Epidemiological Study of Influenza Virus in Brazil from 1996 - 1998

Abstract

The results of the epidemiological study of 828 nasal swabs collected in 1996 and 1997 from 0-85 year old patients with influenza-like illness living in the South and Southeastern regions of Brazil and 594 nasal swabs from two more cities of the Northeast in 1998 are described.

The unpredictable behaviour of influenza virus strains was clearly demonstrated during this study. In 1996 the circulation of three different strains of influenza virus A (H1N1) were detected: A/Taiwan/01/86 (25,40%), A/Bayern/07/95 (4,70%), A/Texas/36/91 (1,60%). The higher incidence of subtype (H1N1) during the influenza season of 1996, when compared with previous years was also demonstrated in our studies, and this fact was observed in the other continents. Also, three different strains of influenza A (H3N2) as follows: A/Wuhan/359/95 (28,60%), A/Johannesburg/33/94 (15,90%), A/Alaska/10/95 (3,20%) and B/Beijing/184/93 (20,60%).

Key Words:
GROG network, influenza virus, epidemiology, virus isolation, identification.
circulated in the South and Southeastern regions of Brazil. Nevertheless, in 1997 only one representative strain of each subtype of influenza A (H1N1) A/Bayern/07/95 (17,10%) , influenza A (H3N2) A/ Wuhan/395/95 (46,30%) and influenza B/Beijing/184/93 (36,60%) was detected.

During 1998 influenza virus A/Sydney/05/97 (H3N2) (80,50 %) predominated in the South and Southeastern regions of Brazil. A/ Sydney/05/97 is an antigenic variant which evolved from the A/Wuhan/ 359/95 virus. In addition, influenza A/Bayern/07/95 H1N1) (2,10 %) circulated only in the city of São José do Rio Preto.

On the other hand, influenza B/Beijing/184/93 was isolated in the South, Southeast and Northeast.

The present study demonstrated that there are regional differences in the circulation of different strains of influenza virus.

The study demonstrated the seasonality of influenza virus in Brazil. Influenza virus A activity started in March with a peak of virus isolation detected in the June-July period. This activity decreased in September as observed during the 1996-98 period.

Even so it was possible to detect sporadic cases of influenza even during other months. Influenza virus type B activity showed an irregular pattern of circulation when compared with influenza virus type A.

Other viruses were isolated from the respiratory tract during this study: parainfluenza virus (0,77%), respiratory syncytial virus (0,63%), adenovirus (0,91%), rhinovirus (0,56%), mumps virus (0,21%) and enterovirus (3,37%).

**Introduction**

Influenza is one of the oldest and most common diseases which affects man. It was first described by Hippocrates in 412 BC (1), and influenza like outbreaks were described since 1173 AD. Clearly tabulated by HIRSCH (2), but the first well-documented pandemic of influenza-like disease was described in 1580 and since this period 31 possible influenza pandemics have been documented (3). It may also be one of the deadliest diseases. Its ability to kill stems from the capacity of this virus to suffer mutations, often producing new strains (antigenic shift) that cause pandemics without being able to restrain them by modern medical science (4). Influenza virus infections are the most frequent cause of medically attended acute respiratory illness (5,6).

Influenza may cause severe illness such as pneumonia, particularly in children, elderly people and persons presenting medical risk factors such as cardio-pulmonary disease, liver illness and immunodeficiencies.

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Influenza virus type A was first isolated from human beings during an epidemic of human influenza that happened in England in 1933 (7) and subtypes have been grouped according to the characteristics of their glycoprotein membrane: hemagglutinin (HA) and neuraminidase (NA).

Influenza A viruses have been shown to produce frequent epidemics of respiratory disease in swine and horses and are also common pathogens of domestic and wild birds and sea mammals (8).

According to the World Health Organization, among the 31 influenza pandemics documented, four occurred in this century. The pandemic of Spanish influenza occurred in 1918, Asian influenza, in 1957, Hong Kong influenza, in 1968 and Russian influenza, in 1977 constitute the landmarks of the drastic consequences of the mutation of influenza virus.

In 1976 a new influenza virus from pigs caused human infection and severe illness although no pandemic in fact occurred (9). Two other non-pandemic new strains were also identified in 1986 (10) and in 1988 (11).

Recently influenza virus type A (H5N1), isolated in Hong Kong from a fatal case of acute respiratory illness, (12) and influenza virus type A (H9N2), isolated from cases of influenza-like illness in Kowloon, Hong Kong Island and China, (13) have changed the picture of influenza virus subtypes that have already infected human beings, and brought out the fundamental role of surveillance.

Influenza virus is a continuing threat to Public Health and was defined as a prototype of emerging infections by the Institute of Medicine’s Comittee on Emerging Microbial Threats to Health in the United States report, published in 1992 (14). The devastating pandemic of Spanish influenza responsible for 20 to 40 million deaths worldwide in 1918 – 1919 (15,16) was key for the development of the International Surveillance Network (17). Influenza is an illness of high impact in Public Health because of the ability of this virus to infect high numbers of people in a short period of time.

The rapid dissemination to different continents, leads to the constant monitoring of influenza. As an uncontrolable disease, the surveillance is the only tool able to detect new strains and to adopt strategies to avoid the dramatic consequences of the dissemination of new strains. Nowadays a total of 110 designated National Influenza Centres, in 82 countries and four Collaborating Centres for Influenza Reference and Research located in London, Atlanta, Melbourne and Tokyo participate in this international network sponsored by the World Health Organization (WHO). The Respiratory Virus Laboratory of Instituto Adolfo Lutz is one of WHO recognized National Centres and, since 1960, has been part of the network of influenza laboratories cooperating with WHO.

In order to contribute to the control of influenza in the world a project of influenza surveillance (GROG-BRAZIL) has been established in Brazil, since By the systematic collection of nasal swabs from patients with the influenza-like syndrome by sentinel physicians, it has been possible to monitor influenza strains circulating in different states of Brazil and compare them with viruses isolated in other regions.

This project was concluded with the support of Pasteur Mérieux Connaught do Brasil, Instituto Adolfo Lutz and in collaboration with the WHO National Influenza Centre, Lyon, France and the Center for Disease Control and Prevention, Atlanta GA USA.

Materials and methods
Collection of Nasal Specimens

A total of 1422 nasal swabs were collected from patients 0 to 85 years old, with influenza like illnesses.

From April to September 1996, nasal swabs from 396 patients were collected from Porto Alegre and Curitiba (Southern region) and São Paulo and Botucatu (Southeastern region). In the
period of February to October 1997, 432 nasal swabs were collected from the South (Curitiba and Porto Alegre) and Southeast (Botucatu, São José do Rio Preto and São Paulo). From March 1998 to January 1999, 594 nasal swabs were collected from the South (Curitiba and Porto Alegre) and Southeast (cities of Botucatu, São Jose do Rio Preto, Itapetininga, São Paulo and Rio de Janeiro) and Northeast (cities of Salvador and Recife).

**Processing Nasal Swabs for Virus Isolation**

Swabs were removed from the collection vial and vigorously agitated in 2.5 mL of cell culture medium on vortex mixer and 1000 U/mL of penicillin and 1000 ug/mL of streptomycin were added.

Virus isolation attempts were done in cell cultures of MDCK (kidney canine, canine, canis familiaris), Hep - 2 (epidermoid carcinoma larynx, human, Hela markers), Vero (african green monkey, Cercopithecus aethiops, kidney), CPF - III (lung fibroblast human), FRhK - 4 (fetal, rhesus monkey kidney, Macaca mulata), and embryonated hen’s eggs.

The Hep-2 cell culture was maintained in Eagle’s minimal essential medium with 2% fetal calf serum, while Vero and MDCK cell cultures were maintained in Eagle’s minimal essential medium with 2 ug of trypsin per mL. The CPF-III culture was maintained in Eagle’s minimal essential medium plus L-15 medium (Leibovitz) with 2% fetal calf serum, and FRhK-4 culture was maintained in medium 199 with fetal calf serum. Two tubes of each cell culture were inoculated with 0.1 mL of specimen per tube and incubated at 35°C in stationary racks. The cells were fed with fresh medium every 3 days. The cultures were examined every 2 days for cytopathic effect or for the presence of hemadsorption, during 14 days. The hemadsorption test was done 7 days after inoculation of clinical specimens in tubes of Vero (guinea pig erythrocytes) and MDCK (chicken erythrocytes) cell cultures.

Virus isolated from cell culture was identified by indirect immunofluorescence monoclonal antibodies of Respiratory Panel 1 Viral Screening & Identification Kit, (Chemicon International Inc., Temecula, CA). The virus isolated from CPF - III cells was submitted to an acid - stability test to differentiate rhinovirus enterovirus. Viruses isolated from embryonated hen’s eggs were identified by hemagglutination inhibition test using specific sera to influenza virus to characterize the type and subtype of influenza viruses.

Isolated viruses were sent to the Influenza Branch at the Center for Diseases Control and Preventions - (CDC), Atlanta, GA, for antigenic and genetic analysis.

**Immunofluorescence assay (IFA)**

Cells from positive cultures were prepared for IFA as described previously (18). Monoclonal antibodies from Respiratory Panel 1 Viral Screening & Identification Kit (Influenza A&B, Respiratory Syncytial Virus, Adenovirus and Parainfluenza Viruses types 1,2 and 3) provided by Chemicon International Inc., Temecula, CA. was used as reagents for IFA. Cells were harvested, washed with sterile PBS (pH 7.2), and were resuspended in a few drops of PBS. Two cell smears on each microscope slide were prepared and then fixed in nonhydrous acetone for 10 minutes at 4°C. Uninfected cells were used as control.

Cells were incubated with 25 uL of the monoclonal antibodies during 30 minutes at 37°C. After washing with PBS, 25 uL of fluorescein conjugated antimouse IgG were added, and incubated for another 30 min. After three washes with PBS, the specimens were examined under a fluorescent microscope with a halogen lamp (Nikon; 100 W). All of the clinical specimens were read by two observers. The doubtful or discrepant samples were restained and read at least twice to check for reproducibility.

In 1998 the immunocapture ELISA test (19) was introduced for the rapid detection
of infection by influenza viruses types A and B and parainfluenza. Briefly, 90 µL of the sample suspension was added to wells of a 96-well microtiter plate containing fixed monoclonal antibodies obtained from the WHO National Influenza Centre, Lyon, France (0.05 µg of anti-NPA and 0.05 µg of anti-NPB for influenza viruses and 0.05 of anti-NH for parainfluenza viruses). Then, 200 µL of phosphate buffered saline (PBS), containing 1% bovine serum albumin, was added to each well.

After the plates were incubated for 2 h at 37°C. After incubation, the wells were washed three times with PBS, and 100 µL of homologous rabbit antiserum at a dilution of 3 hemagglutination inhibition units was added (19). After 1 h of incubation, the wells were washed three times with PBS and 100 µL of the conjugate solution was added. The plates were incubated for 45 min at 37°C and washed three times with PBS and 100 µL of the substrate solution was added.

The plates were incubated in the dark for 30 min at room temperature, and then the reaction was stopped by adding 25 µL of 2 mM sodium azide. The plates were read with a spectrophotometer at 405 and 630 nm. Positive results were defined as follows: (optical density at 405 nm)/(optical density at 630 nm) > 0.25.

**Results**

A total of 1,422 nasal samples have been analysed during the 1996, 1997 and (influenza season) surveillance and 244 (17.15%) were positive for influenza virus. From 396 swabs studied during 1996, 68 (17.2%) were positive to influenza virus by the immunocapture ELISA test, of which 63 could be characterized as being: 20 of influenza virus A of subtype (H1N1), 30 of subtype (H3N2) respectively and 13 as influenza virus type B (Table 1).

<table>
<thead>
<tr>
<th>Regions</th>
<th>Cities</th>
<th>No. of samples studied</th>
<th>influenza virus isolated n°</th>
<th>%</th>
<th>type A subtypes</th>
<th>type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southeast</td>
<td>São Paulo</td>
<td>71</td>
<td>21</td>
<td>16.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Botucatu</td>
<td>76</td>
<td>10</td>
<td>14.1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>São José do Rio Preto</td>
<td>26</td>
<td>6</td>
<td>7.9</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>5</td>
<td>1</td>
<td>20.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>396</td>
<td>68</td>
<td>17.2</td>
<td></td>
<td>16</td>
<td>3</td>
</tr>
</tbody>
</table>

H1N1 * A/Taiwan/01/86 like  
** A/Bayern/07/95 like  
*** A/Texas/36/91 like  
H3N2 * A/Wuhan/359/95  
** A/Johannesburg/33/94 like  
*** A/Alaska/10/95  
B = B/Beijing/184/93

During 1996, influenza virus types A/Taiwan/01/86 (H1N1) and A/Wuhan/359/95 (H3N2) circulated in the Southern and Southeastern regions of Brazil. Also the influenza virus related to A/Bayern/07/95 H1N1) was detected in Curitiba and São Paulo. These studies demonstrated that influenza virus A/Texas/36/91 (H1N1) was bypassed by influenza virus A/Taiwan/01/86 (H1N1) (Table 1). The presence of A/Texas/36/91 was detected only in Curitiba.

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Influenza virus related to A/ Johannesburg/33/94 (H3N2) was detected in the South (Curitiba) and Southeast (São Paulo). Influenza virus related to A/Alaska/10/95, which is a variant of A/Wuhan/359/95, was detected in the Southern (Porto Alegre) and Southeastern (São Paulo) regions of Brazil. With the exception of São Paulo, influenza virus related to B/Beijing/184/93 was prevalent in all cities studied.

Influenza viruses related to A/ Taiwan/01/86 (H1N1), A/Wuhan/359/95 (H3N2) and B/Beijing/184/93 were the most prevalent strains, during 1996, in the majority of regions studied.

Other respiratory viruses were also isolated during 1996: parainfluenza virus (0.75%), adenoviruses (2) (0.05%), rhinovirus (2) (0.50%), mumps virus (1) (0.25%) and respiratory syncytial virus (1) (0.25%).

Among the 432 swabs studied in 1997, 82 (18.52%) were positive to influenza virus by the immunocapture ELISA test, of which 40 could be characterized: as 7 of influenza virus of subtype A (H1N1), 18 of subtype A (H3N2) respectively and 15 influenza virus type B (table 2).

Table 2. Influenza virus isolated and characterized during 1997 in different regions of Brazil.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Cities</th>
<th>n° of samples studied</th>
<th>influenza virus isolated n°</th>
<th>%</th>
<th>A subtype</th>
<th>type</th>
</tr>
</thead>
<tbody>
<tr>
<td>South</td>
<td>Porto Alegre</td>
<td>102</td>
<td>19</td>
<td>18.6</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Curitiba</td>
<td>35</td>
<td>12</td>
<td>34.3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Southeast</td>
<td>São Paulo</td>
<td>225</td>
<td>40</td>
<td>17.8</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Botucatu</td>
<td>54</td>
<td>6</td>
<td>11.1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>São José do Rio Preto</td>
<td>16</td>
<td>5</td>
<td>31.3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>432</td>
<td>82</td>
<td>19.0</td>
<td>7</td>
<td>15</td>
</tr>
</tbody>
</table>

H1N1= A/Bayern/07/95 like \quad H3N2= A/Wuhan/359/95 like \quad B = B/Beijing/184/93 like

The influenza virus related to A/Bayern/07/95 (H1N1), during 1997, was prevalent only in Porto Alegre. On the other hand, in Curitiba, A/Wuhan/359/95 (H3N2) and B/Beijing/184/93, was prevalent in that season. In the southeast, with the exception of São José do Rio Preto where only virus type B/Beijing/184/93 was isolated, the prevalence of virus related to A/Wuhan/359/95 (H3N2) and B/Beijing/184/1997 was observed (Table 2).

Other viruses also isolated during 1997 were as follows: a high frequency of coxsakievirus A 24 was observed (24) (5.55%) coxsakievirus B (2) (0.46%) echovirus (1) (0.23%) respiratory syncytial virus (7) (1.62%) adenovirus (5) (1.15%) rhinovirus (5) (1.15%), parainfluenza virus (3) (0.69%) mumps virus (2) (0.46%).

Among the 594 swabs studied in 1998, 95 (16.0%) influenza viruses were isolated, of which 46 were characterized as being 38 of type A of which - 37 belonging to subtype H3N2, 1 to subtype H1N1 and 8 of type B (Table 3).
Table 3. Influenza virus isolated and characterized during 1998 in different regions of Brazil.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Cities</th>
<th>no. of samples studied</th>
<th>influenza virus isolated</th>
<th>type A subtypes</th>
<th>type B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>no.</td>
<td>%</td>
<td>H1N1</td>
</tr>
<tr>
<td>South</td>
<td>Porto Alegre</td>
<td>125</td>
<td>30</td>
<td>24.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Curitiba</td>
<td>85</td>
<td>10</td>
<td>11.8</td>
<td>0</td>
</tr>
<tr>
<td>Southeast</td>
<td>São Paulo</td>
<td>96</td>
<td>28</td>
<td>29.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Botucatu</td>
<td>127</td>
<td>11</td>
<td>8.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>São José do Rio Preto</td>
<td>26</td>
<td>2</td>
<td>7.7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Itapetininga</td>
<td>74</td>
<td>5</td>
<td>6.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rio de Janeiro</td>
<td>29</td>
<td>4</td>
<td>13.8</td>
<td>0</td>
</tr>
<tr>
<td>Northeast</td>
<td>Salvador</td>
<td>26</td>
<td>5</td>
<td>19.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Recife</td>
<td>6</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>594</td>
<td>95</td>
<td>16.0</td>
<td>1</td>
</tr>
</tbody>
</table>

H1N1 = A/Bayern/7/95 like  H3N2 = A/Sidney/5/97 like  B= B/Beijing/184/93 like

The results demonstrate that influenza virus A/Sydney/5/97 like (H3N2), was prevalent during the 1998 influenza season in Brazil.

Influenza viruses related to A/Bayern/7/95 (H1N1) and influenza B/Beijing/184/93, were also isolated. Both strains were predominant worldwide (Table 3) during that year, also.

Other viruses isolated during 1998 were: enterovirus (21) (3.53%), adenovirus (6) (1.01%), parainfluenza virus (5) (0.84%) and respiratory syncytial virus (1) (0.50%) and rhinovirus (1) (0.16%).

The monthly distribution of influenza viruses isolated - during 1996, 1997 and is described in figure 1. Influenza virus A activity started in March with a peak of infection detected in the June-July period. This activity decreased in September. Influenza virus type B activity showed an irregular pattern of circulation. The influenza strains which circulated in Brazil during 1996 - 98 are presented in Table 4.

Discussion and conclusion

The majority of influenza virus type A (H3N2), isolated in our laboratory, in 1996, was closely related to A/Wuhan/359/95, the recommended vaccine strain for the 1996 - 97 season. This virus was also isolated in Africa, the Americas, Asia and Europe. Most of the influenza virus type A (H1N1) isolated worldwide were closely related to A/Bayern/7/95 which was detected in this study, (20) also.

Influenza A (H3N2) related to A/Johannesburg/33/94 that circulated in Brazil, in 1995, (21) was also detected in 1996.

Influenza A (H3N2) related to A/Alaska/10/95 antigenically related to A/Wuhan/359/95 (H3N2) as determined by genetic analysis (CDC Personal Communication) circulated in Brazil in this period. It was not detected in other regions in the same period.

Influenza virus of type B, isolated in our study ,and the majority of influenza virus type B isolated in Africa, the Americas, Asia, Europe, and Oceania was antigenically similar to B/Beijing/184/93 and to B/ Harbin/7/94.

However, influenza B virus antigenically similar to B/Guangdong/5/94 (related to the earlier reference strain B/Victoria/287),
circulated in China and Hong Kong in 1996 (20). In 1996, influenza virus related to A/Bayern/07/95 (H1N1) was first observed in Brazil and it was the only subtype (H1N1) detected here, in 1997. This strain occurred only in the Southeastern region of Brazil. A vaccine composition of component H1 was included in the 1997-98 season.

In contrast to 1996, during influenza season 1997, only influenza A (H3N2), antigenically related to A/Wuhan/359/95, circulated in Brazil.

The majority of influenza B isolated in the present study was characterized as related to B/Beijing/184/93, included in the vaccine of 1996-97. According to the WHO report, published on 26 September 1997 (22) Influenza activity in the Southern Hemisphere has generally been moderate to severe. Influenza A (H3N2), antigenically similar to A/Wuhan/359/95, and Influenza B, antigenically similar to B/Beijing/184/93 and B/ Harbin/7/94 viruses, circulated in many countries causing outbreaks in Australia, Brazil, Chile, New Zealand and South Africa and viruses related to these were also reported in Argentina, Madagascar and Senegal.

The surveillance of influenza virus types A and B has been followed since 1995. Influenza type A/Johannesburg/33/94 - like (H3N2) circulated in our country during 1995, (21) the H3 component of the 1995 -1996 vaccine. This influenza virus strain was substituted by a new variant of influenza virus related to type A/Wuhan/359/95- like (H3N2), which appear in Brazil in 1996, and has been recommended as the WHO H3 component of the 1996 -97 and the 1997 -98 vaccine.

Influenza virus related to type B/Beijing/184/93, the component of the 1995 -96 vaccine, and the WHO recommended B component of the 1996 -97, 1997-98 and 1998 -99 vaccines, has been prevalent in our country since 1995.

The higher incidence of subtype (H1N1) during influenza season 1996, when compared with previous years, was also demonstrated in our studies, and this fact was also observed in the other continents. Influenza virus related to A/ Johannesburg/33/94 (H3N2) prevalent in 1995 (21), was substituted by A/Wuhan/359/95 in 1996. This strain was also prevalent during the 1997 influenza season. Between this two virus strains a variant of A/Wuhan/359/95 - A/Alaska/10/95 (H3N2) - was also detected during 1996 influenza season. Influenza virus A/Wuhan/359/95 was bypassed by A/Sydney/7/97 during 1998 influenza season in Brasil (Table 4).

**Table 4. Strains of influenza virus isolated in Brazil during 1996 - 98.**

<table>
<thead>
<tr>
<th>Years</th>
<th>Viral strains</th>
<th>1996</th>
<th>1997</th>
<th>1998</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(n=396)</td>
<td>(n=432)</td>
<td>(n=594)</td>
</tr>
<tr>
<td></td>
<td>A/Texas/36/91 (H1N1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A/Bayern/07/95 (H1N1)</td>
<td>3</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>A/Taiwan/01/86 (H1N1)</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A/Alaska/10/95 (H3N2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A/Johannesburg/33/94 (H3N2)</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A/Wuhan/359/95 (H3N2)</td>
<td>18</td>
<td>18</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>A/Sydney/05/97 (H3N2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B/Beijing/184/93</td>
<td>13</td>
<td>15</td>
<td>8</td>
</tr>
</tbody>
</table>

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It was demonstrated by the surveillance in previous years that during the 1995 and 1997 flu season, influenza viruses isolated in Brazil were antigenically related to reference strains selected by the World Health Organization for vaccine composition. Influenza virus A/Taiwan/01/86 (H1N1) predominated during influenza season 1996, in Brazil. Nevertheless, the recommended vaccine composition by WHO based on the finding in the Northern Hemisphere contained the A/Texas/36/91 (H1N1) strain. Again, in 1998 the study brought out differences between influenza virus H3N2, isolated in Brazil, and the H3 component recommended by WHO for the Northern-Hemisphere. The surveillance realized in 1998, showed the prevalence of A/Sydney/5/97, which had been already detected in Australia. It was not included in the vaccine composition of 1997. This new strain spread to Asia, Europa Occidental, and also to United States of America, Argentina and Chile. However, when the countries of Southern Hemisphere received the characterization of the isolates as A/Sydney/5/97 (H3 N2) it had already been circulating in Argentina and Chile in the end of 1997 and was the prevalent strain in South America during the winter of 1998.

Meantime, Public Health authorities had already ordered the vaccine production according to the recommendation of February 1997 which had A/Wuhan/359/95 (H3N2) as H3 component which was prevalent in our country in the 1996-1997 period. This finding shows the need of constant follow up of influenza circulation in our country, so that a more adequate composition of vaccine for each region, should be selected.

Due the findings of the present study, the vaccine recommendation will continue to be made each February, which relates to the composition of vaccines intended for use in the following winter in the Northern Hemisphere (November to April). A second recommendation will be included for the Southern Hemisphere in September, which relates to vaccines that will be used for the following winter in the Southern Hemisphere (May to October) (23).

The present study showed higher incidence of influenza during the autumn and winter season in Brazil (Fig.1). Influenza virus A activity started in March, with a peak of isolation detected in the June-July period. This activity decrease in September, as observed during the 1996-98 period (Fig.1). Although, it was possible to detect influenza virus even during other months, similarly as demonstrated in previous studies (24,25). These sporadic cases indicate the permanent circulation of influenza virus. This finding, emphasizes the importance of the GROG - system first set up in France, in 1984 (26). In Brazil, this project was established in 1995 but the clinical specimens collected in that occasion were sent to WHO National Influenza Centre, Lyon, France for virus isolation attempts (21).
The establishment of the project in Brazil, increased the detection of circulating influenza virus and consequently resulted in more accurate knowledge of the main strains circulating in the country (Table 4).

The proportion of influenza virus isolated did not vary much in the three different years of study being (17.2%) in 1996, (19.0 %) in 1997 and (16. %) in 1998. Influenza virus is usually recovered from 5% to 20% of patients with influenza-like illnesses (3).

Our study emphasizes the importance of maintaining an active surveillance, in order to detect the emergence of new and different strains of influenza virus and also the variation in the prevalence of determined strains of influenza virus in different regions.

Recently, new subtype of influenza virus caused concern all over the world about the possibility of the occurrence of pandemics of influenza.

In May 1977, influenza A (H5N1) virus was isolated from a child who died
with Reye’s Syndrome, in Hong Kong. Prior to this, the H5N1 was known to infect various species of birds, including chickens and ducks. It was first discovered in terns in South Africa, in 1961, and caused death to chickens: in spring, 1997, when thousands of chickens died in Hong Kong after contracting it. (WHO Emerging and other Communicable Disease (EMC), 9 January 1998).

A total of 18 people were infected by H5N1 subtype of virus A. Among them, 6 deaths were confirmed by Health Authorities in Hong Kong, Special Administrative Region of China. The H5 virus, however, did not evolve into a form that is readily transmitted from person to person, and its potential for this kind of transmission remains unknown. Infection with the virus is believed to come through contact with infected birds.

Influenza A (H5N1) virus emerging in Hong Kong on May, 1997 (27) brought out the potential threat of the virus spreading to the human population. Fortunately, it was restricted to that region.

In 1999, Influenza virus A (H9N2) antigenically related to swine influenza virus, isolated in Hong Kong Island, SAR, in 1998, WHO report (13) has been identified in 2 hospitalized children aged 1 and 4 years. One of the children was from Kowloon and the other from Hong Kong Island.

Both of them were hospitalized in early March 1999 due to influenza-like illness with fever 39-40°C. In addition, five cases of H9N2 infection were reported from Guangdong province, in China, and were believed to have been infected through contact with poultry.

These findings, demonstrate how important is to maintain an active surveillance in order to help the Public Health Authorities for the correct measure of prevention and control of influenza epidemics.

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