

Book: Emerging Infectious Diseases: Trends and Issues 2<sup>nd</sup> Ed

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Chapter title: Avian Influenza A(H5N1)

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**Vignette:**

Kumawari is a 30 year old woman who lives in a poor urban neighborhood in Jakarta, Indonesia. Her small concrete block house with a tin roof is similar to those of her 38 extended family members who live nearby. Narrow dirt paths and open sewers separate the houses. Until recently, the small community shared the responsibility for about 20 ducks and 60 chickens. One day, two of the community's hens suddenly became sick. Crouching low with feathers ruffled and unsteady the hens died within 24 hours of first becoming ill. During each of the next ten days, more chickens became sick and died rapidly, many displaying severely discolored, swollen combs and wattles. Fearing that all of her hens would soon die and that her family would not benefit from the nutrition these chickens provide, Kumawari and her 10 year-old daughter Muriara caught two hens and prepared them for their family to eat. Once boiled and fried, several other family members and neighbors consumed the birds.

Four days later, Kumawari woke with a severe headache, fever, nausea, and vomiting. By that afternoon, Muriara was also vomiting, febrile and had developed diarrhea but no

other family members were ill. Chills and muscle aches set in a few hours later and, not having the financial resources to afford medical care, both women remained at home. Extended family members provided nursing care until the fourth day when they could no longer avoid a visit to the hospital. Both received intravenous fluids to treat their dehydration, and that night they both abruptly developed a severe, dry cough and dyspnea. Chest X-rays taken the next morning showed patchy bilateral infiltrates and intravenous broad spectrum antibiotics were begun. As is the practice in many poor hospitals, family members provided much of the nursing care in the hospital.

Over the next three days Kumawari became severely ill and required intubation and mechanical ventilation. A blood test showed leucopenia and thrombocytopenia. She developed pulmonary edema and her chest x-ray showed a virtual "white-out" in all lung fields. On the fourth day of hospitalization, Kumawari died despite aggressive medical care.

Muriara's illness progressed more slowly and she was intubated the day her mother died. As Muriara began to slowly recover several days later, reports began to surface that several members of her extended family and others persons from the community were also ill. The district

hospital outpatient clinic began to see increasing numbers of patients with influenza-like symptoms. While some patients became rapidly ill and were hospitalized, others seemed to have a milder form of illness. Five days after their initial contact with Kumawari and her daughter, two nurses called in sick with vomiting and fever.

An investigation by central disease control authorities began, and the next day, the national public health laboratory reported that a respiratory specimen taken from Kumawari had tested positive for influenza type A, but negative for hemagglutinin subtypes 1 and 3. The specimen was forwarded to a regional WHO reference laboratory and, two days later, the diagnosis of avian influenza A (H5N1) virus infection was made. By this time more than 50 patients, mostly children and young adults, had become ill and 22 had been hospitalized at several hospitals facilities in the area. Local supplies of personal protective equipment (PPE) were rapidly depleted as reports of the first nurse to succumb from the infection reached the news media. International public health authorities mobilized available PPE and antiviral drugs as hundreds of additional persons reported to hospitals. At the same time, infectious disease hospitals in Kuala Lumpur, Malaysia and Bangkok, Thailand announced they had also admitted multiple

patients with suspiciously similar clinical presentations and histories and were awaiting test results. As countries began to impose travel restrictions, the world braced for the public health crisis looming in the difficult months ahead.

**Background/ Reasons for Emergence:**

Wild waterfowl, gulls and shorebirds are the natural reservoir for influenza type A viruses. Influenza A viruses representing all 16 subtypes of Hemagglutinin (HA) and 9 subtypes of neuraminidase (NA) have been isolated from waterfowl[1, 2] Until the emergence of highly pathogenic H5N1, influenza A viruses in waterfowl were considered to be in evolutionary stasis, causing mainly asymptomatic infections [3-6]. In contrast, many influenza A virus subtypes have been documented to cause symptomatic infection in perching birds, marine mammals, horses, pigs, cats and dogs [7-10]. Until 1997 however, only subtypes H1, H2, and H3 had been associated with clinically significant disease in humans.

In 1997 in Hong Kong, 18 human cases of avian influenza A (H5N1) infection and 6 deaths occurred concurrently with outbreaks in domestic poultry [11, 12].

Until this event, avian influenza A viruses had not been associated with severe disease in humans. The fear of the potential of the H5N1 virus to cause a human pandemic prompted the culling of millions of poultry in Hong Kong and the implementation of extensive measures to prevent further spread [13].

Due to their low fidelity polymerase and segmented genome, influenza A viruses are characterized by extreme genetic variability [14, 15]. In addition, cross-species transmission events appear to accelerate the rates of mutations [16-18]. Across much of Asia, it is common practice to both raise and market multiple bird species and pigs in close proximity to humans, creating an ideal environment for the development of new influenza A subtype virus reassortants potentially capable of causing disease in humans [19-22]. In addition, international agribusinesses that maintain production facilities in many Asian countries and produce billions of poultry in crowded conditions may also favor the development and distribution of new avian influenza A virus strains [23, 24]. Finally, the interaction of wild migratory waterfowl with domestic ducks and chickens appears to have contributed to the geographic spread of the H5N1 virus [25-28]. In fewer than 8 years since the virus was identified in Hong Kong, it has

become endemic in much of east Asia and was identified in eastern Europe in October, 2005 [29, 30].

The precursor to the H5N1 virus identified in Hong Kong in 1997 was first detected in geese in Guangdong province of China in 1996 (A/Goose/Guangdong/1/96-like). Despite extensive control measures, new H5N1 reassortants emerged and caused outbreaks among birds in Hong Kong in 2000 and 2001, and probably killed two of three humans in 2002 [31-33]. In 2001, H5N1 viruses were isolated from live wet poultry markets in Vietnam [34]. In 2003, highly pathogenic avian influenza (HPAI) H5N1 viruses began to cause massive mortality in large scale commercial poultry farms in Thailand, Cambodia, China, Indonesia, Japan, Laos, South Korea, and Vietnam[35, 36]. As of February 2004, 23 human H5N1 cases and 18 deaths (78% case fatality rate) had been reported in Vietnam and Thailand[37]. By December 2005, Cambodia, China and Indonesia had joined the list of countries reporting human fatalities, and WHO had recorded 133 confirmed human cases with 68 (51%) deaths[38]. H5N1 is now considered to be endemic among poultry in East and Southeast Asia. Infection and culling has resulted in the deaths of more than 140 million poultry with devastating economic losses to large agribusinesses and to small farmers[39].

## **Description of the virus**

Influenza viruses belong to the family Orthomyxoiviridae and have three antigenic types: influenza A, B and C. Only influenza type A and B viruses are known to cause human disease, and only type A viruses have been documented to cause human pandemics. Influenza virions are enveloped particles of spherical or slightly elongated dimensions measuring from 80-120 nm in diameter. The genome consists of single-stranded, negative-sense RNA in eight gene segments that code for 10 proteins [40].

The major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), play primary roles in transmission and pathogenesis of human influenza viruses. Specific antibody against HA is protective, but minor antigenic changes occur frequently and new strains can infect and cause disease in persons who have antibody against other related but antigenically distinct strains. Antibody to NA may help modify disease severity. Sixteen different HA (differing by at least 30% in their nucleotide homology) and nine different NA subtypes have been identified. Of these, only viruses with combinations of HA 1-3 and NA 1-2 were known to cause severe disease in humans until the occurrence H5N1 infection in human in 1997. Briefly,

neuraminidase promotes the release of virus from infected cells, inhibits the aggregation of new virions and facilitates their spread to other respiratory tract cells [41]. Hemagglutinin mediates receptor-binding and membrane fusion of influenza virus and is the primary target for infectivity-neutralizing antibodies [42]. While the determinants of viral tropism and receptor specificity are thought to be polygenic, hemagglutinin is believed to be the key molecule in pathogenesis.

The receptor specificity of HA is directly relevant to the ability of H5N1 viruses to cross the species barrier [43]. Human influenza viruses bind preferentially to cells with sialic acid receptors containing alpha 2,6-galactose (SAalpha2,6Gal) linkages while avian viruses bind preferentially those containing alpha 2,3-galactose (SAalpha2,3Gal) linkages [44]. However there is evidence that even a single amino acid substitution in the HA gene can significantly alter receptor specificity of avian H5N1 viruses, providing them with an ability to bind to receptors optimal for human influenza viruses[45]. The pandemic implications of such a mutation are enormous. The H1N1 virus that caused a massive pandemic in 1918 was also of avian origin and had acquired a preference for the SAlpha2,6-Galactose receptors [46-48]. Close monitoring of

the genetic evolution and receptor binding preference of H5N1 viruses is a public health priority.

The ability of influenza viruses to replicate in cell culture in the absence of the proteolytic enzyme trypsin has been considered to be an important determinant of the ability of these viruses to cause disease in humans[49]. The presence of multiple basic amino acids at the HA cleavage site is also characteristic of highly pathogenic avian strains[50, 51] and H5N1 viruses have been found to have both of these characteristics for increased pathogenicity. It is interesting to note however, that the 1918 pandemic H1N1 virus had neither characteristic[47].

Since its identification in humans in 1997, the H5N1 virus has undergone rapid evolution demonstrated by development of multiple genotypes[31], antigenic changes[52, 53], increased pathogenicity and extrapulmonary disease [26, 54-56], an extended host range [57, 58], increasing numbers of human clusters [59], and development of resistance to antiviral medications that inhibit the M2 ion channel (adamantanes)[60, 61]. In addition, one case report has documented the development of resistance to the neuraminidase inhibitor oseltamivir [62]. The latter developments are of great public health importance as

antiviral medications are key public health tools to combat a future pandemic [63-65].

### **Epidemiology of Human Infection**

The epidemiology of human infection with avian influenza A (H5N1) virus is incompletely understood but each new case affords an important opportunity to advance what is known about this pathogen. Human influenza is principally transmitted through droplet spread, while fomite and aerosol transmission each have a role in certain situations. While the routes of transmission for H5N1 have not been definitively established, most patients have had direct exposure to infected birds including butchering, consuming incompletely cooked or raw poultry products, and handling fighting cocks or other poultry being commonly reported [66]. Such exposures suggest that pharyngeal or gastrointestinal inoculation of the virus may be an important method of transmission. Even so, contact with sick poultry has not been identified in all cases. Importantly, while chickens infected with H5N1 rapidly develop symptoms that can signal a risk for potential human exposure, domestic ducks can remain apparently healthy while continuing to excrete virus[67, 68]. This has ominous

implications for widespread human exposures in Asia where duck husbandry is very common.

#### Human to Human Transmission

Because the human population has no effective immunity to H5N1 influenza, confirmation of efficient and sustained human-to-human transmission will likely signal the start of the next global influenza pandemic. While transmission directly from infected poultry explains most cases to date, small clusters of human cases are increasingly reported, suggesting the possibility of limited person-to-person transmissions[69]. In Hong Kong in 1997, neutralizing antibodies to H5N1 were found in six of 51 household contacts, one with no clear history of exposure to poultry [70]. In Thailand, transmission from a severely ill child to a family member who provided intensive and prolonged nursing care was reported [71]. Documenting human-to-human transmission is complicated by many factors including the high frequency of potential confounding exposures to ill poultry or surfaces contaminated with feces, delays in the initiation of epidemiologic investigations, and limited availability of clinical specimens of adequate quality. To date, no evidence of sustained person-to-person transmission of H5N1 virus has been identified, but the

potential for the development of this mode of transmission highlights the need for rapid epidemiological investigation of H5N1 case contacts when cases are identified.

The possibility of mild or asymptomatic H5N1 virus infection is supported by epidemiological studies conducted during the 1997 outbreak. One health care worker had no symptoms and one colleague had mild respiratory illness, but both seroconverted for H5N1 antibody[72]. Another study among poultry workers estimated that 10% had been infected [73]. A 2004 community survey in northern Vietnam suggested that mild H5N1 infections may be more common than currently appreciated but this study did not include laboratory confirmation [74]. Limited serosurveys of contacts and those with presumably intense exposures (i.e. poultry cullers) in Thailand and Vietnam during 2004 did not support the widespread occurrence of mild or asymptomatic disease. Therefore, case-finding for H5N1 has focused on severe hospitalized respiratory disease. Early identification of an expanded spectrum of illness with H5N1 infection is of public health importance as it may represent a key change towards a virus with increased pandemic potential.

## **Clinical Presentation and Laboratory Diagnosis**

Most clinical descriptions of H5N1 are from patients hospitalized with severe pneumonia. While most cases in 2004 have been in children or young adults, patients from across the age spectrum were identified in 2005. The incubation period for H5N1 ranges from 2-8 days with a median of 4 days [66, 75]. This appears to be longer than for human influenza viruses in which the incubation period is 1-4 days with a median of 2 days [76]. Nearly all patients present with high fever and systemic influenza-like symptoms such as nausea, headache, and myalgia. Upper respiratory symptoms are not always present. A few case reports have documented atypical syndromes, including patients whose primary or only symptoms are gastrointestinal [77] or neurological [56]. Diarrhea is common and may precede the onset of respiratory symptoms by several days[77]. Clinically significant lymphopenia and mild to moderate thrombocytopenia are common laboratory findings [75]. Lower respiratory tract symptoms are usually found on admission to the hospital with dyspnea developing in a median of 5 days from onset of illness in one group of patients in Thailand [78]. A variety of radiographic abnormalities usually follow closely after the onset of

dyspnea including diffuse, multifocal or patchy infiltrates, interstitial infiltrates, or lobular consolidation. Pleural effusions are uncommon. In many patients, the clinical course worsens over several days with the onset of Acute Respiratory Distress Syndrome (ARDS) and the characteristic diffuse "ground-glass" infiltrates on chest X-ray. Death is commonly preceded by multi-organ failure [66, 79, 80].

Laboratory diagnosis is complicated by the difficulty in obtaining properly collected and well maintained clinical specimens. Often, H5N1 infection has not been suspected until late in the course of illness or even after death.[71] Isolation of H5N1 virus from respiratory specimens using embryonated hen's eggs or tissue cell culture under enhanced biosafety level 3 conditions is the "gold standard." Reverse transcriptase polymerase chain reaction (RT-PCR) testing of respiratory specimens is most frequently used to diagnose H5N1 infection due to its high sensitivity and speed. Throat and lower respiratory tract specimens appear to have higher yield for detecting H5N1 virus than nasal specimens. Stool specimens have tested positive for viral RNA and yielded virus isolates [56, 66].

Serologic testing for evidence of H5N1 antibody is limited by method's technical complexity and the need to

use live H5N1 virus under BSL-3 laboratory conditions. When properly timed acute and convalescent serum samples have been collected, the microneutralization assay with confirmatory Western Blot assay is highly sensitive and specific [81]. The traditional hemagglutination-inhibition test (HI) does not require live virus and effectively detects increases in human influenza antibody in serum. However, HI is insensitive for the detection of human antibody responses to avian hemagglutinin, even in the presence of high titers of neutralizing antibody after confirmed infection. A modified HI test using horse red blood cells has been developed [44] and is being field tested in Indonesia. Rapid antigen influenza diagnostic tests are much less sensitive than PCR methods and are not currently recommended for the purpose of detecting H5N1 [78].

In most cases, religious beliefs and social customs have prevented postmortem analyses. Early reports have found severe pulmonary injury with histopathological changes of diffuse alveolar damage and hyaline membrane formation similar to pneumonia due to human influenza virus infection [82-84]. One autopsy report found evidence of H5N1 viral replication in the lungs and intestinal tract [83].

## **Prevention and Related Cultural and Social Issues**

Across East and Southeast Asia, billions of terrestrial and aquatic poultry are raised annually for personal consumption, sale, ornamental collection, and gaming purposes. In one recent survey in rural Thailand, 74% of households raised at least one type of poultry[85]. In addition, international trafficking in wild Asian birds is an ongoing environmental and potential human health problem [86, 87]. These activities result in frequent, and potentially intense, human exposures as well the distribution of avian influenza viruses across international borders.

In both rural and in poor urban settings, multiple avian species and swine are often raised in close proximity to each other, increasing the risk of cross-species transmissions, and a reassortment event [88, 89]. In addition to the economic importance of these activities, such practices are often deeply rooted in social and religious customs. For example, the consumption of raw duck blood is considered a delicacy in Vietnam but may carry substantial risks of avian influenza infection [90].

While affluent Hong Kong has made substantial progress in controlling avian influenza through farm and market regulations [91], most Asian countries lack human and

financial resources required to significantly improve biosecurity in traditional farming practices. The situation is particularly severe for millions of Asia's poorest citizens where the loss of poultry to H5N1 infection or culling to control the disease can have dire nutritional consequences. The threat of large-scale poultry culling acts as a significant deterrent for villagers to report poultry outbreaks to veterinary authorities. Further, visibly ill chickens are often butchered and eaten by poverty stricken families, a behavior that has been implicated in a growing number of fatal human cases[92].

#### **Influenza Antivirals: Growing resistance**

A small number of antiviral medicines play an important role in the control of influenza infection and are a key component in the pandemic influenza plans of most countries [93, 94]. The adamantane derivatives (amantadine and rimantadine), block the ion channel function of the M2 protein and have been used for treatment and prophylaxis of influenza A viruses for more than 30 years [95, 96]. Prior to 2000, fewer than 2% of all influenza A/H3 viruses isolated worldwide demonstrated resistance to adamantanes[97]. However, recent surveillance has revealed an alarming increase in the incidence of adamantine

resistance with 160/1304 (12.3%) global isolates being resistant in 2004. In China 109 of 149 (73.2%) of influenza H3 isolates in 2004 were found to be resistant[61]. In 2005, 91% of influenza isolates in the US were also resistant leading the US CDC to recommend that adamantanes not be used to treat influenza virus infection during the 2005/06 season [98]. In addition, H5N1 viruses isolated in the 1997 Hong Kong outbreak were uniformly susceptible to adamantanes, but most human and most avian A (H5N1) isolates tested since 2003 have been resistant[61,99]. The disturbing increase in resistance to adamantanes suggests that these drugs will be of limited value in response to a future influenza pandemic.

Oseltamivir and zanamivir belong to a newer class of antiviral agents which inhibit the viral enzyme neuraminidase and have proven to reduce the length and severity of infection from human influenza A and B when early treatment is initiated, reduce viral shedding, and are effective prophylactics[100, 101]. However, the effectiveness of neuraminidase inhibitors used for late treatment of severe H5N1 illness is unknown. The lack of proven effectiveness with late antiviral treatment could be due to induction of proinflammatory cytokines as a major factor in pathogenesis of H5N1 [102]. The ideal dose and

duration of treatment for H5N1 infection has not been established, but research in the mouse model suggests that higher doses and longer than standard treatment periods may be necessary[103]. Due to the widespread resistance of H5N1 to adamantanes, many countries have elected to establish national stockpiles of oseltamivir as one measure of pandemic preparedness[64, 65, 104]. Accordingly, the development of resistance to neuraminidase inhibitors is of major public health importance.

Naturally occurring oseltamivir-resistant influenza viruses have not been identified [105]. Resistant variants appear to develop infrequently during treatment, particularly in young children who receive inadequate weight-based unit doses[106]. Some evidence suggests that these resistant variants may have reduced biological fitness in terms of infectivity, pathogenicity and replicative ability[107, 108]. Of particular concern is the isolation of an H5N1 virus with partial resistance to oseltamivir in a Vietnamese girl in 2005 who was treated for 11 days and survived[62]. A second study from Vietnam reported high-level resistance to oseltamivir in two of eight patients. One of these patients died despite the early initiation of antiviral therapy at currently recommended levels[109]. These findings underscore the

importance of global monitoring for drug resistance and the judicious use of antiviral medications[110].

### **Global Response**

The unprecedented spread and virulence of avian influenza A (H5N1) in poultry and on-going sporadic human infections raise concern that this could develop into the next pandemic influenza virus. An effective response requires political transparency and the cooperation of animal and human health authorities at every level. In most countries the capacity of the veterinary health system lags well behind that of human public health, and significant resources will be required to correct this deficit. In much of Asia, H5N1 is now endemic in poultry, and early hopes of eradication appear unlikely. Coordinated efforts should aim to reduce the amount of virus circulating in domestic poultry flocks, decrease the risk of avian-to-human infection, thereby minimizing the potential for development of an H5N1 strain capable of efficient and sustained human-to-human transmission.

Although new H5N1 cases in humans continue to be identified, they remain relatively uncommon. Control of the infection in poultry through improved biosecurity in farming and marketing is a priority. In response to massive

losses during outbreaks in 2003 and 2004, the commercial poultry sector has taken effective steps to reduce H5N1 infection. However, changing animal husbandry practices in millions of small "backyard" farms in rural and urban settings is a daunting challenge. Systematic poultry surveillance, accurate laboratory diagnosis, separation of domestic poultry from wild birds, rapid culling of infected flocks, strict movement restrictions, and restocking or adequate financial compensation to farmers are key components of an effective control program[111]. Countries that choose to vaccinate poultry as one component of a broader control program must have reliable systems in place to assure vaccine quality, proper administration, monitor efficacy, and long-term funding to sustain the vaccination program.

Public education campaigns to discourage behaviors known to be associated with the risk of bird-to-human transmission are essential to prevent illness and deaths. Likewise, family members and healthcare workers must be educated and equipped with personal protective equipment as appropriate to reduce the risk of human-to-human transmission. As early symptoms of H5N1 infection are highly nonspecific, surveillance for H5N1 infection has focused primarily on severe respiratory illness in

hospitals. Improving laboratory diagnostic capacity to detect H5N1 virus is essential. Development of a rapid and accurate diagnostic H5N1 test that could be conducted in simple hospital laboratories would represent a major advance. Serological surveys should be regularly undertaken to monitor for mild or asymptomatic illness that could suggest the virus has become better adapted to humans.

Each new human case merits thorough investigation. Multiple, sequential clinical specimens should be collected and viruses promptly submitted to a WHO collaborating laboratory. Molecular analysis of the H5N1 genome is essential to monitor for changes in host affinity, genetic reassortment, antigenic drift, antiviral resistance, and to ensure virus strains used to develop pandemic influenza vaccines are current[52]. Reverse genetics has been used to develop nonvirulent H5N1 vaccine strains [112]. Vaccine trials are underway in several countries and one vaccine has been found to be immunogenic at high doses [113]. Clinical research to better describe the natural history of illness, determine transmission risks, and develop more effective treatment protocols is a priority.

H5N1 avian influenza is a threat to animal and human health worldwide. It is possible that we are now witnessing similar events that led to the 1918 influenza pandemic that

claimed at least 40 million lives [47, 48]. A long term, multi-sector approach with sustained funding is needed to control the disease in poultry and prepare for a possible human influenza pandemic. The challenges are many and the time may be short. Global pandemic preparedness planning is urgently needed [114].

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