Results of Surveillance of Influenza in Out-patients Influenza-like Illness (ILI), Inpatients Severe Acute Respiratory Illness (SARI) and Severe Pneumonia cases in Community Based Medical College Hospital, Bangladesh, Mymensingh during the period of May 2007 to May 2012.

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Abstract

Influenza is prevalent in Bangladesh among both adults and children. This observation is proved by recent population-based estimates. A hospital based surveillance study was conducted under the supervision of ICDDRB at Community Based Medical College Hospital, Bangladesh (CBMCH,B). To explore the epidemiology and seasonality of influenza throughout the Mymensingh region and all age groups, we analyzed the data collected during the period of May 2007 to May 2012 for a period of 5 years. The result revealed that out of 1014 patients, 140(14%) were influenza positive by real time RT-PCR. Among the sample-positive patients, 90(64%) were type A and 50(36%) type B. Hemagglutinin subtyping of type A virus detected 7(8%) A/H1 and 42(47%) A/H3, 41(46%) H1N1 pdm09 but no A/H5 or other novel influenza strains. The frequency of influenza cases was highest among children aged under 5 year 44%, while the laboratory confirmed cases were highest among patients aged under 1 year to 30 years 54%. We identified a distinct influenza peak during the rainy season (May to September), highest in July and August. There is a very low presence of influenza in the month of October, November and December (2, 1, 2 flu positive cases in 5 year period respectively). 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 this disease through further studies.

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Introduction

Influenza annually infecting 5–15% of the global population. It is a major public health problem, 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 1968influenza pandemics [5]. The prevalence and burden of influenza are well described for the temperate countries in both

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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 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 countries 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/year). 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 May 2007 to May 2012 for a duration of 5 years.

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 100%.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 May 2007 to May 2012.

Surveillance Methods

To determine the number of influenza positive with influenza like illness (ILI) and severe acute respiratory illness (SARI) case-patients conducted active surveillance in outpatient departments on two consecutive days each month. In addition, to identify novel influenza virus, we collected specimens from SARI case-patients from the hospitals' inpatient wards during those two days initially and throughout the month. After obtaining signed informed consent, the surveillance physicians collected throat and nasal swabs from patients of all age groups visiting outpatient departments of those hospitals 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 SARI, defined as fever (38^{0}C) 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 radiograph 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-piperazineethanesulfonic acid), 1% Penicillin-Streptomycin and Fungizone (250 mg/ml). The field assistants stored the specimens in refrigerators or cool boxes at 2–8^{o}C 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 -70^{0}C 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 andH5 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 3kilometer 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 also consider inpatient SARI and severe pneumonia cases and finally over duration of 5 years we collected 1014 samples. 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 May 2007 and May 2012, we collected specimens from 1014ILI and SARI case-patients. The mean age of under 1 year children case-patients was 6 months, ranging from less than 1 month to 11 months. The mean age above 1 year children to 85 years old person was 43 years. Among the case-patients 638 (63%) were male and 376(37%) were female. Almost 44% of patients were ranges within 5 years of age group. Among all enrolled cases SARI 341(34%), ILI 455(45%), severe pneumonia 218(21%) (Table1).

		Cases	FLU		Total
			Positive	Negative	
Component	SARI	No. of cases	75	266	341
		FLU percentage among 140 positive cases	53.6%	30.4%	33.6%
		Percentage among total 1014 cases	7.4%	26.2%	33.6%
	ILI	No.	51	404	455
		FLU %	36.4%	46.2%	44.9%
		Percentage of total	5.0%	39.8%	44.9%
	Severe pneumonia	No.	14	204	218
		FLU percentage	10.0%	23.3%	21.5%
		Percentage of Total	1.4%	20.1%	21.5%
Total		No.	140	874	1014
		FLU percentage	100.0%	100.0%	100.0%
		Percentage among total 1014 cases	13.8%	86.2%	100.0%

Table 1: Distribution and percentage of case patients among 3 components of cases with flu positive and negative cases.

Proportion of Case Patients with rRT-PCR Confirmed Influenza

Among case-patients 140 (14%) tested positive for influenza (Table 2). The mean age of influenza positive case-patients was 5 months under 1 year age group and 51years among 1 year to 85 year of case.

	ILI, No. (%) N=1014	Total, No. (%) N=1014
Influenza positives	140 (14)	140 (14)
Types		
Influenza A	90 (64)	90 (64)
Influenza B	50 (36)	50 (36)
Subtypes		
Influenza A/H1	7 (8)	7 (8)
Influenza A/H3	42 (47)	42 (47)
Influenza A/H1N1pdm 09	41(46)	41(46)
Influenza A/H5	0	0

Table 2. Proportion of ILI and SARI case patients with influenza virus infection.

Among ILI case patients, children under 5 years of age constituted 44% of the total cases, while the proportion of laboratory-confirmed influenza cases were higher among participants aged 1 to 30 years. Among case patients 638male case-patients 90 (8.9%) had influenza in comparison to 50 (4.9%) of 376 females.

Circulating Strains

Both strains of seasonal influenza A and B virus infections were present among cases. Influenza A/H1, A/H3 and H1N1 pdm09 were present. None of the cases from inpatients or outpatients were influenza A/H5 or novel strains or unsubtypable (Table 2).

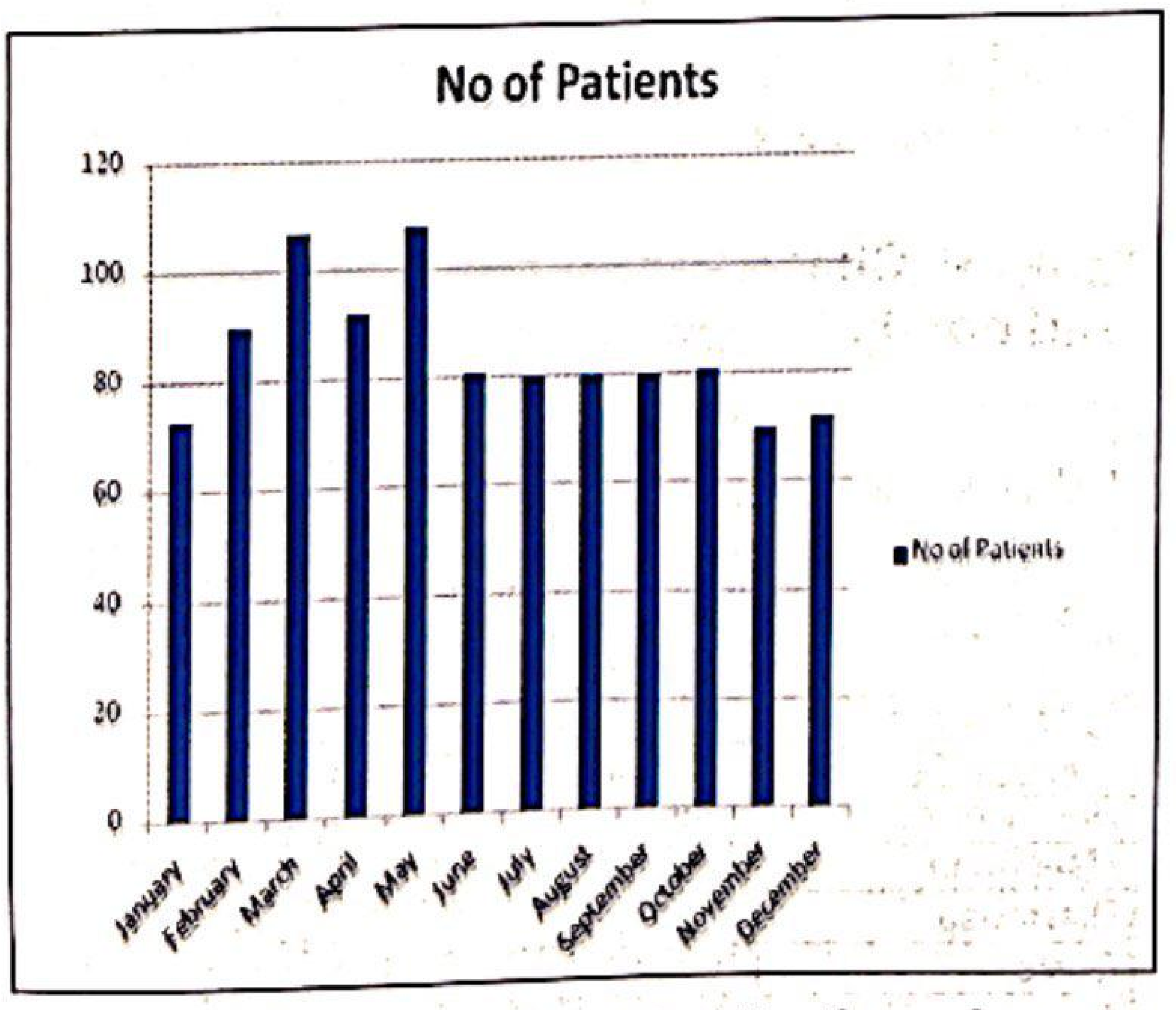

Figure 1: Month wise distribution of case patients

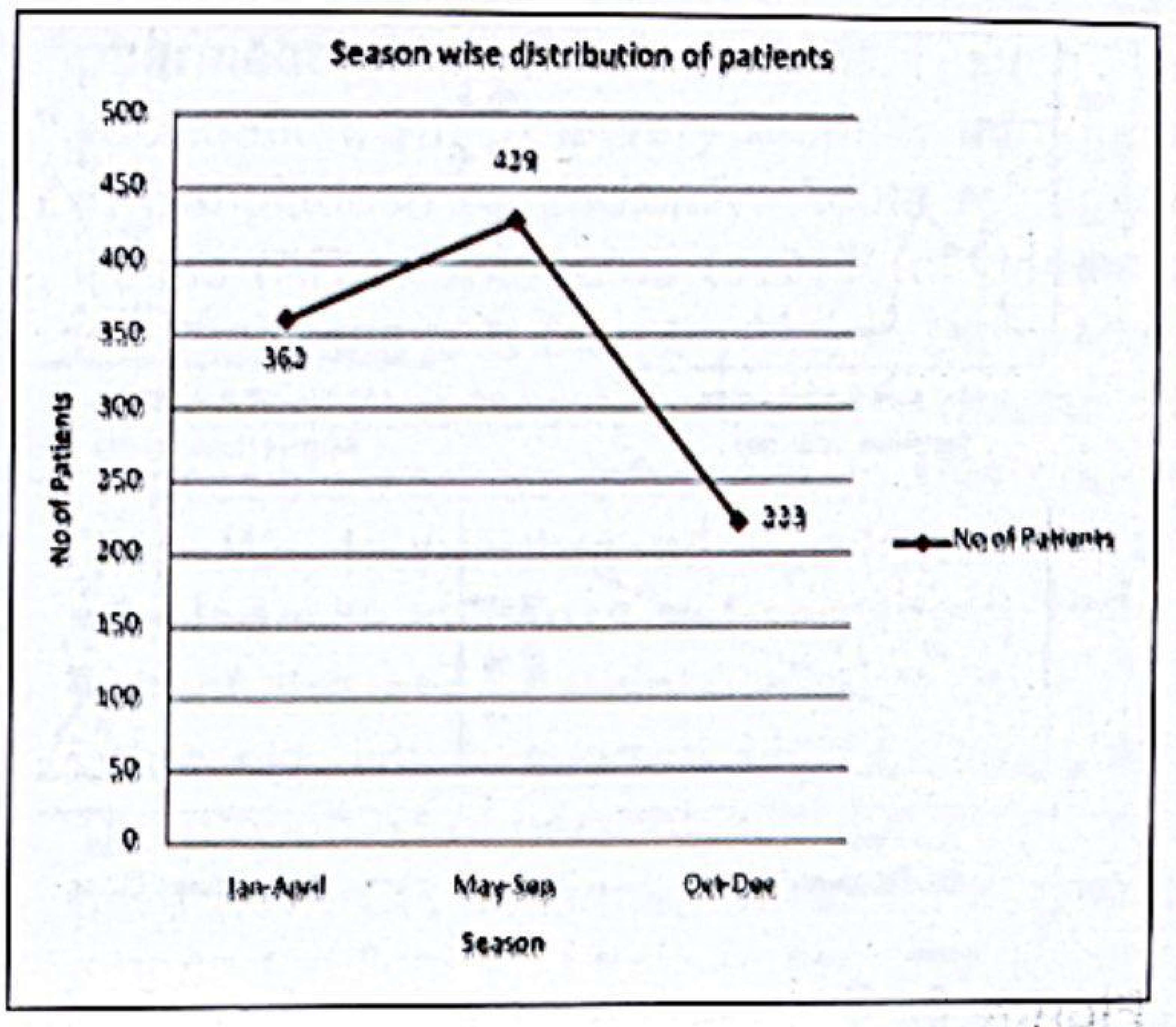

Figure 2: Season wise distribution of case patients

Month	FLU		Total
	Positive	Negative	
January	4	69	73
February	6	84	90
March	10	97	107
April	10	82	92
May	19	89	108
June	15	66	81
July	32	48	80
August	31	49	80
September	8	72	80
October	2	79	81
November	1	69	70
December	2	70	72
Total	**140**	**874**	**1014**

Table 3: Month wise distribution of flu positive and negative cases

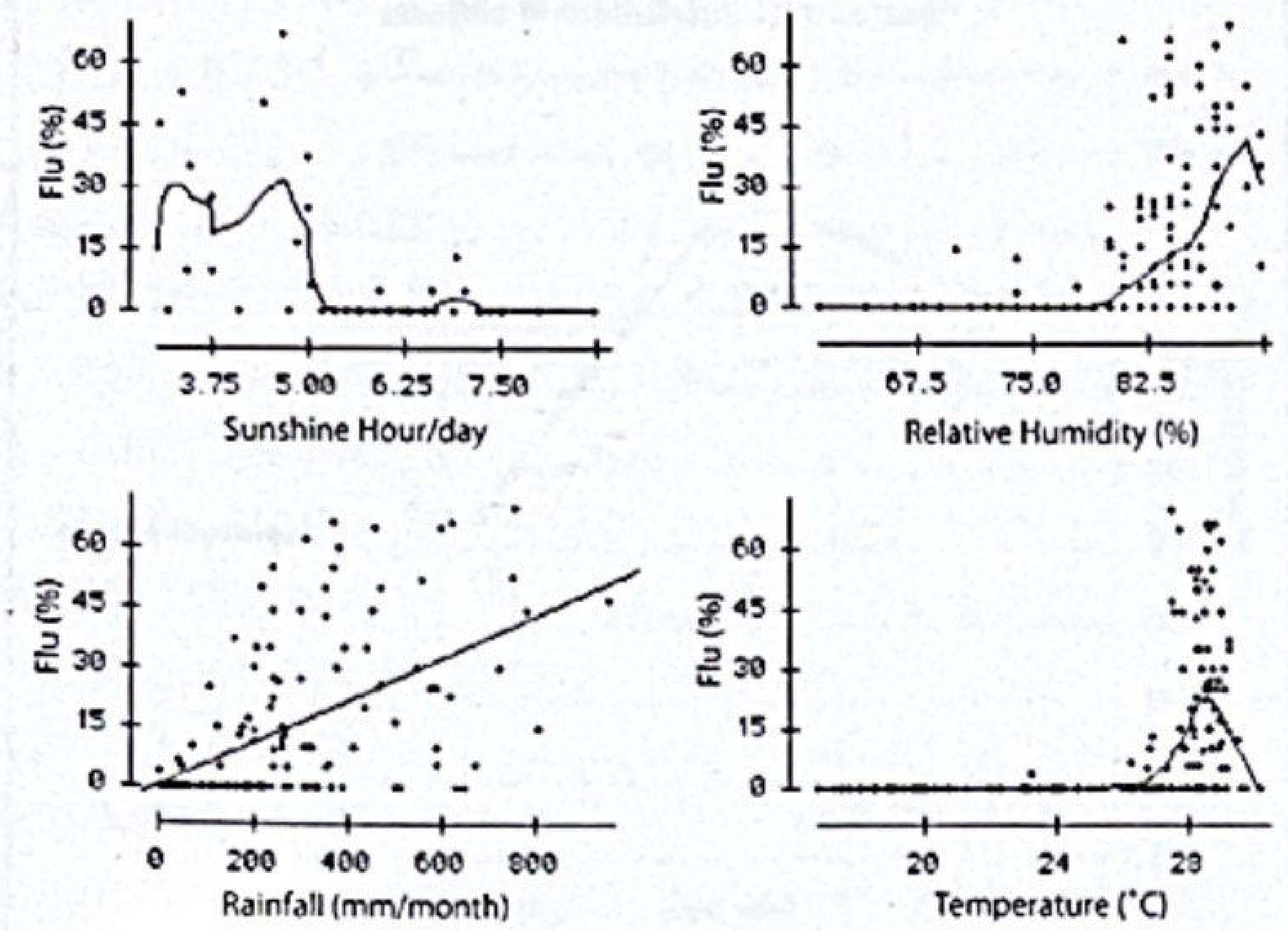

Figure 3. Correlation of percent positivity of influenza with the average monthly rainfall, average temperature, average relative humidity, and average sunlight hours. (50)

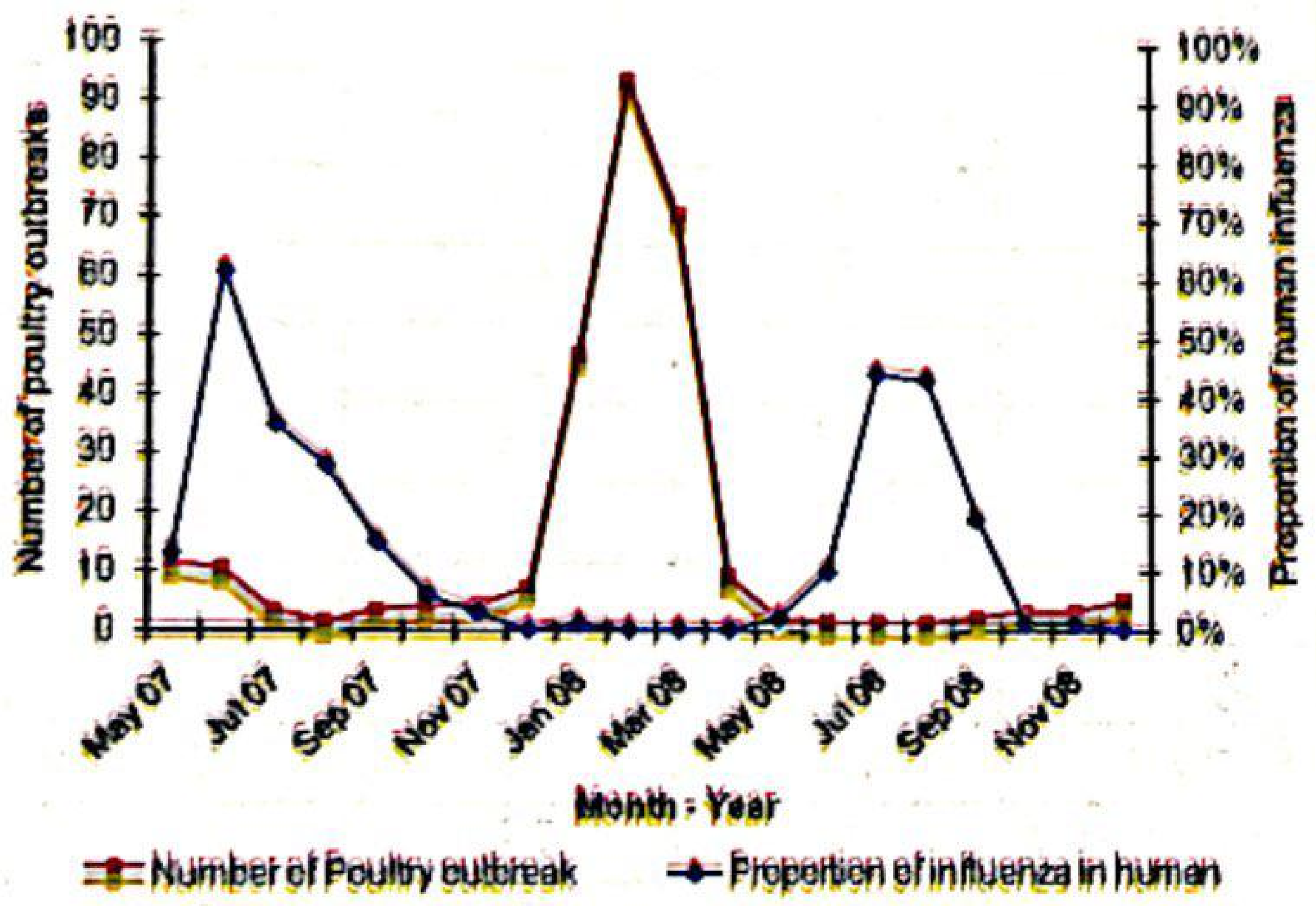

Figure 4. Non-coinciding seasonality of seasonal influenza in human and avian influenza in poultry in Bangladesh. (50)

Seasonality

The influenza activity was seasonal, unimodal and sharply demarcated (Figure 1). Total 140 flu positive samples were collected. The positive samples were collected throughout the year. The largest positive samples were collected in July (23%) and August (22%); July and August comprise 45% of flu positive cases. During the month of May to August 69% of flu cases were identified. Largest positive samples were collected in July (58%); in August (35%), and 5% in June. During the month of peak activity (June to August), 32% of the case-patients tested positive for influenza. In contrast, we identified 20% of influenza infections during September to May in the whole study period. The lowest positive sample were collected in September (8), October (2), November (1), December (2), January (4) and February (6) during the 5 year period of surveillance (Table2, Fig 1, 2). 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. The seasonality of reported outbreaks of H5N1 in poultry in Bangladesh does not coincide with the seasonality of human influenza (Fig-3, 4).

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 case patients. In addition they frequently presented with cough and runny nose (Table 4). Among the case-patients with influenza infection, 41% had documented temperature greater than 38^{0}C during sample collection. The mean temperature was 99.69^{0}F ranging between 97.0 ^{0}F and 105.0 ^{0}F. On auscultation 40% had rhonchi and 40% had crepitations in flu patients. The mental status was normal in 42% of flu positive patients, while the remaining 17% were irritable/less active in 56% or lethargic 2%.Two case patients had cyanosis and they were flu positive. No case patient was unconscious during the time of sample collection.

Table 4. Clinical presentations in influenza and non-influenza case-patients.

Symptoms	Influenza positive cases No. (%) n = 140	Influenza negative cases No. (%) n = 874
Fever*	140 (100)	874 (100)
Cough	137 (98)	832 (95)
Difficulty breathing	63 (45)	527** (60)
Sore throat	41 (29)	105 (12)
Runny nose	94 (67)	481** (55)
Headache	74 (53)	229** (26)
Diarrhoea	10 (7)	93 (11)
Chills	23 (16)	139(18)
Bodyache	70 (50)	199(26)

*Fever was the essential criterion for enrolment.
**Significant.

Treatment Pattern

Typically, influenza positive case patients were managed with supportive care and antibiotics. Out of 1014 case-patients, 752 (74%) were prescribed medications, 11(1%) were referred to other hospitals, 39 (4%) were advised for admission and 122(12%) were referred for laboratory investigations in the hospital laboratory. Among 1014 case-patients who received treatment, 266(26%) 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 SuspectedH5 Cases

Hospital and event surveillance did not identify any clusters of severe acute respiratory illness or novel strains of influenza. Seventy eight percent (792/1014) of patients reported raising poultry in their homes including 13.5% (105/792) of people with confirmed influenza. We collected specimens from 10 health care workers and 12 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 [21,22,24–26,50]. Our findings strengthen 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 May-September 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 [34,47], the findings from this surveillance suggest that human infections with H5or 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 5years 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 initially; therefore we may have missed the peak influenza activity in some places. Another limitation is no enrolment of SARI case-patients from inpatient departments in the initial period 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 confirm that influenza is common among all age groups throughout Bangladesh. Human influenza epidemics mostly occur during the rainy season in Bangladesh which is during a different time of the year than 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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