

Influenza surveillance in Pune, India, 1978–90*

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Continuous surveillance of influenza was carried out in Pune between 1978 and 1990. Most of the cases were identified during investigation of 16 outbreaks of influenza in Pune over this period. The majority of cases were children. Ten of the outbreaks occurred during rainy seasons. A total of 290 isolates consisting of several antigenic variants of influenza type A (H3N2), type A (H1N1), and type B viruses were isolated from throat/nasal swabs that were processed in chick embryos and MDCK cell culture and identified using the haemagglutination inhibition test. These variants circulated every year or in alternate years. Nearly two-thirds of the influenza virus isolates (181 out of 290) were from children aged <10 years. Seasonal analysis indicated that the highest number of isolates (174) were collected during the rainy months of July, August and September, with the maximum number (93) in July.

Introduction

Acute respiratory diseases (ARD) cause enormous morbidity and mortality, particularly in infants and children in developing countries. It is estimated that about 15 million infants and young children die each year in the world from diarrhoea, ARD, and other diseases. Of these deaths, 14 million occur in developing countries, and ARD is responsible for about 4 million deaths and 500–900 million episodes of infection per annum in children (1, 2). In India estimates indicate that 600 000 children under 5 years of age die every year from ARD (3).

Over 300 antigenic types of bacteria and viruses are known to cause ARD, with viruses outnumbering bacteria (4). In particular, influenza viruses cause frequent epidemics and periodic pandemics that affect large segments of the global population, owing to antigenic variation of the viral surface antigens. In view of the public health importance of influenza, WHO initiated a global network of surveillance centres in 1948. Influenza surveillance is carried out throughout the year to study the morbidity and mortality patterns and to detect as early as possible new antigenic variant strains for use in vaccines (5).

In Pune influenza surveillance was initiated by the National Institute of Virology in 1976–77. The present article reports the results of the influenza surveillance conducted on humans in Pune between 1978 and 1990 as an ongoing programme.

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Materials and methods

Selection of dispensaries/hospitals

When influenza surveillance was initiated in Pune, dispensaries and small hospitals were chosen rather than large hospitals because the majority of patients with ARD seek medical aid from these dispensaries rather than from large hospitals. Subsequently, the paediatric departments of large hospitals were also included in the surveillance. The dispensaries/hospitals selected are located in congested localities of the city where the population is of low socioeconomic status. Attendance at the outpatient departments is high. New dispensaries were selected as and when necessary.

Frequency of visits to the dispensaries/hospitals

Regular weekly visits were made to two or three selected dispensaries/hospitals, irrespective of the incidence of ARD in the community. Whenever high incidence was noticed, frequent or daily visits were made to investigate the cases and to collect a sufficient number of specimens. This approach permitted outbreaks to be detected and investigated as early as possible. Influenza outbreaks characteristically arise and wane within a month; it is essential that transient outbreaks are detected as soon as they occur. Our approach also facilitated investigation of sporadic cases during the interepidemic periods. Thus, it was possible to make an overall assessment of the incidence of ARD in the community throughout the year.

Case definition

An individual with one or more of the following conditions, with or without systemic manifestations

(fever, bodyache, headache, malaise, weakness) was taken to be a case of ARD: common cold, pharyngitis, laryngitis, tracheitis, bronchitis, bronchiolitis, pneumonia, or bronchopneumonia.

Clinico-epidemiology

During the course of surveillance over the period 1978–90 (13 years), more than 10 000 cases of ARD among various age groups were investigated in Pune. The majority of cases were children, including infants. Most of the patients were seen during investigations of 16 outbreaks of influenza. Generally, the cases presented with two or three manifestations of respiratory diseases and one or two systemic manifestations. Some cases had only respiratory manifestations.

Collection of specimens

Throat and nasal swabs were collected from ARD cases during the acute phase of their illness (1–4 days) since viruses could be isolated equally easily from either. Because they are easy to collect, nasal swabs were generally taken from infants and young children. Throat/nasal swabs were taken from over 10 000 ARD cases. About 80% of these specimens were cultivated for influenza virus in embryonated chicken eggs (9–11 days' old) and about 30% in Madin–Darby canine kidney cell culture (MDCK) with crystalline trypsin.

By and large, it was not possible to collect paired blood samples from outpatient cases since they frequently refused to give blood. The method described by Kendal et al. was followed with some modifications for isolation of virus, conducting haemagglutination and haemagglutination inhibition tests using guinea-pig red blood cells to identify influenza isolates (6).

Results

Outbreaks investigated

During the period 1978–90, several variants of influenza virus types A and B were isolated during

the 16 outbreaks investigated in Pune, including the following important variant strains: A/USSR/77 (H1N1) in 1978 (7); A/Singapore/6/86 (H1N1) in 1986 (8); and B/Yamagata/16/88-like in 1990 (9).

Virus isolation

A total of 290 influenza virus isolates comprising several variants of influenza type A (H3N2) and A (H1N1) and type B were isolated and identified (Table 1). MDCK cell culture, which was used from 1983, was superior to embryonated chicken eggs for influenza virus isolation (10). However, both methods of cultivation were employed to obtain optimum results. The strains circulated singly or in different combinations depending on the particular outbreak. The variant strains of influenza type A (H1N1), type A (H3N2), and type B circulated regularly either every year or in alternate years.

Nearly two-thirds of the influenza isolates (181 out of 290) were from children aged <10 years. There were some isolates from infants also. Analysis of the isolates, by month, showed the greatest number (174) were from the rainy months (July, August, September) with the maximum number (93) occurring in July (Fig. 1).

Seasonality

Pune has a tropical monsoon climate with a hot season from March to May, a rainy season from June to September (with October as a transitional hot month) and a cool season from November to February. The annual average rainfall is 714.7 mm. Average climate data for Pune are shown in Fig. 1. Of the 16 outbreaks of influenza, 10 occurred in the rainy season, three in the hot season, one in the cool season, and two in February and March.

Correlation coefficients (*r*) were calculated between the number of isolates and each of the following variables: rainfall (mm), relative humidity at noon and in the morning, maximum temperature, and minimum temperature (Table 2).

To construct an equation for the statistically significant independent variables that gives the best

Table 1: Influenza virus isolates from human cases in Pune, 1978–90

Virus strain	No. of isolates													Total
	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	
H1N1	6	29	10	6	1	9	—	—	10	2	1	2	14	90
H3N2	1	—	26	35	1	20	5	6	—	—	2	20	—	116
B	—	2	1	5	—	6	—	23	1	11	6	6	23	84
Total	7	31	37	46	2	35	5	29	11	13	9	28	37	290

Fig. 1. Plots showing correlation between influenza virus isolates from Pune 1978–90, and various seasonal factors.

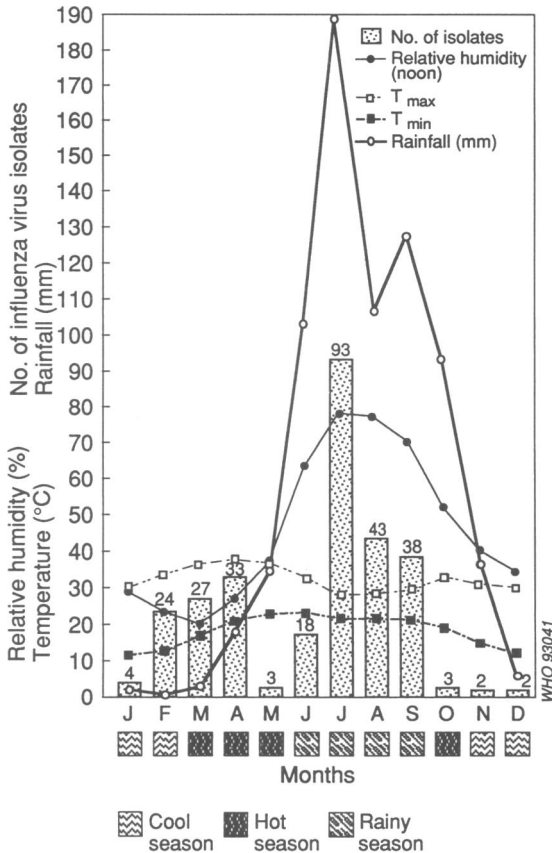


Table 2: Correlation between influenza virus isolates and the variables examined in the study

Correlation between the number of isolates and:	Correlation coefficient (r)	Degrees of freedom	Statistical evaluation
Rainfall	0.697	10	Significant, $P < 0.05$
Relative humidity (noon)	0.572	10	Borderline significance, $0.1 > P > 0.05$
Relative humidity (morning)	0.257	10	Not significant, $P > 0.05$
Maximum temperature (T_{max})	-0.354	10	Not significant, $P > 0.05$
Minimum temperature (T_{min})	0.452	10	Not significant, $P > 0.05$
Temperature difference ($T_{max} - T_{min}$)	-0.608	10	Significant, $P < 0.05$

was also sent to the WHO Collaborating Centre on Influenza Reference and Research, Atlanta, GA, USA, for further characterization of the strains. Most of these isolates were found to be similar to the various strains that were prevalent in the world when they were collected (Table 3). One of the strains (A/India/6263/80 (H1N1)) that was isolated during the July 1980 monsoon outbreak, however, was identified as a minor antigenic variant of the H1N1 strain, and is being used as a reference strain (12).

prediction of the number of isolates, a multiple regression was carried out for rainfall (X_1), temperature difference (X_2) and the number of isolates (Y). The following expression was obtained:

$$Y_{\text{estimate}} = 0.5512X_1 + 3.1738X_2 - 52.564$$

The analysis indicated that in addition to X_1 and X_2 some other unidentified variables that we had not included were responsible for more than half of the variation in the values of Y (11).

Collaboration with WHO

Details on the occurrence of influenza outbreaks and on the strains isolated during the period of surveillance were communicated regularly to WHO. A representative number of isolates from each outbreak

Table 3: Influenza virus isolates from Pune 1978–90 that were identified to be similar to the reference strains shown

Type A (H3N2) strains	Type A (H1N1) strains	Type B strains
A/Victoria/3/75	A/USSR/90/77	B/Hongkong/5/72
A/Texas/1/77	A/Brazil/11/78	B/Singapore/222/79
A/Taiwan/1/79	A/India/6263/80	B/USSR/100/83
A/Bangkok/2/79	A/England/333/80	B/Hong Kong/8/83
A/Oregon/4/80	A/New Caledonia/4/83	B/Kanagawa/2/84
A/Shanghai/31/80	A/Singapore/6/86	B/Texas/1/84
A/Arizona/2/80	A/S. Carolina/6/88	B/Ann Arbor/1/86
A/Philippines/2/82		B/Victoria/2/87
A/Taiwan/16/83		B/USSR/2/87
A/Mississippi/1/85		B/Yamagata/16/88
A/Sichuan/2/87		
A/Sichuan/68/89		
A/Beijing/353/89		

Discussion

The outbreaks caused by variant H1N1 strains in 1978 and 1986 and the B/Yamagata/16/88-like strain in 1990 in Pune were important epidemiologically. The A/USSR/77 (H1N1) strain was first isolated in the USSR in November 1977 and then spread to other countries. This strain was responsible for outbreaks of influenza predominantly among persons aged <25 years; it was found to be similar to the influenza A (H1N1) strains that were prevalent in the world in the period 1946–57. The A/USSR/77 strain therefore infected mainly the non-immune younger age groups who were born after 1950, and it may have appeared because of the recycling of earlier influenza A (H1N1) strains (13). In 1986 a new variant of influenza A (H1N1) strain (A/Singapore/6/86 (H1N1)) was isolated and reported subsequently from a number of locations, including Pune. This strain was readily distinguishable from all the reference A (H1N1) virus variants that had been prevalent between 1978 and 1985. The pattern of circulation of the various influenza virus strains did not vary from area to area of the city (14).

Our continuous surveillance in Pune, which has a tropical monsoon climate, shows that influenza outbreaks occurred predominantly during the rainy months. Only few reports have appeared on the seasonality of influenza outbreaks in the tropics; for example, in Panama and Thailand (15, 16) during the rainy season (15, 16) and in Nigeria and Singapore during the hot and rainy seasons (17, 18).

In countries with a temperate climate, influenza outbreaks occur in winter. This may be related to the improved transmission of airborne viruses at that time of the year and to the impairment of the host's respiratory tract defences due to the seasonal fall in temperature (19). A study of five successive winters of different severity in the United Kingdom indicated that the incidence of influenza was less in mild winters than in severe (20).

Studies conducted under carefully controlled conditions of humidity and temperature in winter and summer have found that the transmission rates of influenza are higher in winter, suggesting that either relative humidity before contact may be a contributory factor or unknown factors associated with winter may be operative (21). Our study has demonstrated that rainfall, relative humidity, and small differences between minimum and maximum temperatures influence the occurrence of influenza outbreaks in the rainy season but that other unidentified factors may also be involved.

A serological survey employing sera from blood donors from Pune was carried out in 1980–81 (22). The findings indicated a broadening of antibody res-

ponse to a greater number of influenza virus strains as the age of the subjects increased, an overall higher prevalence of antibodies to the pandemic strains, and a low-to-moderate prevalence of antibodies to the epidemic strains.

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Résumé

Surveillance de la grippe à Poona, Inde, 1978–1990

Une surveillance continue de la grippe a été réalisée à Poona entre 1978 et 1990. La plupart des cas ont été identifiés au cours des investigations sur les flambées grippales survenues à Poona pendant cette période. Il s'agissait pour la plupart de cas pédiatriques. Dix de ces flambées ont eu lieu au cours de la saison des pluies. Au total, 290 isolements, comprenant plusieurs variants antigéniques des virus grippaux de type A (H3N2), de type A (H1N1) et de type B, ont été obtenus à partir d'écouvillonnages de gorge et d'écouvillonnages nasaux cultivés sur embryons de poulet et sur cellules MDCK, et identifiés par inhibition de l'hémagglutination. Ces variants circulaient soit chaque année, soit une année sur deux. Près des deux tiers des isolements grippaux (181 sur 290) provenaient d'enfants de moins de dix ans. L'analyse saisonnière a montré que le plus grand nombre d'isolements (174) ont été recueillis au cours des mois pluvieux (juillet, août et septembre), avec le maximum (93) en juillet.

Les résultats de cette surveillance continue démontrent que la grippe survient essentiellement au cours de la saison de pluies à Poona, où règne un climat de mousson. Ces observations donnent des renseignements précieux sur les aspects épidémiologiques des maladies respiratoires aiguës et de la grippe, qui seront utiles lors de la planification des programmes de prévention et de lutte.

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