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The first fatal human infection with highly pathogenic avian influenza A (H5N1) virus detected in Bangladesh

Previously six human cases of avian influenza A (H5N1) were identified in Bangladesh between 2008 and 2012; all cases experienced mild respiratory illness. On 12 February 2013, a 23-month old boy from Comilla was hospitalized for febrile convulsions and enrolled in a respiratory virus research study. Laboratory tests conducted on 12 March suggested that the boy had influenza A (H5) infection. This was the 7th case of A (H5N1) identified in Bangladesh. A joint IEDCR-icddr,b outbreak response team investigated to explore the child's exposure history and clinical outcome and to look for additional cases in the community. Several chickens raised in the family's backyard became sick a few days before he became ill; they were slaughtered and the boy played with the carcasses. The child died on February 18 with evidence of severe pneumonia, meningitis and disseminated intravascular coagulation; no other human cases were identified. Viral sequences from the child and



slaughtered chicken confirmed that both were infected with influenza A (H5N1), clade 2.3.2.1. This first identified death from avian influenza infection in Bangladesh presented primarily with neurologic rather than respiratory symptoms and was apparently infected through contact with sick chickens. Clinicians should consider avian influenza infection in the differential diagnosis for any patient with severe respiratory or neurologic infection with a history of contact with sick poultry. All such patients should be treated with oseltamivir.

From 2003 through 29 August 2013, 637 laboratory-confirmed human cases of avian influenza A (H5N1) virus infection have been reported to the World Health Organization from 15 countries with a case-fatality of 59% (1). Influenza A (H5N1) virus has circulated widely among poultry in Bangladesh since 2007 (2). However, only six human cases of influenza A (H5N1) virus infection were detected in Bangladesh during January 2008 to March 2012. All cases suffered from mild respiratory symptoms and were detected through intensive, active surveillance for respiratory infections among communities and poultry market workers (3,4). The Institute of Epidemiology, Disease Control and Research (IEDCR), Ministry of Health and Family Welfare, and icddr,b jointly collaborate in hospital-based surveillance for influenza illness but this surveillance system has not detected any human infections with influenza A (H5N1).

A 23-month old boy from Chaudhagram sub-district, Comilla was admitted for febrile convulsions at Comilla Medical College Hospital on 12 February 2013. An ongoing icddr,b study investigating the association between vitamin D deficiency and respiratory illness among children enrolled the boy as a control subject, as he was not admitted for respiratory complaints. As part of study enrollment, throat and nasopharyngeal samples were obtained to rule out infection with respiratory viruses using real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays. On 12 March 2013, test results from icddr,b's virology laboratory indicated the boy had an influenza A (H5) infection; these results were subsequently confirmed at the National Influenza Center laboratory at IEDCR. A collaborative team from IEDCR and icddr,b investigated to ascertain the child's clinical and exposure histories and identify any additional cases in the community.

A team of epidemiologists, clinicians, virologists, anthropologists and veterinarians conducted a detailed investigation of the case. During the visit to the child's home on 22 March, the outbreak investigation team interviewed the parents and other household members to determine his symptoms, possible exposures, and examine any medical records. Epidemiologists and clinicians also interviewed physicians who treated the child and reviewed hospital records to obtain additional treatment and clinical information.

The boy's parents reported that he developed a fever on the evening of February

11 and they treated him with paracetamol. The next morning the mother observed abnormal rolling of his eyeballs and stiffness in his body so she took him to the local Chaudagram sub-district health complex. Treatment records show that the boy received treatment for febrile convulsions with diazepam during his visit. After the convulsions were controlled, he was referred to the Comilla Medical College Hospital, where he was enrolled in the icddr,b study the same day. Due to crowding in the hospital wards and because his condition seemed to be stable, the child's parents decided to take him back home on 12 February.

He was readmitted on 13 February to the Chaudagram sub-district health complex for diarrhoea and treated with intravenous fluid for dehydration and ceftriaxone, metronidazole and nitazoxanide. He developed shortness of breath and was given oxygen. A chest x-ray conducted on 14 February revealed opacities in the both upper lung fields (Figure 1). On February 15, the boy was referred to a tertiary-level treatment facility.

On 15 February, the parents transferred the child to a private hospital in Comilla City. His breathing difficulty continued and he was noted to have peripheral oedema and peripheral cyanosis. A second digital x-ray confirmed opacities in both upper lung fields suggesting pneumonia. As the child's condition deteriorated he was transported to Dhaka and admitted at a private health care facility where ventilator support was begun at 4:30 am.

The child gradually became unconscious. His difficulty breathing persisted and he had bloody discharge from his nostrils. He was noted to have thrombocytopenia and anaemia and received a packed red blood cell transfusion to counter his blood loss. He was also noted to have leucocytosis

Figure 1: Digital radiography on 15 February demonstrating opacities in both the lung fields



(count: 30,100/mm³; 83% neutrophils, 14% lymphocytes), suggesting a secondary bacterial infection. An examination of his cerebrospinal fluid revealed an elevated cell count (40 cells/cu mm; 60% neutrophils, 40% lymphocytes), reduced sugar (2.5 mmol/L) and elevated protein (200 mg/dl), suggesting bacterial meningitis. He developed bluish-black discoloration of the skin all over the body and was noted to have disseminated intravascular coagulation on the night of 17 February. Based on the very poor prognosis given by the treating physicians and the family's financial constraints, the parents decided to discontinue treatment and return home. The boy died en route from Dhaka to Comilla on 18 February, at around 1:30 am.

None of the child's family or household members developed any symptoms of illness before, during or after the boy got sick. However, they reported that several of the 35 chickens they raised in their backyard became sick and died a few days prior to the onset of the child's illness. When several chickens died, the household members slaughtered the remaining chickens, some of which were apparently sick. While the mother processed one of the sick chickens, the boy played with the liver, gizzard, uterus with developing eggs, and other parts of the chicken. The family consumed some of the chickens immediately and stored the rest in a freezer. The parents provided one frozen chicken to the investigation team for examination and virologic testing.

The boy's biological specimens and tissues from the frozen chicken were sent to the reference laboratory of the Influenza Division at the US Centers for Disease Control and Prevention (CDC), in Atlanta, Georgia, USA, for further testing and virus isolation. On April 6, CDC confirmed the presence of influenza A (H5N1) virus, clade 2.3.2.1, in the child's respiratory swabs. Influenza A (H5N1) virus, clade 2.3.2.1 was also isolated from tissues from the chicken tissues and the genetic sequence was 99.9% homologous to the viral sequence from the child's specimens.

Reported by: Institute of Epidemiology, Disease Control & Research (IEDCR), Ministry of Health and Family Welfare, Government of The People's Republic of Bangladesh; and Surveillance and Outbreak Investigation Research Group, Centre for Communicable Diseases, icddr,b

Supported by: Centers for Disease Control and Prevention, Atlanta, USA

Comments

This report describes the first human fatality from influenza A (H5N1) identified in Bangladesh. Epidemiologic and virologic evidence strongly suggest that this boy was infected from contact with infected poultry. This finding suggests that influenza A (H5N1) continues to circulate in backyard poultry and poses a risk to human health. Exposure to the organs and meat of infected poultry has been implicated in other human cases of illness (5); however, eating cooked meat from infected poultry is considered safe (6).

The vast majority of human influenza A (H5N1) cases reported worldwide have presented for care of respiratory disease. However, this child presented first with what were diagnosed as febrile convulsions; signs of respiratory distress and pneumonia was not diagnosed until later in his illness. The case was detected by chance when he was enrolled in a research study because hospital-based surveillance activities for influenza were developed to capture primary respiratory illness. Although atypical of reported human infections with influenza A (H5N1), neurological illness has been reported from human cases identified in other countries (7) and has been noted to be a more common clinical manifestation of influenza A (H5N1) infection than respiratory symptoms in geese and ducks (8).

This child's death from influenza A (H5N1) was preventable. First, the child's infection could have been prevented by limiting his contact with the blood and organs of the infected poultry. Backyard poultry raisers in Bangladesh frequently have close contact with sick poultry and they are often slaughtered when they are ill so the family can recoup some of their financial investment (9). Because of financial incentives to slaughter sick poultry, and the fact that the majority of poultry illnesses are not transmissible to humans, changing this behaviour will be very difficult for backyard poultry raisers. However, interventions to limit the participation of children in the slaughtering of sick poultry could reduce the risk of infection to this age group, particularly because children may be likely to put their contaminated hands in their mouths or noses.

In addition, after the child became ill, early treatment with oseltamivir may have prevented his death. The government has distributed oseltamivir to all districts in Bangladesh and it can be requested by providers to treat suspected cases of human infection with avian influenza. Providers should consider avian influenza infection in the differential diagnosis for any person with severe febrile illness with respiratory or neurological signs and symptoms who has a recent history of slaughtering or other close contact with sick poultry. Suspected cases should be treated immediately with oseltamivir (10).

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Alert for healthcare providers

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and travelers returning from the Middle East

In April 2012, a new virus was identified as the cause of severe respiratory illness among residents and visitors to the Middle East. The virus has been named the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and as of September 7, 2013, there have been 114 laboratory-confirmed cases of MERS-CoV; approximately 50% of cases have died. Additional information and updates on the MERS-CoV situation are available at the World Health Organization website: http://www.who.int/csr/don/2013_09_07/en/index.html. Researchers are attempting to identify the animal reservoir for the virus. However, person-to-person transmission has been repeatedly identified and many cases are among healthcare workers.

As Hajj pilgrims begin returning to Bangladesh, healthcare providers should be on alert for patients presenting to healthcare providers with severe respiratory illness within 2 weeks of returning from the Middle East. If you identify a patient with fever ($>38^{\circ}\text{C}$), cough and severe respiratory difficulty with onset within 2 weeks of returning from the Middle East, please call the Institute of Epidemiology Disease Control and Research (IEDCR) at 01937110011 or 01937000011 for diagnostic support and clinical advice. Patients suspected to have MERS-CoV should be separated from other patients and hospital visitors as much as possible. Healthcare workers and family members caring for these patients should wear surgical masks and practice frequent handwashing with soap to reduce the risk of transmission.

Avian influenza virus surveillance at live bird markets in Bangladesh, 2007-2012

Highly pathogenic avian influenza (HPAI) A virus (H5N1) has caused repeated outbreaks in chickens raised on both commercial and backyard farms in Bangladesh. The first H5N1 outbreak was identified in poultry on March 2007 and there have been >500 reported outbreaks to date. A total of seven human H5N1 cases have also been reported, including one death. In 2007, icddr,b began avian influenza surveillance in live bird markets (LBMs) to assess avian influenza virus circulation in waterfowl and the environment of LBMs. From October 2007 to December 2012, a total 4,258 waterfowl and 625 pooled environmental samples were tested using real-time reverse transcriptase polymerase chain reaction (rRT-PCR) to detect avian influenza A virus RNA. Samples that tested positive for influenza A were further subtyped for H5-specific RNA. We detected RNA for influenza A virus in 229 (5%) waterfowl and H5 subtype-specific RNA in 90 (2%) waterfowl. Among pooled environmental samples, 280 (49%) were confirmed positive for influenza A virus RNA and 141 (23%) for H5 subtype. Surveillance findings suggest avian influenza A viruses, including the H5 subtype, circulate in domestic waterfowl and environments of LBMs in Bangladesh. LBMs could be targeted for interventions that include regular disinfection and improved biosecurity practices to reduce avian influenza virus transmission and contamination.

Highly pathogenic avian influenza (HPAI) A virus (H5N1) is a serious zoonotic pathogen that poses a threat to human health and the poultry industry. H5N1 has caused a large number of outbreaks in poultry in Asia, Europe and Africa (1). Since 2003, 633 cases of H5N1 infection in humans have been identified, with a case fatality rate of 59% (2). H5N1 was first reported in poultry in Bangladesh in March 2007 and there have been >500 reported H5N1 outbreaks to date, 90% of which have occurred on commercial poultry farms (3). Seven human H5N1 cases, including one death, have been reported in Bangladesh (2,4,5). Although flock mortality rates due to H5N1 infection reach up to 100%, ducks often carry and shed H5N1 without showing any signs of severe illness (6,7). Thus, ducks may act as a reservoir for sustaining and perpetuating H5N1 and transmit infection to other susceptible hosts, particularly chickens (8). Live bird markets (LBM) are a potential source for H5N1 transmission in many Asian countries including Vietnam, Thailand, Egypt, Indonesia, Hong Kong, China and Cambodia (9-15). In Bangladesh, poultry are reared in backyard (flock size: 1-100), small-scale (flock size: 101-500) and commercial (flock size: >500) farms. In backyard farms, multiple poultry species including chickens ducks, geese,

pigeons and quail are reared together, which facilitates transmission among species. Urban markets trade poultry daily and peri-urban markets typically trade poultry twice-weekly and conduct both retail and wholesale trade. Peri-urban markets are those located in areas surrounding cities. Multiple poultry species are typically sold together in urban and peri-urban LBMs, promoting cross-species avian influenza transmission. Assessment of avian influenza virus circulation in waterfowl is important for gaining a better understanding about their role as a reservoir host in the transmission of avian influenza to susceptible poultry species. We conducted surveillance in LBMs to identify avian influenza viruses in waterfowl and environmental samples.

In October 2007, icddr,b began avian influenza surveillance in three peri-urban LBMs in sub-districts of Bangladesh: Chittagong, Rajshahi and Netrokona. Markets were selected based on the high population density of chickens and domestic waterfowl in surrounding areas, extensive interactions between domestic and migratory waterfowl, and proximity to international borders and land ports. In January 2011, we added a peri-urban LBM in the sub-district of Dinajpur. Surveillance teams visited the LBMs monthly to collect samples from waterfowl (ducks and geese). Each month, 20 waterfowl were selected by convenience sampling from each of the four peri-urban LBMs. No more than four waterfowl were selected from any individual poultry raiser. Cloacal swabs were collected from healthy waterfowl. For waterfowl showing signs of illness, both cloacal and tracheal swabs were collected. The poultry raisers of sampled waterfowl were interviewed using a structured questionnaire that included questions about poultry demographics, poultry husbandry, flock size, individual health status of birds, and any poultry mortality in flocks during the preceding seven days. In May 2009, the four peri-urban LBMs began to collect environmental samples and in May 2011, environmental surveillance was extended to the 16 urban LBMs in Dhaka City. Swabs were collected from seven sources (poultry droppings, cages, feed, water, slaughtering sites, market floors and drains) to prepare a pooled environmental sample from each of the 20 LBMs once monthly. Swab samples from individual waterfowl were kept in 2 ml cryovials containing 1 ml viral transport medium and pooled environmental samples were kept in 50 ml falcon tubes containing 10 ml viral transport medium. Individual samples and pooled environmental samples were placed in a cold box and maintained at 2-4°C for up to 72 hours at field sites before being transported to icddr,b where they were transferred to a -80° C laboratory freezer. The icddr,b virology laboratory tested all samples using real-time reverse transcriptase polymerase chain reaction (rRT-PCR) to detect avian influenza A virus RNA. Samples confirmed positive for influenza A were further subtyped for H5-specific RNA, as previously described (16). We used the chi-square test and bivariate analysis to identify demographics and exposure variables associated with laboratory-confirmed test results for influenza A and H5 subtype.

Swab samples were collected from 4,258 waterfowl, the majority of which were ducks (88%). Of the sampled waterfowl, 3,679 (86%) were older than six months and 4,081 (96%) appeared to be healthy when sampled. Most (93%) of the sampled waterfowl were raised on backyard farms where waterfowl were typically allowed to scavenge freely during the day and given a small amount of supplemental feed in the mornings and evenings. The mean size of backyard poultry flocks was 16 (range: 1-100) birds. All sampled waterfowl were domesticated except for one wild bird. Thirty-five percent of the waterfowl samples were collected during colder months (November-February) (Table 1). Poultry mortality during the seven days preceding sample collection was less than 1% during the surveillance period.

Table 1: Demographics and detection of influenza virus type A and H5 subtype among waterfowl from peri-urban live bird markets, Bangladesh, October 2007-December 2012

Characteristics	Sample no. (%)	rRT-PCR positive sample no. (%)	
		Influenza virus A (all subtypes)	H5 subtype
Waterfowl type			
Ducks	3,759 (88.3)	224 (5.9)	87 (2.3)*
Geese	499 (11.7)	5 (1.0)	3 (0.6)
Age of waterfowl			
Juvenile	580 (13.6)	26 (4.5)	9 (1.6)
Adult	3,678 (86.4)	203 (5.5)	81 (2.2)
Farm type			
Backyard (flock size: 1-100)	3,975 (93.3), 16 [†]	202 (5.1)	89 (2.2)
Small scale (flock size: 101-500)	211 (4.9), 116 [†]	21 (9.9)*	0 (0.0)
Commercial (flock size: >500)	72 (1.7), 347 [†]	6 (8.3)*	1 (1.4)
Health status of waterfowl			
Apparently healthy	4,081 (95.8)	224 (5.5)	88 (2.1)
Sick	117 (4.1)	5 (2.8)	2 (1.1)
Seasons in which sampling conducted			
Summer (March-June)	1,360 (31.9)	34 (2.5)	21 (1.5)
Monsoon (July-October)	1,420 (33.3)	90 (6.3)*	38 (2.7)
Winter (November-February)	1,478 (34.7)	105 (7.1)*	31 (2.1)
*p<0.05; † mean flock size			

During the surveillance period, 229 (5%) waterfowl tested positive for influenza A viral RNA and 90 (2%) waterfowl had H5 subtype-specific RNA (Figure 1). The proportion of waterfowl testing positive for H5 subtype varied from year to year ($p < 0.01$) and was highest in 2012 (Table 2). The proportion of waterfowl specimens positive for H5 subtype varied from one site to another ($p < 0.01$) and was highest at the LBM in Chittagong (Table 3). Specimens from ducks were more likely to be positive for H5 subtype than those from geese (odds ratio=3.9, 95% confidence interval: 1.3-19.4). The highest proportion (2.2%) of waterfowl samples testing positive for H5 subtype were those collected from backyard farms. The proportion of waterfowl specimens testing positive for influenza virus type A was highest (7%) for those collected during the winter (December-February) and were higher than those collected in the other two seasons ($p < 0.05$). However, no statistically significant seasonal differences were observed for the proportion of specimens testing positive for H5 subtype (Table 3).

Figure 1: Summary of influenza surveillance findings in waterfowl from Netrokona, Rajshahi, Dinajpur* and Chittagong live bird markets (October 2007–December 2012)

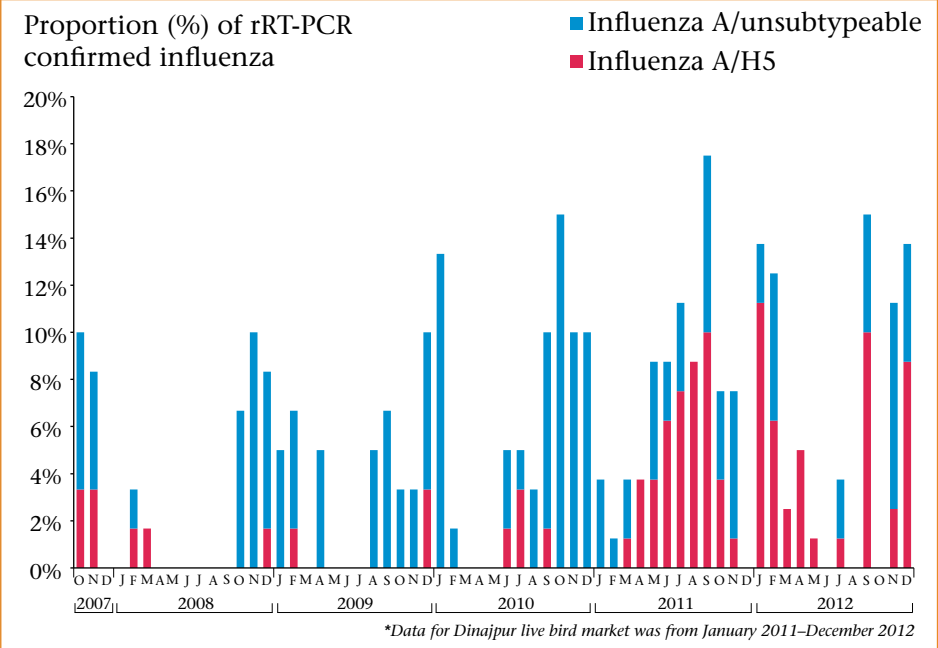


Table 2: Detection of influenza virus type A and H5 subtype in waterfowl from peri-urban live bird markets in Bangladesh by year (October 2007–December 2012)

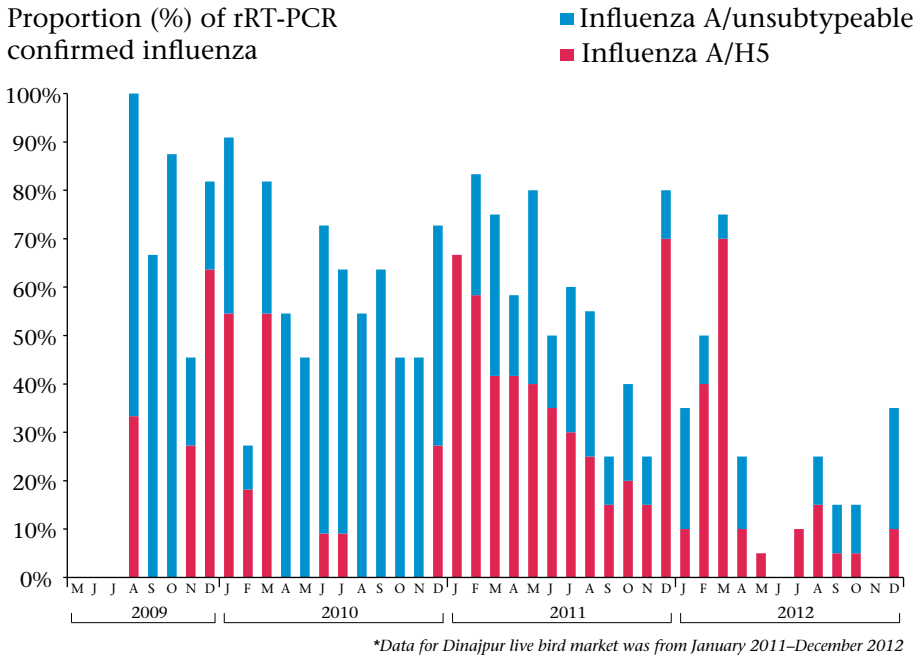
Year	Total no. of collected swabs	No. of samples positive for influenza virus type A (%)	No. of samples positive for H5 subtype (%)	No. of samples positive for influenza A/ unsubtypeable (%)
2007	180	11 (6.1)	4 (2.2)	7 (3.9)
2008	718	18 (2.5)	3 (0.4)	15 (2.0)
2009	720	27 (3.8)	3 (0.4)	24 (3.3)
2010	720	44 (6.1)	4 (0.5)	40 (5.5)
2011	960	66 (6.9)	37 (3.8)	29 (3.0)
2012	960	63 (6.6)	39 (4.0)	24 (2.5)

Table 3: Detection of influenza virus type A and H5 subtype in waterfowl from the peri-urban live bird markets by district (October 2007–December 2012)

Surveil- lance sites	Total collected swabs	No. of sample positive for influenza virus type A (%)	No. of sample positive for H5 subtype (%)	No. of sample positive for influenza A/ unsubtypeable (%)
Netrokona	1,258	69 (5.5)	9 (0.7)	60 (4.8)
Rajshahi	1,260	70 (5.5)	20 (1.6)	50 (4.0)
Dinajpur	480	20 (4.2)	14 (2.9)	6 (1.3)
Chittagong	1,260	70 (5.5)	47 (3.7)	23 (1.8)

From May 2009 to December 2012, a total of 625 pooled environmental samples were tested for influenza A virus and H5 subtype. Among the tested samples, 280 (49%) were positive for influenza virus type A and 141 (23%) were positive for H5 subtype (Figure 2). Environmental samples collected from Dhaka City LBMs were five times more likely to be positive for H5 subtype than those from peri-urban LBMs (95% confidence interval: 2.9-12.4). The proportion of environmental specimens testing positive for H5 subtype was highest (31%) for those collected in the winter and were higher than those collected in the other two seasons ($p < 0.01$).

Figure 2: Summary of influenza A surveillance findings from pooled environmental samples from Dhaka, Netrokona, Rajshahi, Dinajpur* and Chittagong live bird markets (May 2009–December 2012)



Reported by: Zoonotic Diseases Research Group, Centre for Communicable Diseases, icddr,b

Supported by: Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA, USA; Institute of Epidemiology, Disease Control and Research, Ministry of Health and Family Welfare and Department of Livestock Services, Ministry of Fisheries and Livestock, Government of the People’s Republic of Bangladesh

Comments

Surveillance findings from 2007-2012 suggest that influenza A viruses, including the H5 subtype, circulate in domestic waterfowl and in the environments of LBMs in Bangladesh. Over the study period, surveillance identified H5 subtype circulation in waterfowl, however a clear seasonal trend in H5 circulation was not detected. Studies from Southeast Asia indicate that H5N1 circulation in birds peaks during winter months (17). Compared to waterfowl specimens collected during the first four years of surveillance, a greater proportion of waterfowl specimens were found to be positive for H5 subtype in 2011 and 2012. This increase could be due to the introduction

of new clades or an evolution of influenza viruses in poultry in Bangladesh. In fact, in 2011, a new clade, 2.3.2.1, was detected in crows, quail and ducks in Bangladesh (18). Most sampled ducks that were positive for H5 subtype were apparently healthy when samples were collected. The role of ducks in the epidemiology of avian influenza is important as they can carry and shed influenza A viruses, including H5N1, without having clinical signs of severe infection. Epidemiological studies suggest that asymptomatic ducks could be H5N1 carriers, maintaining, perpetuating and transmitting H5N1 to other susceptible avian hosts (6,19). Ducks raised on backyard farms appear to be responsible for spreading avian influenza in the environment and to backyard chickens (8,20). We identified H5 subtype more often in ducks from backyard flocks than ducks from small-scale and commercial farms. In Bangladesh, approximately 50% of chickens are reared on backyard farms where they are typically allowed to live together with ducks (21). These backyard poultry rearing practices could promote H5N1 transmission from ducks to chickens. LBMs may act as a potential source of H5N1 transmission to chickens and may increase the risk of avian-to-avian and avian-to-human transmission. Domestic waterfowl are typically reared with poultry and poultry raisers and local vendors sell chickens, quail, and pigeons alongside waterfowl such as ducks and geese at LBMs. The co-existence of multiple species of birds on backyard farms and in LBMs may promote a suitable environment for sustaining, perpetuating and transmitting avian influenza viruses among poultry in Bangladesh. Surveillance of avian influenza circulation in LBMs from several countries suggests LBMs are potential sources for avian influenza virus transmission (9,10,12-14). Our surveillance data, together with government reports (1) on H5N1 outbreaks in poultry suggest consistent circulation of H5N1 in poultry and LBMs since 2007.

This study is subject to several limitations. Because of the small number of waterfowl kept for sale, we used convenience sampling to select waterfowl. In this study, we were only able to sample a small number of waterfowl reared on small-scale and commercial farms and therefore may have underestimated overall avian influenza virus circulation. Finally, these surveillance data were only collected in five of the 64 districts in Bangladesh.

LBM-based surveillance for avian influenza viruses should be continued and expanded to elucidate the epidemiology of avian influenza in domestic poultry, monitor trends, and detect novel influenza viruses of public health importance. LBMs should be targeted for interventions including regular disinfection and improved biosecurity practices to reduce H5N1 transmission and contamination.

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Improving estimates of diarrhoea prevalence among Bangladeshi children from survey data

Diarrhoea is one of the leading causes of morbidity among children under 5 years and a major cause of childhood mortality in Bangladesh. Accurate estimates of disease burden are important for prioritizing limited resources for disease prevention. Previous studies have shown that the reported prevalence of diarrhoea is underestimated when long recall periods are used. We aimed to improve the accuracy of the estimate of diarrhoea prevalence from a longitudinal study among children under 5 years of age in Bangladesh. We developed a methodology to make a more accurate estimate of diarrhoea prevalence collected from a two-week recall period by using two-day recall data. Using this methodology, we estimated that over the period of two years, 10,176 episodes of diarrhoea had occurred, though only 3,927 episodes were reported. Thus, using a recall period of two weeks resulted in an estimated diarrhoea prevalence of 14% whereas using the proposed methodology resulted in an estimated diarrhoea prevalence of 37%. These results demonstrate that collecting both two-day and two-week recall data would result in a more accurate measurement of diarrhoea prevalence. These results should make policymakers cautious of diarrhoea prevalence estimates based solely on two-week recall data.

Globally, diarrhoea is the second leading cause of death among children under the age of five years, causing 1.5 million deaths each year (1). Although mortality from diarrhoea has decreased over the past two decades, it remains one of the leading causes of morbidity among children under five (2), and in Bangladesh, it remains a major cause of childhood mortality (3). In low income countries like Bangladesh, where public health resources are limited, an accurate measure of diarrhoea in children is important to identify how resources should be utilized to decrease mortality and morbidity of children (4). This is increasingly highlighted by the World Health Organization (WHO) as countries look to measure their progress towards meeting the Millennium Development Goals (5).

The prevalence of diarrhoea among children is commonly measured using data from surveys. Data are collected by asking the primary caregiver, usually the mother, to recall symptoms of diarrhoea among her children for a specified time period varying from one day to two weeks (6-9). Investigators often opt for a longer recall period because as the recall period increases the required sample size for the study decreases thus saving resources (10).

However, several studies have shown that when caregivers are asked to recall an episode of diarrhoea, the estimated frequency of diarrhoeal episodes underestimates the actual frequency more as the recall period increases, suggesting that caregivers may forget episodes if the recall period is too long (4,11-13). Evidence suggests that a two-day recall period produces accurate estimates and that estimates based on a two-week recall period are low due to underreporting (9-12).

The objective of this study was to more accurately estimate the proportion of children who suffer from diarrhoea for a two-week recall period in Bangladesh.

Sanitation, Hygiene, Education, and Water Supply, Bangladesh (SHEWA-B) was one of the largest intensive hand-washing, sanitation, and water-quality promotion programmes implemented in a low-income country (14). icddr,b conducted an assessment of the SHEWA-B programme to measure the effectiveness of the programme interventions on key hygiene behaviours and practices. The complete data collection methods of SHEWA-B are described elsewhere (14). One of the data collection activities of SHEWA-B was a longitudinal survey of diarrhoeal disease in children under the age of five years. This survey was conducted in 68 sub-districts of 19 districts in Bangladesh.

Once a month between September 2007 to August 2009, field workers used a structured questionnaire to interview primary caregivers of children under five years in 1,000 randomly selected households and asked if the children had experienced an episode of diarrhoea (defined as three or more loose stools within a 24 hour period) in the preceding two days and the preceding two weeks. If caregivers reported that children had diarrhoea in the preceding two weeks they were asked to report how many days the diarrhoea lasted.

Using this information, we calculated the proportion of children reported to have a diarrhoeal episode in the past two days and in the past two weeks. We assumed that the prevalence for the two-week recall period was an underestimate, so we used the prevalence from the two-day recall to make a more accurate estimate of the two-week recall prevalence using the following methodology:

First, we created a probability distribution of the duration of diarrhoeal episodes reported by caregivers during the two-week recall period. Next, we estimated the incidence of diarrhoea for each day of the two-day recall period using the prevalence of diarrhoea obtained from the two-day recall data and the distribution of the duration of reported episodes from the survey. We then recalculated the two-week diarrhoea prevalence estimate by using the incidence estimated from the two-day recall period data. Finally, we compared the reported diarrhoea prevalence from the two-week recall data with the recalculated diarrhoea prevalence to determine the recall error,

using the following formula:

$$\text{Recall Error} = \frac{P_{2 \text{ weeks (recalculated)}} - P_{2 \text{ weeks (reported)}}}{P_{2 \text{ weeks (recalculated)}}} \times 100$$

where,

$P_{2 \text{ weeks (reported)}}$ = reported prevalence for a two-week recall period from the survey data

$P_{2 \text{ weeks (recalculated)}}$ = recalculated prevalence for the two-week recall period

Our analysis included data from 24 visits to each of 1,289 children in 1,000 households, which includes 55,798 child-weeks of observation. The mean age of the children in the study was 29 months (standard deviation=13 months). There were 2,796 episodes of diarrhoea reported in the two days preceding the survey from 55,798 child-days of observation, giving a 10% diarrhoea prevalence from a two-day recall period. When asked to recall diarrhoeal episodes in the previous two weeks, caregivers reported 3,927 episodes from 390,586 child-days of observation, resulting in a 14% diarrhoea prevalence from a two-week recall period. Based on the reported data, we found that 88% of the diarrhoea cases lasted for at least two days, 59% for at least 3 days, and 35% for at least 4 days (Figure 1). We estimated that on average 26 new diarrhoeal episodes occurred per day among the 1,289 children, giving an incidence of .02 diarrhoeal episodes per day. Using this methodology, we estimated that 10,176 episodes of diarrhoea had occurred in the two weeks preceding the survey, and that a more accurate estimate of diarrhoea prevalence for this time period was 37% (Figure 2). Therefore, we estimate that the recall error in the SHEWA-B programme was 61%, meaning that SHEWA-B, caregivers reported 61% fewer episodes of diarrhoea than actually occurred when asked to recall diarrhoeal episodes from a two-week recall period.

Reported by: Training Support Group and Water, Sanitation and Hygiene Research Group, Centre for Communicable Diseases, icddr,b

Supported by: United Nations Children's Fund, Department for International Development, UK and Government of the People's Republic of Bangladesh

Comments

In similar studies, Zafar *et al.* estimated that diarrhoeal episodes in Guatemala were underestimated by 31% when comparing a six-day recall period to a two-day recall period (4), and Melo *et al.* estimated that episodes in Brazil were underestimated by 70% using a four-week recall period (8).

Demographic health surveys typically use two-week recall periods for data collection about diarrhoeal episodes. In 2011, the Bangladesh Demographic

Figure 1: Distribution of diarrhoea episode duration (in days) for children under five years in rural Bangladesh obtained from SHEWA-B programme data using a two-week recall period, 2007-2009

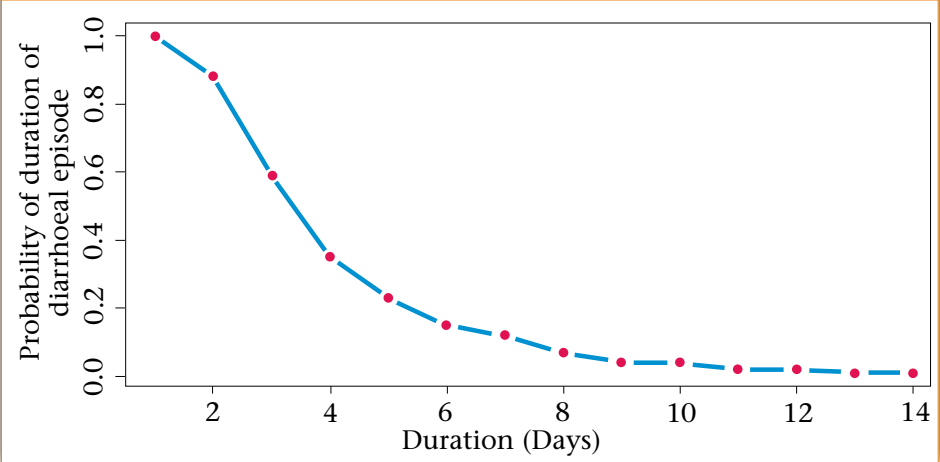
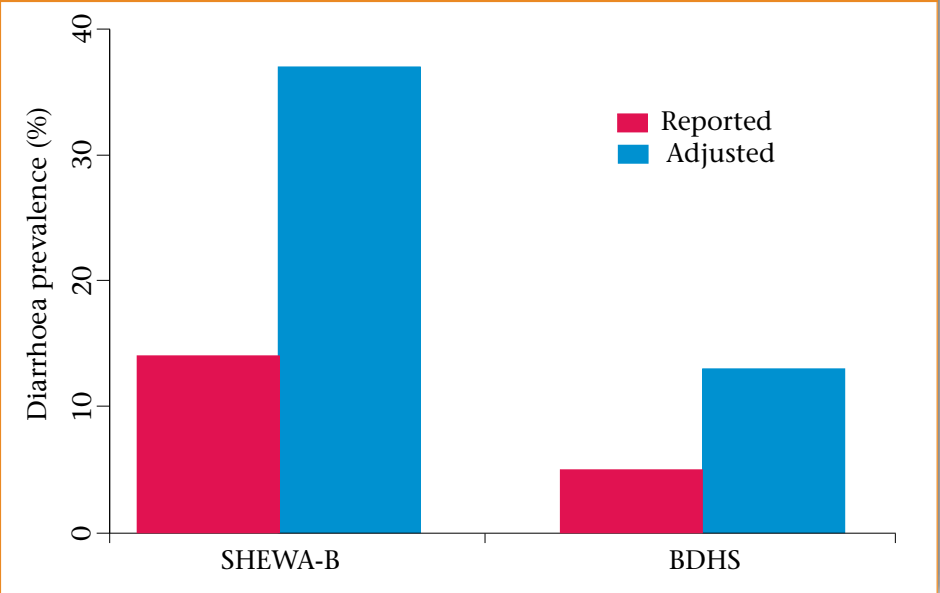


Figure 2: Reported and adjusted diarrhoea prevalence of children under 5 years of age from SHEWA-B (2007-2009) and Bangladesh Demographic and Health Survey (2011) data



and Health Survey (BDHS) used a two-week recall period to estimate the prevalence of diarrhoea. BDHS reported that 5% of children under 5 years of age had diarrhoea in the two weeks prior to the survey (3). However, if this estimate is adjusted using the recall error from the SHEWA-B study, we conclude that a more accurate estimate would be 13% (Figure 2). This demonstrates that childhood morbidity from diarrhoea in Bangladesh may be a substantially greater problem than has previously been reported.

The main limitation of this study is that these data were collected from only rural areas of Bangladesh and the recall error for these areas may not be representative of the entire country. For this reason, the adjusted BDHS diarrhoea prevalence might either overestimate or underestimate the true prevalence because BDHS data are collected from rural and urban areas and are considered to be representative of the whole population in Bangladesh. Nonetheless, given our findings and those of other researchers, the reported diarrhoea prevalence from the BDHS survey data are almost certainly underestimates of the true prevalence of diarrhoea.

Researchers using surveys to estimate prevalence of diarrhoea should consider collecting data using both two-day and two-week recall periods and then adjust estimates from the two-week recall data using the methodology described in this study to increase the accuracy of estimates of diarrhoea prevalence. During the next BDHS, public health officials should consider including a two-day recall period for diarrhoea to the two-week recall period to improve the estimate of diarrhoea prevalence in Bangladesh. Public health officials and policymakers throughout Bangladesh should interpret diarrhoea prevalence estimates based solely on two-week recall data cautiously and account for the fact that they may be substantially underestimated when planning interventions and services.

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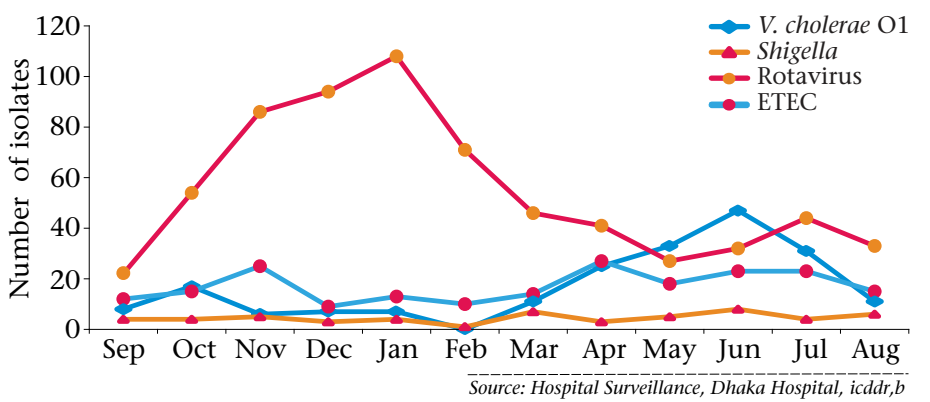
Surveillance updates

With each issue of HSB, updates of surveillance data described in earlier issues are provided. These updated tables and figures represent the most recent observation period available at the time of publication. We hope these updates will be helpful to health professionals who are interested in current patterns of disease and drug resistance in Bangladesh.

Proportion of diarrhoeal pathogens susceptible to antimicrobial drugs: September 2012-August 2013

Antimicrobial agents	<i>Shigella</i> N=54	<i>V. cholerae</i> O1 (N=203)
Mecillinam	85%	Not tested
Ampicillin	57%	Not tested
TMP-SMX	24%	3%
Ciprofloxacin	48%	100%
Tetracycline	Not tested	3%
Azithromycin	80%	100%
Ceftriaxone	100%	Not tested

Monthly isolation of *V. cholerae* O1, *Shigella*, Rotavirus and ETEC: September 2012-August 2013

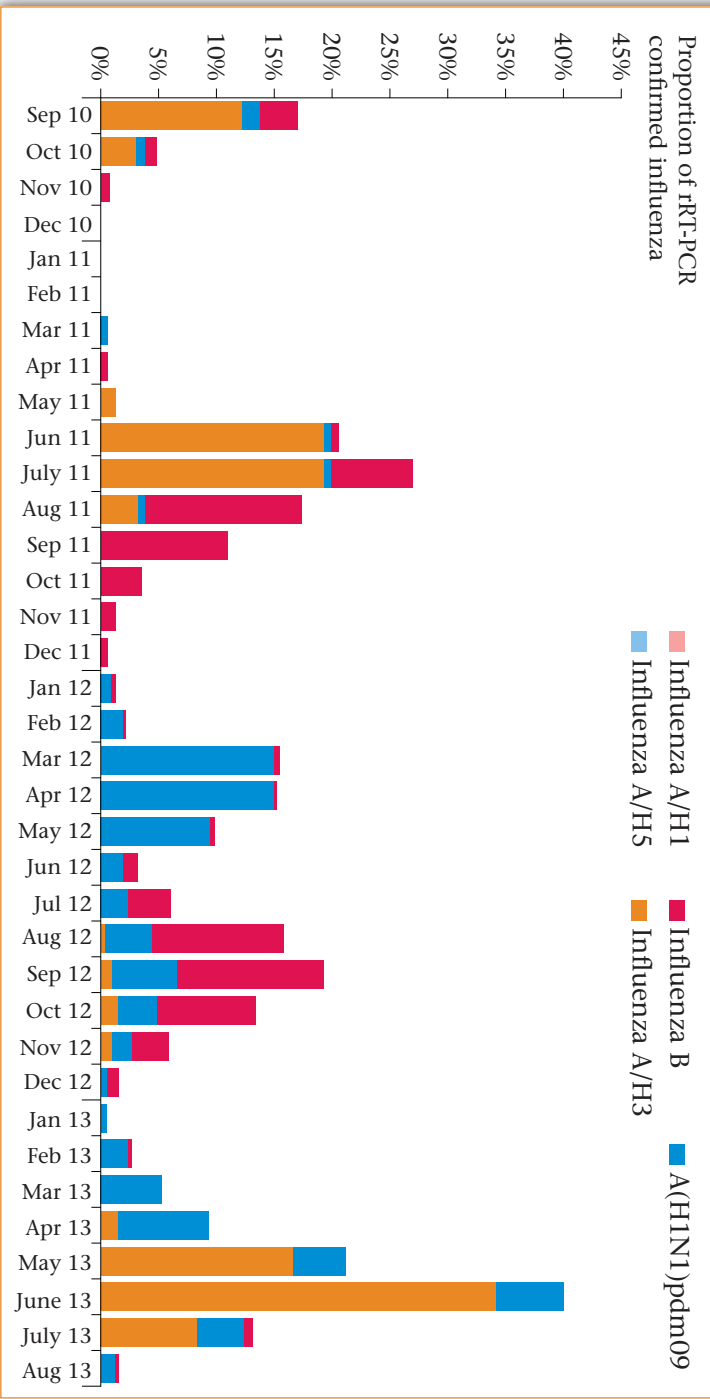


Antimicrobial susceptibility pattern of *S. typhi* among children <5 years during July-September 2013

Antimicrobial agent	Total tested (N)	Susceptible n (%)	Reduced susceptibility n (%)	Resistant n (%)
Ampicillin	41	28 (68)	0 (0)	13 (32)
Cotrimoxazole	41	28 (68)	0 (0)	13 (32)
Chloramphenicol	41	29 (71)	0 (0)	12 (29)
Ceftriaxone	41	41 (100)	0 (0)	0 (0)
Ciprofloxacin	41	2 (5)	39 (0)	0 (0)
Nalidixic Acid	41	2 (5)	0 (0)	39 (95)

Source: icddr,b's urban surveillance in Kamalapur (Dhaka)

Proportion of laboratory-confirmed influenza among hospitalized severe acute respiratory illness (SARI) and outpatient influenza like illness (ILI) cases between September 2010 and August 2013



Source: Patients participating in hospital-based influenza surveillance in Dhaka National Medical College Hospital, Community-based Medical College Hospital (Mymensingh), Jahurul Islam Medical College Hospital (Kishoregonj), Rajshahi Medical College Hospital, Shahbed Ziaur Rahman Medical College Hospital (Bogra), LAMB Hospital (Dinajpur), Bangabandhu Memorial Hospital (Chittagong), Comilla Medical College Hospital, Khulna Medical College Hospital, Jessore General Hospital, Jalalabad Ragib-Rabeya Medical College Hospital (Sylhet) and Sher-e-Bangla Medical College Hospital (Barisal)



Live bird market in Dhaka City

This publication of HSB is funded by icddr,b and its donors who provide unrestricted support for its operations and research. Currently donors providing unrestricted support include: Government of the People's Republic of Bangladesh, Australian Agency for International Development (AusAID), Canadian International Development Agency (CIDA), Swedish International Development Cooperation Agency (Sida) and Department for International Development (UK aid). We gratefully acknowledge these donors for their support and commitment to icddr,b's research efforts.

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Printed by

Dina Offset Printing Press