

spp.) that cohabitated the pond, but showed no external signs of disease, were positive for ranavirus by PCR using primers M153 and M154. Further sequence analyses are ongoing, and additional investigations of other amphibians and fishes are needed.

Live freshwater fish from several countries have been imported into Japan. However, large amounts ($\leq 1,300$ tons in 2007) of live aquaculture products, including eels and other fishes, have been imported from Taiwan into Japan (www.customs.go.jp/tariff/2007_4/data/03.htm). Given that viruses that originate in Japan and Taiwan are similar, the ranavirus we detected was likely imported into Japan in an infected aquatic organism. However, an epidemiologic survey will be necessary to determine the source of the ranavirus in the pond studied. Likewise, this virus may be endemic to Japan, and a survey of native and foreign free-ranging amphibians should be conducted. Molecular analysis of ranaviruses detected in these surveys will be necessary to differentiate endemic viruses from introduced viruses.

Japan is located at middle latitudes and has a temperate climate. This country has long been geologically isolated from Asia. This isolation has resulted in the development of many diverse species of amphibians in Japan; 23 species of the order Caudata and 35 species of the order Anura. Of these species, 49 (84%) are native and 36 (62%) are listed by the Ministry of the Environment as threatened species (8). *R. catesbeiana* frogs were introduced into Japan in 1918 as a food animal, and raising them by aquaculture was widely attempted. Although they are no longer cultured, feral populations have become established throughout Japan (9). Ranavirus in *R. catesbeiana* frogs represents a serious threat to amphibians throughout Japan.

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Maternal Antibody Transfer in Yellow-legged Gulls

To the Editor: Avian influenza viruses (AIVs) are emerging pathogens of concern because they can cause deaths in birds and humans (1). Although wild birds likely contribute to AIV emergence because they are the natural reservoir for all known influenza virus subtypes (1), our understanding of AIV transmission and immunology in natural avian populations is incomplete (2). In this context, the transfer of maternal antibodies is a tool that should be used more often in immunologic analysis. Because antibodies in eggs and hatchlings can reflect the mother's past exposure to pathogens (3,4) and both life stages are more easily sampled than adults, quantifying antibodies found in avian young could help clarify AIV epidemiology.

We determined whether eggs of yellow-legged gulls (*Larus michahellis*) contained antibodies against AIVs. Yellow-legged gulls can host AIVs (C. Lebarbenchon, unpub. data), are abundant, and nest in large, dense colonies in coastal areas. In April 2008, we collected 466 eggs from 2 yellow-legged gull colonies located on the Mediterranean coast: 252 eggs from Gruissan (43.1099°N, 3.1071°E; 350 breeding pairs over 1.5 hectares), and 212 from Villeneuve-lès-Maguelone (VLM; 43.4895°N, 3.8520°E; 400 pairs over 1 hectare). Villeneuve nests formed 2 spatially clustered

subcolonies: VLM1 (129 eggs) and VLM2 (83 eggs). We also collected global positioning system coordinates for VLM1 nests.

Egg yolks were isolated and frozen at -20°C until analysis. Antibodies were obtained by using chloroform extraction (4). The yolk was diluted 1:1 in phosphate-buffered saline and homogenized. An equal volume of chloroform was added, the solution was centrifuged ($6,000 \times g$ for 15 min), and the supernatant was used in the analyses.

Extracts were tested for antibodies against the AIV nucleoprotein by using a commercial competitive ELISA (IDEXX/Institut Pourquier, Montpellier, France). The assay has been validated by using seagull serum (IDEXX, pers. comm.) and chicken egg yolk (5). A subset of samples was tested by using a second commercial competitive ELISA (IDVet, Montpellier, France). Optical density values obtained in the 2 assays were significantly correlated ($r = 0.90$, $df = 39$, $p < 0.001$), and serostatus was consistent across assays.

Overall antibody prevalence was 14% (65/466), indicating exposure to influenza A viruses in these colonies. As expected, antibody prevalence in gulls is higher than the viral prevalence previously estimated by reverse transcription-PCR or virus isolation on fecal samples, i.e., the methods typically used by avian influenza surveillance networks. A spring and summer survey performed on feces from gulls of the Camargue region (east of our colonies) showed that only 0.9% of gulls (2 infected of 228 sampled) were excreting AIVs (C. Lebarbenchon, unpub. data).

Egg antibody prevalence did not differ significantly between colonies. The antibody prevalence of 13.5% found at Gruissan (34 of 252 eggs) was comparable with the 14.5% found at Villeneuve (31 of 214 eggs) (generalized linear model with binomial distribution, $z = 0.4$, $p = 0.8$). The sub-

colonies of Villeneuve also did not differ: 18/129 (14%) in VLM1 compared with 12/83 (14.5%) in VLM2 ($z = 0.02$, $p = 0.9$). There was no evidence of spatial autocorrelation in the distribution of antibody in eggs by using the Moran I spatial statistic. This similarity of antibody prevalence across and within colonies suggests that exposure is dictated by regional rather than local conditions, a hypothesis that should be tested by sampling across a broader range of nest densities and over time.

Our study presents evidence for the presence of antibodies against AIVs in wild bird eggs, and the findings have important practical implications. The difference in prevalence estimated from virus isolation (0.9%) and antibody detection (14%), although expected, highlights the complementary nature of the 2 approaches. Most surveys estimate current infection by virus isolation, which provides information about disease risk in addition to phylogeographic tracking of strains. In contrast, information on antibody prevalence, which shows past and present population exposure and risk, has largely been ignored with few exceptions (e.g., 6,7). Future work could benefit from using both approaches in tandem with modeling to develop an understanding of avian influenza ecology in nature.

Our results also show the generalizable potential of maternal antibody transfer for tracking pathogen exposure in wild birds, notably in the case of recognized emerging zoonoses. Because eggs and hatchlings are proxies of past and present adult pathogen exposure (3,4), the difficult and sometimes disruptive sampling of adults can be circumvented by the rapid and cost-efficient sampling of their young, which will facilitate monitoring efforts. Due to the high intranest correlation in egg antibodies (4), only partial sampling of clutches (e.g., 1 of 3 eggs) is necessary to track pathogen presence and prevalence through space and time. The sampling effects could further be mini-

mized by taking blood samples from young nestlings of a standardized age. Finally, such samples provide abundant material for the simultaneous surveillance of other emerging pathogens of interest, such as *Campylobacter* spp. (8) and West Nile virus (9).

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Prevalence of Human Bocavirus in Human Tonsils and Adenoids

To the Editor: Recently, Longtin et al. (1) reported a high rate (43%) of human bocavirus (HBoV) infection in a group of children chosen to serve as controls in a study of HBoV prevalence among hospitalized children and adults. In contrast, previous reports had found low HBoV prevalence rates of 0%–1% in control groups (2,3). Attempting to explain this surprising difference in rates, Lu et al. (4) suggested that selection of control patients may be related to the difference in rates. The control group used in the Longtin study was primarily (71%) made up of children undergoing elective surgery; previous studies had selected control groups from other sources, including well children on routine visits and outpatients with nonrespiratory symptoms. Because the Longtin study surgeries were mainly tonsillectomies, adenoidectomies, and myringotomies, Lu et al. examined the possibility that tonsillar tissues, which include the adenoids, are sites of persistent HBoV infection. When these researchers extracted DNA from tonsillar lymphocytes obtained from pediatric patients who had undergone tonsillectomies or adenoidectomies, they detected HBoV DNA in 32% of the samples (4). These findings strongly suggest a connection between HBoV and tonsillar tissue. Therefore, we tested a number of tonsillar samples for the presence of HBoV DNA.

Sample acquisition was approved by the Nationwide Children's Hospital Institutional Review Board. Tonsils and adenoids were obtained from 91 patients who underwent elective surgery at Nationwide Children's Hospital from June through September 2004. Patients' ages ranged from 1 to 16 years (median 5.9 years; age was unknown for 4 patients).

Samples consisted of surgically removed tonsil or adenoid tissues. DNA was extracted and its concentration was determined as previously described (5). Two primer sets were used for HBoV detection by using real-time PCR with SYBR Green detection and melting-point determination. We designed primers 3097F (5'-GTC-CAA-TTA-CAT-GAT-CAC-GCC-TAC-TC) and 3420R (5'-TGC-GTC-CAC-AGT-ATC-AGG-TTG-TTG) that targeted the viral protein 1/2 (VP1/VP2) region of HBoV. The nonstructural protein 1 (NP1) region was targeted by using primers 188F and 542R from Allander et al. (6) Each 20- μ L reaction contained SYBR Green JumpStart Taq ReadyMix (Sigma, St. Louis, MO, USA), 4 mmol/L MgCl₂, 250 nmol/L primers, double-distilled H₂O, and 2 μ L of DNA (50–200 ng) cycled on an ABI PRISM 7900HT (Applied Biosystems, Foster City, CA, USA) instrument at 94°C for 2 min, followed by 40 cycles of 94°C for 20 s, 60°C (NP1 primers) or 68°C (VP1/VP2 primers) for 20 s, and 72°C for 14 s. Amplification and melting curves were analyzed with 7900HT version 2.2.1 software (Applied Biosystems); positive samples were verified by sequence analysis. Sequenced VP- and NP1-generated amplicons were 99%–100% identical to HBoV strain ST1 (6). The detection sensitivities of the VP and NP1 assays, determined by using a plasmid construct containing the full HBoV genome, were 1–5 and 5–10 gene copies/reaction, respectively.

Our testing identified HBoV DNA in 5 (5.5%) of the 91 children who underwent elective tonsillectomy/adenoidectomy. Ages ranged from 1.9 to 4.6 years (median 3.4 years). The reason for the much lower HBoV prevalence in this group of children, compared with prevalences found in studies by Longtin et al. and Lu et al., is unclear. Lu et al. (4) reported a much higher HBoV rate of lymphocytes from adenoids (56%) than from tonsils (16%). Although we did not know