Surveillance of Influenza in Outpatient ILI Cases in Community Based Medical College Hospital, Bangladesh.

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Abstract

Recent population-based estimates in Bangladesh suggest that influenza is prevalent in children. To explore the epidemiology and seasonal variability of influenza among all age groups of patients. A hospital based surveillance study was conducted under the supervision of ICDDRB at Community Based Medical college hospital, with an objective to provide guidelines for prevention & control of influenza. We conducted influenza-like illness and severe acute respiratory illness sentinel surveillance in Community Based Medical College Hospital during January 2008 - December 2008. The result reveled that out of 186 patients, 17 (9.1%) of which were influenza positive by real time RT-PCR. Among the sample-positive patients, 10 (58.8%) were type A and 7 (41.2%) were type B. Hemagglutinin subtyping of type A viruses detected 7 (70%) A/H1 and 3 (30%) A/H3, but no A/H5 or other novel influenza strains. The frequency of influenza cases was highest among children aged under 5 years (47%), while the proportions of laboratory confirmed cases was also highest among participants aged under 5 years (47%). We identified a distinct influenza peak during the rainy season (June-August). Our surveillance data confirms that influenza is prevalent throughout Mymensingh, affecting a wide range of ages and causing considerable morbidity and hospital care. A unimodal influenza seasonality may allow Mymensingh, Bangladesh to time annual influenza prevention messages and vaccination campaigns to reduce the national influenza burden. To scale-up such national interventions, we need to quantify the national rates of influenza and the economic burden associated with this disease through further studies.

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Introduction

Influenza is a major public health concern, annually infecting 5-15% of the global population, resulting in an estimated 250,000 to 500,000 deaths per year[1,2]. In the United States the proportion of the population infected with influenza ranges between 5-20% resulting in an average of 36,000 annual deaths[3,4]. The number of deaths in the United States related to these annual influenza epidemics during 1974-1994 was many times greater than the number of deaths caused by the 1957 and 1968 influenza pandemics[5]. The prevalence and burden of influenza are well described for the temperate countries in both the northern and southern hemispheres[4-13]. In those countries the seasonal peaks of influenza occur distinctly during the cold seasons[2,12,14-17].Typically, elderly people and

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children aged under 5 years have the highest influenza morbidity and mortality and vaccination campaigns target these groups [2,6,7,18]. In contrast to countries in temperate climates, much less is known about the epidemiology and seasonality of influenza in tropical countries. In recent years, there has been increasing data on the potential magnitude of influenza burden in sub-tropical and tropical areas. However these were predominantly sporadic outbreak reports or hospital-based studies from wealthier tropical countries[19,20]. What has been lacking are data from surveillance in the tropics, although a few courtiers are notable exceptions. El Salvador, for example, reported repeated annual influenza epidemics during the rainy seasons[21]. Hospital surveillance in Kenya found 248 (38%) influenza positives out of 660 collected samples[22]. In Thailand the incidence was highest among the elderly over 55 years of age with epidemics occurring during June-September with an occasional increase of circulation during January and February. Thailand also quantified an annual influenza incidence ranging from 64-91 cases per 100,000 persons during 1993 and 2002[23,24]. Surveillance data from Pune and Chennai in India suggested that 5-12% of the influenza like illness (ILI) cases were due to influenza, especially during the rainy season[25-27]. Recent improvements in surveillance and laboratory capacity have allowed Bangladesh, a populous country with widespread outbreaks of H5N1 in poultry, to study the epidemiology and seasonality of human influenza and identify potentially novel strains, such as influenza A/(H5N1) and novel influenza A/(H1N1)[28,29]. During 2004, the International Centre for Diarrhoeal Disease Research (ICDDR,B) established population based influenza surveillance in children younger than five years old in Kamalapur, a low income urban neighborhood in the capital city, Dhaka. After two years of surveillance investigators reported that 14% of children with acute respiratory infections had respiratory isolates that tested positive for influenza (84.5 episodes/1000 child/years). The surveillance suggested that influenza season occurred during April through September[30,31]. This surveillance system also identified the one human case of infection with influenza A/(H5N1) in Bangladesh[32]. Based on the knowledge gained from the Kamalapur study, investigators from ICDDR,B, the Institute of Epidemiology, Disease Control and Research (IEDCR) of the Government of Bangladesh and Centers for Disease Control and Prevention (CDC), United States, collaborated to broaden influenza surveillance in this country. The primary objective was to understand the epidemiology and seasonality of influenza strains in Bangladesh from all areas and all age groups in the country. Aims included quantifying the prevalence of influenza infections among persons seeking care at the outpatient department of these hospitals, identifying circulating influenza virus strains, exploring seasonality, and characterizing clinical manifestation of influenza. In addition to these we also intended to identify novel influenza viruses among hospitalized case-patients. These data are important

for public health decisions to prevent influenza in Bangladesh. Here we present the surveillance data from January 2008 to December 2008.

Methods

Ethics Statement

The Ethical Review Committee (ERC) of International Centre for Diarrhoeal Disease Research (ICDDR,B) reviewed and approved the protocol (#2007-002) on 22 March 2007. All the surveillance participants provided written informed consent during enrollment.

Surveillance Sites and Personnel

Community Based Medical College Hospital is one of the study centre of a national hospital-based influenza surveillance system, which included six government and six non-government hospitals located throughout Bangladesh in all six divisions directly conducted by ICDDR,B(International Centre for Diarrhoreal Disease Research, Bangladesh). This hospital treats between 300 and 400 patients daily in outpatient departments. Its inpatient capacity is 550 beds with an average monthly bed occupancy

rate of 80% to 90%. A physician is recruited from the hospitals' existing staff to oversee surveillance activities. ICDDR,B field assistants were deployed to help the surveillance physicians collect data and transport biological samples to the Virology Laboratory of ICDDR,B in Dhaka. In this report the patient were enrolled in the surveillance started from January 2008 to December 2008.

Surveillance Methods

To determine the number of influenza positive case, the survey was conducted on two consequtive days each month among ILI cases. In addition, to identify novel influenza virus, we collected specimens from SARI case-patients from the hospitals' inpatient wards during those two days. After obtaining signed informed consent, the surveillance physicians collected throat and nasal swab from patients of all age groups visiting outpatient departments of those hospitals with influenza like illness (ILI), defined as subjective fever and (cough or sore throat). We also collected samples from the patients admitted in the medicine and pediatrics inpatient departments who met the case definition of severe acute respiratory illness (SARI), defined as fever (380C) and (cough or sore throat) and (shortness of breath or difficulty breathing). We excluded the children aged less than 5 years of age from inpatient SARI surveillance because childhood pneumonia is very common among this age group and samples from these cases, of which a large proportion may have been positive for other respiratory viruses, were expected to overwhelm our laboratory throughput. The surveillance physicians only collected specimens from those patients whose symptom onset was within seven days as virus can be more efficiently detected in respiratory specimens during the acute stage of infection. The surveillance physicians also performed physical examinations and recorded demographic and clinical information from the patients on a structured form. They collected data on demographics, potential work exposures for health care workers and poultry workers, travel history, clinical presentations, admission and discharge dates, symptoms, signs, provisional diagnosis, outcome of the admitted patients, available laboratory investigations, chest radiogram and treatment. The surveillance physicians collected specimens from up to 20 patients every month from each site. First from inpatient department the surveillance physicians collected samples from all eligible SARI case-patients. Then they moved to outpatient departments and collected samples until a total of 20 samples are collected. Immediately after collection, nasal and throat swabs were placed together in a single vial with viral transport media containing DMEM (Dullbecco's modified Eagle medium), 2.5% BSA (Bovine serum albumin) fraction V, 1% Glutamine, 2% HEPES (4-(2-hydroxyethyl)-1-piperazinee-thanesulfonic acid), 1% Penicillin Streptomycin and Fungizone (250 mg/ml). The field assistants stored the specimens in refrigerators or cool boxes at 2-8oC in the field sites until transported. Within 72 hours after collecting specimens, study personnel transported them in cool boxes to the Virology Laboratory of ICDDR,B. The specimens were aliquoted in a BSL-2 (bio-safety level - 2) safety cabinet and were stored in freezers at or below -700C until analysis. For influenza testing, we performed real time reverse transcriptase polymerase chain reaction (rRT-PCR) [33]. Influenza A viruses were further subtyped with H1, H3 and H5 primers provided by Influenza Division at CDC. We strictly maintained the quality control of the laboratory testing. During rRT-PCR the ribonucleoprotein (RNP) was assessed to see whether the samples contain sufficient human cells. CDC periodically sent unknown samples to ICDDR,B laboratory and asked for the laboratory results for verification and all the test results were correct. We also periodically shipped randomly selected subsets of specimens to CDC for external verification, identification of unsubtypable influenza virus, nucleotide sequencing and anti-viral resistance testing using pyro-sequencing. In addition to regular active surveillance, we also sought to identify clusters of severe acute respiratory illness defined as 3 or more patients aged .5 years admitted with severe acute respiratory illness, who live within a 30 minute walk (or within 3 kilometer radius) and

who developed symptoms within 7 days of each other. To identify clusters of SARI, the surveillance physicians enlisted all the SARI cases, more than 5 years of age, in a registers throughout the month and looked for clusters based

on above mentioned case-definition. We tested specimens of any hospitalized patient meeting the SARI case definition with history of exposure to a known or suspected H5N1 outbreak in poultry on priority basis and collected acute and convalescent serum for serologic testing at CDC.

Sample Size and Data Analysis

We assumed that an influenza virus was present in at least 1% of the population that has influenza-like-illness. We targeted to collect, 20 ILI samples each month and finally over 12 months we collected 186 samples. We did not consider inpatient SARI cases during sample size calculations since the SARI surveillance was designed to identify novel virus and clusters of severe respiratory illness cases. Therefore the SARI component of the surveillance was not representative. We collected data from Department of Livestock of Government of Bangladesh to compare the seasonality of poultry outbreaks with influenza A/(H5N1) and human seasonal influenza. We analyzed the data using SPSS and performed correlation analysis, two-way contingency tables with chi-square test or Fisher's exact test for association, chi-square test for trend and logistic regression to study the association of proportion of laboratory confirmed influenza with different variables.

Results

Demographics

Between January 2008 and December 2008, we collected specimens from 186 ILI case-patients. The mean age of under 1 year children case-patients was 2 months, ranging from less than 1 month to 11 months. The mean age above 1 year children to 85 years old person was 14. Among the case-patients 100 (53.8%) were male and 86 (46.2%) were female. The highest number of patients were enrolled in the study from less than 1 month (38 case patients) and within 1 year (20 patients). Almost 47% of patients are ranges within 5 years of age group.

Proportion of Case Patients with rRT-PCR Confirmed Influenza

Among ILI case-patients 17 (9.1%) tested positive for influenza (Table 1). The mean age of influenza positive ILI case-patients was 2 months under 1 year age group and 14 years among 1 year to 85 year of case.

Table 1. Proportion of ILI case patients with influenza virus infection.

	ILI, No. (%) N=186	Total, No. (%) N=186
Influenza positives	17 (9)	17 (9)
Types		
Influenza A	10 (59)	10 (59)
Influenza B	7 (42)	7 (42)
Subtypes		
Influenza A/H1	7 (70)	7 (70)
Influenza A/H3	3 (30)	3 (30)
Influenza A/H5	0	0

Among ILI case patients children under 5 years of age constituted 47% of the total cases, while the proportion of laboratory-confirmed influenza cases were higher among participants aged 2 to 10 years. Among outpatient ILI 100 male case-patients 11(11%) had influenza in comparison to 6 (7%) of 86 females.

Circulating Strains

Both strains of seasonal influenza A and B virus infections were present among ILI cases. Influenza A/H1 and A/H3 were present. None of the cases from inpatients or outpatients were influenza A/H5 or novel strains or unsubtypable (Table 1).

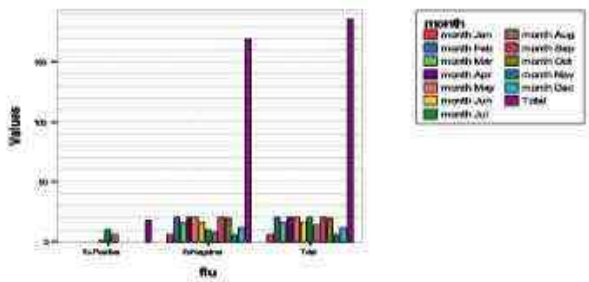

Figure:1 Seasonality of influenza in Mymensingh, Bangladesh.

Seasonality

The influenza activity was seasonal, unimodal and sharply demarcated (Figure 1). Surprisingly the positive samples were collected during June to August (100%). Largest positive samples were collected in July (58%); in August (35%), and 5% in June. During the month of peak activity, 34% of the ILI case-patients tested positive for influenza. In contrast, we identified no influenza infections during September to May 2008. The unimodal peak was concurrent with the rainy season in Bangladesh. Higher percent positivity appears concurrent with months during which there were low hours of sunlight and elevated temperatures, relative humidity, and rainfall (Figure 2). The seasonality of reported outbreaks of H5N1 in poultry in Bangladesh does not coincide with the seasonality of human influenza (Figure 3).

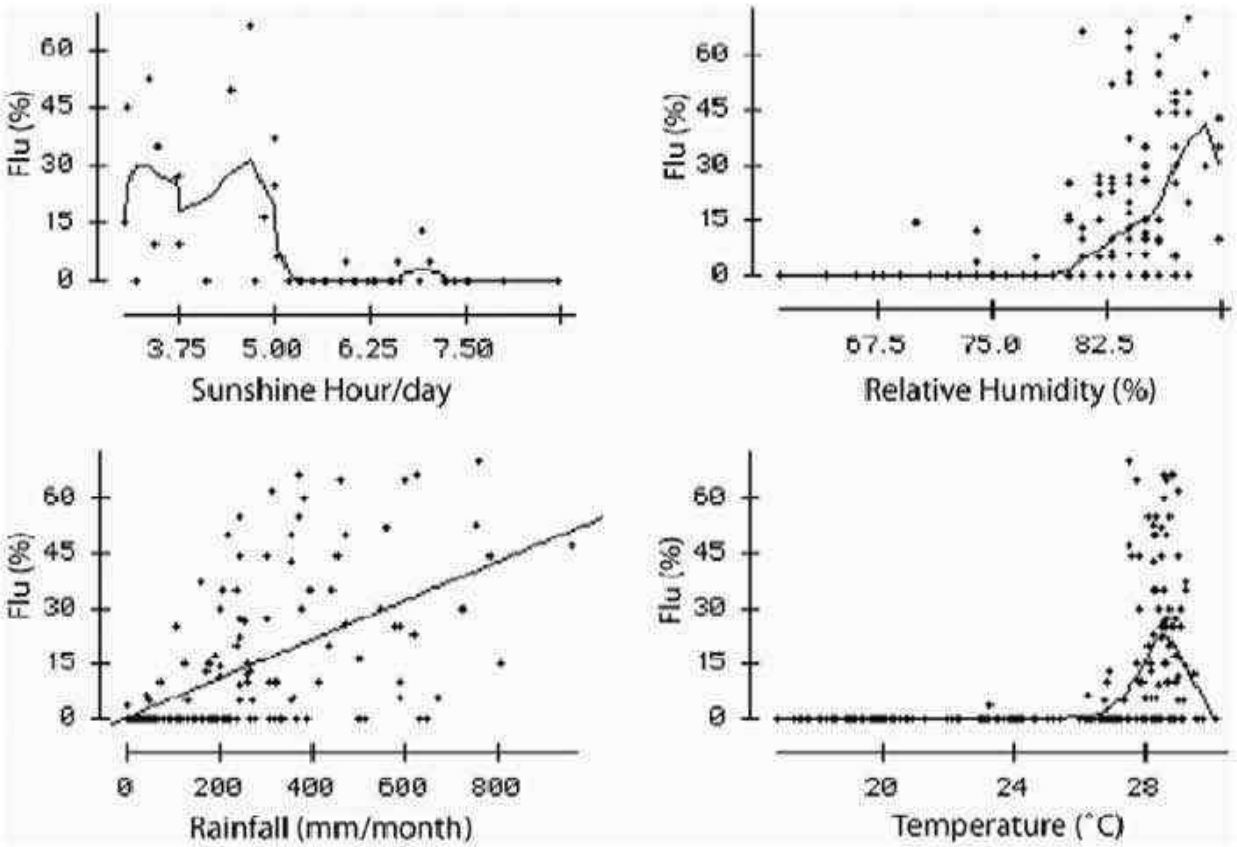

Figure 2. Correlation of percent positivity of influenza with the average monthly rainfall, average temperature, average relative humidity, and average sunlight hours.

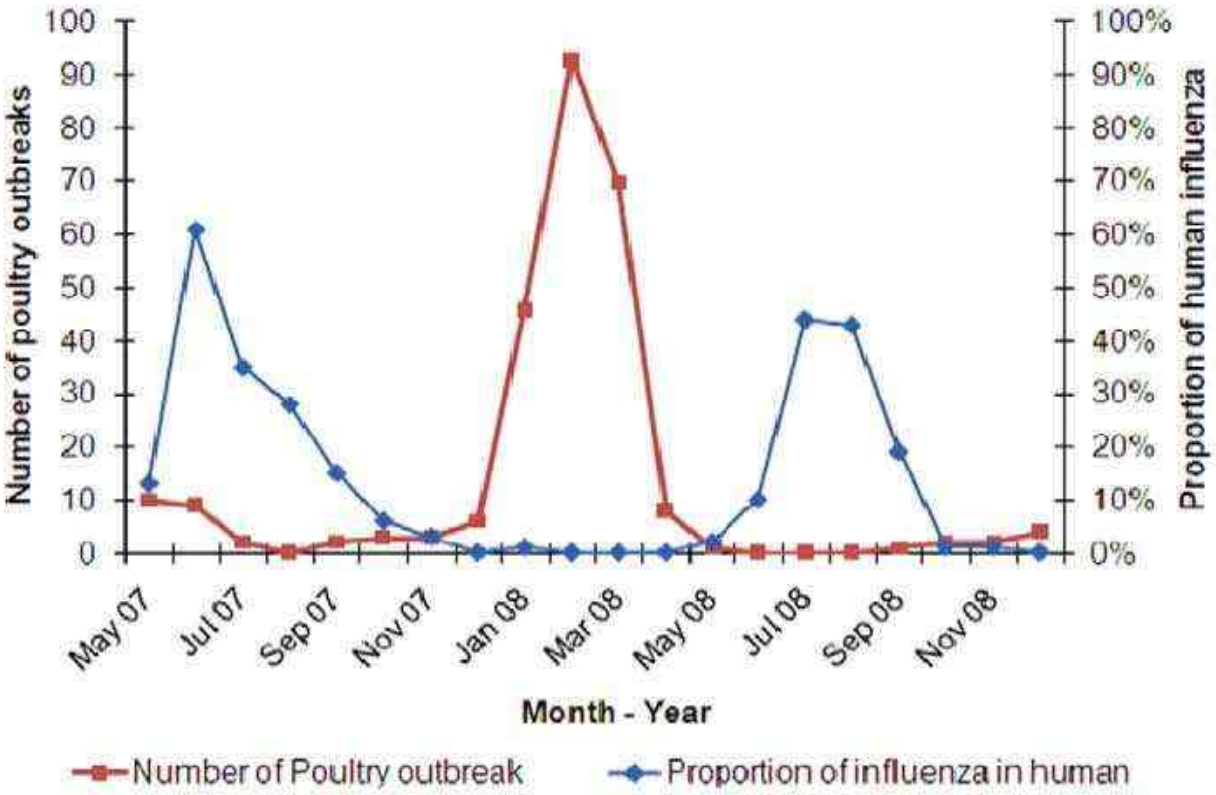

Figure 3. Non-coinciding seasonality of seasonal influenza in human and avian influenza in poultry in Bangladesh. (50)

Clinical Presentations

Influenza confirmed case-patients presented with classic clinical signs and symptoms of disease. Fever was essential criterion in the case-definitions and so was present in all ILI case patients. In addition they frequently presented with cough and runny nose (Table 2). Among the case-patients with influenza infection, 41% had documented temperature greater than 380C during sample collection. The mean temperature was 99.2 0F ranging between 97.4 0F and 104.4 0F. On auscultation 9% had rhonchi and 13% had crepitations. The mental status was normal in 82% of case-patients, while the remaining 17% were irritable, less active or lethargic. Two case patients had cyanosis. No case patient was unconscious during the time of sample collection.

Table 2. Clinical presentations in influenza and non-influenza case-patients

Symptoms	Influenza positive ILI No. (%) n = 17	Influenza negative ILI No. (%) n = 169
Fever*	17 (100)	169 (100)
Cough	14 (82)	137 (81)
Difficulty breathing	5 (29)	65** (38)
Sore throat	1 (6)	20 (12)
Runny nose	15 (88)	116** (88)
Headache	4 (24)	19** (11)
Diarrhoea	2 (12)	19 (11)
*Fever was the essential criterion for enrolment. **Significant.		

Treatment Pattern

Typically, influenza positive case patients were managed with supportive care and antibiotics. Out of 186 ILI case-patients, 74 (39.78%) were prescribed medications, 8(4.3%) were referred to other hospitals, 17 (9.1%) were advised for admission and 87(46.8%) were referred for laboratory investigations in the hospital laboratory. Among 186 ILI case-patients who received treatment, 53 (71%) received antibiotics. Analysis showed that there was an increasing linear trend between prescribing antibiotics and the severity of disease.

Health Care Workers, Poultry Exposures, and Suspected H5 Cases

Hospital and event surveillance did not identify any clusters of severe acute respiratory illness or novel strains of influenza. Fifty eight percent (108/186) of patients reported raising poultry in their homes including 7% (8/108) of people with confirmed influenza. We collected specimens from 8 health care workers and no poultry workers during surveillance. No health care worker was found positive for influenza virus.

Discussion

This surveillance data confirms that influenza is prevalent throughout Mymensingh territory, affects all age groups, and causes considerable morbidity. These data are in agreement with recently published papers from ICDDR,B, Bangladesh, El Salvador, Kenya, Thailand and India that also demonstrated prevalent seasonal influenza epidemics in the tropics[36-43]. Our findings strengthened the data highlighting seasonal influenza as a countrywide contributor to respiratory disease burden and it is important to include tropical countries in global influenza prevention activities. The unimodal and distinct seasonality of human influenza in Bangladesh provides an opportunity to explore measures to prevent influenza by non-pharmaceutical interventions, such as annual hand washing campaign, respiratory hygiene campaigns and pharmaceutical interventions, such as vaccination, which is very recently introduced in Bangladesh sporadically. Acute respiratory illness (ARI) contributes to 21% of deaths of children aged less than 5 years in Bangladesh and contributes largely to the 31% deaths due to possible serious infections in this country[34]. We found that all age groups were affected with influenza in Bangladesh. Nearly half of the case-patients were less than 5 years old, which suggest high rates of ARI. The proportion of persons with symptoms who have rRT-PCR confirmed influenza is greater among toddlers and teen-agers, and lower in the youngest children and oldest adults, where influenza causes illness severe enough to seek hospital visits or admissions. Higher proportions of influenza among school-aged children suggest that school-based non-pharmaceutical interventions or vaccination may be worth considering for preventing influenza in Bangladesh. Causing over 40% of acute respiratory illness in its peak, influenza could be an important cause of ARI in Bangladesh. Preventing influenza could contribute to mortality reduction in children under five and achieving the millennium development goal of reducing infant and childhood mortality (MDG-4)[35]. Influenza virus subtypes found in Bangladesh were similar to viruses that circulated around the globe during 2007-08[1,2]. However our surveillance did not found any evidence of year round circulation. We expect to have more information on this with subsequent years of surveillance. Surveillance data suggest that June-August was the peak influenza season in Mymensingh, Bangladesh which is offset from the influenza A (H5N1) season in poultry. The seasonality is consistent with reported seasonality of influenza infection from the population based surveillance in the Kamalapur neighborhood of Dhaka city, where the peak season was April to September during 2004-2006[30]. This time of the year is typically considered the rainy season in Bangladesh and during these two seasons of surveillance these months had higher rainfall as evident in the weather data from Bangladesh meteorological department. We found influenza positivity was concurrent with increased rainfall, temperature, and relative humidity consistent with recently

published papers on influenza and climate[44,45]. It is possible that, during the monsoons, people spend more time indoors in small poorly ventilated spaces which may increase influenza transmission. In contrast, the influenza A (H5N1) season in poultry occurs during October-March which is the time when wild birds migrate through Bangladesh[46]. Our surveillance system did not identify human infection with influenza A/H5 or other novel influenza strains in Bangladesh. Although a significant proportion of Bangladeshis do not routinely seek medical care for respiratory illness[47], the findings from this surveillance suggest that human infections with H5 or other novel influenza viruses were not commonly occurring during the study period. The seasonality of human seasonal influenza does not coincide with the seasonality of H5N1 influenza in poultry, which might reduce opportunities for reassortment of avian strain with a human strain in Bangladesh[48,49]. This surveillance has some important limitations. Our surveillance does not estimate the incidence and prevalence of influenza and so provides limited information on the burden of disease in the population. Duration of surveillance is also a limitation. This paper covered 12 months of surveillance data. We will be able to comment more robustly on the epidemiology and seasonality of influenza in Bangladesh after gathering a few more years of surveillance data. We conducted the surveillance in two consecutive days in each month; therefore we may have missed the peak influenza activity in some places. Another limitation is no enrolment of SARI case-patients from inpatient departments than anticipated. In May 2009 we amended the surveillance protocol and started obtaining comprehensive epidemiologic information about this important age group that is at high risk of complications from influenza disease and sampling hospitalized children with severe pneumonia aged less than 5 years. Another limitation was that our case definition for clusters was very specific. Identifying three or more cases from the same locality within 7 days of symptom onset was probably appropriate for early detection of large outbreaks, but not sensitive enough to capture small outbreaks. Findings from this surveillance confirmed that influenza is common among all age groups throughout Bangladesh. Human influenza epidemics mostly occur during the rainy season in Bangladesh which differs from the poultry influenza A (H5N1) avian influenza season. The identification of seasonality in human influenza activity which is different from that of H5N1 activity in poultry provides public health agencies with an opportunity to time annual prevention strategies and conduct surveillance for unusual ILI or SARI outbreaks out of season. Continued and enhanced hospital surveillance focusing on severe hospitalized influenza cases is important to characterize severe disease and identify novel respiratory viruses. Studies on the epidemiology and financial burden of influenza are needed to determine the public health and economic burden of this disease and to guide public health interventions intended to reduce influenza infection in Bangladesh.

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