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# INFLUENZA SURVEILLANCE IN RIO DE JANEIRO BETWEEN 1980-1981: A VIROLOGICAL AND SEROLOGICAL STUDY

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*Laboratory surveillance of Influenza has shown a low virus activity in Rio de Janeiro during 1980 and 1981. A few influenza A (H3N2) viruses were isolated in both years during the winter months. Serological investigations showed that this subtype has circulated mostly among children under 10 years of age. No H1N1 virus was isolated but an increase in the proportion of adults with antibody to this virus was noted in sera collected in 1981. Influenza B virus was isolated from children in the spring of 1981 and again an increase was noted in the proportion of adults with antibody to this virus.*

Influenza is a truly international disease that in its epidemic form recognizes no territorial boundaries and spreads with ease from country to country, often affecting a large proportion of the population irrespective of age.

There are two closely related reasons for maintaining surveillance of influenza: epidemiologically, there is the need to know at any one time the extent and severity of epidemics; virologically, it is desirable to know the antigenic composition of the viruses isolated in such episodes. Undoubtedly, the more important of the two is the virus isolation.

The prevalence of influenza can be measured indirectly by the collection and analysis of information on mortality. Such information is available in many of the developed countries and has been found to correlate closely with virological evidence of the presence of influenza viruses circulating in the population.

Another way to measure indirectly the prevalence of influenza is using morbidity figures based on notifications of acute respiratory illness (Stuart-Harris, 1979).

In countries where mortality and morbidity data are not available, serological surveys may be used to measure the impact of influenza over an epidemic period. This may be done by testing randomly collected or blood-bank sera for variations in the proportions with antibody or in the geometric mean titres (Candeias & Pereira, 1972; McGregor et al., 1979).

This paper describes influenza surveillance in Rio de Janeiro during 1980 and 1981, by virus isolation and by studies on the prevalence of antibodies to the prevalent variants.

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## MATERIAL AND METHODS

Between January 1980 and December 1981, 592 specimens for virus isolation were taken from people, most of whom were children, with acute respiratory tract diseases within three days of the onset of symptoms.

Serum samples were collected during the same period from persons of all ages with various clinical conditions. These sera were stored frozen at – 20°C until tested.

After collection throat swabs, were sent to the laboratory in virus transport medium (VTM: Eagle's Minimal Essential Medium (MEM) plus 0.2% Bovine Serum Albumin) at 4°C and inoculated into tissue cultures within two hours of collection after the addition of penicillin (1,000 U/ml), streptomycin (5,000 μg/ml) and amphotericin B (10 μg/ml). Primary and secondary cultures of rhesus monkey kidney (RMK) and a canine kidney cell line (MDCK) were used. RKM cell were maintained in Eagle's MEM without serum and MDCK cells in the same medium, plus 1.5 μg/ml of crystalline trypsin (BDH Chemicals Ltd.) after prior washing of the cell monolayer with serum free medium.

Cultures were incubated at 33°C on a rotating drum. MDCK culture fluids were tested for haemagglutination using guinea-pig and chicken erythrocytes one week after inoculation, or before this time if the cell monolayer was destroyed. RMK cultures were tested for hemadsorption using guinea-pig erythrocytes at five to seven day intervals up to 21 days.

Positive MDCK or RMK culture fluids were inoculated in embryonated chicken eggs. After 3 days incubation at 33°C, allatoic and amniotic fluids were harvested and isolates were identified by double immune diffusion (DID) and haemagglutination inhibition (HI).

Standard antigens and sera for DID were obtained from the National Institute of Allergy and Infectious Disease (Maryland), and those used in HI test were supplied by the WHO Collaborating Centers for Influenza in CDC (Atlanta) and CPHL (London).

To confirm the identification, all the isolates obtained during this time were sent to WHO Collaborating Centers afore-mentioned.

Human sera were treated with receptor destroying enzyme (RDE) and tested by haemagglutination inhibition (HI) by a standard microtitre procedure. The viruses used in HI tests were A/Brazil/11/78 (H1N1) – allantoic fluid. A/Bangkok/1/79 (H3N2) – allantoic fluid and B/Singapore/222/79 – ether extracted allantoic fluids.

Reagents used in HI test (RDE and antigens) were obtained from WHO Collaborating Center for Influenza in CDC (Atlanta).

All the techniques used for virus identification of antibody titrations were described in Advanced Laboratory Techniques for Influenza Diagnosis – CDC. Immunology series no. 6 – Procedural Guide.

## RESULTS

*Influenza A:* Eight influenza A viruses were isolated from 592 clinical specimens during the two years. All of them were subtype H3N2 antigenically homogenous, cross-reacting with the A/Bangkok/1/79 and A/Texas/1/77 reference strains and referred as "intermediate". The positive specimens were from seven children less than five years old and from one adult. Seven were detected from May to August 1980 and one in May 1981. No H1N1 virus was isolated during this period. The overall isolation rate for influenza A

virus was 1.7%. The serological study done in 887 human sera (all ages) showed that H3N2 viruses circulated mainly in the under 10 years age group, as can be observed by the increased percentage with significant titres ( $\geqslant 40$) when comparing the two years (Fig. 1).

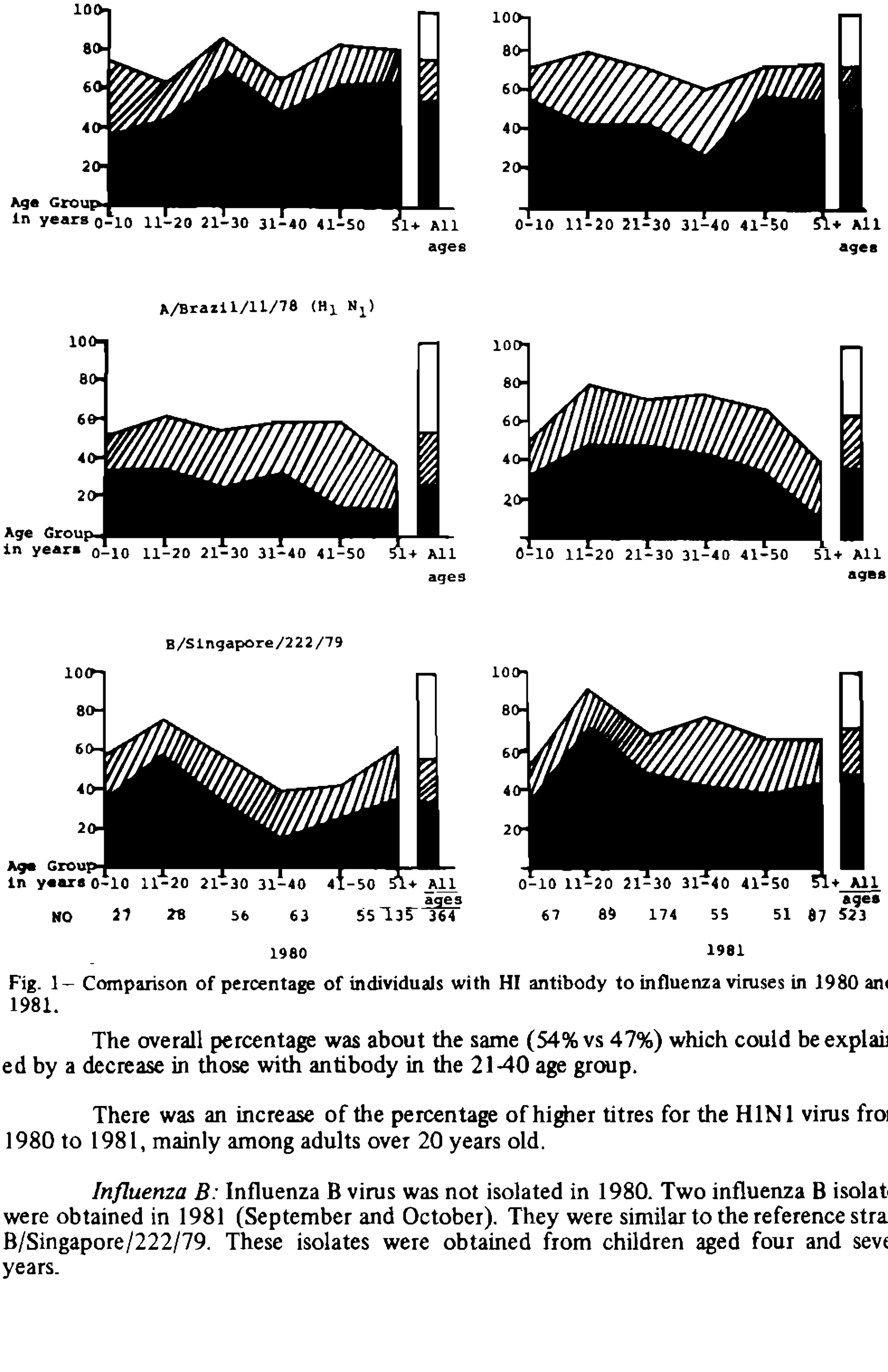


Fig. 1– Comparison of percentage of individuals with HI antibody to influenza viruses in 1980 and 1981.

The overall percentage was about the same (54% vs 47%) which could be explained by a decrease in those with antibody in the 21-40 age group.

There was an increase of the percentage of higher titres for the H1N1 virus from 1980 to 1981, mainly among adults over 20 years old.

*Influenza B:* Influenza B virus was not isolated in 1980. Two influenza B isolates were obtained in 1981 (September and October). They were similar to the reference strain B/Singapore/222/79. These isolates were obtained from children aged four and seven years.

In the serological study there was no change in the percentage with high titres in children under 10 years but there were increases in the age groups 11 and 40 years old (Fig. 1). There was an increase of 13% in the overall percentage of people with high titre antibody.

## DISCUSSION

Influenza activity was low in Rio de Janeiro during 1980 and 1981. A significant circulation of influenza virus is not suggested either by the low virus isolation rate or the serological results. Morbidity figures collected during these two years have not shown an outbreak of acute respiratory infections during the periods in which influenza virus was isolated (Sutmoller et al., 1983). This is in agreement with the World Health Organization observations that influenza activity has been low in countries of the Northern hemisphere, the only exception being the United States where a considerable increase occurred in both morbidity and mortality due to the H3N2 subtype (WHO, 1982).

Our influenza A isolates were obtained mostly from children, since we have directed our investigation towards the age group where morbidity and mortality by acute respiratory infection is highest (WHO, 1980). These viruses circulated unchanged for two successive years during the later fall and winter. The serological investigations confirm that the H3N2 subtype circulated specially among those under 10 years of age. This was not suprising because a previous serological study (Chaves, Nascimento & Pereira, 1982) had shown that antibodies to A/Texas/1/77 (H3N2) were infrequent in the first months of 1980. Unpublished data from another Influenza laboratory in Rio de Janeiro, in which the majority of specimens were from adults, showed that there too only H3N2 strain were isolated (R.D. Machado, personal comunication).

No H1N1 virus was isolated during this period but the serological investigation suggests that this strain may have circulated among adults. Chaves, Nascimento & Pereira (1982) showed that H1N1 virus appeared in Rio de Janeiro in 1979 among under 20 years age groups, and in the begining of 1980 about 50% of this group had antibodies to this virus.

The lower isolation rate of influenza B virus is in agreement with the serological results for the under 10 age group.

This study will be continued, with the inclusion of morbidity data and examination of many more clinical specimens from adults in order to obtain a better monitoring system of the circulation of influenza virus in Rio de Janeiro.

## RESUMO

Dados laboratoriais indicam que a atividade do vírus influenza durante os anos de 1980 e 1981 foi limitada no Rio de Janeiro. Poucas amostras de vírus influenza A (H3N2) foram isoladas durante o inverno em ambos os anos. Estudos sorológicos indicam que esta variante do vírus H3N2 circulou principalmente entre crianças menores de 10 anos. Nenhum vírus H1N1 foi isolado, mas sua circulação foi detectada pelo aumento dos títulos de anticorpos específicos nos soros de adultos coletados em 1981. O vírus influenza B foi isolado de crianças durante a primavera de 1981, e nos soros de adultos coletados em 1981 houve uma elevação nos títulos de anticorpos também para este vírus

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