The Journal of Infectious Diseases

SUPPLEMENT ARTICLE

Mild Respiratory Illness Among Young Children Caused by Highly Pathogenic Avian Influenza A (H5N1) Virus Infection in Dhaka, Bangladesh, 2011

Apurba Chakraborty,[1,2] Mahmudur Rahman,[1] M. Jahangir Hossain,[2,4] Salah Uddin Khan,[2] M. Sabbir Haider,[1] Rebeca Sultana,[2] Nadia Ali Rimi,[2] M. Saiful Islam,[2] Najmul Haider,[2] Ausraful Islam,[2] Ireen Sultana Shanta,[2] Tahmina Sultana,[2] Abdullah Al Mamun,[2] Nusrat Homaira,[2,5] Doli Goswami,[2] Kamrun Nahar,[2] A. S. M. Alamgir,[1,3] Mustafizur Rahman,[2] Khondokar Mahbuba Jamil,[1] Eduardo Azziz-Baumgartner,[6] Natosha Simpson,[6] Bo Shu,[6] Stephen Lindstrom,[6] Nancy Gerloff,[6] C. Todd Davis,[6] Jaqueline M. Katz,[6] Andrea Mikolon,[2,6] Timothy M. Uyeki,[6] Stephen P. Luby,[2,6] and Katharine Sturm-Ramirez[2,6]

[1]Institute of Epidemiology, Disease Control and Research, [2]International Centre for Diarrhoeal Diseases Research (icddr,b), and [3]World Health Organization, Dhaka, Bangladesh; [4]Medical Research Council Unit, The Gambia; [5]UNSW, Sydney, Australia; and [6]Centers for Disease Control and Prevention, Atlanta, Georgia

Background. In March 2011, a multidisciplinary team investigated 2 human cases of highly pathogenic avian influenza A(H5N1) virus infection, detected through population-based active surveillance for influenza in Bangladesh, to assess transmission and contain further spread.

Methods. We collected clinical and exposure history of the case patients and monitored persons coming within 1 m of a case patient during their infectious period. Nasopharyngeal wash specimens from case patients and contacts were tested with real-time reverse-transcription polymerase chain reaction, and virus culture and isolates were characterized. Serum samples were tested with microneutralization and hemagglutination inhibition assays. We tested poultry, wild bird, and environmental samples from case patient households and surrounding areas for influenza viruses.

Results. Two previously healthy case patients, aged 13 and 31 months, had influenzalike illness and fully recovered. They had contact with poultry 7 and 10 days before illness onset, respectively. None of their 57 contacts were subsequently ill. Clade 2.2.2.1 highly pathogenic avian influenza H5N1 viruses were isolated from the case patients and from chicken fecal samples collected at the live bird markets near the patients' dwellings.

Conclusion. Identification of H5N1 cases through population-based surveillance suggests possible additional undetected cases throughout Bangladesh and highlights the importance of surveillance for mild respiratory illness among populations frequently exposed to infected poultry.

Keywords. Avian influenza; HPAI; outbreaks; Bangladesh; influenza A virus; H5N1 subtype.

From November 2003 to July 2016, 854 confirmed human cases of infection with highly pathogenic avian influenza (HPAI) A (H5N1) virus have been reported worldwide [1], with a case fatality proportion of 53%. As of December 2011, a total of 523 HPAI H5N1 poultry outbreaks from 52 out of 64 districts had been reported in Bangladesh [2, 3] (Figure 1). The only confirmed human case of HPAI H5N1 virus infection in Bangladesh before this report was identified in 2008 in a child with mild illness through a population-based active surveillance for respiratory and febrile illness conducted in Kamalapur, Dhaka, where the child lived [4]. The event suggested that Bangladesh's routine hospital-based influenza surveillance might systematically miss the detection of mild HPAI H5N1 cases.

In Bangladesh, persons with mild illness do not usually attend hospitals for primary care, with about 7–17 cases of influenza associated influenza-like illness in patients <5 years of age per 100 person-years of hospital attendance [5, 6]. In contrast, another study conducted in a rural area during the 2010 influenza season reported that 51% of patients with severe acute respiratory infection (SARI) sought care in a hospital or clinic [7]. Bangladesh's routine hospital-based surveillance for influenza started in 2007 in 6 public and 6 private hospitals widely distributed across the country. In these sentinel sites, a subset of patients with influenza-like illness seeking care as outpatients and hospitalized patients with SARI are enrolled in the surveillance and tested for influenza using real-time reverse-transcription polymerase chain reaction (rRT-PCR) of nasopharyngeal swab samples [8]. Since inception of this program, the sampling scheme has been adjusted over time, based on laboratory testing capacity and available resources.

From September 2010 to June 2011, all identified case patients with SARI and the first 5 with severe pneumonia (among children aged <5 years) in each month from each of the hospital were being tested for influenza. No sampling was being performed among

Presented in part: International Conference on Emerging Infectious Diseases, Atlanta, Georgia, 11–14 March 2012.

Correspondence: A. Chakraborty, MBBS, MPH, Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois, 1603 W. Taylor St (MC 923), Chicago, IL 60612 (apurba.dr@gmail.com).

The Journal of Infectious Diseases® 2017;216(S4):S520–8

DOI: 10.1093/infdis/jix019

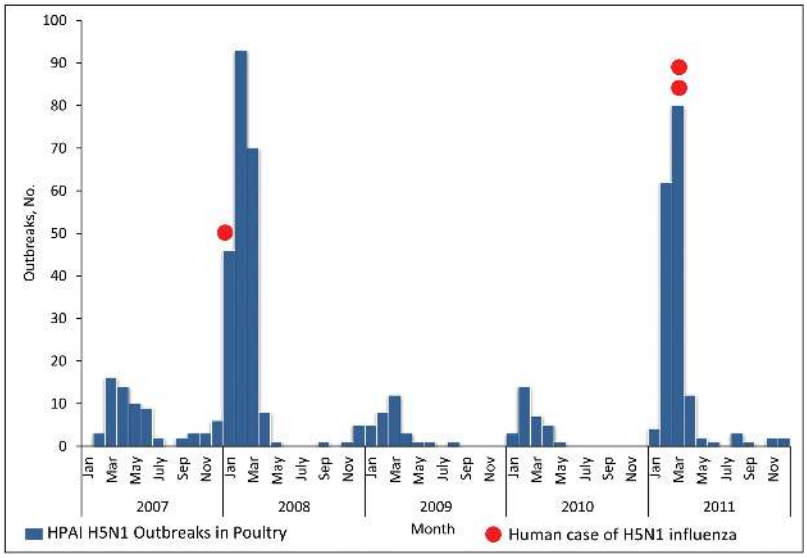

Figure 1. Monthly human cases of highly pathogenic avian influenza (HPAI) H5N1 virus infection and H5N1 HPAI outbreaks among poultry reported in Bangladesh from March (Mar) 2007 to December 2011 (523 outbreaks). Abbreviations: Jan, January; Sep, September; Nov, November.

patients with influenzalike illness seeking care in the outpatient department. On the other hand, through the longitudinal population-based surveillance in Kamalapur, >6600 households (or 30 000 participants) with children <5 years old, selected using a stratified cluster sampling from a population of 200 000 residents in an area of 4 km^2, were under active surveillance for influenza since 2004 [9, 10]. Twice every week, teams collect information about specific illness signs during the previous 7 days, visiting each household and using standardized calendar questionnaires. As part of this surveillance, from every fifth child with acute respiratory or febrile illness, a nasopharyngeal wash (NPW) specimen is collected and tested for influenza virus, using rRT-PCR and culture in Madin-Darby canine kidney cells. In the wake of pandemic influenza, enhanced surveillance was started in this surveillance area in 2009, which included collection and rRT-PCR testing of NPW specimens for influenza viruses from all children aged <5 years presenting with fever or respiratory illness [10].

Two human cases of influenza A (H5N1) virus infection were detected through the Kamalapur surveillance system in 2011, the first on 13 March (case A) and another on 15 March (case B). Within 24 hours after the first case was detected, the Institute of Epidemiology, Disease Control and Research of the government of Bangladesh and the International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr,b) formed a multidisciplinary outbreak investigation team consisting of epidemiologists, clinicians, veterinarians, virologists, and social scientists to conduct an outbreak investigation.

The objectives of this investigation were to confirm HPAI H5N1 virus infection, to assess the possible source(s) of infection and modes of transmission, to search for possible additional cases due to human-to-human transmission of HPAI H5N1 virus, and to contain further spread. The outbreak investigation team also collected clinical data to characterize illness severity and duration of symptoms and signs.

METHODS

Epidemiological Investigation

Using a structured questionnaire, we collected the age, sex, and clinical history during the 14 days before NPW specimen collection of the patients who tested positive for influenza A subtype H5 viral RNA by rRT-PCR. Because both patients were <5 years old, we interviewed their parents as their proxies.

Using a structured questionnaire and open ended in-depth interviews, we explored the potential exposures of the case patients, including touching, handling, slaughtering or butchering, or being in close proximity to (within 1 m) well-appearing, sick, or dead poultry or wild birds and uncooked poultry products during the 2 weeks before the onset of illness in the child. We inquired about the patients' travel history and possible close exposure to humans with acute respiratory illness or to a person who had died of respiratory illness of unknown cause during the same period. We also explored the history of poultry or wild bird sickness or die-offs in the neighboring area in Kamalapur during the 4 weeks before illness onset. For patient A in particular, the investigation extended to her paternal grandparent's house in a village 30 km southeast of Dhaka, where the patient went 5 days before illness onset and stayed for 3 days. We used a structured questionnaire to ask households in the village whether they had seen sick poultry or birds within 4 weeks of patient A's illness onset.

We defined a case patient's potential infectious period as 1 day before the onset of illness to 1 week after the last identification of

H5 viral RNA in follow-up NPW specimens. Those who came within 1 m of a case patient during the infectious period were identified as close contacts. We instructed the case patient's family members to communicate with surveillance staff if any close contact became ill. Surveillance field workers also monitored close contacts for respiratory symptoms during their weekly visits to case patient households. Using a structured questionnaire, we interviewed close contacts to determine whether they experience fever, cough, runny nose, sore throat, or difficulty breathing during the same period.

Sample Collection and Laboratory Investigation

We collected follow-up NPW specimens from the case patients (Figure 2). On the first day of the investigation, we collected NPW specimens from the patients' asymptomatic parents. We collected paired serum samples from both patients and their family caregivers, with the first serum sample obtained within 15 days of the patient's symptom onset and the second collected 3 weeks after the first. We collected a single serum sample from contacts between 21 to 90 days after the last exposure to the 2 case patients.

All NPW and nasopharyngeal swab specimens were tested with rRT-PCR at the Institute of Epidemiology, Disease Control and Research and icddr,b for influenza A and B viruses. Specimens positive for influenza A specimens were further tested for subtypes H1, H3, H1pdm09, and H5 [11]. All clinical specimens were also shipped frozen to the Centers for Disease Control and Prevention (CDC), Atlanta for virus isolation, molecular characterization, testing for antiviral resistance, and serology. Human serum samples were tested by means of microneutralization and hemagglutination inhibition assays for antibodies to 2 contemporary HPAI H5N1 viruses circulating in Bangladesh in 2011 [12, 13]. One of the virus strains isolated from patient A (clade 2.2.2.1) was used as antigen to detect clade 2.2.2.1-specific antibody. An HPAI A (H5N1) virus isolated from a crow (clade 2.3.2.1a) in 2011 was also included to assess seroconversion across clades detected in Bangladesh [14]. The geometric mean neutralizing antibody titers were calculated using 2 replicate tests with a starting serum dilution of 1:10. According to World Health Organization (WHO) criteria, seroconversion was defined as a ≥4-fold rise in neutralization antibody titer for H5N1 virus based on testing of an acute and convalescent serum specimen, with the convalescent neutralizing antibody titer ≥40 [12]. A seropositive result was defined as an HPAI H5N1 virus neutralizing antibody titer ≥40 (equivalent to the WHO protocol criterion of ≥80) [15, 16].

We collected poultry and wild bird samples from the case patient households, their neighboring area, and the locations visited by the case patients during the 2 weeks before their illness. We collected swab samples from the frozen chicken meat and viscera stored in the freezer of patient A's house. We collected chicken fecal samples from 2 live bird markets located close to the houses of the case patients in the Kamalapur neighborhood. In each of the 2 markets, we pooled swab samples collected from 10 broiler chickens and from 10 indigenous chickens. We collected swab samples from all birds in viral transport medium.

Swab samples from chickens, ducks, quails, crows, and environment were tested for influenza viruses by rRT-PCR at the icddr,b and the CDC. Virus isolation and full molecular characterization of resultant virus isolates were done at the CDC. Handling of infectious materials was performed in compliance with biosafety level 3 containment, including enhancements required by the US Department of Agriculture and the Select Agents program [17].

Ethics Statement

All case patients and their families provided written informed consent to participate in the Kamalapur respiratory and febrile illness surveillance, approved by the icddr,b Ethical Review

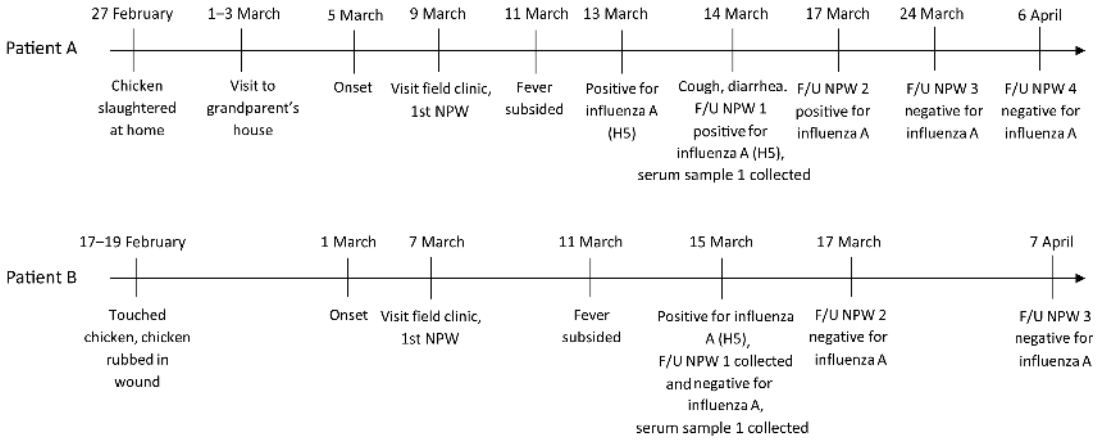

Figure 2. Timeline of events for 2 human cases of highly pathogenic avian influenza A (H5N1) virus infection in Bangladesh, February–March 2011. Abbreviations: F/U, follow-up; NPW, nasopharyngeal wash specimen.

Committee before the outbreak. The committee reviewed and approved a protocol for H5N1 outbreak investigations. All human study participants gave informed verbal consent for participation in the investigations. We sought informed verbal consent from the parents before obtaining information about minors.

RESULTS

Epidemiological Findings

Patient A

Patient A, a 13-month-old girl, developed a cough on 5 March 2011, followed by fever and loose stool (Figure 2). She visited the icddr,b Kamalapur field clinic on 9 March. At examination, she had fever (39°C) with otherwise normal findings (Table 1). A project physician classified her as having a case of suspected enteric fever and collected NPW and blood specimens from her according to the protocol for the ongoing respiratory and febrile disease surveillance [18, 19]. No organism was isolated from blood culture. The NPW specimen tested positive by rRT-PCR for influenza A (H5N1) on 13 March. On 14 March, the patient's parents reported that she had been afebrile since 11 March, but she still had cough and loose stool. Follow-up NPW specimens from patient A collected on 14 and 17 March tested positive for influenza A (H5). She was treated with oseltamivir, starting from 16 March, because she was still coughing. She never had severe illness or required hospitalization, and she recovered fully.

Seven days before her illness onset, patient A's mother bought 7 chickens from a door-to-door vendor. The vendor slaughtered, defeathered, and skinned the chickens inside patient A's home while patient A was present. Her father handled the chickens, washed his hands afterward only with water, and then held his daughter in his lap.

Patient A's great-grandmother, who had a previous diagnosis of bronchial asthma, was had fever, cough and respiratory distress during patient A's visit to her house. The great-grandmother recovered a week later after taking bronchodilators prescribed by a physician. In patient A's grandparents' village, 10 of the 31 surrounding households interviewed had sick poultry, and 8 families reported poultry deaths within the 4 weeks before patient A's illness onset. There were also reports of crow die-offs in the village, and the investigative team observed 1 sick and 5 dead crows in the village during their visit.

We interviewed 27 of 28 close contacts of patient A. Among them, only 1, a healthcare worker, reported any respiratory symptoms at the time of the interview. A NPW specimen collected from the symptomatic healthcare worker within 24 hours of symptom onset tested negative for influenza viruses. One contact, a neighbor, was not available for an interview despite repeated attempts.

Patient B

Patient B, a 31-month-old boy, experienced cough and rhinorrhoea on 1 March 2011 (Figure 2), followed by conjunctivitis, fever, vomiting, and loose stool (Table 1). On 7 March, the physician at the Kamalapur Field Clinic found the child's tonsils to be inflamed, diagnosed tonsillitis and conjunctivitis, and collected an NPW specimen, following the enhanced surveillance for influenza [10]. A blood sample was collected, and no organism was isolated from the blood culture. The NPW specimen tested positive for influenza A (H5) on 15 March (Figure 2). Patient B did not receive antiviral treatment because >7 days had elapsed since symptom onset. He did not become severely ill or require hospitalization, and he recovered uneventfully.

Ten days before his illness, patient B had touched live poultry while his mother purchased a chicken from a roaming vendor. Blood from the chicken was smeared on his hand when the chicken was eviscerated. The next morning, he cut his hand with the knife his mother was using to prepare the raw chicken meat, and his mother pressed her hand on his wound to stop the bleeding without washing her hands.

Many of their neighbors raised ducks, chickens, and quails in the yard where the child played. Of the 21 families interviewed,

Table 1. Clinical Features in 2 Human Cases of Highly Pathogenic Avian Influenza A (H5N1) Infection in Bangladesh, February–March 2011

Patient	Age, mo	Sex	Date of Illness Onset	Symptoms	Signs on Day of Clinical Visit	Chest Radiographic Findings	Duration of Symptoms, d	Date of NPW Specimen Collection
A	13	Female	5 March 2011	Fever, cough, and loose stool	Temperature, 39°C; pulse rate, 140/min; respirations, 50/min; blood pressure, 90/60 mm Hg; SpO_2, 99%; chest clear at auscultation	Normal	22	9 March 2011
B	31	Male	1 March 2011	Fever, cough, runny nose, conjunctivitis, vomiting and diarrhea	Temperature, 38.8°C; respirations, 32/min; pulse rate, 140/min; lungs clear at auscultation; SpO_2, 98%; tonsils enlarged and congested, with pus point present		12	7 March 2011

Abbreviations: NPW, nasopharyngeal wash; SpO_2, saturation of peripheral oxygen.

5 families reported ill and dead poultry during the previous 4 weeks. The mother took the child along with her to a live poultry market during the week before his illness. He did not come in contact with any person with acute respiratory illness. Households of the case patients were 1 km apart from each other, and they did not come in contact with each other during the study period. Among the 26 of 29 close contacts of patient B whom we interviewed, 4 reported developing respiratory symptoms. Three close contacts were not available for interview, but they were reportedly in good health.

Laboratory Findings

The CDC isolated HPAI H5N1 viruses both in tissue cell culture and in embryonated eggs from NPW specimens obtained from the 2 case patients and performed full genome sequencing of the isolates. The virus isolate from patient A was designated as A/Bangladesh/3233/2011 (Global Initiative on Sharing All Influenza Data [GISAID] accession Nos. EPI314772–EPI314779) and the isolate from patient B was designated as A/Bangladesh/5487/2011 (GISAID accession Nos. EPI448088–EPI448095) (Figure 3). Phylogenetic analysis of the hemagglutinin gene sequence from the 2 isolated HPAI H5N1 viruses indicated that they belonged to the clade 2.2.2.1 lineage and were closely related to HPAI H5N1 virus isolates collected from birds in Bangladesh and in neighboring countries in recent years (Figure 3).

The 2 isolated HPAI H5N1 viruses were 99% identical to each other for each gene segment. No evidence of reassortment with human influenza A viruses was identified in either isolated HPAI H5N1 viruses, and all 8 gene segments in each virus isolate were of avian origin. The viruses were sensitive to the neuraminidase inhibitors, oseltamivir, zanamivir and peramivir by functional antiviral susceptibility assays (data not shown). NPW specimens from the 6 healthy family members of the case patients, collected within 10 days after the onset of illness in the case patients, tested negative for influenza A by rRT-PCR.

Patient A met the WHO serology criteria for a confirmed H5N1 case (Table 2) [12]. Patient B did not meet the WHO criteria for seroconversion but was considered seropositive based on a microneutralization antibody titer ≥40 against A/Bangladesh/3233/2011 virus in a convalescent specimen collected >14 days after symptom onset and a titer of ≥40 (but not achieving 80, equivalent to WHO protocol criteria of 160) in the horse-red blood cell hemagglutination inhibition assay (Table 2).

Paired serum samples were obtained from the mothers of both patients and the grandmother of patient A. We could obtain only a single serum sample from the case patients' fathers and the great-grandmother of patient A, because they did not agree to provide a second blood sample. None of the case patients' family members (n = 7) or the contacts who had febrile or respiratory symptoms and consented to provide serum sample (n = 3) had any detectable virus-neutralizing antibodies or any hemagglutination inhibition specific antibodies against the H5N1 virus isolated from patient A. All serum samples from this study were seronegative by both microneutralization and hemagglutination inhibition assays when tested against A/crow/BD/1061/2011 virus (H5N1 clade 2.3.2.1a).

Influenza A (H5N1) virus was detected by rRT-PCR from a pool of chicken fecal samples collected from 2 live bird markets located within 500 m of the 2 case patients' houses in Kamalapur. Phylogenetic analysis of the hemagglutinin from the isolated HPAI H5N1 virus indicated that it belonged to clade 2.2.2.1 (GISAID accession No. EPI869846). Influenza A (H5N1) virus was also detected by rRT-PCR from the fecal sample collected from a sick crow and a pooled sample of 5 dead crows in the village of patient A's grandparents. Phylogenetic analysis of the hemagglutinin from the HPAI H5N1 viruses detected in this pool indicated that it belonged to clade 2.3.2.1a (GISAID accession No. EPI869847). All other samples collected from animals or animal products during this investigation (ie, swab samples from the meat and viscera of the frozen chickens stored in the freezer [n = 7], cloacal swab samples from ducks [n = 33], oropharyngeal swab samples from chickens [n = 45] and a quail, and swab samples from the poultry sheds of households [n = 14]) tested negative for influenza A (H5N1) virus with rRT-PCR.

DISCUSSION

Our study suggests that direct contact with infected poultry was the likely source of infection for the 2 clinically mild pediatric cases of HPAI H5N1 virus infection in Dhaka detected through a population-based active surveillance, and there was no evidence of subsequent person-to-person transmission. Both case patients had illness onset within 5 days of each other and attended the same health clinic. There was no evidence that they were otherwise epidemiologically linked to each other. We could not ascertain whether they were exposed to poultry from the same source. Nevertheless, the 2 HPAI H5N1 viruses isolated from clinical specimens were identified as clade 2.2.2.1, closely related to each other and the HPAI H5N1 viruses circulating among poultry in Bangladesh during the same time period (Figure 3).

Although other members of the case families were exposed to poultry, none had any evidence of H5N1 virus infection. There was no evidence of HPAI H5N1 virus infection among the 3 identified contacts with acute respiratory illness who underwent serology. HPAI H5N1 viruses are uncommonly transmitted person to person [20]. Although in different outbreaks clusters of HPAI H5N1 virus infection have been reported, those resulted from limited, nonsustained human-to-human transmission among family members, mostly following close, prolonged, unprotected contact with severely ill patients [21, 22].

Both case patients had a history of contact with poultry in their households, 7 and 10 days before the onset of illness for

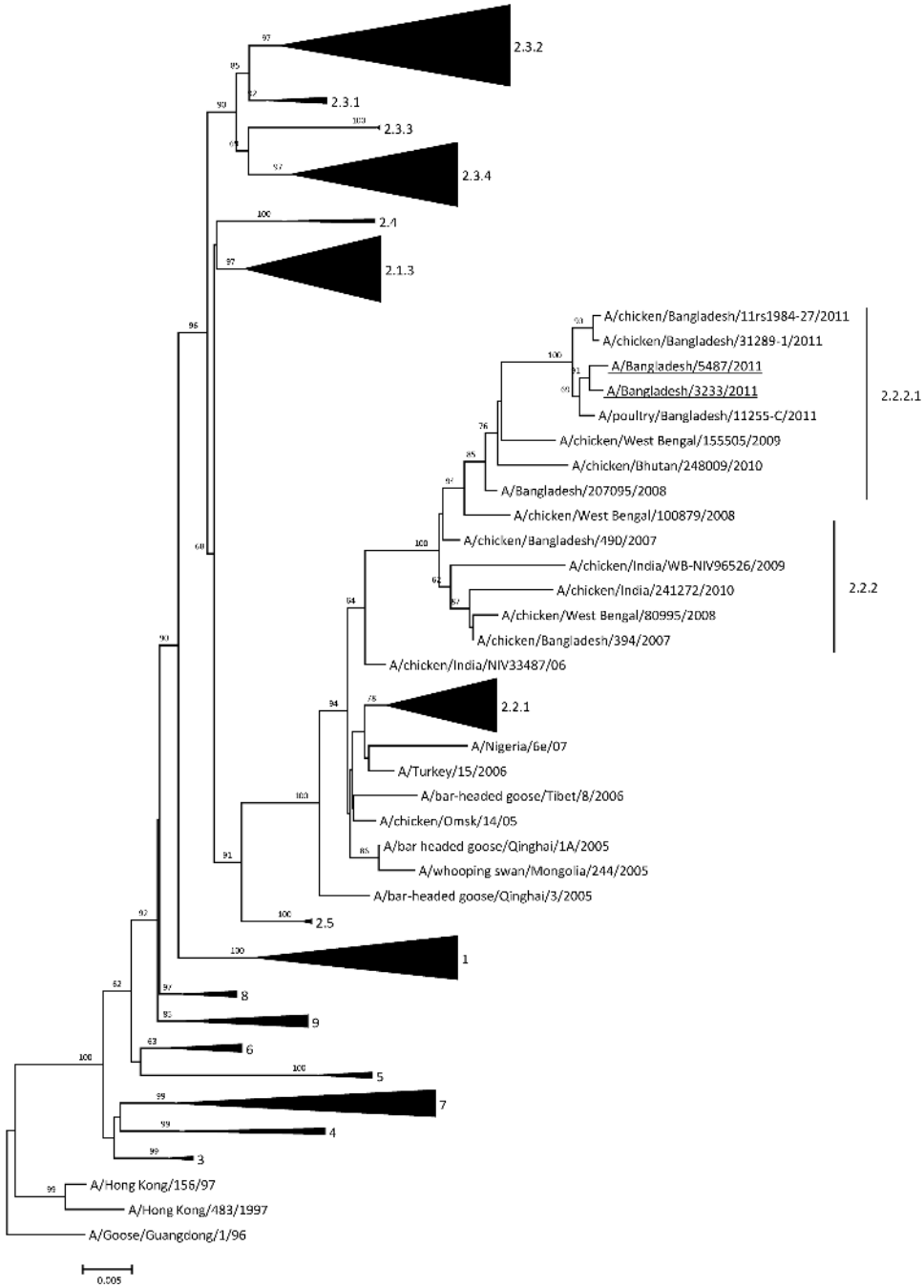

Figure 3. Neighbor-joining phylogenetic tree of H5N1 virus hemagglutinin sequences. Bootstrap values calculated from 500 replicates are shown above each branch. The viruses identified in 2 human cases are underlined.

patients A and B, respectively (Figure 2). Patient A also had possible exposure to poultry 3–5 days before her illness onset when she visited her grandparent's house, and patient B visited a live poultry market with his mother during the week before his illness onset. Visiting a live poultry market has been identified as a risk factor for HPAI H5N1 virus infection [23, 24]. Therefore, considering multiple potential exposures for these case patients, their incubation periods are estimated to be 3–10 days, consistent with findings in HPAI H5N1 case patients reported from a study in China, who had multiple exposures to poultry and had an overall median incubation period of 5 days (range, 2–7 days) and a median maximum incubation period of 11.5 days (range, 7–14 days) [25].

Although patient A met the WHO serology criteria for a confirmed HPAI H5N1 case by demonstrating 4-fold rise in antibody titers, patient B did not [12]. The first serum sample

Table 2. Results of Serological Testing of Case Patients, Their Family Members, and Symptomatic Contacts for Antibodies to Highly Pathogenic Avian Influenza A (H5N1) Virus Clades 2.2.2.1 and 2.3.2.1a

		A/Bangladesh/3233/2011, E2			A/crow/Bangladesh/1061/2011, E2		
Sample Collection Date (in 2011) by Subject	Time After Onset of Illness (for Patients), d	Microneutralization Geometric Mean Titer	Hemagglutination Inhibition Geometric Mean Titer	Serology Result	Microneutralization Geometric Mean Titer	Hemagglutination Inhibition Geometric Mean Titer	A/crow/Bangladesh/1061/2011 (H5N1 clade 2.3.2.1a): Serology Result
Patient A							
14 March	9	5	5	Seroconversion	5	5	Seronegative
17 March	12	5	5		Not tested	Not tested	
24 March	19	8	10		Not tested	Not tested	
6 April	32	40	10		5	5	
Father of patient A							
14 March	…	5	5	Seronegative	5	5	Seronegative
Mother of patient A							
14 March	…	5	5	Seronegative	5	5	Seronegative
10) April		5	5		5	5	
Great-grandmother of patient A							
18 March	…	6	5	Seronegative	5	5	Seronegative
Grandmother of patient A							
18 March	…	5	5	Seronegative	5	5	Seronegative
18 April	…	5	5		5	5	
Clinic staff contact of patient A							
27 April	…	5	5	Seronegative	Not tested	Not tested	Not tested
Patient B							
15 March	15	45	57	Seropositive	5	5	Seronegative
17 March	17	80	40		Not tested	Not tested	
7 April	38	113	40		5	5	
Mother of patient B							
15 March	…	5	6	Seronegative	5	5	Seronegative
7 April	…	5	7		5	5	
Aunt of patient B							
26 May	…	5	5	Seronegative	5	5	Seronegative
Father of patient B							
16 March	…	6	5	Seronegative	5	5	Seronegative
Neighbor contact of patient B							
9 June	…	5	5	Seronegative	Not tested	Not tested	Not tested

from patient B was collected 15 days after symptom onset and was, therefore, not an acute serum specimen; the delay between symptom onset and acute serum sample collection may explain why there was detection of <4-fold increase in H5N1 virus antibody titer when compared with the convalescent sample. Indeed, HPAI H5N1 virus neutralizing antibody titers were detectable in patient B's first serum samples. Nevertheless, not all HPAI H5N1 virus-infected patients may mount a detectable 4-fold rise in antibody titer. The 2 identified case patients described here had only moderate antibody titers after infection (Table 2) [13].

Detection of 3 cases of mild HPAI H5N1 illness through population-based surveillance since 2008 [4] suggests the platform's superior sensitivity to detect such events when compared with national sentinel site surveillance, which focuses on more severe cases. Although active population-based surveillance in this community ensured that children with acute respiratory infection are tested for influenza, only 0.03% of children aged <5 years receive care for influenzalike illness and are tested at national influenza surveillance sentinel sites [5, 7, 26, 27]. Indeed, the widespread practice of backyard poultry raising [28, 29] and HPAI H5N1 virus outbreaks occurring among poultry in commercial and backyard poultry farms throughout the country suggest that similar cases may remain undetected throughout Bangladesh in communities where there is no active population based-surveillance.

Influenza A (H1N1)pdm09 virus was in circulation among humans while these children were ill with HPAI H5N1 virus infection [30], and cocirculation of HPAI H5N1 virus and low pathogenic avian influenza A(H9N2) virus among poultry and wild birds raises the concern of reassortment between

these viruses in coinfected persons [31, 32]. In a population with extensive exposure to poultry likely to be infected with avian influenza A virus with minimal biosafety measures [29], these clinically mild cases highlight the potential for coinfection with both human and avian influenza A viruses. Without a sensitive and robust surveillance system, reassortment would be challenging to detect in its early stages. This evidence suggests that Bangladesh is a possible high-risk environment for such viral reassortment, and active influenza surveillance should continue among persons with exposure to avian influenza A viruses.

Worldwide, most surveillance for HPAI H5N1 virus infection in humans is focused on hospitalized and severe cases [33–35]. Milder cases are, therefore, less likely to be detected, investigated, and reported. Although serosurveys suggest that groups at higher risk have zero to low seropositivity, serosurveys can miss prior infections as antibody titers wane over time [36]. Repeated detection of mildly symptomatic cases in children through this population-based active surveillance and their survival suggest that the case fatality proportion reported among human cases of HPAI H5N1 virus infection might be overestimated [1]. Clinically mild HPAI H5N1 cases have been more frequently reported among young children, and the HPAI H5N1 case fatality proportion is lower in young children than in adults [37].

Our study had several limitations; we defined as contacts persons who came close to the case patients within 7 days after last identification of H5 viral RNA in NPW specimens of case patients. This might have resulted in inclusion of persons who came close to the case patients when they were not infectious as potential contacts. In addition, we were not able to collect all of the specimens from all identified close contacts, which might have reduced our ability to identify additional secondary cases, especially if contacts had mild symptoms.

Detection of these mild cases through active community surveillance suggest that additional cases might remain undetected in HPAI H5N1 endemic areas under current passive surveillance using sentinel sites. Public health authorities might want to explore the potential value of enhancing surveillance for mild illness from HPAI H5N1 virus infection among humans during the typical avian influenza season in poultry [38]. Interventions to promote safe slaughtering practice and limit close contact with poultry should be developed to reduce the risk of avian-to-human transmission of HPAI H5N1 virus in Bangladesh. The case fatality proportion of HPAI H5N1 influenza among humans should be reassessed using surveillance systems able to detect mild cases in areas where HPAI H5N1 viruses are endemic among poultry.

Notes

Acknowledgments. The icddr,b acknowledges with gratitude the commitment of the CDC to the center's research efforts and is thankful to the governments of Bangladesh, Canada, Sweden, and the United Kingdom for providing core/unrestricted support. We also thank the case patients, their family members, and the community for extending their cooperation during this investigation, and e thank W. Abdullah Brooks, Aaron Storms, and Kathy Hancock for their contribution in conducting the investigation.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Financial support. This work was supported by the Influenza Division of the CDC (cooperative agreements U01CI000628 and U51GH001209).

Supplement sponsorship. This work is part of a supplement sponsored by the Centers for Disease Control and Prevention.

Potential conflicts of interest. J. K. reports grants from Juvaris BioTherapeutics (2007–2013) and Glaxo-Smith-Kline (2009–2014), outside the submitted work. She also has US patents #7959929 (canine influenza vaccine) and #6169175 (recombinant influenza M2 construct vaccine) issued, and US patent application #20100158939 (pandemic influenza vaccine). All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. World Health Organization. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003–2015. http://www.who.int/influenza/human_animal_interface/EN_GIP_20150501CumulativeNumberH5N1cases.pdf. Accessed 30 May 2015.
2. Biswas PK, Christensen JP, Ahmed SS, et al. Avian influenza outbreaks in chickens, Bangladesh. Emerg Infect Dis **2008**; 14:1909–12.
3. World Organization for Animal Health. Update on highly pathogenic avian influenza in animals (type H5 and H7). 2017. http://www.oie.int/en/animal-health-in-the-world/update-on-avian-influenza/2011/. Accessed 15 March 2017.
4. Brooks WA, Alamgir AS, Sultana R, et al. Avian influenza virus A (H5N1), detected through routine surveillance, in child, Bangladesh. Emerg Infect Dis **2009**; 15:1311–3.
5. National Institute of Population Research and Training (NIPORT), Mitra and Associates, and ICF International. Bangladesh Demographic and Health Survey 2014. Dhaka, Bangladesh, and Rockville, Maryland: NIPORT, Mitra and Associates, and ICF International, **2014**.
6. Azziz-Baumgartner E, Alamgir AS, Rahman M, et al. Incidence of influenza-like illness and severe acute respiratory infection during three influenza seasons in Bangladesh, 2008–2010. Bull World Health Organ **2012**; 90:12–9.
7. Nasreen S, Luby SP, Brooks WA, et al. Population-based incidence of severe acute respiratory virus infections among children aged <5 years in rural Bangladesh, June–October 2010. PLoS One **2014**; 9:e89978.
8. Zaman RU, Alamgir ASM, Rahman M, et al. Influenza in outpatient ILI case-patients in national hospital-based surveillance, Bangladesh, 2007–2008. PLoS One **2009**; 4:e8452.
9. Brooks WA, Breiman RF, Goswami D, et al. Invasive pneumococcal disease burden and implications for vaccine policy in urban Bangladesh. Am J Trop Med Hyg **2007**; 77:795–801.
10. Azziz-Baumgartner E, Rahman M, Al Mamun A, et al. Early detection of pandemic (H1N1) 2009, Bangladesh. Emerg Infect Dis **2012**; 18:146–9.
11. World Health Organization. CDC protocol of real time RTPCR for influenza A (H1N1) 2009. http://www.who.int/csr/resources/publications/swineflu/CDCRealtimeRTPCR_SwineH1Assay-2009_20090430.pdf?ua=1. Accessed 30 May 2015.
12. World Health Organization. Serological diagnosis of influenza by microneutralization assay. Geneva, Switzerland: World Health Organization, **2010**. http://www.who.int/influenza/gisrs_laboratory/2010_12_06_serological_diagnosis_of_influenza_by_microneutralization_assay.pdf. Accessed 28 February 2014.
13. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol **1999**; 37:937–43.
14. Khan SU, Berman L, Haider N, et al. Investigating a crow die-off in January–February 2011 during the introduction of a new clade of highly pathogenic avian influenza virus H5N1 into Bangladesh. Arch Virol **2014**; 159:509–18.
15. Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC. Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/duck/Singapore/97 vaccine. Virus Res **2004**; 103:91–5.

16. World Health Organization. WHO case definitions for human infections with influenza A (H5N1) virus. http://www.who.int/influenza/resources/documents/case_definition2006_08_29/en/. Accessed 28 February 2014.
17. US Department of Health and Human Services, Centers for Disease Control and Prevention (CDC) and National Institutes of Health. Biosafety in microbiological and biomedical laboratories (BMBL). Washington, DC: HHS Publication, **2001:**21-1112. http://www.cdc.gov/biosafety/publications/bmbl5. Accessed 14 March 2017.
18. Brooks WA, Hossain A, Goswami D, et al. Bacteremic typhoid fever in children in an urban slum, Bangladesh. Emerg Infect Dis **2005**; 11:326–9.
19. Brooks WA, Goswami D, Rahman M, et al. Influenza is a major contributor to childhood pneumonia in a tropical developing country. Pediatr Infect Dis J **2010**; 29:216–21.
20. Imai M, Herfst S, Sorrell EM, et al. Transmission of influenza A/H5N1 viruses in mammals. Virus Res **2013**; 178:15–20.
21. Kandun IN, Wibisono H, Sedyaningsih ER, et al. Three Indonesian clusters of H5N1 virus infection in 2005. N Engl J Med **2006**; 355:2186–94.
22. Ungchusak K, Auewarakul P, Dowell SF, et al. Probable person-to-person transmission of avian influenza A (H5N1). N Engl J Med **2005**; 352:333–40.
23. Mounts AW, Kwong H, Izurieta HS, et al. Case-control study of risk factors for avian influenza A (H5N1) disease, Hong Kong, 1997. J Infect Dis **1999**; 180: 505–8.
24. Zhou L, Liao Q, Dong L, et al. Risk factors for human illness with avian influenza A (H5N1) virus infection in China. J Infect Dis **2009**; 199:1726–34.
25. Huai Y, Xiang N, Zhou L, et al. Incubation period for human cases of avian influenza A (H5N1) infection, China. Emerg Infect Dis **2008**; 14: 1819–21.
26. Bangladesh Bureau of Statistics. Population and housing census 2011: national report. Vol 1, Analytic report. Dhaka, Bangladesh: Bangladesh Bureau of Statistics, **2015**.
27. Azziz-Baumgartner E, Alamgir ASM, Rahman M, et al. Incidence of influenza-like illness and severe acute respiratory infection during three influenza seasons in Bangladesh, 2008–2010. Bull World Health Organ **2012**; 90:12–9.
28. Sultana R, Nahar N, Rimi NA, et al. Backyard poultry raising in Bangladesh: a valued resource for the villagers and a setting for zoonotic transmission of avian influenza. A qualitative study. Rural Remote Health **2012**; 12:1927.
29. Sultana R, Rimi NA, Azad S, et al. Bangladeshi backyard poultry raisers' perceptions and practices related to zoonotic transmission of avian influenza. J Infect Dev Ctries **2012**; 6:156–65.
30. World Health Organization. FluNet. **2011**. http://apps.who.int/globalatlas/dataQuery/default.asp. Accessed 14 March 2017.
31. Negovetich NJ, Feeroz MM, Jones-Engel L, et al. Live bird markets of Bangladesh: H9N2 viruses and the near absence of highly pathogenic H5N1 influenza. PLoS One **2011**; 6:e19311.
32. Shanmuganatham K, Feeroz MM, Jones-Engel L, et al. Genesis of avian influenza H9N2 in Bangladesh. Emerg Microbes Infect **2014**; 3:e88.
33. Uyeki TM. Global epidemiology of human infections with highly pathogenic avian influenza A (H5N1) viruses. Respirology **2008**; 13:S2–9.
34. Lai S, Qin Y, Cowling BJ, et al. Global epidemiology of avian influenza A H5N1 virus infection in humans, 1997–2015: a systematic review of individual case data. Lancet Infect Dis **2016**; 16:e108–18.
35. Kandun IN, Tresnaningsih E, Purba WH, et al. Factors associated with case fatality of human H5N1 virus infections in Indonesia: a case series. Lancet **2008**; 372:744–9.
36. Toner ES, Adalja AA, Nuzzo JB, Inglesby TV, Henderson DA, Burke DS. Assessment of serosurveys for H5N1. Clin Infect Dis **2013**; 56:1206–12.
37. Oner AF, Dogan N, Gasimov V, et al. H5N1 avian influenza in children. Clin Infect Dis **2012**; 55:26–32.
38. Durand LO, Glew P, Gross D, et al. Timing of influenza A(H5N1) in poultry and humans and seasonal influenza activity worldwide, 2004–2013. Emerg Infect Dis **2015**; 21:202–8.