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## Probable Person-to-Person Transmission of Avian Influenza A (H5N1)

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### ABSTRACT

#### BACKGROUND

During 2004, a highly pathogenic avian influenza A (H5N1) virus caused poultry disease in eight Asian countries and infected at least 44 persons, killing 32; most of these persons had had close contact with poultry. No evidence of efficient person-to-person transmission has yet been reported. We investigated possible person-to-person transmission in a family cluster of the disease in Thailand.

#### METHODS

For each of the three involved patients, we reviewed the circumstances and timing of exposures to poultry and to other ill persons. Field teams isolated and treated the surviving patient, instituted active surveillance for disease and prophylaxis among exposed contacts, and culled the remaining poultry surrounding the affected village. Specimens from family members were tested by viral culture, microneutralization serologic analysis, immunohistochemical assay, reverse-transcriptase–polymerase-chain-reaction (RT-PCR) analysis, and genetic sequencing.

#### RESULTS

The index patient became ill three to four days after her last exposure to dying household chickens. Her mother came from a distant city to care for her in the hospital, had no recognized exposure to poultry, and died from pneumonia after providing 16 to 18 hours of unprotected nursing care. The aunt also provided unprotected nursing care; she had fever five days after the mother first had fever, followed by pneumonia seven days later. Autopsy tissue from the mother and nasopharyngeal and throat swabs from the aunt were positive for influenza A (H5N1) by RT-PCR. No additional chains of transmission were identified, and sequencing of the viral genes identified no change in the receptor-binding site of hemagglutinin or other key features of the virus. The sequences of all eight viral gene segments clustered closely with other H5N1 sequences from recent avian isolates in Thailand.

#### CONCLUSIONS

Disease in the mother and aunt probably resulted from person-to-person transmission of this lethal avian influenza virus during unprotected exposure to the critically ill index patient.

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**D**URING THE FIRST MONTHS OF 2004, outbreaks of highly pathogenic avian influenza caused by influenza A (H5N1) virus were recognized in eight Asian countries.<sup>1,2</sup> The poultry outbreaks receded and then reappeared in July in five countries, with human cases recognized in Vietnam and Thailand.<sup>3</sup> As of November 11, 2004, there had been 44 documented human infections and 32 deaths (mortality, 73 percent), sparking fears that this lethal pathogen might cause a pandemic.

Since the first avian influenza outbreak, in 1997,<sup>4</sup> there has been concern that the influenza A (H5N1) virus might either mutate and adapt to allow efficient transmission during the infection of mammals or reassort its gene segments with human influenzaviruses during the coinfection of a single host, resulting in a new virus that would be both highly lethal and transmissible from person to person. Such events are believed to have preceded the influenza pandemics of 1918, 1957, and 1968.<sup>5</sup> Several lines of evidence indicate that the currently circulating influenza A (H5N1) viruses have in fact evolved to more virulent forms since 1997, with a higher mortality among human cases,<sup>1,4</sup> different antigenic properties,<sup>6</sup> a different internal gene constellation,<sup>7</sup> and an expanded host range.<sup>8,9</sup>

In most of the human cases to date, the patients had well-documented exposure to sick or dying poultry,<sup>10-12</sup> but there have been several episodes of possible person-to-person spread. Two health

care workers who cared for patients in Hong Kong in 1997 were later found to have antibodies to hemagglutinin H5, and one recalled having had a respiratory illness after exposure to one of the patients.<sup>13</sup> Two family clusters in Vietnam in 2004 were considered to be compatible with bird-to-human spread, although limited person-to-person spread could not be ruled out.<sup>12</sup>

We report the results of an investigation into a family cluster of influenza A (H5N1) virus infections. This cluster was unusual in that one of the infected family members lived in a distant city but provided direct, in-hospital care for the index patient, highlighting the possibility of person-to-person transmission.

METHODS

PATIENTS

The index patient was an 11-year-old girl who lived with her aunt and who presented to a clinic with fever, cough, and a sore throat on September 2, 2004. She was admitted to the hospital on September 7 with a temperature of 38.5°C and moderate dyspnea. Initial testing identified lymphopenia and thrombocytopenia (Table 1) and a left-lower-lobe infiltrate on chest radiography (Fig. 1A). Because of progressive respiratory distress, hypoxemia, and shock, she was transferred to the provincial hospital the next day with a diagnosis of viral pneumonitis or the den-

**Table 1. Clinical and Epidemiologic Features of the Family Cluster of Avian Influenza (H5N1).\***

Patient	Age	Date of Fever Onset	Date of Pneumonia Diagnosis	Findings on Admission				Antiviral Treatment	Respiratory Isolation	Testing for Hemagglutinin H5	Outcome
				Total White-Cell Count	Absolute Lymphocyte Count	Platelet Count	Chest Radiograph				
Girl (index patient)	11	Sept. 2	Sept. 7	4500	1350	150,000	Right-lower-lobe consolidation	No	No	Inadequate sample	Died Sept. 8
Mother	26	Sept. 11	Sept. 17	2300	667	90,000	Bilateral lower-lobe consolidation	No	No	Positive (RT-PCR of lung tissue)	Died Sept. 20
Aunt	32	Sept. 16	Sept. 23	5400	1296	230,000	Left-lower-lobe consolidation	Yes	Yes	Positive (RT-PCR of oropharyngeal swab)	Survived; discharged Oct. 7

\* RT-PCR denotes reverse-transcriptase–polymerase chain reaction. All dates are 2004.

gue shock syndrome. A serum sample was negative for antibodies to dengue virus. Despite mechanical ventilation, administration of broad-spectrum antibiotics, and fluid resuscitation, the patient died three hours after admission to the provincial hospital.

The index patient's mother was a 26-year-old woman who lived in another province. She provided bedside care for her daughter in the hospital for 16 to 18 hours on September 7 and 8. She began to have fever and headache three days later and spent a night in her daughter's village before returning to her home. On September 17, she was admitted to a hospital in her own province with fever and severe dyspnea. She had lymphopenia and thrombocytopenia (Table 1) and bilateral interstitial infiltrates on chest radiography (Fig. 1B). Pneumonia and progressive respiratory failure were diagnosed, and she died on September 20.

The index patient's aunt was a 32-year-old woman who lived with her niece. She provided bedside care for her niece for 12 or 13 hours on September 7 and noted the onset of fever, myalgia, and chills on September 16. An upper respiratory infection was diagnosed at a clinic on September 19, but she had progressive difficulty breathing and was admitted to the district hospital on September 23 with a temperature of 39.7°C, lymphopenia (Table 1), and left-lower-lobe consolidation (Fig. 1C). On the day of admission, an investigating team suspect-

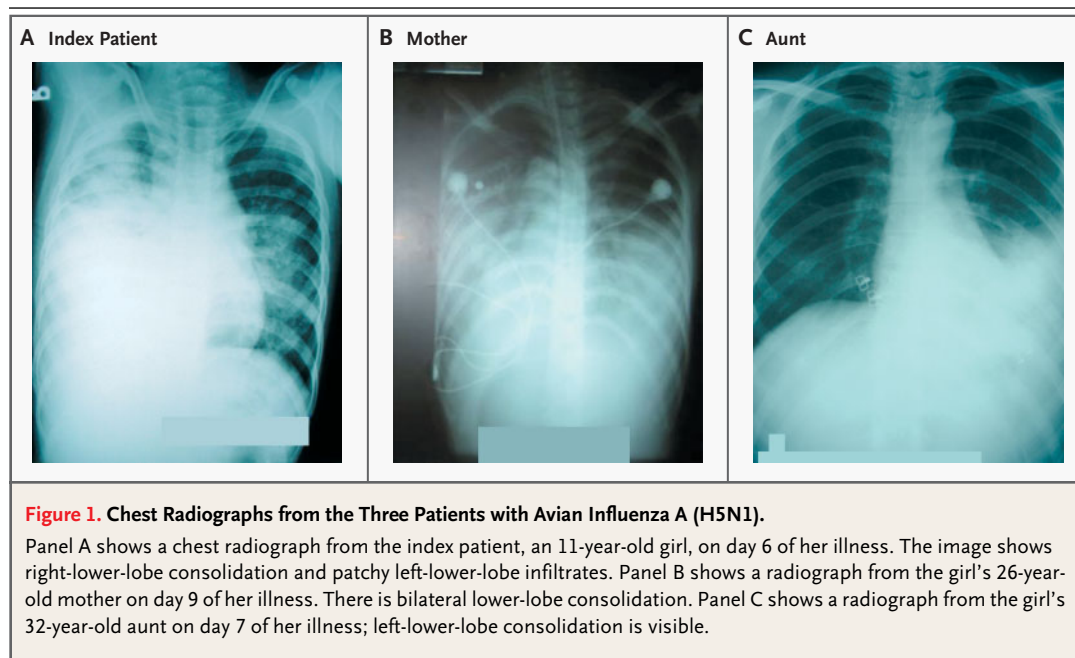
ed avian influenza, obtained respiratory specimens for testing, initiated treatment with oseltamivir, and instituted full isolation precautions. Despite moderate dyspnea and hypoxemia, her condition gradually improved, and she was discharged on October 7.

#### EPIDEMIOLOGIC INVESTIGATIONS

Under the nationwide surveillance system established in Thailand in early 2004, patients who were hospitalized with pneumonia or influenza and who had been exposed to ill poultry were reported to the Thai Ministry of Public Health. Because the mother had no exposure to poultry and the index patient's exposure was not initially reported, this cluster was recognized only coincidentally, during the investigation of another pneumonia-related death at the hospital where the mother had died.

Public health staff interviewed all family members on multiple occasions, especially those with possible exposure to sick or dying poultry, and developed and cross-checked several written timelines of events. Medical records were reviewed for the time of onset and progression of the illnesses.

All household members, other family contacts, exposed neighbors, and exposed health care workers were placed under active surveillance for fever and respiratory symptoms for 14 days. All remaining poultry were culled.



**LABORATORY INVESTIGATIONS**

By the time this family cluster was recognized, the index patient had died and her body had been cremated, and the mother had died and her body had been embalmed; therefore, appropriate specimens for influenza A (H5N1) testing were not easily obtained. Serum from the index patient and the aunt was tested for antibodies to H5. With the permission of the family, an autopsy was performed on the mother. Nasopharyngeal and oropharyngeal swabs were obtained from the aunt and other household members.

Specimens were submitted for testing at the Thai National Institute of Health and the virology laboratory at Siriraj Hospital, Mahidol University, in Bangkok, and at the Centers for Disease Control and Prevention (CDC), in Atlanta. Specimens in transport medium were tested by conventional reverse-transcriptase–polymerase-chain-reaction (RT-PCR) analysis and real-time RT-PCR and by cell culture and hen's-egg inoculation for viral isolation, including two or three blind passages, as previously described.<sup>14-16</sup> Antibody testing was performed at Siriraj Hospital and at the CDC by means of microneutralization and enzyme-linked immunosorbent assays, with confirmation by Western blotting.<sup>17</sup>

Fragments of the hemagglutinin gene containing sequences encoding the receptor-binding site and fragments of other genes were amplified by RT-PCR from RNA samples extracted from embalmed lung tissue from the mother and from the aunt's nasopharyngeal swab. RT-PCR was performed with the use of random hexamers as primers for DNA synthesis and specific primers for RT-PCR, and the products were sequenced directly. The nucleotide sequences were analyzed with Phylogeny Inference Package software and the use of a maximal-parsimony algorithm.

Formalin-fixed, paraffin-embedded lung-tissue blocks from the mother were examined by routine staining with hematoxylin and eosin and were tested with a monoclonal antibody specific for influenza A nucleoprotein by means of a colorimetric immunohistochemical assay.<sup>18</sup>

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**RESULTS**


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**EPIDEMIOLOGIC FINDINGS**

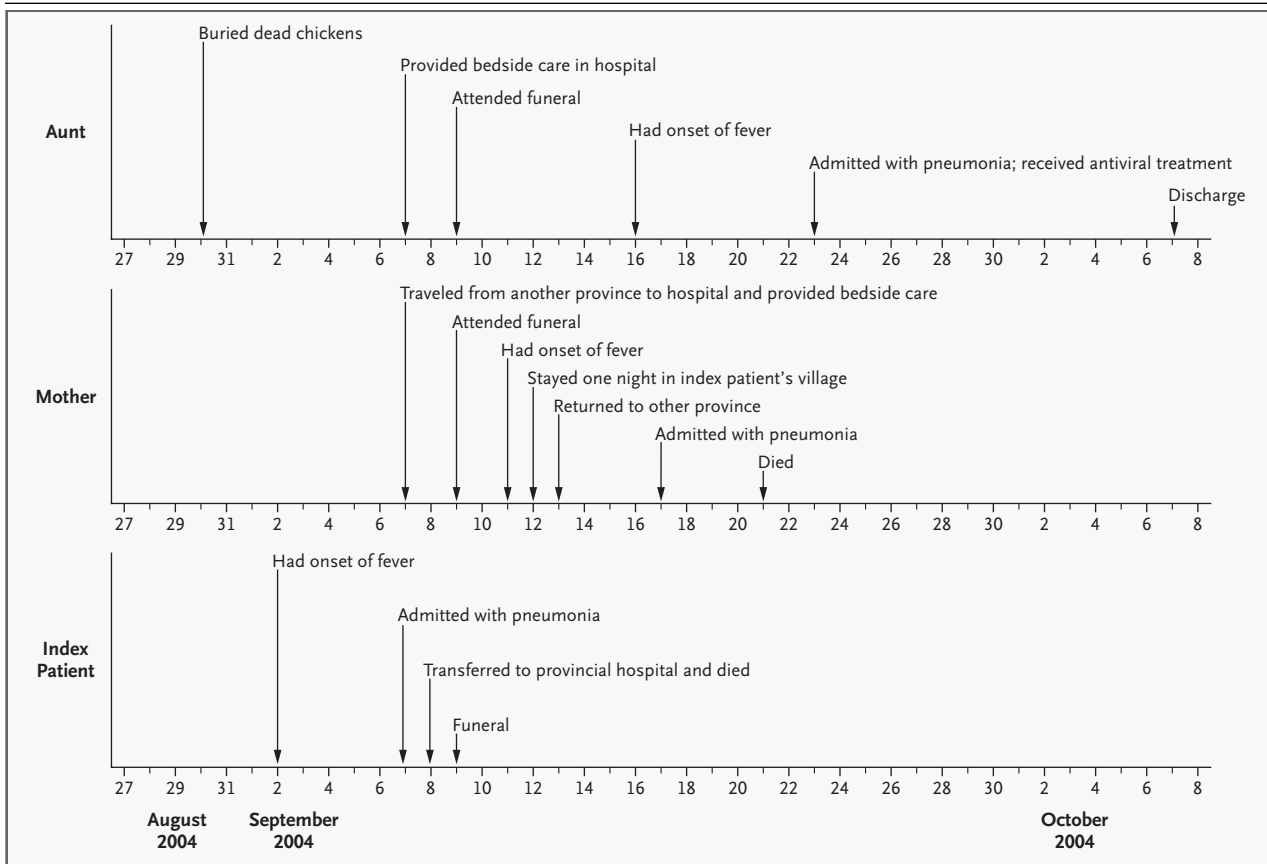
Interviews of the aunt, the other surviving family members, and neighbors permitted reconstruction of the timing of relevant exposures and the onset of

illness in the index patient and her mother and aunt (Fig. 2). The last of the free-ranging household chickens died on August 29 or 30, after progressive illness and death among the flock during the preceding weeks. The index patient was not known to have had direct contact with the sick or dying birds, but she played and slept in the area under the elevated house, where the chickens were also often present. The aunt buried the last five chickens on August 29 or 30, using plastic bags on her hands for protection. None of the three patients or other members of the household had any recognized exposure to poultry from the time these chickens were buried through the end of September.

From the time the index patient became ill until the arrival of her mother at the hospital, the aunt provided much of her care, including bedside care for 12 or 13 hours on September 7. The girl's mother lived in a Bangkok suburb with her husband, but they drove to the province (a four-hour trip) on learning of her daughter's hospitalization. They stopped at the household for less than 10 minutes to pick up a document and arrived at the hospital at about midnight. The mother then provided bedside care for the next 16 to 18 hours, and nurses later reported that she sat on the bed, hugged and kissed her daughter, and wiped secretions from her mouth.

After the girl's death, the mother and aunt went to the grandparents' village, 40 km from their home village, for the three-night funeral. Poultry in this village had died from avian influenza six months earlier, and all the remaining poultry in the village and surrounding area had been culled. Therefore, there was no exposure to live or dead poultry, including raw chicken or eggs, during the course of the funeral.

After noting fever on September 11, the mother returned to the aunt's village, as did the aunt. The mother spent one night there and returned to Bangkok the following morning. The mother worked in a garment factory and lived in a nearby apartment. There were no chickens at the apartment or at the factory. Her husband and others could recall no exposure to live or dead poultry in the two weeks preceding her illness. The aunt had had no known exposure to poultry since August 30, when she had buried the last of the dead chickens. Her husband and the immediate neighbors disinfected the house after her niece died by cleaning and spraying with a chlorine bleach solution, and they culled and buried the remaining neighborhood chickens.



**Figure 2. Timeline of Pertinent Exposures and Dates of Illness in the Three Patients.**

The index patient, who lived with her aunt, was not known to have had direct contact with the sick or dying chickens, but she played and slept in an area where the chickens were also often present. The mother lived and worked in a province four hours' drive from the index patient's village. The three-night funeral took place in a different, unaffected village.

#### LABORATORY DATA

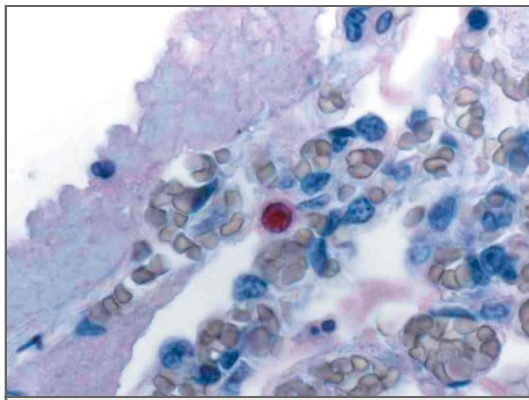
RT-PCR analysis of an oropharyngeal swab from the aunt indicated that it contained influenza A nucleoprotein and that the sequence was most closely related to an influenza A (H5N1) virus isolated from a chicken in Thailand in early 2004. A nasopharyngeal swab from the aunt was also weakly positive for the influenza A nucleoprotein gene. None of the available specimens yielded influenzaviruses on tissue culture or egg inoculation.

Serum obtained from the index patient on day 6 of her illness and from the aunt on day 8 of her illness were negative for antibodies to H5 on micro-neutralization analysis, but a convalescent-phase specimen obtained from the aunt on day 21 was positive.

Specimens of lung tissue obtained from the mother after her body had been embalmed were

positive for influenza A (H5N1) by RT-PCR in the Siriraj Hospital laboratory and at the CDC. Pathological findings included diffuse alveolar damage and interstitial pneumonia in the lung; cholestasis, congestion, and hemophagocytic activity in the liver; and congestion and depletion of lymphoid cells in the spleen. Immunohistochemical analysis of paraffin-embedded specimens of lung tissue from the mother revealed influenza-specific staining of multiple epithelial cells, which were sloughed within the airways (Fig. 3).

Sequencing of RT-PCR products from the mother and the aunt revealed that all the viral genes were avian and were closely related to other H5N1 sequences in Thailand (Fig. 4). The receptor-binding site of the encoded hemagglutinin was similar to those of other H5 hemagglutinins (amino acid positions 91, 130 through 134, 149, 151, 179, 186,



**Figure 3. Specimen of Lung Tissue from the Index Patient's Mother.**

Immunohistochemical analysis of the specimen shows interstitial pneumonitis and a single epithelial cell containing intranuclear influenza A viral antigens (red) and an antinucleocapsid antibody. Amplification of nucleic acid from this tissue specimen confirmed the presence of influenza A (H5N1) virus.

190, 191, and 220 through 225), including amino acid positions 222 and 224 (226 and 228 in the H3 numbering system). These amino acids are important determinants of the receptor-binding preference (i.e., 2,3-linked vs. 2,6-linked sialic acid)<sup>19</sup>; the receptor-binding pattern identified was avian-specific. The virus contained a 20-amino-acid deletion at the stalk of neuraminidase and the amantadine-resistance mutation in matrix M2, similar to previously described genotype Z viruses.<sup>20</sup> Sequences of the virus from the aunt were more limited because of the small sample available, but a sequence of 709 bases (nucleotides 480 to 1189) of the hemagglutinin gene was the same in the viruses from the mother and aunt, except for one synonymous substitution at nucleotide 936.

#### DISCUSSION

We believe that the most likely explanation for the family clustering of these three cases of avian influenza is that the virus was transmitted directly from the infected index patient to her mother and to her aunt. Person-to-person spread of avian influenza A (H5N1) strains has been the focus of intense concern. Ongoing surveillance for such an event across Asia has so far yielded no evidence of efficient per-

son-to-person spread. In this context, it is reassuring that no further transmission of the virus has been detected and that the available characterization of the virus from this cluster showed no adaptive change in the receptor-binding site from the avian 2,3-linked pattern toward the 2,6-linked pattern of the human sialic acid receptor. Furthermore, phylogenetic analysis of all the genomic segments showed that the H5N1 virus from this family cluster belongs to the prevalent genotype Z and that there was no reassortment with human influenza viruses. These findings confirmed that the virus was not a new variant that has gained the ability to transmit itself from person to person more efficiently.

Other explanations for this cluster are possible, although we believe they are less likely. The diagnosis in the index patient could not be confirmed virologically, but the clinical features — pneumonia with lymphopenia and thrombocytopenia and rapid progression to the acute respiratory distress syndrome and death — and the exposure to sick and dying poultry correspond to all the cardinal features of previously reported cases in humans.<sup>11,12</sup> Antibodies to H5 were not detected but would not yet be expected in serum collected six days after the onset of illness.<sup>21</sup> The confirmation that the clinically similar illnesses that followed in her mother and aunt were caused by influenza A (H5N1) provides strong support that this pathogen also caused the disease in the girl.

It was fortuitous for the investigation that the mother lived in a distant city, where she had no exposure to poultry, and traveled to the affected province only to care for her daughter. She had prolonged, direct, unprotected exposure to her critically ill daughter and had not had known exposure to poultry or poultry products. Her 10-minute visit to the affected household on September 7 and her return to that household on September 12, after the onset of her fever, are unlikely sources of her exposure.

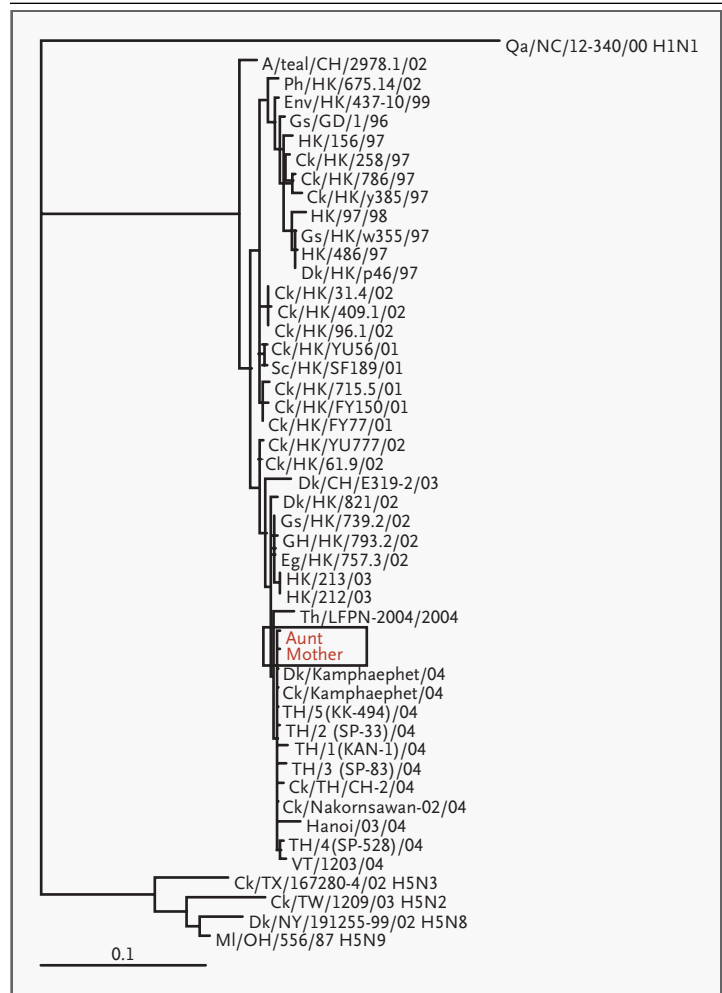
The illness in the aunt also probably resulted from transmission from the index patient. Her last recognized exposure to poultry was 17 days before the onset of her illness — a period that is longer than the accepted incubation period, which ranges from 2 to 10 days.<sup>10-12</sup> She was exposed to the index patient from the onset of the girl's illness through the first day of her hospitalization. We think the bedside exposure to the index patient

best explains the time and source of infection. It is also possible that the aunt was infected by the mother (her sister), rather than by the index patient, but this exposure would have had to have occurred during the first one or two days of the mother's illness, when she had only mild symptoms.

Direct transmission of avian influenza from person to person has probably occurred before. In addition to one of the Hong Kong health care workers, who had mild symptoms, and the Vietnamese family clusters discussed above, there were three probable secondary infections among family members of poultry workers in an outbreak of conjunctivitis caused by avian influenza A virus (H7N7).<sup>22</sup> Recent experimental infection of cats lends further biologic plausibility to the transmission of H5N1 among mammals.<sup>23</sup> The current family cluster is unique in that the secondary infections resulted in severe disease and death and in that the epidemiologic circumstances and laboratory findings made it possible to rule out transmission from poultry. The infection of close contacts with no further chains of transmission suggests that the virus has not adapted to efficient human spread, but this should not be a rationale for complacency.

Since the emergence of avian influenza H5N1 virus in 1997, the virus has gone through many reassortment events, resulting in the emergence of several genotypes. The sequences of the hemagglutinin and neuraminidase genes in the currently circulating genotype Z viruses differ significantly from those of the 1997 viruses.<sup>20</sup> This finding suggests that the virus may become more efficient in infecting humans, either by acquiring genetic material from a human influenza virus through reassortment or by adapting its receptor-binding site. It has been shown that a single amino acid substitution at position 226 or 228 of the hemagglutinin gene could change the receptor-binding preference from avian-specific 2,3-linked sialic acid to human-specific 2,6-linked sialic acid, which is believed to be a major determinant of the host range of epidemic and epizootic influenza A viruses.<sup>19</sup>

Although this family cluster was recognized late and partly by chance, the investigation of the cluster was immediate, specimens were obtained and shared with the World Health Organization network, the patients were isolated and treated, the contacts were given antiviral prophylaxis, exposed



**Figure 4. Phylogenetic Trees of Partial Sequences of the Hemagglutinin Gene, Showing the Genetic Relatedness of the Influenza A (H5N1) Virus Associated with Person-to-Person Transmission in the Family Cluster to Other Recently Isolated Influenza A (H5N1) Viruses.**

The sequences from the mother and the aunt are marked in a box. Nucleotides 273 through 1248 of the hemagglutinin gene (*HA*), 88 through 944 of the neuraminidase gene (*NA*), 938 through 1031 of the polymerase basic protein 2 gene (*PB2*), 1120 through 1218 of the polymerase basic protein 1 gene (*PB1*), 616 through 728 of the polymerase acidic protein gene (*PA*), 304 through 385 of the nucleoprotein gene (*NP*), 242 through 386 of the matrix gene (*M*), and 553 through 654 of the nonstructural gene (*NS*) were used for the analyses. The length of each horizontal line is proportional to the minimal number of nucleotide differences required to join nodes. The length of the reference line represents a 10-nucleotide difference per 100 nucleotides. The trees are rooted to H1N1 sequences. The sequences without specified H and N numbers belong to the H5N1 subtype. Ck denotes chicken, CH China, Dk duck, Eg egret, Env environment, GD Guangdong, GH gray heron, Gs goose, HK Hong Kong, MI mallard, NC Nanchang, NY New York, OH Ohio, Ph pheasant, Qa quail, Sc silky chicken, Th Thailand, TW Taiwan, TX Texas, and VT Vietnam.

persons were put under active surveillance, and poultry in the surrounding area were culled. If influenza A (H5N1) remains endemic for months to years in the eight countries that contain more than 30 percent of the world's human population, it is likely that such clusters will appear again, and it will be necessary to investigate each one rapidly and thoroughly to determine whether a critical change in the virus has occurred.

The 1918 influenza pandemic, also hypothesized to have originated from an animal influenza-virus adapted to human transmission,<sup>24</sup> killed more people in a single year than the epidemic of black death (now believed to have been bubonic plague, caused by *Yersinia pestis*) in the Middle Ages killed in a century. One author has attributed the 1918 death toll in part to the disregard for public health on the part of a government intently focused on World War I.<sup>25</sup> The person-to-person transmission of one of the most lethal human pathogens in the modern

world should serve as a reminder of the urgent need to prepare for a future influenza pandemic.

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