AIV were detected. Our study shows the non-essential role of wild geese in the spread of the AIV over long distances and reaches the conclusion that geese are probably not natural reservoirs for the primary viruses. However, further inquiry of AIV in wild goose populations is required. Studies of wild geese and AIV ecology will allow us to obtain more information about pathogen-host relationships and to make arrangements for the maintenance of wild goose population.

Perez-Ramirez E, Lebarbenchon C, Feare CJ. 2011. Avian influenza in wild birds in Europe, Asia and Africa. In 'Pandemic Influenza Viruses: Science, Surveillance and Public Health.' (Eds SK Majumdar, FJ Brenner, JE Huffman, RG McLean, AI Panah, PJ Pietrobon, SP Keeler and S Shive) pp. 112-129.

(Abstract)

Since the recognition that influenza viruses in wild birds can be significant factors in both human and poultry health, the distribution and life history of these viruses have been studied in various parts of the world. The emergence of highly pathogenic avian influenza virus (HPAIV) Asian lineage H5N1 simulated more intensive development of local and international networks of surveillance (e.g. Gaidet *et al.*, 2007b, Dauphin *et al.*, 2010). Such surveillance systems have provided “early warning” signals for the spread and introduction of HPAIV into new geographic areas. Furthermore, they provided valuable data concerning low pathogenic avian influenza viruses (LPAIV) in wild birds. The subtypes of LPAIV that circulate commonly in wild waterbirds undergo annual and seasonal changes in their incidence within populations. Spillover of LPAIV into domestic poultry usually causes no or only mild sign of disease but H5 and H7 viruses sometimes transform genetically into HPAIV forms that can cause high mortality in poultry. Such highly pathogenic viruses have until recently not survived for long periods as a result of targeted control measures, and possibly intrinsic features of their replication and transmission, and have usually been confined to highly localised areas. In this chapter we provide an overview on the current knowledge on avian influenza virus in wild birds in Europe, Asia and Africa. We focus mainly on LPAIV circulation in the three continents and then provide insight on the

occurrence of HPAIV H5N1 in wild birds and its potential role in the spread of the disease as well as identify future research needs.

Cappelle J, Gaidet N, Iverson SA, Takekawa JY, Newman SH, Fofana B, Gilbert M. 2011. Characterizing the interface between wild ducks and poultry to evaluate the potential of transmission of avian pathogens. *International Journal of Health Geographics* **10**, 60-69.

(Abstract)

Background: Characterizing the interface between wild and domestic animal populations is increasingly recognized as essential in the context of emerging infectious diseases (EIDs) that are transmitted by wildlife. More specifically, the spatial and temporal distribution of contact rates between wild and domestic hosts is a key parameter for modeling EIDs transmission dynamics. We integrated satellite telemetry, remote sensing and groundbased surveys to evaluate the spatio-temporal dynamics of indirect contacts between wild and domestic birds to estimate the risk that avian pathogens such as avian influenza and Newcastle viruses will be transmitted between wildlife to poultry. We monitored comb ducks (*Sarkidiornis melanotos melanotos*) with satellite transmitters for seven months in an extensive Afro-tropical wetland (the Inner Niger Delta) in Mali and characterise the spatial distribution of backyard poultry in villages. We modelled the spatial distribution of wild ducks using 250-meter spatial resolution and 8-days temporal resolution remotely-sensed environmental indicators based on a Maxent niche modelling method.

Results: Our results show a strong seasonal variation in potential contact rate between wild ducks and poultry. We found that the exposure of poultry to wild birds was greatest at the end of the dry season and the beginning of the rainy season, when comb ducks disperse from natural water bodies to irrigated areas near villages.

Conclusions: Our study provides at a local scale a quantitative evidence of the seasonal variability of contact rate between wild and domestic bird populations. It illustrates a GIS-based methodology for estimating epidemiological contact rates at the wildlife and livestock interface integrating high-resolution satellite telemetry and remote sensing data.

Avian Influenza 2010

Sakoda, Y., S. Sugar, D. Batchluun, T.-O. Erdene-Ochir, M. Okamatsu, N. Isoda, K. Soda, H. Takakuwa, Y. Tsuda, N. Yamamoto, N. Kishida, K. Matsuno, E. Nakayama, M. Kajihara, A. Yokoyama, A. Takada, R. Sodnomdarjaa, and H. Kida. 2010. Characterization of H5N1 highly pathogenic avian influenza virus strains isolated from migratory waterfowl in Mongolia on the way back from the southern Asia to their northern territory. *Virology* **406**:88–94.

(Abstract)

H5N1 highly pathogenic avian influenza (HPAI) viruses were isolated from dead wild waterfowl at Khunt, Erkhel, Doityn Tsagaan, Doroo, and Ganga Lakes in Mongolia in July 2005, May 2006, May 2009, July 2009, and May 2010, respectively. The isolates in 2005 and 2006 were classified into genetic clade 2.2, and those in 2009 and 2010 into clade 2.3.2. A/whooper swan/Mongolia/6/2009 (H5N1) experimentally infected ducks and replicated systemically with higher mortality than that of the isolates in 2005 and 2006. Intensive surveillance of avian influenza in migratory waterfowl flying from their nesting lakes in Siberia to Mongolia in every autumn indicate that HPAI viruses have not perpetuated at their nesting lakes until 2009. The present results demonstrate that wild waterfowl were sporadically infected with H5N1 HPAI viruses prevailing in domestic poultry in the southern Asia and died in Mongolia on the way back to their northern territory in spring.

Fuller TL, Saatchi SS, Curd EE, Toffelmier E, Thomassen HA, Buermann W, DeSante DF, Nott MP, Saracco JF, Ralph CJ, Alexander JD, Pollinger JP, Smith TB. 2010. Mapping the risk of avian influenza in wild birds in the US. *BMC Infectious Diseases* **10**, 187-200.

(Abstract)

Background: Avian influenza virus (AIV) is an important public health issue because pandemic influenza viruses in people have contained genes from viruses that infect birds. The H5 and H7 AIV subtypes have periodically mutated from low pathogenicity to high pathogenicity form. Analysis of the geographic distribution of AIV can identify areas where reassortment events might occur and how high pathogenicity influenza might travel if it enters wild bird populations in the US. Modelling the number of AIV cases is important because the rate of co-infection with multiple AIV subtypes increases with the number of cases and co-infection is the source of reassortment events that give rise to new strains of influenza, which occurred before the 1968 pandemic. Aquatic birds in the orders Anseriformes and Charadriiformes have been recognized as reservoirs of AIV since the 1970s. However, little is known about influenza prevalence in terrestrial birds in the order Passeriformes. Since passerines share the same habitat as poultry, they may be more effective transmitters of the disease to humans than aquatic birds. We analyze 152 passerine species including the American Robin (*Turdus migratorius*) and Swainson's Thrush (*Catharus ustulatus*).

Methods: We formulate a regression model to predict AIV cases throughout the US at the county scale as a function of 12 environmental variables, sampling effort, and proximity to other counties with influenza outbreaks. Our analysis did not distinguish between types of influenza, including low or highly pathogenic forms.

Results: Analysis of 13,046 cloacal samples collected from 225 bird species in 41 US states between 2005 and 2008 indicates that the average prevalence of influenza in passerines is greater than the prevalence in eight other avian orders. Our regression model identifies the Great Plains and the Pacific Northwest as high-risk areas for AIV. Highly significant predictors of AIV include the amount of harvested cropland and the first day of the year when a county is snow free.

Conclusions: Although the prevalence of influenza in waterfowl has long been appreciated, we show that 22 species of song birds and perching birds (order Passeriformes) are influenza reservoirs in the contiguous US.

Cappelle J, Girard O, Fofana B, Gaidet N, Gilbert M. 2010. Ecological Modeling of the Spatial Distribution of Wild Waterbirds to Identify the Main Areas Where Avian Influenza Viruses are Circulating in the Inner Niger Delta, Mali. *EcoHealth* 7, 283-293.

(Abstract)

Predicting areas of disease emergence when no epidemiological data is available is essential for the implementation of efficient surveillance programs. The Inner Niger Delta (IND) in Mali is a major African wetland where >1 million Palearctic and African waterbirds congregate. Waterbirds are the main reservoir of Avian Influenza Viruses (AIV). Our objective was to model their spatial distribution in order to predict where these viruses would be more likely to circulate. We developed a generalized linear model (GLM) and a boosted regression trees (BRT) model based on total aerial bird counts taken in winter over 6 years. We used remotely sensed environmental variables with a high temporal resolution (10 days) to predict the spatial distribution of four waterbird groups. The predicted waterbird abundances were weighted with an epidemiological indicator based on the prevalence of low pathogenic AIV reported in the literature. The BRT model had the best predictive power and allowed prediction of the high variability of waterbird distribution. Years with low flood levels showed areas with a higher risk of circulation and had better spatial distribution predictions. Each year, the model identified a few areas with a higher risk of AIV circulation. This model can be applied every 10 days to evaluate the risk of AIV emergence in wild waterbirds. By taking into account the IND's ecological variability, it allows better targeting of areas considered for surveillance. This could enhance the control of emerging diseases at a local and regional scale, especially when resources available for surveillance programs are scarce.

Avian Influenza 2019 and earlier

2009

Si Y, Skidmore AK, Wang T, de Boer WF, Debba P, Toxopeus AG, Li L, Prins HHT. 2009. Spatio-temporal dynamics of global H5N1 outbreaks match bird migration patterns. *Geospatial Health* 4, 65-78.

(Abstract)

The global spread of highly pathogenic avian influenza H5N1 in poultry, wild birds and humans, poses a significant pandemic threat and a serious public health risk. An efficient surveillance and disease control system relies on the understanding of the dispersion patterns and spreading mechanisms of the virus. A space-time cluster analysis of H5N1 outbreaks was used to identify spatio-temporal patterns at a global scale and over an extended period of time. Potential mechanisms explaining the spread of the H5N1 virus, and the role of wild birds, were analyzed. Between December 2003 and

December 2006, three global epidemic phases of H5N1 influenza were identified. These H5N1 outbreaks showed a clear seasonal pattern, with a high density of outbreaks in winter and early spring (i.e., October to March). In phase I and II only the East Asia Australian flyway was affected. During phase III, the H5N1 viruses started to appear in four other flyways: the Central Asian flyway, the Black Sea Mediterranean flyway, the East Atlantic flyway and the East Africa West Asian flyway. Six disease cluster patterns along these flyways were found to be associated with the seasonal migration of wild birds. The spread of the H5N1 virus, as demonstrated by the space-time clusters, was associated with the patterns of migration of wild birds. Wild birds may therefore play an important role in the spread of H5N1 over long distances. Disease clusters were also detected at sites where wild birds are known to overwinter and at times when migratory birds were present. This leads to the suggestion that wild birds may also be involved in spreading the H5N1 virus over short distances.

2008

Brown, J. D., D. E. Stallknecht, and D. E. Swayne. 2008. Experimental Infection of Swans and Geese with Highly Pathogenic Avian Influenza Virus (H5N1) of Asian Lineage. *Emerging Infectious Diseases* **14**:136-142.

(Abstract)

The role of wild birds in the epidemiology of the Asian lineage highly pathogenic avian influenza (HPAI) virus subtype H5N1 epizootic and their contribution to the spread of the responsible viruses in Eurasia and Africa are unclear. To better understand the potential role of swans and geese in the epidemiology of this virus, we infected 4 species of swans and 2 species of geese with an HPAI virus of Asian lineage recovered from a whooper swan in Mongolia in 2005, A/whooper swan/Mongolia/244/2005 (H5N1). The highest mortality rates were observed in swans, and species-related differences in clinical illness and viral shedding were evident. These results suggest that the potential for HPAI (H5N1) viral shedding and the movement of infected birds may be species-dependent and can help explain observed deaths associated with HPAI (H5N1) infection in anseriforms in Eurasia.

Bin Muzaffar, S., J. Y. Takekawa, D. J. Prosser, D. C. Douglas, B. Yan, Z. Xing, Y. Hou, E. C. Palm, and S. H. Newman. 2008. Seasonal movements and migration of Pallas's Gulls *Larus ichthyaetus* from Qinghai Lake, China. *Forktail* **24**:100–107.

(Abstract)

We studied the seasonal movements and migration of ten Pallas's Gulls *Larus ichthyaetus* from Qinghai Lake to assess migratory routes and stopover areas. Each individual was captured and equipped with an 18 g solar-powered Platform Transmitter Terminal (PTT) to track its movements from September 2007 to May 2008. Six individuals remained near Qinghai Lake until the PTTs stopped transmitting. Three individuals flew 50–330 km from Qinghai Lake to nearby salt lakes. One

individual departed on 8 December and flew over 1,700 km south-west to arrive at coastal Bangladesh on 9 January 2008. Two individuals flew in October to the Brahmaputra River in Assam, India, remaining in the area for at least one month until one stopped transmitting. The second individual travelled southwest to coastal Bangladesh. Of the two individuals overwintering in Bangladesh, one remained for 67 days before migrating north. The second bird departed after 96 days, and it returned to Qinghai on 10 May 2008 after 48 days in migration. Both individuals that overwintered in coastal Bangladesh arrived much later than the outbreaks of Highly Pathogenic Avian Influenza (HPAI H5N1) in poultry in 2007. This disparity in timing would tentatively suggest that this species was not involved in long-distance movements of the virus. Instead, the converse may be true: previous work demonstrates the potential for virus spill-over from poultry into gulls and other wild bird species upon arrival into locations with widespread HPAI H5N1 outbreaks and environmental contamination.

2006

Chen, H., G. J. D. Smith, K. S. Li, J. Wang, X. H. Fan, J. M. Rayner, D. Vijaykrishna, J. X. Zhang, L. J. Zhang, C. T. Guo, C. L. Cheung, K. M. Xu, L. Duan, K. Huang, K. Qin, Y. H. C. Leung, W. L. Wu, H. R. Lu, Y. Chen, N. S. Xia, T. S. P. Naipospos, K. Y. Yuen, S. S. Hassan, S. Bahri, T. D. Nguyen, R. G. Webster, J. S. M. Peiris, and Y. Guan. 2006. Establishment of multiple sublineages of H5N1 influenza virus in Asia: Implications for pandemic control. *PNAS* **103**:2845–2850.

(Abstract)

Preparedness for a possible influenza pandemic caused by highly pathogenic avian influenza A subtype H5N1 has become a global priority. The spread of the virus to Europe and continued human infection in Southeast Asia have heightened pandemic concern. It remains unknown from where the pandemic strain may emerge; current attention is directed at Vietnam, Thailand, and, more recently, Indonesia and China. Here, we report that genetically and antigenically distinct sublineages of H5N1 virus have become established in poultry in different geographical regions of Southeast Asia, indicating the long-term endemicity of the virus, and the isolation of H5N1 virus from apparently healthy migratory birds in southern China. Our data show that H5N1 influenza virus, has continued to spread from its established source in southern China to other regions through transport of poultry and bird migration. The identification of regionally distinct sublineages contributes to the understanding of the mechanism for the perpetuation and spread of H5N1, providing information that is directly relevant to control of the source of infection in poultry. It points to the necessity of surveillance that is geographically broader than previously supposed and that includes H5N1 viruses of greater genetic and antigenic diversity.

2004

Tracey, J. P., R. Woods, D. A. Roshier, P. West, and G. R. Saunders. 2004. The role of wild birds in the transmission of avian influenza for Australia: an ecological perspective. *Emu* **104**:109-124.

(Abstract)

Waterbirds, particularly Anatidae, are natural reservoirs for low-pathogenic avian influenza and have been implicated as the primary source of infection in outbreaks of highly pathogenic avian influenza. An understanding of the movements of birds and the ecology of avian influenza viruses within the wild bird population is essential in assessing the risks to human health and production industries. Marked differences in the movements of Australian birds from those of the Northern Hemisphere emphasises the danger of generalising trends of disease prevalence to Australian conditions. Populations of Anatidae in Australia are not migratory, as they are in the Northern Hemisphere, but rather display typical nomadic traits, sometimes moving large distances across continental Australia in response to flooding or drought. There is little known regular interchange of anatids between Australia and Asia. In contrast, species such as shorebirds and some seabirds are annual migrants to Australia along recognised flyways from breeding grounds in the Northern Hemisphere. Movement into Australia by these species mainly occurs into the north-west and along the east coast over the Pacific Ocean. These species primarily arrive during the Australian spring and form large aggregations along the coastline and on inland wetlands. Other Australian migratory species (passerines, bee-eaters, dollar-birds, cuckoos, doves) regularly move to and from Asia through the Torres Strait Islands. The disease status of these birds is unknown. The movements of some species, particularly anatids and ardeids, which have ranges including Australia and regions where the virus is known to occur, have been poorly studied and there is potential for introduction of avian influenza subtypes via this route. Avian influenza viruses are highly unpredictable and can undergo reassortment to more pathogenic forms. There is insufficient knowledge of the epidemiology and transmission of these viruses in Australia and broad-scale surveillance of wild birds is logistically difficult. Long-term studies of anatids that co-habit with Charadriiformes are recommended. This would provide an indication of the spatial and temporal patterns of subtypes entering Australia and improve our understanding of the ecology of endemic viruses. Until such time as these data become available, Australia's preparedness for avian influenza must focus on biosecurity at the wild bird–poultry interface.

Hubálek, Z. 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of Wildlife Diseases* **40**:639-659.

(Abstract)

The potential for transport and dissemination of certain pathogenic microorganisms by migratory birds is of concern. Migratory birds might be involved in dispersal of microorganisms as their biological carriers, mechanical carriers, or as carriers of infected hematophagous ectoparasites (e.g., ixodid ticks). Many species of microorganisms pathogenic to homeothermic vertebrates including humans have been associated with free-living migratory birds. Migratory birds of diverse species can play significant roles in the ecology and circulation of some arboviruses (e.g., eastern and western equine encephalomyelitis and Sindbis alphaviruses, West Nile and St. Louis encephalitis flaviviruses), influenza A virus, Newcastle disease virus, duck plague herpesvirus, *Chlamydophila psittaci*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, *Campylobacter jejuni*, *Salmonella enterica*, *Pasteurella multocida*, *Mycobacterium avium*, *Candida* spp., and avian hematozoans. The efficiency of dispersal of pathogenic microorganisms depends on a wide variety

of biotic and abiotic factors affecting the survival of the agent in, or disappearance from, a habitat or ecosystem in a new geographic area.