

BRIEF REPORT

Fatal Avian Influenza A (H5N1) in a Child Presenting with Diarrhea Followed by Coma

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SUMMARY

In southern Vietnam, a four-year-old boy presented with severe diarrhea, followed by seizures, coma, and death. The cerebrospinal fluid contained 1 white cell per cubic millimeter, normal glucose levels, and increased levels of protein (0.81 g per liter). The diagnosis of avian influenza A (H5N1) was established by isolation of the virus from cerebrospinal fluid, fecal, throat, and serum specimens. The patient's nine-year-old sister had died from a similar syndrome two weeks earlier. In both siblings, the clinical diagnosis was acute encephalitis. Neither patient had respiratory symptoms at presentation. These cases suggest that the spectrum of influenza H5N1 is wider than previously thought.

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N Engl J Med 2005;352:686-91.
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FORTY-FIVE CASES OF INFLUENZA A (H5N1) WERE REPORTED IN HUMANS during 2004, of which 33 were fatal. All the patients presented primarily with severe respiratory illnesses.¹ We report an additional fatal case of influenza H5N1, diagnosed by isolating the virus from cerebrospinal fluid, fecal, throat, and serum specimens, in a boy who presented with severe diarrhea but no apparent respiratory illness, followed by rapidly progressive coma, leading to a clinical diagnosis of acute encephalitis. Two weeks earlier, his sister had died of a similar illness. These cases suggest that the clinical spectrum of influenza H5N1 is wider than previously thought, and therefore they have important implications for the clinical and public health responses to avian influenza.

CASE REPORTS

PATIENT 1

A previously healthy nine-year-old girl presented to a hospital in Dong Thap Province in southern Vietnam on February 1, 2004, with a four-day history of fever, watery diarrhea without blood or mucus (daily frequency of stools exceeding 10 times), and increasing drowsiness. She had no respiratory symptoms. On admission, she had a temperature of 38.5°C, a weak pulse of 120 beats per minute, a blood pressure of 80/60 mm Hg, and a score of 9 on the Glasgow Coma Scale (where scores range from 3 to 15, with lower scores indicating reduced levels of consciousness). The results of a physical examination and routine hematologic and biochemical measurements, including measurement of blood glucose levels, were otherwise normal. A chest radiograph also was normal

(Fig. 1A). Neuroimaging studies were not performed. Culture and parasitologic examination of stool specimens did not reveal enteric pathogens. Examination of the cerebrospinal fluid showed no white cells and normal levels of glucose and protein. Bacterial cultures of blood and cerebrospinal fluid were negative. The differential diagnosis was septicemia from a gastrointestinal source or acute encephalitis. She was treated with intravenous fluids, acetaminophen, ceftriaxone, gentamicin, and mannitol. During the next few hours, her hemodynamic condition stabilized, but the coma worsened, with the Glasgow Coma Scale score decreasing to 5. Despite aggressive support, including intubation and ventilation, the girl died on February 2, 2004. Acute

encephalitis of unknown origin was reported as the cause of death. No autopsy was performed.

PATIENT 2

The four-year-old brother of Patient 1 presented to the same hospital on February 12, 2004, with a two-day history of fever, headache, vomiting, and severe diarrhea. His stools (daily frequency, 10 times) were watery without blood or mucus. On admission, he was alert, and the results of physical examination were unremarkable. The results of the laboratory evaluation are shown in Table 1. A chest radiograph was normal (Fig. 1B). Culture and parasitologic examination of stool specimens did not show enteric pathogens. Enteric fever was diagnosed, and the pa-

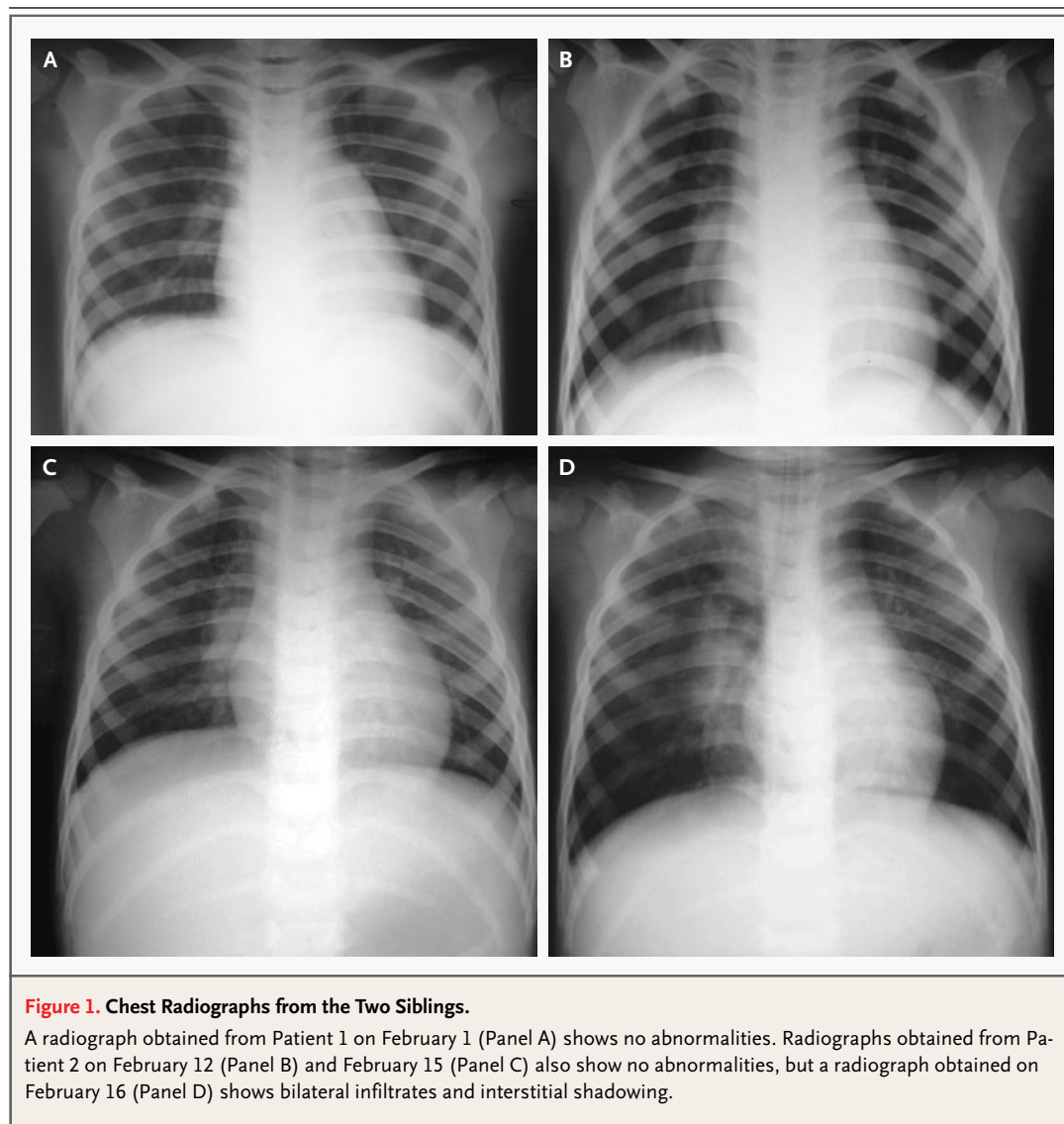


Table 1. Hematologic and Blood Chemical Values for Patient 2.*

Variable	February 12	February 15	February 16	Normal Range
Leukocyte count (cells/mm ³)	7,300	3,600	2,500	5500–15,500
Neutrophils (%)	85	32	10	23–45
Lymphocytes (%)	NA	68	88	35–65
Platelet count (cells/mm ³)	314,000	100,000	30,000	250,000–550,000
Prothrombin time (sec)	NA	15.1	14.2	11.0–13.5
Serum				
Glucose (mmol/liter)†	NA	5.0	NA	3.9–6.4
Alanine aminotransferase (U/liter)	1,020	NA	NA	<55
Aspartate aminotransferase (U/liter)	400	NA	NA	<50
Lactate (mmol/liter)	NA	5.14	NA	0.5–2.2
Arterial blood				
pH	NA	7.27	7.38	7.38–7.42
Partial pressure of carbon dioxide (mm Hg)	NA	19.8	21.6	35–45
Partial pressure of oxygen (mm Hg)	NA	129.4‡	192.2§	70–100
Bicarbonate (mEq/liter)	NA	9.0	12.5	22.0–26.0
Alveolar–arterial oxygen gradient (mm Hg)	NA	60	500	20–60

* Laboratory data on February 12 were obtained at the Dong Thap provincial hospital; data on February 15 and 16 were obtained at the pediatric referral hospital in Ho Chi Minh City. NA denotes not available.

† To convert the values for glucose to milligrams per deciliter, divide by 0.05551.

‡ The patient was receiving 30 percent oxygen.

§ The patient was receiving 100 percent oxygen.

tient was treated with intravenous fluids, acetaminophen, ceftriaxone, and gentamicin.

As a result of increasing diarrhea and drowsiness, the patient was transferred to a pediatric referral hospital in Ho Chi Minh City on February 15. On admission there, he was coughing and had a temperature of 38°C, a blood pressure of 80/30 mm Hg, a respiratory rate of 36 breaths per minute, and a Glasgow Coma Scale score of 12. Auscultation of the lungs revealed mild crackles and wheezes bilaterally. The laboratory findings are summarized in Table 1. Blood cultures were negative for bacteria. The chest radiograph was normal (Fig. 1C). Neuroimaging studies were not performed. The admission diagnosis was septicemia from a gastrointestinal source, and intravenous fluids, ceftazidime, and amikacin were started.

The patient had a generalized convulsion and became comatose (Glasgow Coma Scale score, 7) 12 hours after admission, and he was transferred to the intensive care unit with a clinical diagnosis of encephalitis, where he was treated with phenobarbital and mannitol. Laboratory analysis of cerebrospinal fluid obtained by means of a slightly traumatic lumbar puncture showed 1 white cell per cubic

millimeter, normal glucose levels, and increased protein levels (0.81 g per liter). The number of erythrocytes was not assessed. Bacterial culture of cerebrospinal fluid was not performed. Respiratory failure developed over the next 12 hours, the patient was intubated, and mechanical ventilation was instituted. A chest radiograph obtained on February 16 showed bilateral infiltrates (Fig. 1D). The patient died on February 17, 2004. Acute encephalitis of unknown origin was reported as the cause of death. No autopsy was performed.

METHODS AND RESULTS

DIAGNOSTIC SPECIMENS

No diagnostic specimens were available from Patient 1. Patient 2 was included in an ongoing study of the causes of acute encephalitis, and hence throat and rectal swabs and cerebrospinal fluid and serum specimens were stored at –80°C.

VIROLOGIC INVESTIGATIONS

All virologic investigations were done at the Oxford University Clinical Research Unit, Hospital for Tropical Diseases, in biosafety level 2 and 3 culture facil-

ities and molecular diagnostic facilities consisting of three physically separated laboratories — one for the preparation of reagents, one for the extraction of nucleic acids, and one for nucleic acid amplification.

Initial routine analyses of cerebrospinal fluid included real-time polymerase-chain-reaction (PCR) assays to identify herpes simplex viruses and enteroviruses^{2,3} and capture enzyme-linked immunoassays (Venture Technologies) to identify IgM specific for dengue virus and IgM specific for Japanese encephalitis virus. The results of these analyses were negative.

As part of the encephalitis study, stored cerebrospinal fluid and serum specimens were inoculated onto C6/36 mosquito cells, baby-hamster-kidney (BHK-21) cells, Vero cells, and human rhabdomyosarcoma cells in the biosafety level 2 laboratory. Primary culture of cerebrospinal fluid and serum specimens from Patient 2 was done in April and September 2004, respectively. Nonspecific cytopathic effects, consisting of ballooning and detachment of cells, were observed in Vero and BHK-21 cells within one week after the inoculation of cerebrospinal fluid and in rhabdomyosarcoma cells on blind passage. All three cell lines inoculated with the patient's serum showed cytopathic effects on primary culture. Immunofluorescence and PCR assays were negative for herpes simplex viruses type 1 and 2, varicella-zoster virus, enteroviruses, dengue viruses, and Japanese encephalitis virus.

Further efforts at identification were delayed until late October. At that time, the stored viral isolates were inoculated onto LLC-MK2 and Madin-Darby canine-kidney cells to further assess the cell tropism, and both cultures showed cytopathic effects within one week. At this time, an influenza virus was suspected, and this suspicion was confirmed by influenza A-specific reverse-transcriptase (RT) PCR of culture supernatant. A highly pathogenic strain of avian influenza A was considered the likely cause in view of the broad tropism of the virus, and hence all further cultures and hemagglutination-inhibition assays were done in the biosafety level 3 laboratory. The viral isolates were subtyped as influenza A (H5N1) virus by serotype-specific RT-PCR assays and hemagglutination-inhibition assays, as described previously.^{1,4} For the hemagglutination-inhibition assays, reference antiserum against H1N1 (A/New Caledonia/20/99), H3N2 (A/Panama/2007/99 and A/Korea/770/02), and H5N3 (A/Duck/HK/820/80) viruses was used.

Subsequently, stored throat and rectal swabs

were inoculated onto Madin-Darby canine-kidney cells on separate occasions, and influenza A (H5N1) virus was also isolated from these specimens. Finally, viral RNA was detected directly in stored cerebrospinal fluid and serum specimens and in throat and rectal swabs by a real-time RT-PCR assay targeted at a conserved region of the matrix gene (for details see the Supplementary Appendix, available with the full text of this article at www.nejm.org). Quantitative estimations revealed viral loads of 85,000 and 64,000 copies of complementary DNA per milliliter of serum and cerebrospinal fluid, respectively, and 180,000 and 98,000 copies per milliliter of viral-transport medium in throat and rectal swabs, respectively.

EPIDEMIOLOGIC INVESTIGATION

After the diagnosis was established in November 2004, the hamlet and house of the two children were surveyed and their parents and neighbors were interviewed. The family lived in a one-room house. The parents had no other children. Water from a nearby canal was used for washing and, after boiling, for drinking. Patient 1 swam regularly in this canal, as did other children in the neighborhood. At the time of the children's illnesses, the family owned apparently healthy fighting cocks. Many chickens and ducks were present in the hamlet and canal during early 2004, but none were ill. All were culled in February as part of routine measures to contain the outbreak of influenza H5N1 in poultry. The parents did not handle poultry from markets. Before the children were admitted, they were cared for by both parents and several close relatives. No febrile illnesses were reported in the parents, close relatives, or other residents of the hamlet.

DISCUSSION

We report a fatal case of influenza A (H5N1) in a child who presented with severe diarrhea, followed by convulsions and coma, and who received a diagnosis of acute encephalitis. The diagnosis of influenza H5N1 was established by isolating the virus from stored cerebrospinal fluid, serum, throat, and rectal specimens. The possibility of laboratory contamination can be ruled out, for the following reasons: all clinical specimens were cultured on separate occasions, weeks or months apart; viral RNA was subsequently detected directly in all stored specimens; and the H5N1 virus was not isolated from other patients' specimens during the same period.

Although the possibility remains unproven because of the lack of specimens from Patient 1, the temporal relationship and similarity of illnesses render it likely that the two patients died of the same disease.

These cases have important clinical, scientific, and public health implications. In both cases, the clinical presentation led to diagnoses of gastrointestinal infection and acute encephalitis, which alone or in combination are common clinical syndromes in southern Vietnam. Patient 1 had no respiratory symptoms and a normal chest radiograph less than 24 hours before she died. Although Patient 2 showed signs of pneumonia during the last day of his life, a respiratory illness was not considered his most relevant clinical problem. Recently, another patient with influenza H5N1 was described with an initial presentation of fever and diarrhea alone.⁵ These cases emphasize that avian influenza A (H5N1) should be included in the differential diagnosis of a much wider clinical spectrum of disease than previously considered and that clinical surveillance of influenza H5N1 should focus not only on respiratory illnesses, but also on clusters of unexplained deaths or severe illnesses of any kind. Awareness of the full clinical spectrum is essential to appropriate management of the illness, since treatment with antiviral agents is likely to be beneficial only when it is started early in the course of illness.

Encephalitis and encephalopathy are rare complications of infection with human influenza viruses, and the pathogenesis remains unclear.⁶⁻⁸ Although viral RNA has been detected in some cases, reports of isolation of influenza virus from cerebrospinal fluid are extremely rare.⁷⁻⁹ By contrast, avian influenza A (H5N1) viruses replicate systemically in poultry, affecting multiple organs, including the central nervous system.¹⁰ Furthermore, the H5N1 strain implicated in the 1997 Hong Kong outbreak causes encephalitis in experimentally infected mice without prior host adaptation.¹¹⁻¹⁶ Recent studies in ducks and mice show that the capacity of H5N1 strains to cause systemic illness, including central nervous system involvement, is increasing.^{17,18} The currently circulating strain of H5N1 has also been shown to cause encephalitis in tigers and leopards.¹⁹ These reports suggest that avian influenza A (H5N1) virus is progressively adapting to mammals and becoming more neurologically virulent.

In our patient, systemic infection was evidenced by the viremia, which is rarely reported in humans with influenza,²⁰⁻²³ and by the isolation of virus

from cerebrospinal fluid and rectal specimens. It is likely that hepatitis and metabolic acidosis were also secondary to disseminated viral infection. Since imaging of the brain or histologic analyses were not performed in either patient, we cannot be sure whether they had encephalopathy or true encephalitis. However, the presence of virus in the cerebrospinal fluid of Patient 2 strongly suggests that the virus had a causative role in his coma. The precise mechanism of this role needs to be addressed by appropriate investigations of future patients with similar presentations. Although the lumbar puncture was traumatic, the marginal increase in the protein level and the near-absence of white cells in the cerebrospinal fluid argue against blood contamination sufficient to explain the similarity of the viral loads in the serum and cerebrospinal fluid.

Assuming that the two children died of the same illness, why influenza H5N1 presented in this similar atypical manner in these two siblings remains an enigma. On the basis of the combination of influenza, elevated aminotransferase levels, and coma, a diagnosis of Reye's syndrome in Patient 2 could be considered. However, the finding of normal blood glucose levels, the absence of aspirin use, and the evidence of disseminated infection with a highly pathogenic virus as a plausible cause of the illness argue against this diagnosis. Further research is needed to determine whether host factors, which may determine a person's susceptibility to disseminated or central nervous system infection, or a particularly neurologically virulent strain of virus is involved.

The routes of transmission in our patients are unclear. Epidemiologic investigations did not reveal exposure to ill poultry. In view of recent data suggesting that ducks infected with the current H5N1 strain shed large amounts of virus, the source of transmission may have been domestic ducks present in the canal near the children's house. Water from this canal was used for washing, and Patient 1 was reported to have swum regularly in this canal. Direct transmission from sister to brother appears unlikely, considering the interval between their illnesses. Only if the incubation period was unusually long could sister-to-brother transmission be implicated. Nevertheless, the isolation of virus from a rectal specimen is a major source of concern, since it highlights a potential route of human-to-human transmission, especially in combination with crowded living conditions and diarrhea.

In conclusion, our cases emphasize a hitherto

unsuspected broad clinical spectrum of disease attributable to avian influenza A (H5N1) virus and provide important information for treatment and future clinical surveillance. The presence of viable virus in the feces of our patient has important im-

plications for transmission, infection control, and public health.

Supported by the Wellcome Trust.

We are indebted to Le Van Tan for laboratory support and to Professor Malik Peiris for critical review of the manuscript.

REFERENCES

- Hien TT, Liem NT, Dung NT, et al. Avian influenza A (H5N1) in 10 patients in Vietnam. *N Engl J Med* 2004;350:1179-88.
- Ryncarz AJ, Goddard J, Wald A, Huang ML, Roizman B, Corey L. Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. *J Clin Microbiol* 1999;37:1941-7.
- Beld M, Minnaar R, Weel J, et al. Highly sensitive assay for detection of enterovirus in clinical specimens by reverse transcription-PCR with an armored RNA internal control. *J Clin Microbiol* 2004;42:3059-64.
- Identification of influenza isolates by hemagglutination inhibition. In: WHO manual on animal influenza diagnosis and surveillance. Geneva: World Health Organization, 2002:28-36. (WHO/CDS/CSR/NCS/2002.5.)
- Apisarnthanarak A, Kitphati R, Thongphubeth K, et al. Atypical avian influenza (H5N1). *Emerg Infect Dis* 2004;10:1321-4.
- Morishima T, Togashi T, Yokota S, et al. Encephalitis and encephalopathy associated with an influenza epidemic in Japan. *Clin Infect Dis* 2002;35:512-7.
- Steininger C, Popow-Kraupp T, Laferl H, et al. Acute encephalopathy associated with influenza A virus infection. *Clin Infect Dis* 2003;36:567-74.
- Maricich SM, Neul JL, Lotze TE, et al. Neurologic complications associated with influenza A in children during the 2003-2004 influenza season in Houston, Texas. *Pediatrics* 2004;114:e626-e633.
- Fujimoto S, Kobayashi M, Uemura O, et al. PCR on cerebrospinal fluid to show influenza-associated acute encephalopathy or encephalitis. *Lancet* 1998;352:873-5.
- Shortridge KF, Zhou NN, Guan Y, et al. Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. *Virology* 1998;252:331-42.
- Gao P, Watanabe S, Ito T, et al. Biological heterogeneity, including systemic replication in mice, of H5N1 influenza A virus isolates from humans in Hong Kong. *J Virol* 1999;73:3184-9.
- Dybing JK, Schultz-Cherry S, Swayne DE, Suarez DL, Perdue ML. Distinct pathogenesis of Hong Kong-origin H5N1 viruses in mice compared to that of other highly pathogenic H5 avian influenza viruses. *J Virol* 2000;74:1443-50.
- Lu X, Tumpey TM, Morken T, Zaki SR, Cox NJ, Katz JM. A mouse model for the evaluation of pathogenesis and immunity to influenza A (H5N1) viruses isolated from humans. *J Virol* 1999;73:5903-11.
- Nishimura H, Itamura S, Iwasaki T, Kurata T, Tashiro M. Characterization of human influenza A (H5N1) virus infection in mice: neuro-, pneumo- and adipo-tropic infection. *J Gen Virol* 2001;82:971.]
- Park CH, Ishinaka M, Takada A, et al. The invasion routes of neurovirulent A/Hong Kong/483/97 (H5N1) influenza virus into the central nervous system after respiratory infection in mice. *Arch Virol* 2002;147:1425-36.
- Tanaka H, Park CH, Ninomiya A, et al. Neurotropism of the 1997 Hong Kong H5N1 influenza virus in mice. *Vet Microbiol* 2003;95:1-13.
- Chen H, Deng G, Li Z, et al. The evolution of H5N1 influenza viruses in ducks in southern China. *Proc Natl Acad Sci U S A* 2004;101:10452-7.
- Guan Y, Poon LL, Cheung CY, et al. H5N1 influenza: a protean pandemic threat. *Proc Natl Acad Sci U S A* 2004;101:8156-61.
- Keawcharoen J, Oraveerakul K, Kuiken T, et al. Avian influenza H5N1 in tigers and leopards. *Emerg Infect Dis* 2004;10:2189-91.
- Stanley ED, Jackson GG. Viremia in Asian influenza. *Trans Assoc Am Physicians* 1966;79:376-87.
- Naficy K. Human influenza infection with proved viremia: report of a case. *N Engl J Med* 1963;269:964-6.
- Lehmann NI, Gust ID. Viraemia in influenza: a report of two cases. *Med J Aust* 1971;2:1166-9.
- Khakpour M, Saidi A, Naficy K. Proved viraemia in Asian influenza (Hong Kong variant) during incubation period. *Br Med J* 1969;4:208-9.

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