We identified 2 poultry workers with conjunctivitis caused by highly pathogenic avian influenza A(H7N3) viruses in Jalisco, Mexico. Genomic and antigenic analyses of 1 isolate indicated relatedness to poultry and wild bird subtype H7N3 viruses from North America. This isolate had a multibasic cleavage site that might have been derived from recombination with host rRNA.

Although wild birds might be infected with influenza A(H7) viruses, outbreaks among poultry are rare. Human infection with influenza A(H7) virus is rare but has been documented after direct contact with infected birds (1). Conjunctivitis or upper respiratory tract symptoms developed in patients infected with this virus, and outcomes ranged from mild disease to death (1,2). In North America, 6 persons infected with influenza A(H7) virus have been reported; all patients recovered (2–6). We report the cases of 2 poultry workers with conjunctivitis caused by highly pathogenic avian influenza (HPAI) A(H7N3) viruses during poultry-related outbreaks in Jalisco, Mexico (5).

The Study

In June 2012, outbreaks of (HPAI) A(H7N3) virus in poultry on farms throughout Jalisco State were reported by the National Service for Health, Safety, and Food Quality in Mexico (7,8). A 32-year-old poultry worker who reported irritation in her left eye was examined at a clinic in Jalisco on July 7. Bilateral conjunctival swab specimens were collected and sent to the Institute for Epidemiologic Diagnosis and Reference (InDRE) in Mexico City, where H7 subtype virus infection was confirmed by real-time reverse transcription PCR (RT-PCR). Both patients were treated symptomatically and recovered without sequelae (5). We describe characteristics of the virus isolated from the 32-year-old woman.


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1These authors contributed equally to this article.
2Deceased.
Nucleotide sequences of 8 influenza A gene segments from a virus isolate were generated by semiconductor next-generation sequencing with Ion PGM (Life Technologies, Carlsbad, CA, USA) and MBTuni12 and MBTuni13 primers as described (9) at InDRE/Instituto Nacional de Enfermedades Respiratorias and by RT-PCR of overlapping fragments of each gene by using H7N3 subtype and avian influenza virus–specific primers at the Centers for Disease Control and Prevention. Sequences were aligned and phylogenetic trees were constructed from each gene alignment by using a neighbor-joining approach implemented in MEGA5 (www.megasoftware.net/) with 1,000 bootstrap replicates. Genomic sequences confirmed that the conjunctivitis was caused by infection with an HPAI A(H7N3) virus closely related to HPAI A(H7N3) viruses collected during poultry outbreaks in Jalisco State (Figure 1, Appendix, wwwnc.cdc.gov/EID/article/19/9/13-0087-F1.htm). The full genome of 1 isolate was deposited in GenBank under accession no. CY125725–32. Like reported avian A(H7N3) virus sequences from Jalisco, the human isolate had a multibasic cleavage site indicative of an HPAI A virus (7) (Figure 2). Genetic similarity of nucleotides at the cleavage site suggested that this region was inserted into the H7 HA gene at the site of HA0 protein cleavage by nonhomologous recombination of host rRNA from an unknown source (7). Comparison of this protein sequence motif with other HPAI and low pathogenicity avian influenza (LPAI) H7 viruses showed that this sequence indicated a novel cleavage site not observed in influenza A virus HA gene sequences (Figure 2). However, multiple arginine amino acids in this motif would be predicted to result in a highly pathogenic phenotype in chickens.

Phylogenetic trees of HA and neuraminidase (NA) genes indicated high similarity of HPAI A(H7N3) viruses detected in Mexico and LPAI viruses collected from wild birds and poultry in North America (Figure 1). HA genes clustered with LPAI A(H7N9) viruses from turkeys, geese, and guinea fowl in the United States during 2011 (10). The N3 NA genes grouped with LPAI viruses of various subtypes, clustering most closely with viruses collected from wild birds in the midwestern United States in 2009. Internal genes also clustered with LPAI viruses from various subtypes collected primarily in California in 2010 (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/9/13-0087-Techapp1.pdf). An exception was the polymerase acidic gene, which was most closely related to an H11N9 subtype virus from Mississippi.

HA and other protein gene alignments were assessed for putative markers of virulence, mammalian adaptation, receptor-binding specificity, and antiviral drug resistance. Besides the multibasic cleavage site, the virus had typical avian consensus amino acid residues in the HA protein at positions involved in preferential receptor binding to avian sialic acid receptors (amino acids Q226 and G228 by H3 numbering). Avian consensus sequences at other
motifs/amino acid positions in proteins of interest were identified, suggesting that the virus had not accumulated described mammalian host adaptive mutations or known virulence markers.

Antigenic characterization was performed by using a panel of ferret antisera in hemagglutination inhibition (HI) tests with turkey erythrocytes as described (11). The HI assay demonstrated relatedness of HPAI A(H7N3) virus with its oseltamivir-resistant counterpart with mutation H274Y (N2 numbering) were identified, suggesting that the virus had not accumulated virulence markers or known antiviral drug resistance associated with human conjunctivitis demonstrated that these viruses replicated efficiently in eye and respiratory tract tissues (15).

Although further studies are needed to investigate in vivo transmissibility of this virus, direct transmission of this virus from infected poultry to humans remains a threat and warrants use of personal protective equipment (including goggles for eye protection) and monitoring persons at risk to prevent additional cases in humans. Health authorities should consider avian influenza A virus infection in patients who have conjunctivitis or influenza-like illness and contact with poultry in areas with known avian influenza outbreaks.

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References


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Technical Appendix

Technical Appendix Figure (following pages). Phylogenetic trees of A) polymerase basic 2, B) polymerase basic 1, C) polymerase acidic, D) nucleoprotein, E) matrix, and F) nonstructural protein genes of highly pathogenic avian influenza (HPAI) A(H7N3) virus isolated from a poultry worker with conjunctivitis in Jalisco State, Mexico, July 2012, and other influenza viruses. HPAI A(H7N3) viruses from Mexico indicated in boldface. Bootstrap values >60 are shown above or below branches in each tree. *Indicates viruses detected in 2011 or 2012. Scale bars indicate nucleotide substitutions per site.
C

A/mallard/Wisconsin/2575/2009 H3N2
A/mallard/Wisconsin/2719/2009 H4N2
A/b.-w.teal/Guata/CP04-05/2010 H3N8
A/mallard/Cali/1350/2010 H7N5
A/Mexico/IndRE7216/2012 *
A/Div/PA/145565/2001 H7N3
A/mallard/Dela/418/2005 H7N3
A/env/Indiana/0603285/2008 H7N3
A/American black duck/Illinois/0354/2009
A/n pintail/Alberta/6/2009 H3N8
A/env/Cal/7451/2010 H7N3
A/mallard/Missouri/0126/2005 H11N3
A/n shoveler/Wash/44249-763/2000 H7N7
A/n shoveler/Ca/44287-164/2007 H7N7
A/rudturnstone/Dela/780/2006 H7N3
A/mallard/Alaska/44187-130/2008 H7N3
A/mallard/InteriorAlaska/6M09864/2006
A/mallard/ALB/114/1999 H7N9
A/dk/Guangdong/G1/1998 H7N3
A/rudturnstone/Dela/Eey/135/1996 H7N3
A/mallard/Alberta/34/2001 H7N1
A/mallard/Alberta/27/2001 H7N3
A/mallard/Alberta/22/2001 H7N3
A/mallard/Alberta/24/2001 H7N3
A/turkey/Minnesota/1/1988 H7N9
A/turkey/Minnesota/35429/1986 H7N9
A/emu/TX/25414/1996 H7N2
A/mallard dk/Alberta/435/1995 H7N0
A/mallard/Alberta/195/1989 H7N3
A/mallard/Ohio/421/1997 H7N8
A/mallard/Alberta/243/2006 H7N3
A/rudturnstone/NJ/563/2006 H7N2
A/mallard dK/ALB/48/1976 H7N3
A/dk/Alberta/49/1976 H7
A/mallard dK/ALB/224/1977 H7N5
A/turkey/Oregon/1571 H7N3

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