

The use of immuno-double-diffusion tests in epidemiological studies of influenza in Spain

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The immuno-double-diffusion (IDD) test was used to detect antibodies against the prevalent influenza A (H3N2) viruses in sera collected weekly in Madrid from 1972 to 1975. A total of over 14 000 sera were tested. The proportion of sera positive in the IDD test was found to reflect the level of recent influenza A infection of the population.

The methods available for the detection of antibodies to influenza viruses include haemagglutination-inhibition (HI), neuraminidase-inhibition, and complement-fixation tests. These tests are often cumbersome because of the need to dilute the sera and in the case of HI tests to remove non-specific inhibitors. The immuno-double-diffusion (IDD) test (2, 3, 5) has been employed for the detection of antibodies to the antigenic components of influenza viruses, and has been shown to be effective and sensitive for the serodiagnosis of influenza infection with human paired sera (3). Use of this test made it possible to detect and identify antibody to the nucleoprotein (NP), the neuraminidase (NA), and the haemagglutinin (HA) antigens of influenza A virus in convalescent serum samples. The antibodies to NP and NA that were detected by IDD were apparently short-lived (3) and thus their presence appeared to be a useful indicator of recent influenza infection.

This paper describes studies to assess the value of the IDD test in the serological surveillance of influenza. Results obtained in Madrid by the routine application of this method over four years are described and related to information on the incidence of clinical influenza in Spain obtained by the public health services.

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MATERIALS AND METHODS

Serum collection

Serum samples were obtained from a Madrid blood bank (Instituto Nacional de Hematología) which serves the city and province of Madrid, an area about 8000 km². From 1972 to 1975, approximately 100 serum samples were collected each week for IDD tests from donors in the age range of 20–65 years.

Immuno-double-diffusion test

This test was performed in agar gel on a glass slide or in a plastic immunoplate (Hyland Laboratories, California, USA) as described by Schild et al. (3), the antigen used being purified and concentrated influenza virus disrupted by the addition of sarcosyl detergent to a final concentration of 1%. There were six peripheral wells and one central well containing the antigen. The production of one or more visible precipitin lines after staining the plate was taken as evidence of positivity.

To evaluate the reproducibility and accuracy of our results, 25 sera (15 IDD positive and 10 IDD negative) were tested on 10 different occasions with different batches of plates and antigens. Reference sera were included in each set of tests.

The influenza A virus strains used, included the following: A/Hong Kong/1/68 (H3N2), A/England/42/72 (H3N2), A/Port Chalmers/1/73 (H3N2), and A/Madrid/1492/74 (H3N2), a strain isolated in Madrid in the winter of 1974 that is antigenically similar to A/Scotland/840/74 (H3N2) (6).

Viruses were cultivated in the allantoic cavity of 10-day-old embryonated eggs and concentrated and purified as described by Schild et al. (3). The final

virus preparations contained $4-5 \times 10^6$ haemagglutinin units/ml, and 10–15 mg of protein/ml.

Epidemiological data

The number of reported cases of influenza (which is a notifiable disease in Spain) as well as the number of deaths from the three main respiratory disease headings in list B of the ICD (1)—influenza, pneumonia and bronchopneumonia, and bronchitis, emphysema, and asthma—were used as the basis for evaluating the epidemiological impact of influenza. The data on cases of influenza were obtained from the existing influenza surveillance system for the city and province of Madrid, covering a population of nearly 4 million.

RESULTS AND DISCUSSION

Fig. 1 shows the weekly influenza notifications for Madrid from mid-1971 to mid-1975 compared with the maximum and median numbers of influenza cases for the previous five years—the “normal epidemic area”. The median values of the previous five years were considered as the “non-epidemic” level. In addition, the method of Serfling (4) for the analysis of mortality data was applied in order to obtain a curve for the non-epidemic level of influenza, calculating expected values from a sinusoidal function, where the constant and the amplitude are exponential functions of time: (a) the length of cycle is one year (weekly intervals of $2\pi/52$); (b) the amplitude is equal to half the range between the minimum and maximum values in non-epidemic years, and (c) the phase angle is $3\pi/2$ to fit the minimum value of the influenza year with the -1 value of the sine function.

We considered that an epidemic level of influenza

had been reached when the weekly rate of increase of cases was more than double that of the previous week and the number of cases was above that represented by the non-epidemic curve. This method, based only on the number of notified cases, proved to be of value and correlated well with mortality figures, but has the disadvantage of not being etiologically based. Clinically mild influenza outbreaks might be missed and other respiratory infections may be counted as influenza.

The results of IDD tests performed on the human sera collected between 1972 and 1975 are shown in Table 1. Most (93.9%) of the sera recorded as positive gave only one precipitin line, which was identified, by reference to a specific hyperimmune serum, as corresponding to the line produced by antibody to influenza A nucleoprotein antigen. A further 6% gave two precipitin lines identified as corresponding to antibodies to NP and NA. A small number of sera (<0.2%) gave three precipitin lines, the third line corresponding to antibody to HA. There was considerable annual variation in the proportion of sera positive by IDD tests.

Fig. 2 illustrates the proportions of sera found to be seropositive in IDD tests, by 4-week periods from 1972 to 1975, and also the weekly incidence of influenza in Madrid over the same period (log scale). There was, in general, a good correlation between the periods of high incidence of influenza and periods when a high proportion of sera were positive by the IDD test.

The antibodies to influenza antigens detected by IDD in individual sera were found to persist for only 2–3 months after natural infection and could be detected as early as a week after the onset of illness in virologically confirmed cases (R. Nájera, unpublished data, 1975). The proportion of sera positive in the IDD test can therefore be regarded as a cumulative index of recent infection.

Table 1. Results of IDD tests

Year	No. of sera examined	IDD positive sera							
		One line (NP)		Two lines (NP + NA)		Three lines (NP + NA + HA)		Total	
		No.	%	No.	%	No.	%	No.	%
1972	4 548	571	12.55	70	1.54	1	0.02	642	14.11
1973	3 865	255	6.60	17	0.44	0	0	272	7.04
1974	3 470	471	13.57	20	0.57	2	0.05	493	14.21
1975	3 100	536	17.29	10	0.32	0	0	546	17.61
Total	14 983	1833	12.23	117	0.78	3	0.02	1953	13.07

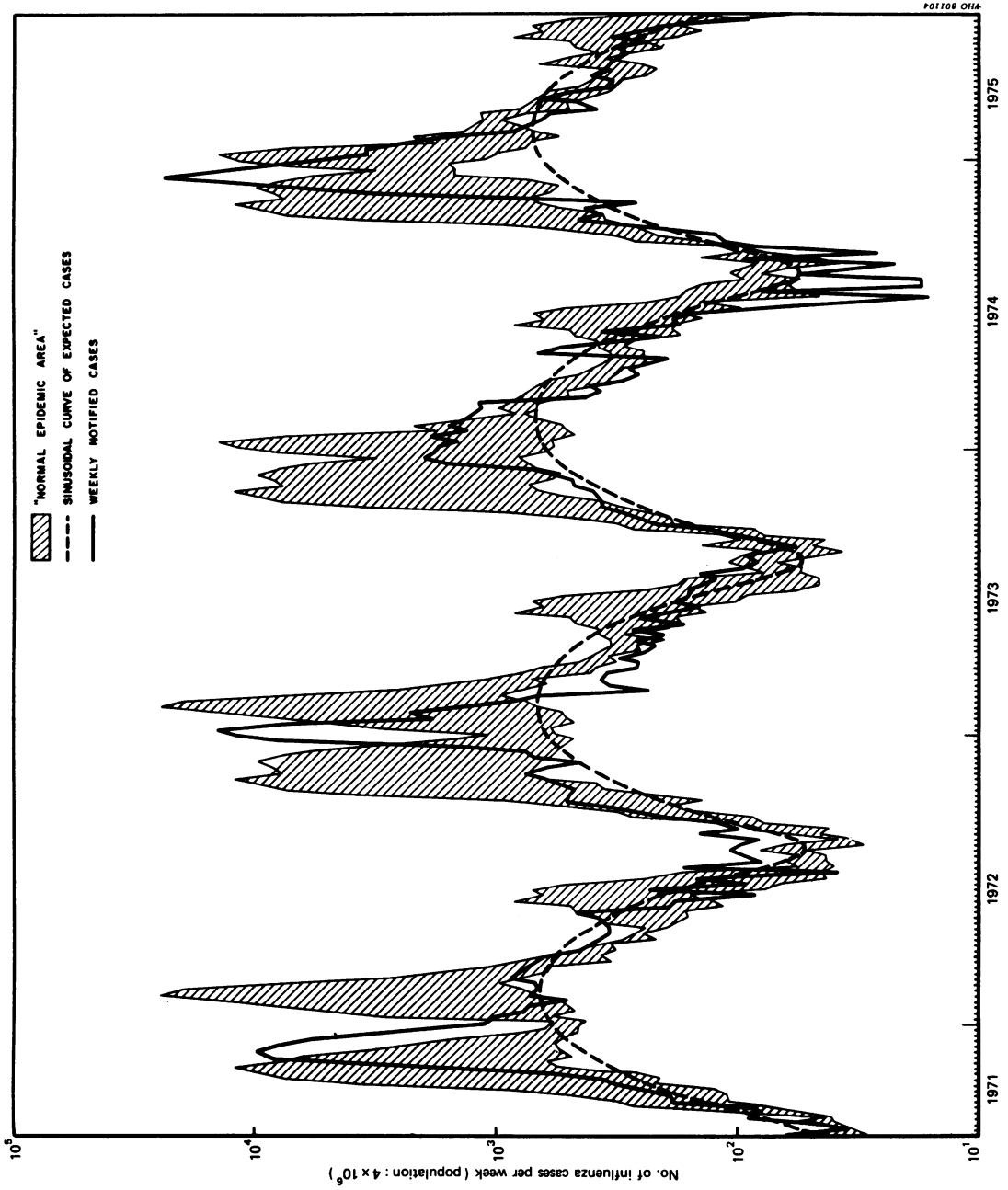


Fig. 1. Incidence of influenza in Madrid, 1971-75.

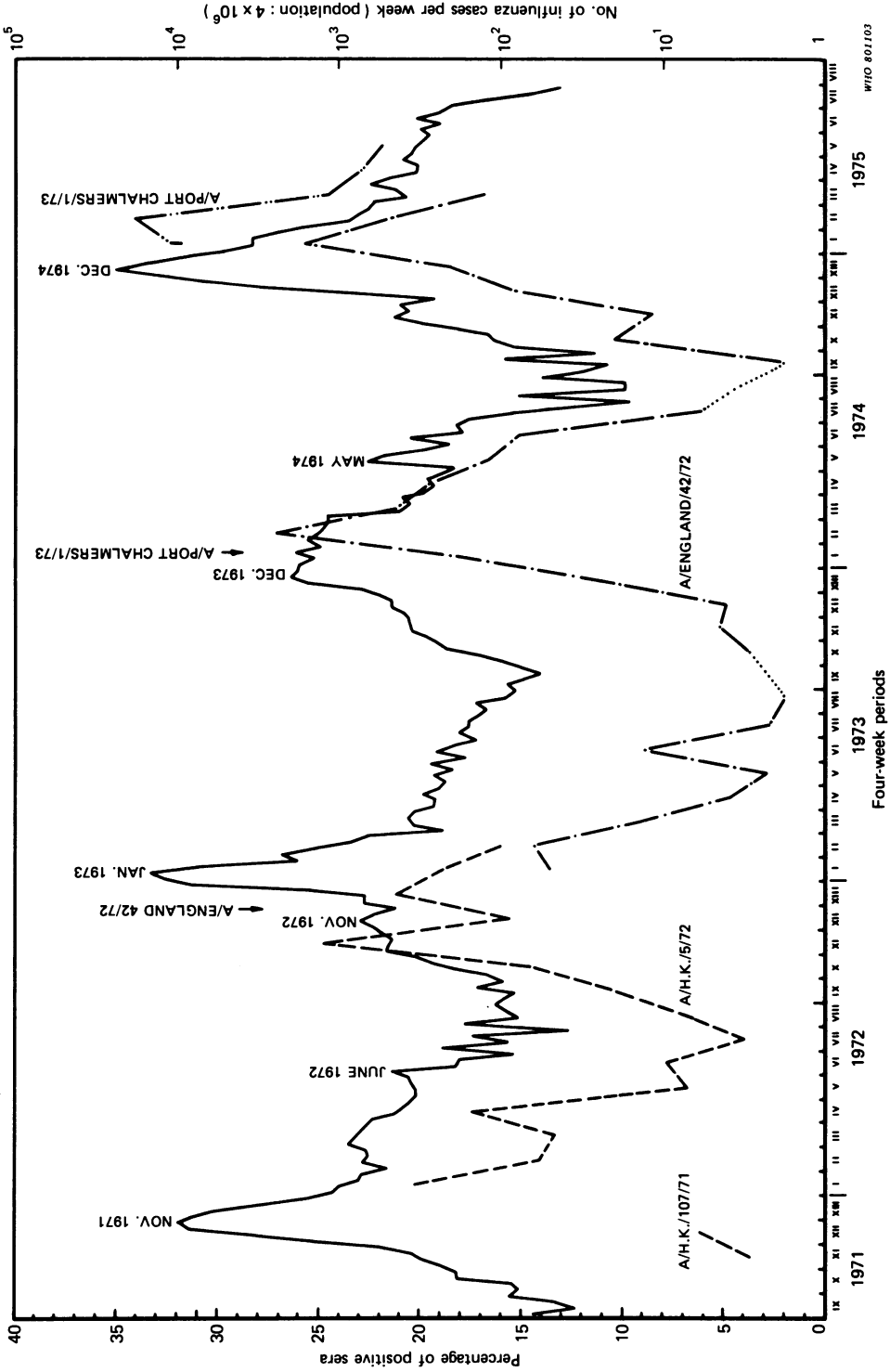


Fig. 2. Incidence of influenza (solid line) and percentage of sera positive for influenza antibodies (broken line), Madrid 1971-75.

High levels of seropositivity were observed during the epidemics that commenced in January 1973 and December 1974, as well as after the period of high incidence of influenza which occurred in December 1973. This indicates that an increase in the number of notified cases to twice the non-epidemic level is accompanied by an increase in the proportion of sera found positive by IDD tests. The highest level of seropositivity observed in December 1974 to January 1975

coincided with the occurrence of the highest number of notified cases. It was concluded that the use of the IDD test to monitor human sera may provide useful information on the recent prevalence of influenza A infection in the community. Nevertheless this information should be regarded as complementary to information provided by virus isolation studies and serological diagnosis based on paired sera.

RÉSUMÉ

UTILISATION DES ÉPREUVES D'IMMUNODIFFUSION DOUBLE DANS DES ÉTUDES ÉPIDÉMIOLOGIQUES SUR LA GRIPPE EN ESPAGNE

Les épreuves d'immunodiffusion double (IDD) ont été effectuées sur des échantillons de sérum prélevés toutes les semaines dans une banque de sang de Madrid, de 1972 à 1975, et les proportions de sérums positifs ont été comparées au nombre de cas cliniques enregistrés par le système de surveillance de la grippe dans la même zone. Les anticorps détectés par l'IDD ne persistaient que deux à trois mois et étaient donc considérés comme un indice cumulatif d'infection récente. Les résultats de l'étude ont montré qu'une

augmentation de deux fois, par rapport au niveau non épidémique, du nombre des cas de grippe signalés s'accompagnait d'un accroissement du niveau de la séropositivité. Bien que les épreuves IDD puissent donc fournir des renseignements utiles sur la prévalence de l'infection récente par le virus grippal A, ces renseignements doivent être considérés comme des compléments aux données provenant des études par isolement du virus et du diagnostic sérologique fondé sur des paires de sérums.

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