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Novel Reassortant Highly Pathogenic Avian Influenza (H5N8) Virus in Zoos, India

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Highly pathogenic avian influenza (H5N8) viruses were detected in waterfowl at 2 zoos in India in October 2016. Both viruses were different 7:1 reassortants of H5N8 viruses isolated in May 2016 from wild birds in the Russian Federation and China, suggesting virus spread during southward winter migration of birds.

Since 1996, the hemagglutinin (HA) gene of subtype H5N1 highly pathogenic avian influenza (HPAI) viruses has evolved into multiple phylogenetic clades (*1*). During 2010, subtype H5N8 virus, bearing an H5N1 backbone and polymerase basic (PB) protein 1 (PB1), nucleoprotein (NP), and neuraminidase (NA) genes from non-H5N1 virus, emerged in China (*2*). In January 2014, a novel reassortant HPAI (H5N8) virus was detected in poultry and wild birds in South Korea (*3*) and subsequently spread to other counties in Asia and Europe before reaching North America by the end of 2014 (*4*). Because the H5N8-associated outbreaks coincided with bird migration routes, movement of wild waterfowl was suspected in intercontinental spread (*5*). Therefore, understanding the source and spread of the virus is a critical requirement for guidance of control measures. We report analysis of the genome of HPAI (H5N8) viruses isolated from waterfowl (domestic duck [*Anas platyrhynchos domesticus*] and painted stork [*Mycteria leucocephala*]) at 2 zoos in India in October 2016.

Twenty avian influenza viruses were isolated from 83 samples from National Zoological Park, Delhi, and Gandhi Zoological Park, Gwalior, Madhya Pradesh, India, in October 2016. The viruses were subtyped as H5N8 using reverse transcription PCR and real-time RT-PCR (online Technical Appendix 1, https://wwwnc.cdc.gov/EID/article/23/4/16-1886-Techapp1.pdf). One representative isolate each from Delhi (A/duck/India/10CA01/2016) and Madhya Pradesh (A/painted stork/India/10CA03/2016) were processed for pathogenic and molecular characterization. A detailed

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description of the methods for the intravenous pathogenicity index test and genetic analysis used are provided in online Technical Appendix 1. Nucleotide sequences were deposited in the GISAID EpiFlu database (http://www.gisaid.org) under accession nos. EP1858833–EP1858848.

Both isolates were highly pathogenic based on amino acid sequence at the HA cleavage region (PLREKRRKR/GLF), which was corroborated by using an intravenous pathogenicity index test of 3.00 (Delhi isolate) and 2.96 (Madhya Pradesh isolate). Amino acid markers in the neuraminidase protein and matrix protein 2 indicated sensitivity to neuraminidase inhibitors and amantadines. Markers for mammalian virulence and poultry adaptation, such as E627K and D701N in PB2 and amino acid deletion in nonstructural protein (NS) 1 (position 80–84), were absent in the H5N8 viruses. However, 42S and 13P mutations in NS and PB1 genes (*6*) associated with increased virulence of the virus to mice were present. The PB1-F2 protein was truncated because of nucleotide mutation C35A, leading to premature termination after 11 aa.

Except the polymerase acidic (PA) and NP genes, all other gene segments of both isolates shared high nucleotide identity, ranging from 99.2% to 99.5%. The nucleotide identity of the PA and NP gene was 95.8% and 94.8%, respectively, suggesting involvement of 2 gene pools of H5N8 virus in the waterfowl outbreaks at Delhi and Madhya Pradesh.

In the HA gene phylogeny, the India isolates clustered with H5N8 viruses from other countries in Asia and Europe within group B (intercontinental group B) (online Technical Appendix 1 Figures 1–8). A similar grouping pattern was observed in the neuraminidase and nonstructural (NS) gene phylogenies. Further, within intercontinental group B, the isolates shared >99% nucleotide sequence identity with H5N8 viruses isolated in Uvs-Nuur Lake (located at the Mongolia–Russia border) and Qinghai Lake, China, in May 2016 (online Technical Appendix 1 Table 2). However, PB1, PB2, and matrix protein genes grouped with low pathogenic avian influenza (LPAI) viruses isolated in Eurasia and H5N8 viruses isolated in Qinghai Lake, Uvs-Nuur Lake, and Tyva Republic (Russian Federation).

In the PA phylogeny, although the Delhi virus grouped with LPAI viruses isolated in Mongolia and Vietnam and viruses isolated in Qinghai Lake, Uvs-Nuur Lake, and Tyva Republic, the Madhya Pradesh virus shared close relationship with LPAI viruses from Eurasia. In the NP gene phylogeny, although the Delhi virus shared close relationship with the Eurasia group of LPAI viruses, whereas the Madhya Pradesh virus and H5N8 viruses from Qinghai Lake, Uvs-Nuur Lake, and Tyva Republic are closely related to the Eurasia 2 LPAI viruses. These results suggest that both isolates are 7:1 reassortant of the Tyva Republic and Uvs-Nuur Lake H5N8 viruses reported previously (*7*) with different gene constellations. A median-joining network analysis indicated that, even though the contemporary H5N8 viruses isolated from wild birds in Qinghai Lake, Uvs-Nuur Lake, and Tyva Republic are not the direct ancestors, closely related precursor gene pools are source of the H5N8 viruses that caused outbreaks in waterfowls at the 2 zoos in India (online Technical Appendix 1 Figure 9).

The outbreak in waterfowls at both zoos coincided with winter migration of birds to India (September–March). The Uvs-Nuur Lake is an important habitat for 46 resident waterfowl species and 215 different species of birds migrating southward from Siberia (*8*). Therefore, different waves of migration of the wild birds might be the source of introduction of the H5N8 virus at the 2 zoos in India, as suggested by the observed spread of H5N1 clade 2.2 and 2.3.2.1c viruses (*9*,*10*).

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Acute Tetraplegia Caused by Rat Bite Fever in Snake Keeper and Transmission of *Streptobacillus moniliformis*

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We report acute tetraplegia caused by rat bite fever in a 59-year old man (snake keeper) and transmission of *Streptobacillus moniliformis*. We found an identical characteristic bacterial pattern in rat and human samples, which validated genotyping-based evidence for infection with the same strain, and identified diagnostic difficulties concerning infection with this microorganism.

Human infections by *Streptobacillus moniliformis* are assumed to be caused by rats on the basis of epidemiologic information. We provide genotyping-based evidence for infection with the same bacterial strain in rat and human samples and highlight diagnostic difficulties concerning this microorganism and its potential for life-threatening consequences.

A 59-year-old man was admitted to Centre Hospitalier Universitaire de Tours (Tours, France) because he was unable to stand and had acute progressive onset of dyspnea and a 15-day history of fever and arthralgia (left knee, right wrist) but no signs of rash. He was sedated, mechanically ventilated, and admitted to the intensive care unit. The patient had a temperature of 39°C, a pulse rate of 63 beats/min, and a blood pressure of 126/68 mm Hg.

After discontinuation of sedation, physical examination showed cervical pain, flaccid tetraplegia, and sensitivity at the T4 level. His knees and left wrist were swollen and had joint effusions. There was little available information for the patient because he could not speak and had no known social contacts. Blood tests showed an increased leukocyte count (15×10^9 cells/L), predominantly neutrophils, and an increased C-reactive protein level (125 mg/L).

The patient was given antimicrobial drugs (amoxicillin and cloxacillin) after blood and synovia (knee) sampling. Cervical magnetic resonance imaging showed C5–T1 vertebral osteomyelitis and an epidural abscess with consecutive compression of the spinal cord (C5–T1) (Figure). Surgical spinal decompression and vertebral stabilization were not attempted because of extensiveness of injury and flaccid tetraplegia. Transthoracic and transesophageal echocardiograms showed no features of endocarditis. Blood cultures showed negative results. Joint effusions contained a culture-negative inflammatory liquid and uric acid crystals. The patient was given a tracheotomy and continuously ventilated.

A final diagnosis was obtained by sequencing the 16S rRNA gene obtained directly from synovia. An 897-nt partial 16S rRNA sequence showed 99.0% identity with sequences of *S. moniliformis* (GenBank accession nos. JQ087393 and CP001779).

The patient was a snake keeper who bred rats for snake food. He reported snake bites but not rat bites. We sampled his snakes (*Boa constrictor* and *Elaphe* sp.) and 1 of his feeder rats (*Rattus norvegicus*) by obtaining swab and biopsy specimens from oral cavities of all animals. All cultures were polymicrobial. We used desorption/ionization time-of-flight mass spectrometry (Bruker Daltonique,

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