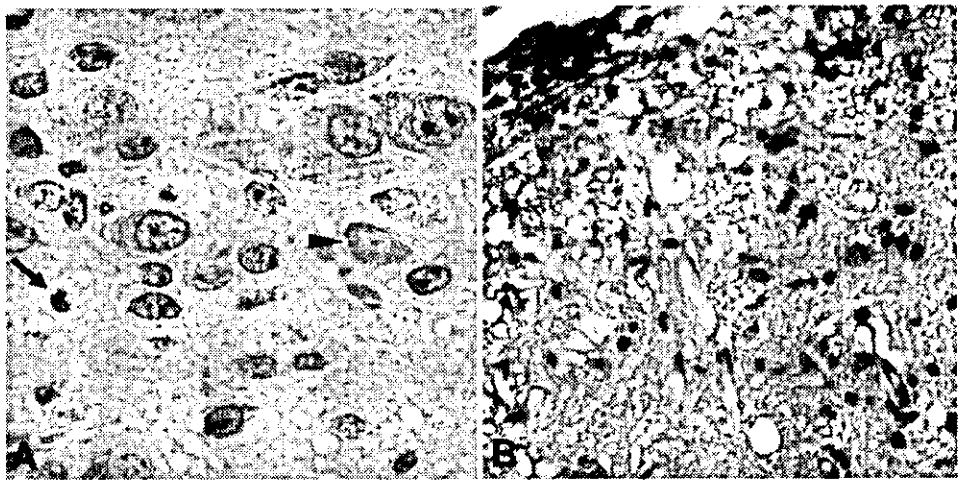

**Fig. 5** Antigen-positive lesions in the vestibular nucleus of a mouse inoculated with HK483 on day 3 p.i. (A) and the pterygopalatine ganglion on day 5 p.i. (B). The antigen is detected not only in the neurons but also in glial and satellite cells. Late-infected cells become positive in the nucleus and cytoplasm (*arrowheads*) and early-infected cells only for nucleus (*arrows*). Immunohistochemistry (peroxidase/diaminobenzidine) for the nucleoprotein of influenza A virus counterstained with methylgreen

spreads within lumen containing extracellular proteases [9]. The distributions of the antigen-positive cells in mice inoculated with H5N1 (HK483) and H1N1 (PR8) viruses correlate well with this concept.

Virus titers in the brain and lungs differed markedly in mice inoculated with either a 2-μl ($2\times10^5$ PFU; present study) or 20-μl ($10^6$ PFU, [15]) volume. In mice inoculated with 20 μl, a high level replication of H5N1 virus was recognized only in the lungs on day 1 p.i. Viral replication in the brain started at a low level on day 2 p.i. and increased on day 3 p.i. In contrast, virological analysis of the mice inoculated with a 2-μl volume disclosed an almost simultaneous replication of H5N1 virus in CNS and in the lungs, although virus antigen-positive cells were detected 1 day earlier in the brain. This discrepancy in histological detection of virus-infected cells and virus titration can be explained by the sensitivity of these methods. From these lines of evidence, we con-

**Table 2** Viral involvement in the murine central nervous system. Two mice were inoculated with 20-μl inoculum of H5N1 virus ($2\times10^5$ PFU/mice) and examined on days 4 and 7 p.i., respectively

| Area | Day p.i. | | Area | Day p.i. | |
|---|---|---|---|---|---|
| | 4 | 7 | | 4 | 7 |
| Olfactory bulb | 2+ | +/− | Pretectum | 2+ | 2+ |
| Olfactory ventricle | 2+ | − | Superior colliculus | 2+ | + |
| Lateral olfactory tract | 2+ | +/− | Inferior colliculus | − | 2+ |
| Anterior olfactory nuclei | + | +/− | Nuclei of lateral lemniscus | + | 2+ |
| Olfactory tubercle | + | +/− | Oculomotor nucleus | + | + |
| Nucleus of diagonal band | 2+ | +/− | Trochlear nucleus | + | + |
| Septum | + | +/− | Pontine nuclei | 2+ | + |
| Preoptic area | + | 2+ | Pontine tegmental reticular nucleus | 2+ | 2+ |
| Hypothalamic nuclei | + | 2+ | Pontine reticular formation | 2+ | 2+ |
| Amygdaloid nuclei | 2+ | +/− | Perifacial region | 3+ | 3+ |
| Piriform cortex | + | +/− | Perilolivary region | 2+ | 3+ |
| Entorhinal cortex | 2+ | +/− | Other region | + | 2+ |
| Hippocampus | 2+ | +/− | Pedunculopontine nucleus | + | 2+ |
| Lateral ventricle | 2+ | +/− | Superior olivary complex | + | 3+ |
| Corpus callosum | 2+ | − | Nuclei of trigeminal nerve | | |
| Cerebral neocortex | 2+ | + | Motor | + | 2+ |
| Thalamic nuclei | | | Principal sensory | 2+ | 3+ |
| Mediodorsal nucleus | + | − | Spinal trigeminal | 3+ | 3+ |
| Ventral posteromedial nucleus | + | + | Facial nucleus | +/− | 2+ |
| Lateral geniculate body | + | − | Ventral cochlear nuclei | + | + |
| Reticular nucleus | 2+ | +/− | Vestibular nuclei | 2+ | 3+ |
| Caudate putamen | 2+ | +/− | Nucleus of solitary tract | 3+ | 3+ |
| Globus pallidus | + | 2+ | Dorsal motor nucleus of vagus nerve | + | 2+ |
| Zona incerta | + | + | Perihypoglossal nucleus | + | 2+ |
| Subthalamic nucleus | 2+ | + | Hypoglossal nucleus | +/− | 2+ |
| Entopeduncular nucleus | 2+ | + | Cuneate/External cuneate nucleus | 2+ | + |
| Substantia nigra | + | 2+ | Gracile nucleus | − | + |
| Ventral tegmental area | 2+ | 2+ | Inferior olive | + | + |
| Red nucleus, magnocellular | − | + | Medullary reticular formation | 2+ | 3+ |
| Periruber reticular formation | + | 2+ | Cervical spinal cord | + | 3+ |
| Cerebellar cortex | 2+ | + | | | |
| Cerebellar nuclei | + | 2+ | | | |

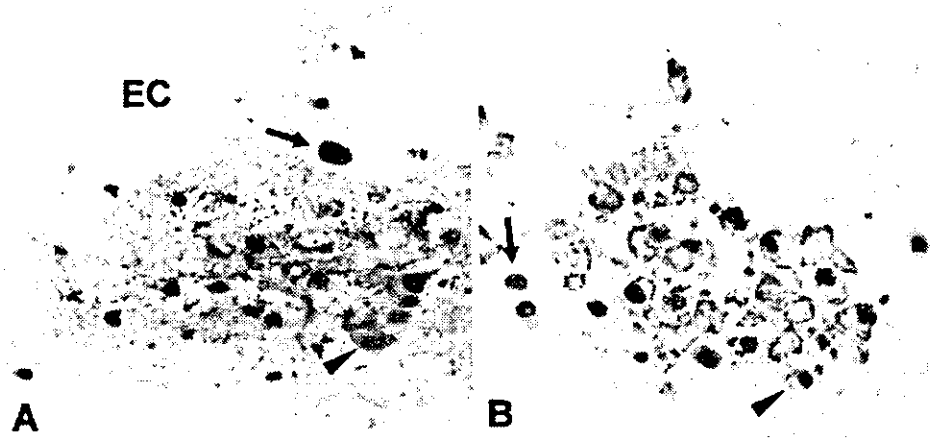


**Fig. 6** Histology of the pons of an infected mouse inoculated with HK483 virus on day 3 p.i (**A**) and day 5 p.i. (**B**). **A** A neuron (*arrowhead*) becomes necrotic in the vestibular nucleus. Granulocytic infiltration (*arrow*) is observed. **B** Inflammatory cell infiltration is observed within subarachnoid space (*SA*) and white matter with spongiosis

sidered that the virus infection starts independently in the CNS and lungs in mice inoculated with a 2-μl volume.

The histological characteristics of H5N1 virus-infected CNS are neuronal degeneration and necrosis with granulocytic infiltration. These changes are consistent with viral encephalitis, although typical characteristics of viral encephalitis, such as vascular cuffing and glial nodules, were not conspicuous. The early neuronal changes are rather difficult to identify and the immunohistochemical detection of the nucleocapsid proteins is helpful in making a correct diagnosis.

The distribution of infected cells in the CNS supports the new concept of H5N1 virus penetration into the CNS through direct neural spread from the cranial nerve endings innervating the respiratory tract, as proposed by Park et al. [16]. A hematogenous route might play some roles in viral dissemination in the inoculated mice since virus was isolated from the blood. However, the early appearance of infected cells in the CNS, other than in the respiratory tract, suggests that the neural spread from the nasal cavity was more predominant than the hematogenous spread. Neural spread is observed in infection of herpes simplex virus [22], rabies virus [14], cercopithecine virus-1 (B virus) [2] and Venezuelan equine encephalitis virus [21].

The neuroarchitectural relationship of the infected cells observed in mice inoculated with a 20-μl volume at 4 and 7 days p.i, suggests the possibility of viral spread through the neural tissue and cerebrospinal fluid (CSF) in the CNS (Table 2). The afferent nerve endings may initially play an important role in viral spread because of

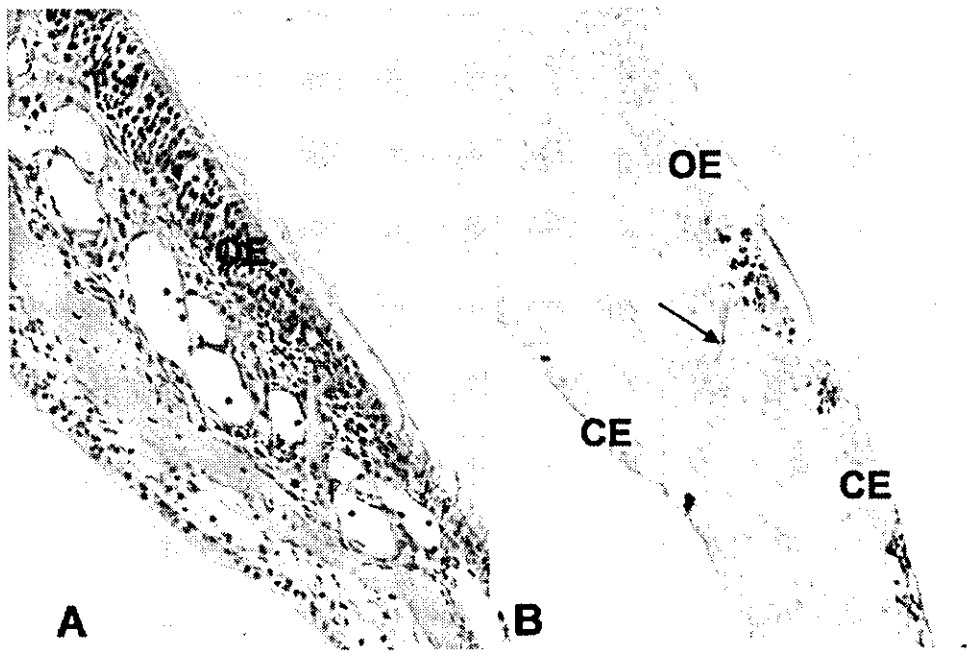


**Fig. 7** Histology (**A**, hematoxylin-eosin) and immunohistochemistry for the viral antigen (**B**) of the nasal cavity of the mouse inoculated with 2 μl of HK483 virus suspension on day 3 p.i. Positive cells are observed in and beneath (*arrow*) the olfactory epithelium (*OE*) and in some columnar epithelium (*CE*). Immunohistochemistry (peroxidase/diaminobenzidine) for the nucleoprotein of influenza A virus counterstained with methylgreen

the early appearance of virus-infected cells in the sensory nuclei, which suggests a retrograde transport of H5N1 virus. Thereafter, virus is transmitted through trans-neuronal pathways. The nucleoprotein of influenza virus appears initially in the nucleus and subsequently in both the nucleus and cytoplasm [9]. The frequency of nucleus-positive and cytoplasm-negative glial cells, in relation to nucleus-positive and cytoplasm-positive neurons, suggests that these glial cells get the virus from infected neurons.

Ependymal cell infection of H5N1 virus, recognized in this study, can cause virus dissemination through the CSF. The clinical significance of ependymal cell involvement remains to be clarified, and further studies, especially by examination of cytological changes of and viral detection in CSF obtained from patients infected with this virus, are needed to resolve this point.

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# Avian Influenza A (H5N1) in 10 Patients in Vietnam

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## ABSTRACT

### BACKGROUND

Recent outbreaks of avian influenza A (H5N1) in poultry throughout Asia have had major economic and health repercussions. Human infections with this virus were identified in Vietnam in January 2004.

### METHODS

We report the clinical features and preliminary epidemiologic findings among 10 patients with confirmed cases of avian influenza A (H5N1) who presented to hospitals in Ho Chi Minh City and Hanoi, Vietnam, in December 2003 and January 2004.

### RESULTS

In all 10 cases, the diagnosis of influenza A (H5N1) was confirmed by means of viral culture or reverse transcriptase–polymerase chain reaction with primers specific for H5 and N1. None of the 10 patients (mean age, 13.7 years) had preexisting medical conditions. Nine of them had a clear history of direct contact with poultry (median time before onset of illness, three days). All patients presented with fever (temperature, 38.5 to 40.0°C), respiratory symptoms, and clinically significant lymphopenia (median lymphocyte count, 700 per cubic millimeter). The median platelet count was 75,500 per cubic millimeter. Seven patients had diarrhea. In all patients, there were marked abnormalities on chest radiography. There was no definitive evidence of human-to-human transmission. Eight patients died, one patient has recovered, and one is recovering.

### CONCLUSIONS

Influenza A (H5N1) infection, characterized by fever, respiratory symptoms, and lymphopenia, carries a high risk of death. Although in all 10 cases the infection appears to have been acquired directly from infected poultry, the potential exists for genetic reassortment with human influenzaviruses and the evolution of human-to-human transmission. Containment of influenza A (H5N1) in poultry throughout Asia is therefore urgently required.

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INFLUENZA A VIRUS INFECTS A VARIETY OF animals, including humans and birds.[1] Although the natural reservoir for all known subtypes of influenza A (hemagglutinins H1 through H15 and neuraminidases N1 through N9) is wild waterfowl, only three subtypes are currently circulating among humans (H1N1, H1N2, and H3N2). However, during the past few years, several subtypes of avian influenza A have been shown to cross the species barrier and infect humans. During an outbreak of a highly pathogenic influenza A (H5N1) virus among poultry in Hong Kong in 1997, 6 of 18 people with confirmed infection died.[2] After this outbreak, prevention policies and early detection strategies were put into place, and no new cases of H5N1 were detected in Hong Kong until February 2003, when two cases were reported, one of which resulted in death.[1] Possibly as a result of heightened surveillance, avian influenza A (H9N2) viruses were also isolated from children in Hong Kong in 1999, but this infection resulted in only mild, self-limiting illnesses.[3,4]

A total of 89 human infections with influenza A (H7N7), including 1 resulting in the death of a Dutch veterinarian, occurred during the extensive outbreak in 2003 that decimated the Dutch poultry industry.[5,6] During late 2003 and early 2004, there were reports of large outbreaks of H5N1 among poultry throughout Asia (including South Korea, Japan, Indonesia, Vietnam, Thailand, Laos, Cambodia, and China). In January 2004, there was confirmation that influenza A (H5N1) virus had been isolated from patients who had died of a respiratory illness in Hanoi and Ho Chi Minh City, Vietnam. We report the epidemiologic, clinical, and radiographic features in 10 patients with confirmed influenza A (H5N1) infection.

## METHODS

### PATIENTS

We identified 10 patients in Vietnam with avian influenza A (H5N1) virus infection as confirmed by culture or reverse-transcriptase polymerase chain reaction (RT-PCR). Four of the patients (Patients 1, 2, 3, and 4) were admitted to the National Hospital for Pediatrics in Hanoi between December 27, 2003, and January 14, 2004. The other six patients (Patients 5, 6, 7, 8, 9, and 10) were admitted to the Hospital for Tropical Diseases in Ho Chi Minh City between January 20 and January 30, 2004. Epidemiologic data were collected through interviews of the patients and their relatives. Data on vital signs, physical findings, and routine laboratory tests were obtained by means of a retrospective review of the hospital records (for Patients 1, 2, 3, and 4) and from prospectively recorded case notes (for Patients 5, 6, 7, 8, 9, and 10).

### RADIOLOGIC ASSESSMENT

Chest radiographs were obtained in all patients during hospitalization and were reviewed by experienced clinicians. The radiologic findings were categorized with attention to unilateral or bilateral changes; focal, lobar, or patchy consolidation; airspace infiltrates; air bronchograms; pleural effusions; and volume loss with or without shift.

### MICROBIOLOGIC EVALUATION

Blood cultures were obtained from all patients on admission to the hospital. Throat and nasal swabs and, when appropriate, tracheal aspirates were collected in viral transport medium (Minimum Essential Medium Eagle with Hanks' salts, supplemented with 0.5 percent gelatin and antibiotics [Sigma-Aldrich]) or phosphate-buffered saline and stored at −80°C. In the four patients in Hanoi, swabs were obtained and stored in a collection and transport system for viruses and chlamydiae (Multi-Microbe Medium Collection and Transport System [M4RT], Remel). Virus was cultured in monolayers of Madin–Darby canine kidney cells. Isolated virus was identified by means of immunofluorescence and hemagglutination-inhibition assays, as previously described.[1] Rapid influenza tests (Capillia Flu A/B Test [Nippon Becton Dickinson] or QuickVue [Quidel]) were used according to the manufacturers' instructions to test nose and throat swabs from six patients.

### RNA EXTRACTION AND RT-PCR

RNA was extracted from 140 μl of nasal and throat swab samples in phosphate-buffered saline or viral transport medium with the use of a viral RNA kit (QIAamp, Qiagen) and a double elution with 2× 40 μl of buffer; 5 μl of the RNA extract was analyzed in the RT-PCR assay. Reverse-transcriptase reactions contained 2 μl of 5× first-strand buffer, 2.5 μM random hexamer primers (Roche Diagnostics), 40 units of RNase inhibitor (RNase OUT, Invitrogen), and 1 μl (200 units) of reverse transcriptase (Superscript II, Invitrogen). Reverse-transcriptase reactions were performed at room temperature for 10 minutes, then at 42°C for 30 minutes and at 70°C for 5 min-

utes; 2 μl of the cDNA was used for amplification in the PCR assays. The reactions (total volume of 25 μl) contained 2.5 μl of 10× PCR Gold buffer, 2.5 mM magnesium chloride, 0.4 mM deoxynucleoside triphosphates (Roche Diagnostics), 0.8 mM of each of the two primers, and 0.5 unit of AmpliTaq Gold DNA polymerase (Applied Biosystems).

The samples from Patients 1, 2, 3, and 4 were tested with the primer set for the H5 gene (forward primer H5-1: GCCATTCCACAACATACACCC; reverse primer H5-2: TAAATTCTCTATCCTCCTTTCCAA) with an expected product size of 358 bp,[2] and the primer set for the N1 gene (forward primer N1-1: TTGCTTGGTCAGCAAGTGCA; reverse primer N1-2: TCTGTCCATCCATTAGGATCC) with an expected product size of 615 bp.[7] Thermal cycling for these reactions was performed under the following conditions: 94°C for 3 minutes; 40 cycles of 94°C for 30 seconds, 45°C (for H5) or 55°C (for N1) for 30 seconds, and 72°C for 1 minute; then 72°C for 7 minutes.

The samples from Patients 5, 6, 7, 8, 9, and 10 were tested with two different primer sets that are specific for the influenza A subtype H5 gene: primer pair H5-1 and H5-2 (as described above) and primer pair H5b (forward primer H5/515: CATACCCAACAATAAAGAGG; and reverse primer H5/1220: GTGTTCATTTTGTTAATGAT, with an expected product size of 708 bp). For samples from these six patients, the influenza A N1 gene was amplified with the primers described above, except that there was a modification in the N1-1 primer: TTGCTTGGTCAGCAAGTGCT. All patients were tested for the H1 subtype of influenza A (with the forward primer H1: AGCAAAAGCAGGGGAAAATAA and the reverse primer H1: GCTATTTCTGGGGTGAATCT; expected size of the PCR product, 729 bp) and the H3 subtype of influenza A (with the forward primer H3: AGCAAAAGCAGGGGATAATTC and the reverse primer H3: TGCCTGAAACCGTACCAACC; expected product size, 1143 bp).

Thermal cycling for all amplifications, except for that of the influenza A N1 gene fragment, was 95°C for 10 minutes (preamplification hot start); 10 cycles of 95°C for 30 seconds, 55°C for 30 seconds (decreased by 1°C per cycle), and 72°C for 1 minute; and 40 cycles of 95°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1 minute. For the N1 gene fragment, thermal cycling conditions were 95°C for 10 minutes; 10 cycles of 95°C for 30 seconds, 60°C for 30 seconds (decreased by 1°C per cycle), and 72°C for 1 minute; and 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute.

Products were analyzed on a 2 percent agarose gel. Precautions for the avoidance of cross-contamination were strictly observed. All samples were obtained and transported in individual sealed bags. The preparation of RT-PCR mixtures, nucleic acid extractions, and amplification and analysis of PCR products were performed in three separate laboratories. Aerosol-resistant filter tips (A.R.T., Molecular BioProducts) were used throughout all laboratory procedures. Negative controls were included during the RNA extraction, reverse transcription, and PCR amplification.

## RESULTS

### PATIENTS AND CONTACT HISTORY

The mean age of patients (four of whom were female and six male) was 13.7 years (range, 5 to 24); none had any known, clinically significant preexisting medical conditions. Patients 8, 9, and 10 were smokers. The patients were from both rural and urban parts of Vietnam (Fig. 1). Seven of the patients were children attending school. Patients 8, 9, and 10 came from the same district in Lam Dong Province, were from the K'hor ethnic group, and worked as subsistence farmers. There was no contact among the patients before hospitalization.

For eight of the nine patients in whom a history could be obtained, there was clear evidence of either direct handling of poultry (chickens or ducks) or exposure to sick poultry in the week before the onset of illness (Table 1). The median time between exposure and the onset of illness was 3 days (range, 2 to 4) and the median time between the onset of illness and hospitalization was 5.9 days (range, 3 to 8). None of the patients had been involved in the organized culling of poultry.

There were similar illnesses reported in relatives of Patients 1 and 2. Patient 1 became sick on December 25, 2003, was admitted to the hospital on December 27, and died on December 30. Her mother became sick on January 1, 2004, was admitted to the hospital on January 5, and died on January 9. Influenza A (H5N1) infection was confirmed in the mother (who is not included in this report). There was no illness reported in the father or sibling of Patient 1. The seven-year-old sister of Patient 2 reportedly died of a respiratory illness on the day Patient 2 was admitted to the hospital, but no clinical

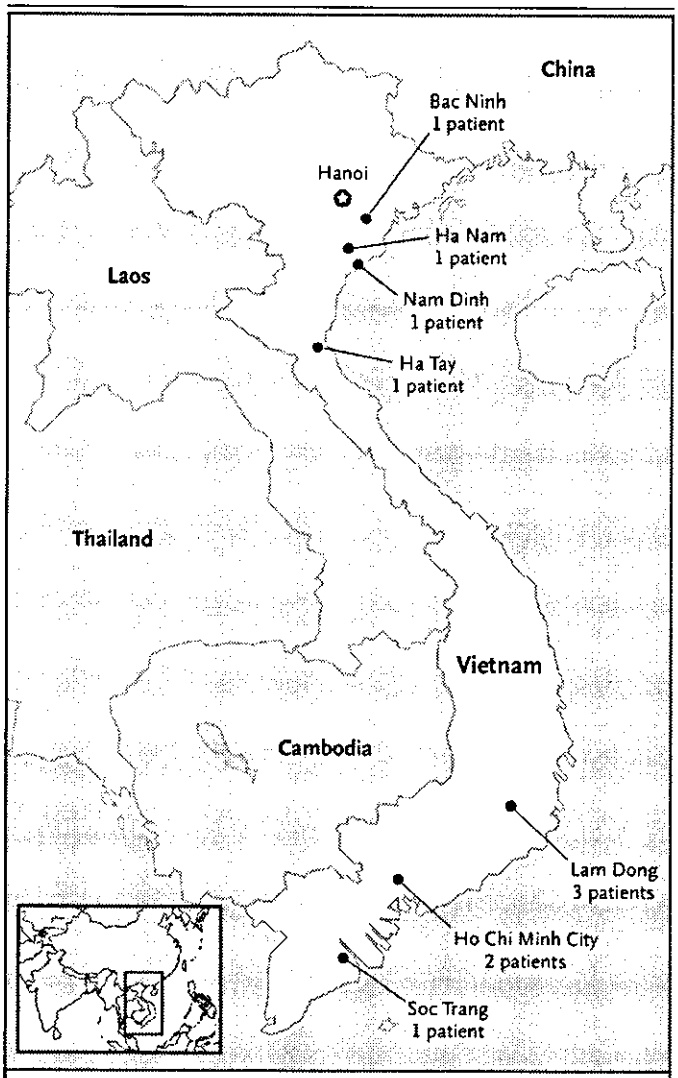


**Figure 1. Map of Vietnam Showing the Reported Cases of Avian Influenza A (H5N1).**

details are available. There was no illness reported in the parents or two other siblings of Patient 2. No illness during the preceding two weeks or during hospitalization was reported in any family member or contacts of the other patients.

Infection-control measures were initiated in both hospitals as soon as it was suspected that this illness was caused by influenza A (H5N1). No negative-pressure isolation facilities were available. Patients 1 and 2 were admitted before influenza A (H5N1) was suspected, and therefore their cases were managed with universal precautions but without additional infection-control measures.

#### CLINICAL AND OTHER FEATURES

The clinical features of the patients with confirmed infection with influenza A (H5N1) virus are summarized in Table 2. All patients presented with fever, shortness of breath, and a cough; in five patients, there was a history of sputum production, and in three of these patients, the sputum was blood-stained. Two patients reported pleuritic pain. Diarrhea was reported in seven of the patients. Bleeding from the nose and gums was noted in one patient on the fourth day of illness. No patient had a sore throat, conjunctivitis, rash, or a runny nose. Physical examination in nine patients revealed fever, rapid respiratory rate (median, 55 breaths per minute; range, 28 to 70), respiratory distress, and crackles on examination of the chest.

#### LABORATORY ASSESSMENT

The results of the basic laboratory tests performed on admission are shown in Table 3. The median total leukocyte count on admission was 2100 per cubic millimeter (range, 1200 to 3400). The median total lymphocyte count was 700 per cubic millimeter (range, 250 to 1100). The median ratio of CD4-positive cells to CD8-positive cells (measured in Patients 5, 7, 8, 9, and 10) was 0.70 (range, 0.59 to 1.08). The median platelet count was 75,500 per cubic millimeter (range, 45,000 to 174,000). Measurements of alanine aminotransferase and aspartate aminotransferase levels on admission were available in six patients and were elevated in five of them (Patients 1, 4, 5, 6, and 10); one patient (Patient 10) had an elevated serum creatinine concentration on admission. Three patients (Patients 7, 8, and 9) had marked hyperglycemia that developed during hospitalization.

#### MICROBIOLOGIC ASSESSMENT

Blood cultures were all negative. *Staphylococcus aureus* was isolated from a tracheal aspirate from Patient 1, and *Haemophilus influenzae* from a tracheal aspirate from Patient 2. The diagnosis of influenza A (H5N1) was confirmed through the isolation of the virus from postmortem lung tissue from Patient 1 and throat swabs from Patient 2. In all other patients, the diagnosis was made by means of RT-PCR with the use of primers specific to H5 and N1 in samples

Table 1. **Epidemiologic Data.**

| Patient No. | Location in Vietnam | Occupation | Epidemiologic Information |
|---|---|---|---|
| 1 | Ha Nam | Student | Family members are farmers who do not keep poultry, but many chickens in neighborhood unexpectedly died in the preceding 2 wk; mother died of influenza A (H5N1) Jan. 9, 2004; father and younger sibling healthy. |
| 2 | Nam Dinh | Not available | No information available on exposure to sick poultry; 7-yr-old sister died of acute respiratory illness on Dec. 29, 2003; parents and two other siblings healthy. |
| 3 | Bac Ninh | Student | Family members are farmers who kept chickens, which died unexpectedly 5 days before onset of illness; parents and older sibling healthy. |
| 4 | Ha Tay | Student | Family members are farmers who kept chickens, which died 2 wk before onset of illness; chickens died in patient's house and neighbors' houses during week before onset of illness; parents and 7 other siblings healthy. |
| 5 | Ho Chi Minh City | Student | Patient bought duckling as pet and cared for it in her house for 5 days; duck had diarrhea and died, patient buried it, dug it up a day later and reburied it; both patient and brother handled duck; patient also ate barely cooked eggs (Vietnamese delicacy) 2 days before onset of illness; neighbors kept 40 chickens, but no illness reported in these birds; fever developed in patient 3 days after she bought duck; no other poultry or animals at home; no other household members or relatives sick. |
| 6 | Ho Chi Minh City | Student | Frequently attended cockfights, held roosters and chickens; no illness reported in the chickens or in 20 people involved in cockfighting; patient walked through live-poultry market 50 m from house on his way to school. |
| 7 | Soc Trang | Student | Extensive exposure, including handling of 10 dead or dying chickens in patient's homestead; father and patient prepared dead chickens for eating (removed feathers, washed, cut meat) 3 days before onset of illness; no other household members or relatives sick; no other poultry or animals at home. |
| 8 | Lam Dong | Farmer | Direct handling of 50 chickens, including dead chickens, at home (which was also a restaurant); patient and father prepared chickens for eating; no other household members or relatives sick; no other poultry or animals at home. |
| 9 | Lam Dong | Farmer | Direct handling of chickens in patient's homestead 3 days before onset of illness; he prepared dead chickens for eating; no one else in family sick. |
| 10 | Lam Dong | Farmer | Direct handling of sick ducks and chickens in patient's home; many sick poultry in the district; no other illness in family. |

obtained from nasal and throat swabs. Among the patients hospitalized in Ho Chi Minh City, the results on RT-PCR with the use of the H5b (H5/515F and H5/1220R) primer pair were positive in all six patients tested (Fig. 2), whereas the results of RT-PCR with the H5-1 and H5-2 primer pair were positive in four of the six patients tested. None of the patients' samples were positive with the use of primers specific for influenza H1 or influenza H3. The median duration of illness at the time when RT-PCR confirmed the presence of avian influenza A (H5N1) was 6 days (range, 5 to 12).

Influenza A antigens were detected in two of the six patients who were tested. In one patient (Patient 2), virus was isolated from a sample obtained on day 7 of illness but could not be isolated from a sample obtained on day 15.

### RADIOLOGIC ASSESSMENT

All chest radiographs were abnormal on admission to the hospital (Fig. 3). The major abnormalities included extensive infiltration bilaterally, lobar collapse, focal consolidation, and air bronchograms. No pleural effusions were noted. All patients had dramatic worsening of findings on chest radiography during hospitalization. Pneumothorax developed in Patients 2 and 4 while they were receiving mechanical ventilation.

### TREATMENT AND OUTCOME

All patients were treated empirically with broad-spectrum antibiotics on admission. Patients 1, 2, 3, and 4 received 5 mg of methylprednisolone per kilogram of body weight per day, and Patients 5, 7, and 8 received 1 to 2 mg of methylprednisolone per kilo-

Table 2. Clinical Characteristics of the Patients on Admission.

| Variable | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Days between exposure to poultry and onset of illness | — | — | — | — | 3 | 2 | 3 | 4 | 3 | 3 |
| Days since onset of illness | 3 | 7 | 7 | 5 | 8 | 6 | 5 | 6 | 5 | 7 |
| Sex | Female | Male | Male | Female | Female | Male | Female | Male | Male | Male |
| Age (yr) | 12 | 5 | 10 | 8 | 8 | 13 | 16 | 18 | 24 | 23 |
| Cough | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Dyspnea | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Sputum | No | No | No | No | Yes | No | Yes | Yes | Yes | Yes |
| Diarrhea | Yes | No | No | No | Yes | Yes | Yes | Yes | Yes | Yes |
| Rash | No | No | No | No | No | No | No | No | No | No |
| Myalgia | No | No | No | No | No | No | No | No | No | No |
| Conjunctivitis | No | No | No | No | No | No | No | No | No | No |
| Fever | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Temperature (°C) | 39.5 | 38.8 | 39.0 | 38.5 | 38.5 | 39.6 | 40.0 | 40.0 | 39.5 | 38.7 |
| Blood pressure (mm Hg) | 90/60 | 112/54 | 105/80 | 80/40 | 104/64 | 110/70 | 110/60 | 100/60 | 110/60 | 120/80 |
| Respiratory rate (breaths/min) | 65 | 70 | 64 | 60 | 40 | 40 | 40 | 60 | 50 | 28 |
| Crackles | Yes | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes |
| Wheeze | No | No | No | No | No | Yes | No | No | No | No |
| Other | Enlarged liver | — | — | Bleeding gums | — | — | — | — | — | — |

gram four times a day for one, three, and four days, respectively. Five patients were treated with the neuraminidase inhibitor oseltamivir (35 mg twice daily in Patient 5 and 75 mg twice daily in Patients 7, 8, 9, and 10) for up to five days. Ribavirin was given to Patient 3 (800 mg three times a day) and Patient 4 (400 mg three times a day). The antiviral treatment was started on day 5 of illness in Patients 4, 7, 9, and 10; day 6 in Patients 6 and 8; day 11 in Patient 3; and day 12 in Patient 5. Patients 5, 6, 7, 8, 9, and 10 received ranitidine.

Patient 5 required continuous positive airway pressure with supplemental oxygen for the first seven days after admission but was subsequently weaned from this support and given maintenance therapy with 40 percent oxygen. Patients 1, 2, 3, 4, 6, 7, 8, and 9 required mechanical ventilation during the first 48 hours after admission. In all these patients, there was a dramatic deterioration of gas exchange despite pressure-controlled ventilation, high end-expiratory pressures, and a fraction of inspired oxygen of 1.0. Patients 7 and 8 were also treated with dopamine and norepinephrine for hypotension. Patient 7 had a small gastrointestinal hemorrhage on the third day of hospitalization. Patients 7 and 8 had marked hyperglycemia requiring insulin treatment to normalize the blood glucose levels.

Despite a prolonged, severe illness, Patient 5 survived with no major sequelae. Eight other patients died, and one patient is recovering, for a case fatality rate of 80 percent among patients in our series. The median time to death from the onset of illness was 9 days (range, 6 to 17). Neither during the period when these patients were hospitalized nor subsequently was any illness reported in a health care worker or laboratory staff member.

## DISCUSSION

To date, there have been 20 confirmed cases of human infection with influenza A (H5N1) in Vietnam and Thailand; 16 of the infected patients have died. We describe the clinical features of 10 cases of confirmed avian influenza A (H5N1) in patients admitted to referral hospitals in Vietnam.

Table 3. Laboratory Values at Presentation.*

| Variable | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Hemoglobin (g/dl) | 13.4 | 12.6 | 12.4 | 12.3 | 11.3 | 13.4 | 11.9 | 14.5 | 15.8 | 17.6 |
| Leukocyte count (per mm³) | 2,100 | 3,400 | 2,800 | 1,900 | 1,200 | 2,700 | 3,000 | 1,700 | 1,900 | 2,100 |
| Lymphocyte count (per mm³) | 1,100 | 710 | 860 | 250 | 300 | 900 | 500 | 500 | 800 | 700 |
| Neutrophil count (per mm³) | 850 | 2,410 | 1,900 | 780 | 700 | 1,300 | 2,500 | 1,100 | 1,100 | 1,300 |
| Platelet count (per mm³) | 45,000 | 174,000 | 135,000 | 91,000 | 117,000 | 81,000 | 70,000 | 69,000 | 62,000 | 62,000 |
| CD4:CD8 ratio | NA | NA | NA | NA | 0.71 | NA | 0.62 | 0.75 | 0.59 | 1.08 |
| ALT level (U/liter) | 53.7 | NA | NA | 265 | 354 | 254 | 47 | NA | NA | 89 |
| AST level (U/liter) | 278 | NA | NA | 1,217 | 320 | 1,058 | 20 | NA | NA | 110 |
| Serum creatinine (μmol/liter) | 50 | 64 | NA | 27 | 34 | 14 | 71 | 89 | 43 | 121 |
| Serum glucose (mmol/liter) | NA | NA | NA | NA | NA | NA | 19.0 | 13.5 | 11.7 | 4.9 |
| Oxygen saturation during receipt of 40% oxygen (%) | 50 | 70 | 86 | 50 | 95 | 85 | 67 | 81 | 80 | 90 |
| Day of illness on which PCR for H5N1 performed | 5 | 7 | 9 | 6 | 12 | 6 | 5 | 6 | 5 | 7 |
| Viral culture | + | + | NA | NA | Pending | Pending | Pending | Pending | Pending | Pending |
| Influenza antigens | NA | NA | NA | NA | + | – | – | + | – | – |
| Blood culture | – | – | – | – | – | – | | – | – | – |
| Outcome | Died (day 6) | Died (day 17) | Died (day 14) | Died (day 7) | Recovered | Died (day 9) | Died (day 14) | Died (day 9) | Died (day 6) | Recovering |

* Normal ranges are as follows: hemoglobin concentration, 13 to 18 g per deciliter; leukocyte count, 4000 to 11,000 per cubic millimeter; neutrophil count, 2200 to 8250 per cubic millimeter; lymphocyte count, 1500 to 4000 per cubic millimeter; CD4:CD8 ratio, 1.4 to 2.0; platelet count, 150,000 to 400,000 per cubic millimeter; alanine aminotransferase (ALT) level, below 37 U per liter; aspartate aminotransferase (AST) level, below 40 U per liter; serum creatinine concentration, 82 to 106 μmol per liter; and serum glucose concentration, 3.9 to 6.4 mmol per liter. NA denotes not available, a plus sign positive, and a minus sign negative. To convert the values for creatinine to milligrams per deciliter, divide by 88.4. To convert the values for glucose to milligrams per deciliter, divide by 0.05551.

The prominent clinical features on admission were those of a severe influenza syndrome with fever, cough, diarrhea, and shortness of breath. The estimated time between the exposure to poultry and the onset of illness suggests an incubation period of two to four days. Diarrhea was present in 7 of the 10 cases. The most striking laboratory findings were marked lymphopenia and thrombocytopenia with a pronounced inversion of the CD4:CD8 ratio in the five patients in whom it could be measured. A recovery of the lymphocyte count and CD4:CD8 ratio was observed only in the two patients who survived. Liver and renal dysfunction or impaired glycemic control was prominent in six of the patients. The patients were all children or young adults.

These clinical presentations were similar to those in the 1997 outbreak of influenza A (H5N1) in Hong Kong, although diarrhea was a more prominent feature in the Vietnamese patients. In addition, the oldest patient in this series was 24 years old, whereas one patient was 54 years old and one was 60 years old in the 1997 outbreak in Hong Kong.[2] However, the mortality in our series was significantly higher than that in the 1997 outbreak.

Eight of the nine patients from whom a clear history could be taken reported close contact with poultry during the week before the onset of illness. A retrospective study after the Hong Kong outbreak showed that visiting poultry markets before the onset of illness was the only significant risk factor.[8] The contact in six of the current cases involved direct handling of chickens or ducks (holding, killing,

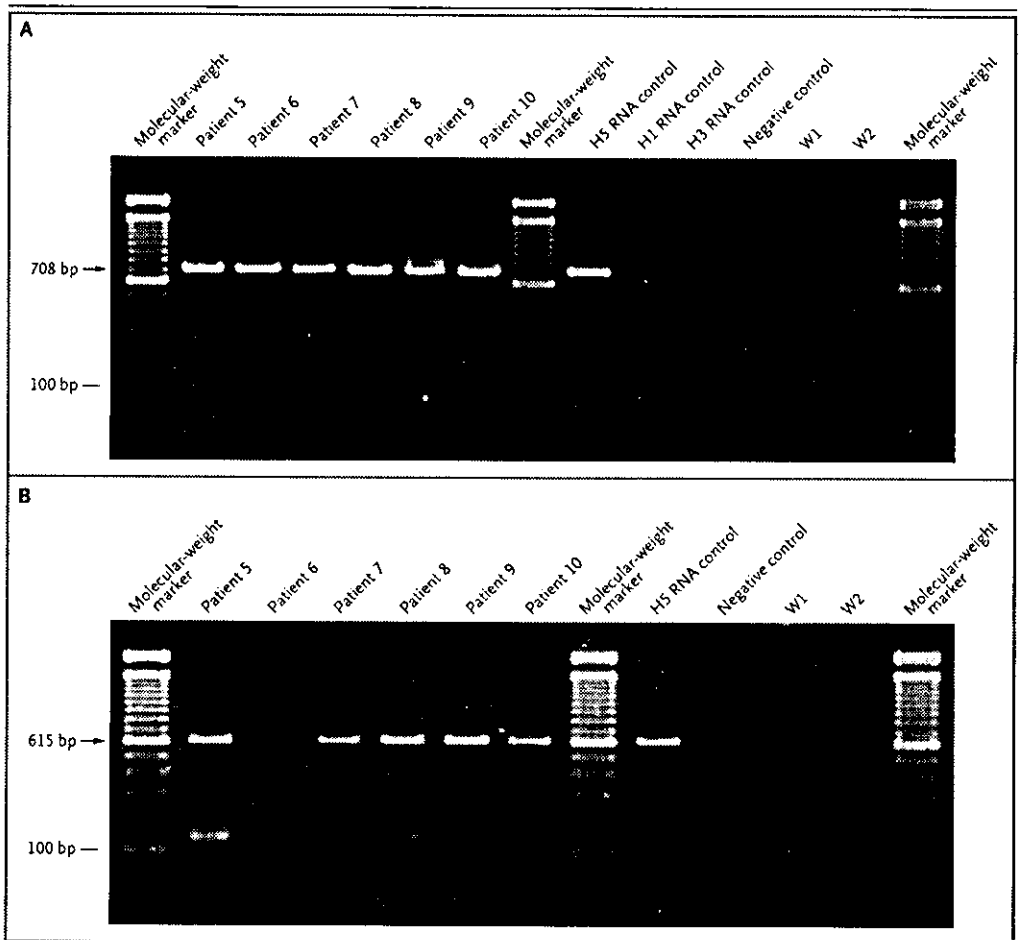


**Figure 2. RT-PCR Specific for the H5 Gene of Influenza A (H5N1) Virus, Performed with the Use of the H5b Primers (Panel A), and RT-PCR Specific for the N1 Gene, Performed with the Use of the Modified N1 Primers (Panel B).**

Nasal or throat swabs from Patients 5, 6, 7, 8, 9, and 10 were used, as well as an H5 RNA control (A/HK/213/2003 [H5N1]), an H1 RNA control (A/New Caledonia/20/99 [IVR-116; H1N1]), and an H3 RNA control (A/Panama/2007/99 [N1B-41; H3N2]), all cultured in embryonated chicken eggs, and a negative control (RNA-extraction control), W1 (water control for reverse transcriptase), and W2 (water control for PCR).

or defeathering them or preparing them to be eaten) within the patient's home environment or small homesteads nearby, where a relatively small number of chickens were kept. This finding suggests that direct contact is the primary route of bird-to-human transmission. None of the patients were involved in the organized culling of poultry or worked on large poultry farms. This observation, if confirmed, may have important implications for our understanding of the transmission of this virus and potential immunity to it.

The available information on the two family clusters is compatible with bird-to-human transmission from a common source, but there is currently not enough information to rule out limited human-to-human transmission within the family. There was no illness reported in family members of the other eight patients, even though other family members seemed to have had very similar exposure to poultry — for example, the brother of Patient 5 and the fathers of Patients 7 and 8. There is evidence from the 1997 outbreak in Hong Kong that the avian influenza A (H5N1) virus may have been transmitted from human to human but that transmission could

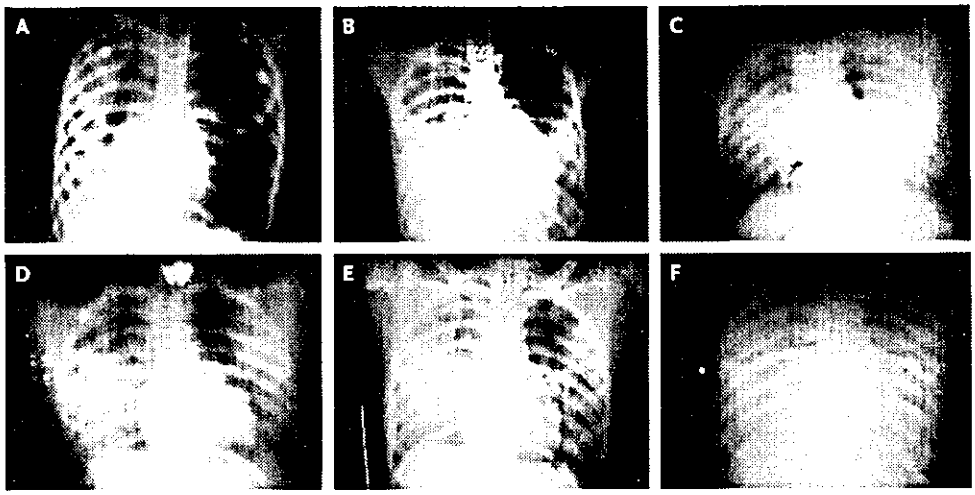


**Figure 3. Chest Radiographs.**

Radiographs from Patient 5 (Panel A), Patient 7 (Panel B), and Patient 9 (Panel C) show widespread consolidation, collapse, and interstitial shadowing. In Panels D, E, and F, three chest radiographs show the progression in Patient 8 on days 5, 7, and 10 of illness, respectively.

not be sustained among humans.[9,10] The absence of any report to date of a similar illness among the health care workers who cared for these patients, despite the lack of full droplet and respiratory infection-control measures early in the outbreak, is reassuring. We cannot rule out the possibility of mild or subclinical infection in persons exposed to either ill poultry or ill persons. Detailed seroepidemiologic studies of the individual family members, health care workers, and others at risk would be necessary in order to assess whether and to what extent human-to-human transmission has occurred.

Oseltamivir was administered to five of the patients, four of whom died. Treatment with the drug may have been started too late to be effective, although one of the two surviving patients did not start oseltamivir therapy until the 12th day of illness. At that point, she was still antigen-positive and PCR-positive for the virus. There seemed to be no benefit from the oral administration of ribavirin (in Patients 3 and 4). In vitro sensitivity testing of a limited number of strains of influenza A (H5N1) virus isolated from patients in Vietnam have shown that they are resistant to amantadine and rimantadine, so these drugs should not be recommended for treatment. Six of the seven patients who were treated with corticosteroids died. This experience is inadequate to permit the establishment of treatment recommendations with respect to corticosteroids; more aggressive treatments may have been used in patients with a greater severity of illness. Our experience suggests that supportive care may be the only option available. Controlled clinical studies are needed to assess the role of antiviral drugs and corticosteroids in the treatment of influenza A (H5N1) virus infections.

Rapid testing for influenza antigens in a small number of patients on admission was less sensitive than RT-PCR for the diagnosis of influenza A (H5N1). Our experience in this small number of cases suggests that the low sensitivity of the rapid diagnostic tests for influenza may limit their usefulness for the reliable detection of influenza A (H5N1) in humans, especially if patients present relatively late in the course of illness and if other strains of influenza A are circulating simultaneously. The H5b primer pair yielded positive RT-PCR results in all six patients tested in this small series, as compared with positive results in four of six with the use of the H5-1 and H5-2 primers on the same samples. The N1 primers used resulted in nonspecific RT-PCR products and required modification to yield specific results. Further evaluation of the two H5 primer systems is being undertaken. The sensitivity of the RT-PCR methods, which were designed for the identification of influenza A (H5N1) virus from cul-

ture, is unknown, and we urgently need new, properly evaluated, sensitive diagnostic tests.

The clinical findings (fever, cough, diarrhea, shortness of breath, rapid respiratory rate, lymphopenia, and abnormalities on chest radiography) and a history of close contact with poultry may be more helpful in identifying patients with influenza A (H5N1) infection than the results on rapid diagnostic tests for influenza. We do not know whether the clinical syndrome described on the basis of these 10 patients is representative of the true clinical spectrum of the disease, since each of these patients was admitted to a referral hospital. The extent of mildly symptomatic disease in the community remains unknown. Increasing the availability of serologic tests, molecular diagnostic procedures, and viral culture throughout Asia would help considerably.

As documented in Hong Kong in 1997 and 2003, and now in Vietnam and Thailand, the avian influenza A (H5N1) virus clearly has the ability to jump between species and cause devastating illness in humans. Widespread efforts to control the poultry outbreak and increased surveillance among poultry and humans should therefore be our highest priority. It is reassuring that to date there has been no evidence of efficient human-to-human transmission of influenza A (H5N1) virus in either the 1997 or the 2004 outbreak. However, the continued circulation of virulent avian influenza A (H5N1) virus increases the possibility of the reassortment of this virus with other circulating human influenza A viruses and increases the threat of a global influenza pandemic.

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## APPENDIX

The members of the World Health Organization International Avian Influenza Investigative Team are as follows: N. Bhat (Centers for Disease Control and Prevention), P. Brudon (World Health Organization), P. Calain (World Health Organization), A. Curns (Centers for Disease Control and Prevention), R. Doran (World Health Organization), K. Fukuda (Centers for Disease Control and Prevention), T. Grein (World Health Organization), P. Horby (World Health Organization), S. Itamura (National Institute of Infectious Diseases, Tokyo, Japan), N. Miranda (World Health Organization), T. Uyeki (Centers for Disease Control and Prevention).

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微生物の基礎知識

# ヒトの鳥インフルエンザA（H5N1）感染症

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## はじめに

2004年1月，山口県の養鶏所のニワトリが大量死したことに端を発し，わが国でも高病原性鳥インフルエンザA（H5N1）（以下H5N1と略）が大きな社会問題となった．本症は主に鳥の疾患であるが，まれにヒトにも重篤な感染症を起こす．日本ではヒトの感染例は出ていないが，今後出現する可能性もあり，臨床医も無関心ではいられない状況になっている．

## 1．高病原性鳥インフルエンザとは[1)]

A型インフルエンザウイルスは，ヒト以外に鳥類，ウマ，ブタ，海獣類などにも感染する．このうち鳥類に感染症を起こすものは「鳥インフルエンザウイルス」と呼ばれ，羽毛の乱れや産卵低下などをきたす軽症型と，「高病原性鳥インフルエンザ」と呼ばれる重症型とがある．なお，「高病原性」とは鳥に対する病原性を表しており，ヒトへの病原性を示すものではない．高病原性株は，H5およびH7亜型に属すものが知られている．

H5N1はニワトリに対して高病原性であり，感染すると肉冠の出血・壊死，脚部皮下出血，下痢などを起こし，短時間にほぼ100%が死亡する[2, 3)]．OIE（国際獣疫事務局）によると，2004年9月の時点においてもベトナムやタイを中心に家禽類からH5N1の検出報告が続いている．

ウイルスは鶏糞中に多量に排泄され，汚染した器物や感染鳥を介して養鶏場から養鶏場へと容易に拡散し，大規模な被害をもたらす．野生の水鳥（特にカモ）は一般に鳥インフルエンザに抵抗性であることから，野鳥が国境を越えてウイルスを運ぶ可能性が指摘されているが，今回のH5N1の流行にどのように関与しているのか十分解明されていない．

## 2．H5N1のヒトへの感染事例

A型インフルエンザウイルスは，その粒子表面タンパク質であるヘマグルチニン（hemagglutinin; HA）とノイラミニダーゼ（neuraminidase; NA）の抗原性の違いによりHAはH1～H15，NAはN1～N9の亜型に分類される．この中で主にヒトに流行を起こしてきたのはH1N1，H2N2，H3N2であり，H5N1のヒトへの感染はこれまで例が無かった．

初めてH5N1のヒトへの感染が報告されたのは，1997年香港においてである．この事例は感染者が18人（うち6人死亡）で，いずれも鳥からの感染と推定された[4)]．香港行政府は感染が疑われる150万羽以上の家禽を殺処分し，感染は終息した．

次いで2003年2月，中国南部を旅行した香港の家族3人（うち2人死亡）の感染が報告された．

2004年1月には，ベトナムとタイでヒト感染例が確認された．以後断続的に報告が続き，同年9月の時点まででベトナム27人（うち20人死亡），タイ13人（うち9人死亡）[5)]となっている．日本でも同年1月から2月にかけ山口，大分，京都で鳥の感染報告があったが，幸いヒトへの感染は無く終息している．

ちなみに，鳥インフルエンザのヒトへの感染事例として，他にH9N2とH7N7などが知られているが，いずれもH5N1ほどの重篤な流行を起こしていない．前者は香港で1999年と2003年に各1

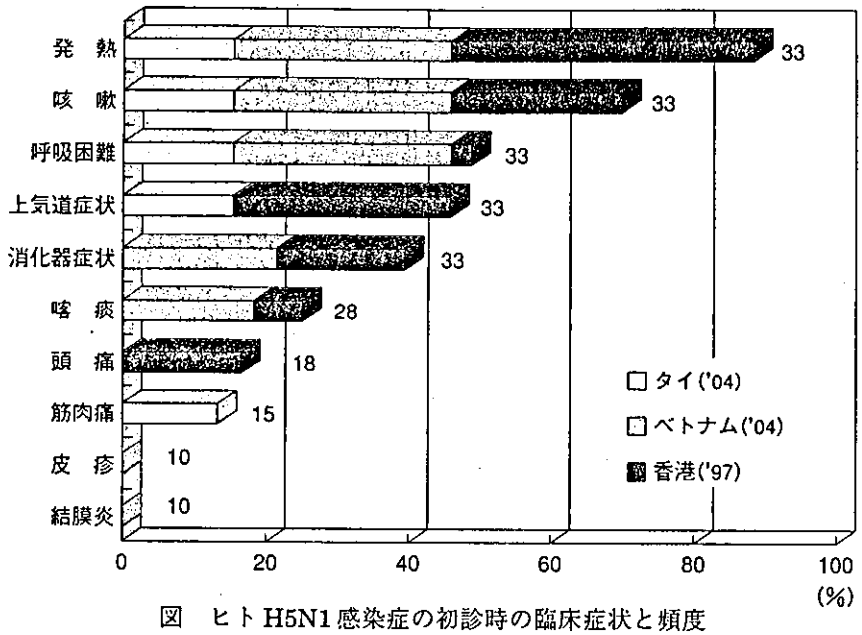


図 ヒトH5N1感染症の初診時の臨床症状と頻度
(数字は検討症例数)[6, 9~11]

名の軽症者が報告された．後者は2003年オランダで報告され，養鶏業者とその家族を含む80人以上に結膜炎などの症状が見られ，獣医師1名が呼吸器症状で死亡した[1]．

### 3. H5N1感染の機序

鳥インフルエンザウイルスがヒトに感染しにくい理由の1つとして，鳥とヒトとの細胞表面レセプターの糖鎖構造の相違が指摘されている[6]．すなわち，ヒトのインフルエンザウイルスはヒト細胞表面のα-2,6結合のシアル酸を，鳥インフルエンザウイルスはトリ細胞表面のα-2,3結合のシアル酸をそれぞれ認識するため，宿主特異性が生じるとされる．しかしレセプター特異性だけでは，時として鳥からヒトへの感染が成立する事実が説明できない．近年，鳥インフルエンザウイルスのレセプター認識の特異性変化に関する報告[7]や，ヒトの気道上皮にもトリ型のレセプターを持つ線毛細胞が存在するとする報告[8]などがなされ，この分野の基礎的研究が進んでいる．

現時点では鳥インフルエンザはヒトには効率よく感染せず，感染成立にはウイルス(感染鳥)との濃厚な接触が必要と考えられている．流行が散発している東南アジアでは，鶏や野鳥が生きたまま売買されるなどヒトと動物が濃厚に接触しながら生活する文化があり，鳥から感染する機会も多いと推定される．

### 4. 臨 床 像

先述のとおり，ヒトのH5N1感染症はこれまで香港，ベトナムならびにタイから報告されてきた．これらのうち，学術誌に報告された計33例(香港18例[6, 9]，ベトナム10例[10]，タイ5例[11])を用いて文献的にその臨床像を検証した．なお，nは検証に用いた症例数を示す．

患者背景：年齢は1歳～60歳(n=33)と幅広いが，中央値は10.0歳で就学児童に多い．小児に多い理由は不明である．男女比は1:0.8とやや男性に多い．85%は基礎疾患の無い健常者である．

感染の成立：症例の大部分は鳥(病気・死亡鳥を含む)との接触歴があるが，接触歴のはっきりしない例，記載の無い例もある．潜伏期間は，鳥との接触から発症までとした場合2～4日(n=6)である．

初診時の症状：初診時の臨床症状の内訳と頻度を図に示した．発熱，咳嗽，呼吸困難など非特異的な症状が主である．体温は37.8～40.0℃(n=22，平均39.2±0.6℃)，呼吸数は51.7±14.0回／分(n=10)，胸部聴診上50%(n=28)でcrackleが聴取された．初診時すでに呼吸不全を呈しているも

のが多いと推察される．

初診時の検査所見：記載のあるものについてみると，末梢血白血球数，リンパ球ならびに血小板の減少，ALT，ASTの上昇が認められる．LDH，CRP，$PaO_2$などに関しては記載が無い．

臨床経過：タイの5例（死亡例）について臨床経過の記載がある[11]．これによると発熱，咳嗽，上気道症状で発症し，2～6日目には呼吸困難が出現，1週間以内に人工呼吸器が装着され，8～29日には全例死亡している．

胸部X線所見：記載のある26例について検討した．全例で肺炎像を認め，その所見は両側性肺炎（34%），一側性肺炎（12%），多発斑状影（12%），ARDS（73%）など多彩（重複あり）であり，気胸（15%），胸水（15%），肺胞出血（4%）の合併もあった．

予後：香港，ベトナム，タイにおける死亡者数／感染者数はそれぞれ6／18，20／27，9／13，であり[5]，単純に合計するとその死亡率は60%となる．すべての感染例が報告されているとは考えにくく，正確な死亡率は明らかでないが，重症化した場合の死亡率は非常に高いといえる．死亡例は経過中に呼吸不全，腎不全，心不全，凝固異常，消化管出血，多臓器不全，ショックなどを合併していた．

## 5. 病　理

H5N1感染症による死亡例の剖検の報告[12, 13]によると，肺においては，肺胞腔内の水腫，出血，フィブリン析出，マクロファージの集積，II型肺胞上皮の増生，間質へのリンパ球浸潤，びまん性肺胞障害（diffuse alveolar damage: DAD）などが見られる．肺以外では，明らかな異常を認めない例から，肝細胞壊死，急性尿細管壊死など全身性の病変を認めた例まで多彩である．共通しているのは骨髄やリンパ組織，気管支などにおいて血球貪食症候群の所見が見られることである．このような病理像はウイルス感染が引き金となって惹起されたサイトカインの異常産生など過剰な免疫反応が原因と推測されている[12~14]．

## 6. 診　断

### 6.1 臨床診断

ヒトの高病原性鳥インフルエンザ感染症は2003年の感染症法の改訂により四類感染症に追加され，診断した医師は直ちに届出を行うこととなった．この中で本疾患感染のサーベイランスのための「疑い例の報告の基準[15]」が示されており，臨床診断の参考になる（表1）．

なお，各医療施設では届出の前にインフルエンザ迅速診断キットを用いて検査することが多いと思われる．H5N1はA型インフルエンザであるため，キットでA型と判定でき，臨床診断の参考になるが，偽陰性の場合もあるため注意が必要である．また，現在市販されているキットではH5N1などの亜型はもちろん診断できない．

H5N1感染症にはまだ明確な臨床診断基準が無いが，WHOはサーベイランスに関するガイドラ

表1　高病原性鳥インフルエンザに関する患者サーベイランスの基準等について[15]（抜粋）
厚生労働省健康局結核感染症課

1. 高病原性鳥インフルエンザウイルスへの感染が疑われる者の報告基準
　下記（1）または（2）に該当するものであって，発熱等のインフルエンザ様の症状がある者
（1）高病原性鳥インフルエンザウイルスに感染している又はその疑いのある鳥（鶏，あひる，七面鳥，うずら等）との接触歴を有する者
（2）高病原性鳥インフルエンザが流行している地域へ旅行し，鳥との濃厚な接触歴を有する者

2. 対応
（1）医療機関：上記「1. 疑い例の報告の基準」に当てはまる患者を診療した場合には，「四類感染症発生届」をもって速やかに最寄の保健所に「疑い例」として提出するとともに，検査に必要な検体を確保すること
（2）保健所：医療機関から（1）についての疑い例の報告があった場合には，当該保健所は地方衛生研究所と調整の上，速やかに検体を地衛研に搬入するとともに，必要に応じ患者の感染源等に関する調査を行うこと
（3）以下省略

表2 ベトナムで用いられた症例定義（WHO[16, 17]）

検査中症例（Patient under investigation）
38℃以上の発熱＋咳嗽，咽頭痛，息切れのうち1つ以上を有するもので，経過観察中で診断検査実施中の患者
可能性のある症例（Possible case）
以下のいずれか
I．38℃以上の発熱＋咳嗽，咽頭痛，息切れのうち1つ以上を有するもので，かつ以下の1つ以上に該当するもの
a. ウイルス亜型までは判明していないが，検査によるA型インフルエンザの証拠がある
b. 発症前7日以内に，A/H5インフルエンザ確定例と感染性のある期間中*の接触歴がある
c. 発症前7日以内に，病気で死亡した鳥との接触歴がある
d. 発症前7日以内に，高病原性鳥インフルエンザ（HPAI）感染が疑われているヒトもしくは動物から得られた検体を取り扱う研究機関で働いていた
II．原因不明の急性呼吸器疾患による死亡例であり，かつ以下の1つ以上に該当するもの
a. HPAIの発生が疑われている，もしくは確定している地域に居住している
b. 発症前7日以内に，A/H5インフルエンザ確定例と感染性のある期間中*の接触歴がある
確定例に準ずる限定的な証拠のある例（Probable case）
38℃以上の発熱＋咳嗽，咽頭痛，息切れのうち1つ以上を有するもので，かつインフルエンザA/H5感染に関する限定的な検査上の根拠がある（単一血清検体でH5特異抗体の検出）
確定例（Confirmed case）
以下の1つ以上の検査結果が得られている
a. インフルエンザA/H5ウイルスの分離培養陽性
b. インフルエンザA/H5のPCR陽性
c. インフルエンザA/H5モノクローナル抗体を用いた免疫蛍光抗体検査（IFA）陽性
d. インフルエンザA/H5特異抗体価がペア血清にて4倍の上昇

＊感染性のある期間：発症の1日前から発症後7日まで

イン[16]の中で，2004年初頭の流行時ベトナムで使用された症例定義を引用しているので参考のため表2[17]に示す．

### 6.2 ウイルス学的診断

WHO[16]は，H5N1感染症の確定例とは，その生死に関わらず以下のウイルス学的検査結果を1つ以上満たすものとしている．すなわち，(1) インフルエンザA/H5ウイルス培養陽性，(2) インフルエンザA/H5に対するポリメラーゼ連鎖反応（PCR法）陽性，(3) H5モノクローナル抗体を用いた免疫蛍光抗体法（IFA）でH5抗原陽性，(4) ペア血清検体でH5特異的抗体が4倍以上の上昇，のいずれかである．

## 7. 治療薬，ワクチン

ベトナムで流行していたH5N1は，M2蛋白阻害剤（アマンタジン，リマンタジン）の耐性遺伝子を持つことが報告され，これらの薬剤が無効である可能性がある．ノイラミニダーゼ阻害剤はin vitroでH5N1に有効であるとされるので，現段階では診断したらできるだけ早くノイラミニダーゼ阻害剤を開始すべきである[11]．1997年の香港ではアマンタジンが，2004年のベトナム，タイではオセルタミビルが主に使用されたが，その臨床的有効性については未だ十分検証されていない．これまでの報告例を見る限り，使用開始時期が遅い印象があり，早期に使用した場合の有用性も未知である．

感染予防法として最も有効なものはワクチンであるが，まだ日本で臨床使用できるH5N1ワクチンはなく，研究開発が進められている．

## 8. 感 染 対 策

### 8.1 感染鳥との接触を避ける

感染予防の最大のポイントは感染鳥との接触を避けることである．したがって動物集団の中でH5N1が確認されている国では生きた鳥を扱う市場や養鶏場などに立ち入らないことが重要である．

ウイルスに感染したと思われる家禽類を殺処分し，流通を停止するなどの行政対応はこれまでの

海外の流行時にもそのつど実施され効果をあげている.

### 8.2 H5N1のヒト—ヒト感染はあるか

ヒト—ヒト感染により発症したことが明らかな事例は無いが，一定の割合で感染が起こることを示すデータがある．Bridges[18]らは，1997年香港でH5N1に感染したヒト症例を診療した医療スタッフの抗H5N1抗体価陽性率は3.7%で，診療しなかったスタッフより有意に高かったと報告した．また，Katz[19]らは，H5N1感染者の接触者における抗H5N1抗体価陽性率を調査したところ，家族が11.8%，一緒に旅行した者3.8%，職場の同僚0%で，濃厚接触によりヒト—ヒト感染(不顕性感染)が起こりうるとした．以上よりH5N1は，効率は悪いもののヒト—ヒト感染も成立すると考えるべきで，特に医療現場での感染者のケアにおいては十分な感染対策が必要である.

### 8.3 院内での感染対策の実際

現時点では本疾患が効率的にヒト－ヒト感染するとは考えられていないことから，通常の個室で入院管理可能と考えられる[20]が，米国CDCはH5N1感染の疑いあるいは確定例の診療においては，SARS(重症急性呼吸器症候群)に準じた感染対策を推奨している[21]．すなわち患者は陰圧個室に収容し，手洗いなど厳密な標準予防策に加え，「飛沫」，「接触」，「空気」の各感染経路別予防策を実施する．具体的には手袋，ガウン，ゴーグル，N-95マスクなど個人防御用具を装着して診療にあたる.

ウイルスは加熱(56℃3時間ないし60℃30分)や次亜塩素酸，グルタルアルデヒド，アルコール，ヨード系を含む通常病院内で使用される殺菌消毒薬に感受性であり，これらを適切に使用する.

また，インフルエンザ流行期には，標準予防策に加え，発熱・咳嗽などインフルエンザ様症状を呈する患者にはマスク着用を奨め，同時にこのような患者の診療にあたるスタッフはマスクを着用する「レスピラトリーエチケット[22]」を実施するなど，普遍的な感染対策も重要である.

## おわりに

鳥集団の中での継続的なH5N1の流行を背景に，しばしばヒトへの感染が見られる現在の状況が続くと，H5N1とヒトインフルエンザウイルスの遺伝子の交換(遺伝子再集合)の機会が増え，全く新しいウイルスが出現する可能性がある．こうして効率良くヒトに感染する新型ウイルスが出来上がると，人類が全く免疫を持たないため世界的大流行(パンデミック)になる可能性がある．今後の監視と対策が必要である.

本総説には文部科学省の平成15年度科学技術振興調整費による「高病原性鳥インフルエンザ対策に関する緊急調査研究」の一環としてベトナムで実施した調査資料の一部を使用した.

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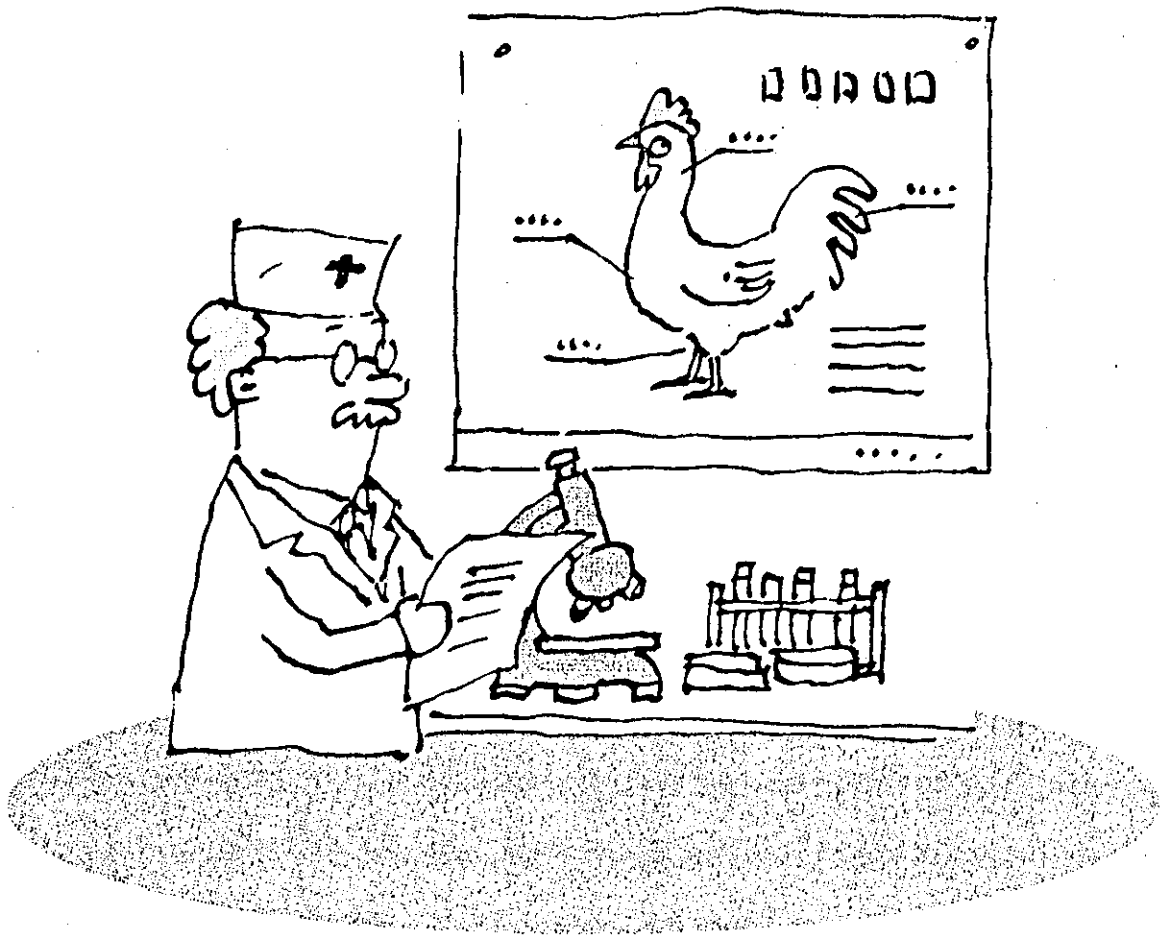

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