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Isolation of Influenza Viruses and Influenza Epidemics in the Taiwan Area, 1999-2002

Introduction

Influenza virus is a most infectious, and one of the most active pathogenic microorganisms in the Taiwan Area each year. It causes in every winter upper respiratory infections in many elderly persons and young children. Some may even die of serious complications. It, therefore, is a threat to the life of many. Influenza viruses come in three types, A, B and C. Types A and B infect humans primarily. Type A influenza virus was first isolated by Smith, Andrews, Laidlaw, et al. in 1933; and type B in 1936 by Francis[(1)]. In 1940, Burnet found that influenza virus could be multiplied in embryonated hen's eggs. Studies of the specific features of the virus and development of attenuated vaccines thus began. That vaccines were protective was first verified in the 1950's.

The genetic substance of influenza virus is a single ply RNA of the orthomyxovirus family. For differences in surface antigen hemagglutinin (H) and neuraminidase (N), the virus can be further classified into several subtypes[(2)]. The influenza viruses isolated in humans are primarily

hemagglutinins H1, H2 and H3, and neuraminidases N1 and N2. Hemagglutinin is to help virus contact cell surfaces; neuraminidase is to help virus enter cells[3]. Type B virus is more stable than type A; its chances of antigen mutation are smaller. Unlike type A virus, which infects both humans and animals such as pigs and avian, type B virus infects humans only[4,5]. Virus are named by 1) virus type, 2) where it is first isolated, 3) serial number of virus for laboratory testing, 4) year isolated, and 5) subtype of strain. The 2002 vaccine of type A Moscow virus strain, for instance, is named A/Moscow/10/99 (H3N2).

Both the hemagglutinin and neuraminidase of influenza virus may mutate periodically. Changes in gene sequences will have impact on the immune system of the host. The host can no longer identify the virus to react immunologically. Several large-scale outbreaks in the recent years were due mainly to a series of point mutation of the RNA coding of hemagglutinin. Antigen changes of influenza are primarily in two ways, antigenic drift, which produces point mutation at certain gene sections. Changes are relatively mild, and the virus strain is still of the same type. For instance, in 1999, A/Beijing/252/95 (H1N1) was the major strain of infection; at the end of 1999, A/New Caledonia/20/99 (H1N1) strain was isolated and was the main strain of infection in 2000. It was then chosen to be the strain of vaccination in 2000. Another mutation is antigenic shift, which is more serious than antigen drift, and may, by reorganizing gene sections, bring about the production of new subtypes of viruses. The immune system of the host can no longer identify the strain, and person-to-person infection will soon spread out to cause pandemics. The last antigenic shift occurred in 1968. H2N2 type A virus, after ten years of infection, was totally replaced by H3N2 as a result of antigenic shift[6].

For the mutability of influenza viruses and the fact that the infection can be transmitted rapidly droplets between individuals, strict and continuous surveillance of the viruses is most essential. The Center for Disease Control set up in 1998, in collaboration with 11 medical centers and teaching hospitals, laboratories of viral infections to jointly establish a surveillance system of influenza, and to understand the activities of influenza viruses and their antigenic mutation in Taiwan. In coordination with the WHO Global Surveillance System of Influenza, information on mutation of virus strains in Taiwan is periodically forwarded to the standard laboratory of the WHO for reference in deciding the vaccine to be used for the year. In this way, the threat of influenza to the health of the population is expected to be minimized.

Materials and Method

Sources of Specimens

Specimens came from patients under either outpatient or hospital care in all medical centers and teaching hospitals, and patients of the 300 some sentinel clinics throughout the country. When a person had several or all of the following symptoms, fever higher than 38.5 ℃, coughing, sore throat, headache, muscle pain and running nose, within three to five days after onset, pharyngeal swab of his/her upper respiratory tract specimen was collected, and sent to any viral laboratory in virus transportation culture medium under low temperature for the cultivation, isolation and assessment of virus.

Cultivation and Isolation of Influenza Viruses

The pharyngeal swab and the 1 ml transportation medium were thoroughly mixed to allow viruses to settle in the medium. The fluid was collected, and filtered through 0.45 μm filter. 100 μl of the specimen was collected and inoculated on MDCK cell strain, cultured for 3-10 days, and

centrifuged at 3,000 rpm for 15 minutes to collect the upper fluid. The sedimented cells suspected of infection were dyed by the indirect immunofluorescence assay (IFA) method for microscopic examination. Appearance of apple-blue fluorescence on the cells indicated influenza virus positive.

Assessment of the Subtypes of Influenza Viruses

Assessment of the types of influenza viruses was made by the hemagglutinin inhibition method using the WHO Influenza Diagnostic Kit. A 96-hole U-shape disc was used. 50 μl of virus fluid was placed in the first row of the disc, and diluted with D-PBS solution. 0.75% of GP RBCs was then placed in each hole. The disc was left under 4 ℃ for 45 minutes to record the HA titers of viruses. After the virus titer was decided, the virus fluid was diluted to 8 HA unit per 50 μl for hemagglutinin inhibition test. 25 μl of standard antibody of each type was added to the first row of the disc, and diluted with D-PBS. 25 μl of virus fluid of diluted antigen concentration of 8 HA/50 μl was added to each hole, and shook the disc slightly to allow virus and antibody to mix. The disc was left for 10 minutes, added 50 μl of 0.75 GP RBCs, and left under 4 ℃ for 45 minutes for the recording of HI titer.

Results and Discussion

Isolation of Influenza Viruses

In the three years between July 1999 and July 2002, 2,821 strains of influenza viruses had been isolated in the Taiwan Area, 1,581 strains of type A, accounting for 56.04% of all; and 1,240 strains of type B, accounting for 43.96% of all. By month, patients concentrated around end of winter and early spring, between December and February of the following year, occupying around 48.53% of all isolated strains (Figure 1). The peaks of influenza

infections in Taiwan correspond to peaks of other countries in the north hemisphere. In Japan for instance, peaks of infections in 1996-2001 were around the 48th week (December) and the 10th week of the following year (March). In the US, in the period between 1976 and 2001, 80% of the strains were isolated in the months between December and February of the following year[8]. In the last three years in Taiwan, the number of influenza virus strains isolated each year had increased year by year; 793 in 1999/2000, 945 in 2000/2001, and 1,083 in 2001/2002. Possible reasons were that the accuracy of the diagnostic and specimen-collection skills of the sentinel physicians had improved; the laboratory testing skills of the laboratories had improved; and the mutation of the influenza virus strains during the study period was greater. This part will be discussed later.

Onset of infection was different in the northern, central, southern and eastern parts of Taiwan. Each year, infection began earlier in the northern and eastern parts, around early December, to reach a peak in February of the following year. In the central and southern parts of the island, infection started in late December to reach a peak in mid-February of the following year, and declined thereafter. Infection began in the central and southern parts always two to three weeks later than the northern and eastern parts, and the infection period was shorter. Possible reasons were climatologic, that it was warmer in the winter in the central and southern parts than the northern and eastern parts; and that rainfall was less. For a lower population density in the eastern part of the island, only one laboratory was under contract, the number of positive cases isolated was fewer, 194 strains in three years, accounting for only 6.87% of al strains isolated. The northern part of the island, for its high population density, had the most number of strains isolated, 1,317 in three years, accounting for 46.69% of all (Figure 2).

Influenza epidemics in Taiwan though are more frequent in late winter and early spring, small-scale outbreaks also occur in April-June. The virus type in the April outbreak, primarily type B, is different from the type, primarily type A, in winter, as shown in Figure 2. This fact indicates that both types A and B coexist each year. When the intensity of infection of the major type declines, another type becomes more active. As the weather is turning warmer, the number of cases infected is thus smaller. By statistics, the virus type of the small-scale outbreaks in April-June can be used to predict the major virus types of the next infections (Figure 3). Though influenza viruses can be isolated in Taiwan any time of the year, by past experience, the epidemic season of influenza in Taiwan is around December to June of the following year.

Analysis of the Antigenic Mutation of Influenza Viruses

Virus types of the winter epidemics of influenza in Taiwan in the period between 1999 and 2002 were different each year, as shown in Figure 3. The type isolated in the winter of 1999/2000 was primarily A(H1N1); it was type B in 2000/2001; and it was A(H1N1) again in 2001/2002. During this period, the virus types of influenza in Taiwan were extremely different from those of the European countries and the US; they were relatively similar to those of some Asian countries such as Japan and Hong Kong (Table 1). In 1999/2000 and 2001/2002, the major strain isolated in Taiwan and Japan was A(H1N1). The primary virus type in Hong Kong in these two years though was also type A, fairly equal numbers of H1N1 and H3N2 were isolated. In 2000/2001 in most Asian countries, type B was the primary type; whereas in the US in 1999/2000 and 2001/2002, it was A(H3N2); and in 2000/2001, it was A(H1N1). In Europe in 2000/2001, A(H1N1) was the major type; and in 2001/2002, it was A(H3N2)[7-10]. Types and intensity of influenza infections in countries of the north hemisphere are, for ethnic and climatic differences, not necessarily

the same. Continuous surveillance of indigenous infections of influenza is most critical to its prevention and control.

By virus types, the A(H1N1) virus strain isolated in November 1999 through February 2000 in Taiwan and other Asian countries was A/Beijing/262/95(H1N1)-like strain transforming into A/New Caledonia/20/99(H1N1)-like. The New Caledonia strain was a newly isolated strain in 1999; many people had not developed antibodies against it yet, a relatively large-scale epidemic thus occurred in 1999/2000. The World Health Organization announced in February 2000 to use New Caledonia as one of the vaccines for 2000/2001, and again also for the A(H1N1) strain of 2002/2003[(11)]. The New Caledonia strain has been the major composition of vaccines recommended by the WHO for the last three years. The virus strain isolated in Taiwan in 2001/2002 was primarily A/New Caledonia/20/99(H1N1)-like. The New Caledonia strain seems to be an extremely strong virus strain. By 2002, many had still been infected by it for the lack of antibodies.

Of the types A and B influenza viruses in Taiwan in the last three years, type B had more antigenic mutation. In December 2000, the type B virus of the Yamagata lineage had mutated from B/Beijing/184/93-like to B/Sichuan/379/99-like, and started an outbreak in 2000/2001. In March 2002, type B virus of the Victoria lineage, B/Hong Kong/330/2001-like, was isolated. The virus started a small-scale outbreak in April-June. When the Hong Kong strain was first isolated in March in the northern part of the island, the type B virus in the southern part was still the Sichuan strain. It was only until May that the Hong Kong strain was isolated in the southern part. The virus seemed to have traveled from the north to the south. By July 2002, both the Sichuan and the Hong Kong strains, two type B strains of different lineage, existed in

Taiwan concurrently. They continued to be isolated (Table 2).

A fact worth noticing is, type A (H3N2) virus though has never caused any large-scale outbreaks, it can be isolated sporadically anytime of the year. Since 2000, the A(H3N2) type has always been the A/Moscow/10/99 (H3N2)-like. It has been relatively stable as compared with A(H1N1) and type B virus.

In February 2002, A(H1N1) virus was isolated one by one in Egypt, Israel, the UK and the US. By comparing their gene sequences, experts were of the opinion that these virus strains were a new virus resulted from reorganization of the gene sequences of the existing A(H1N1) and A(H3N2). The Center for Disease Control of the Department of Health made an analysis of the gene sequences of the neuraminidase of all already isolated type A H1 virus strains. No A(H1N1) virus was found. A(H1N2) virus had been isolated between December 1988 and March 1989 in six cities in China. It did not start any serious outbreaks. The A(H1N2) virus detected this year, its hemagglutinin (H) was found similar to the H1 of A(H1N1), and its neuraminidase (N) was similar to the N2 of A(H3N2). The World Health Organization thus decided that the currently available vaccines should be protective against this new A(H1N2) virus[12].

The World Health Organization has announced that the 2002/2003 influenza strain composition would be A/New Caledonia/20/99(H1N1)-like, A/Moscow/10/99(H3N2)-like, and B/Hong Kong/330/2001-like. These three strains had been isolated in Taiwan in the first half of 2002. The vaccines against influenza to be used this year therefore should cover these three strains. These vaccines should be protective against the influenza viruses currently existing in Taiwan.

Conclusion

Since the establishment of the Center for Disease Control of the Department of Health in July 1999, surveillance of influenza has been a priority. The long-term surveillance can, in addition to informing the World Health Organization for the selection of vaccine strains, detect the mutation of the indigenous influenza virus types in Taiwan and thus to establish a model for predicting the epidemics. The northern part of the island for instance, though the weather has been relatively warm this summer, the number of influenza viruses isolated is not necessarily significantly less than what it used to be in July through August in the past years. After a small-scale type B outbreak in April, the virus isolated in July was primarily A(H3N2). As all types of influenza viruses are still active in the summertime, the infection should be closely watched.

The mutability of influenza viruses and their transmission by droplets from person to person, and the relatively stable status of the viruses in the last two years might bring about a sudden appearance of new virus types. Strict and continuous surveillance therefore is most essential. Through effective vaccination, influenza can be prevented.

Sources: Research and Laboratory Testing Division, Resource Service Division, CDC, and viral infection laboratories under contract

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Figure 1. Influenza Activities by Month, Taiwan, 1999-2001

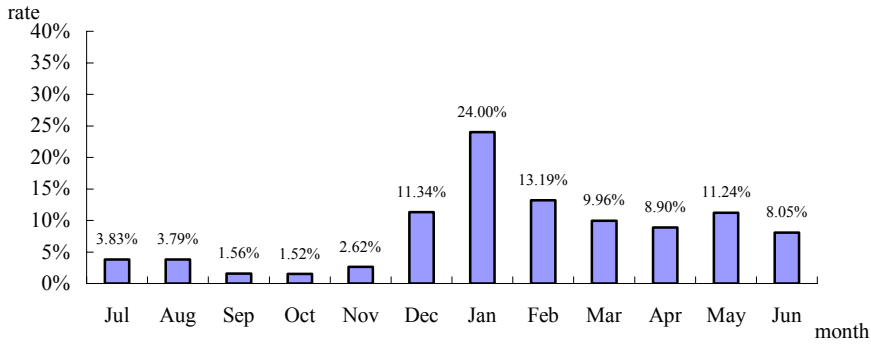
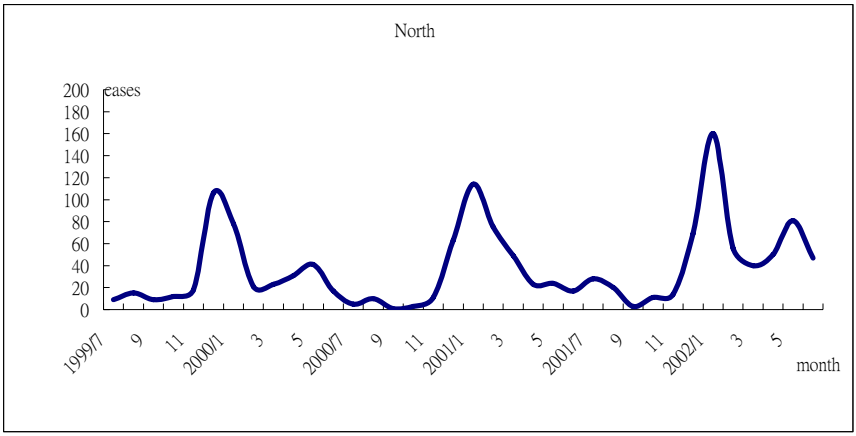
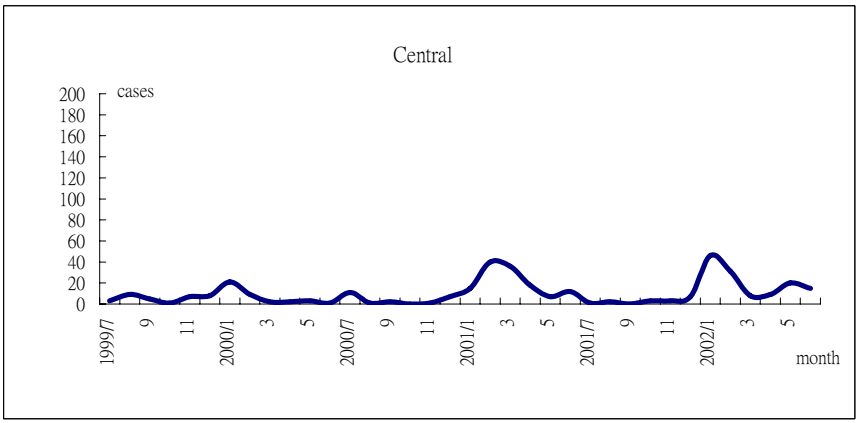
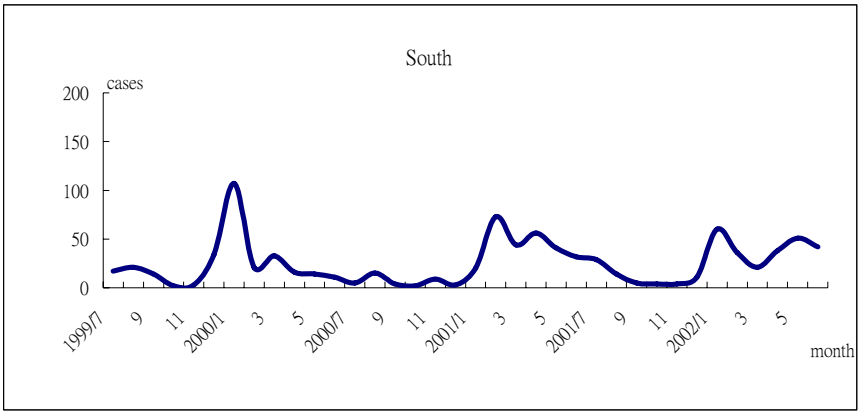
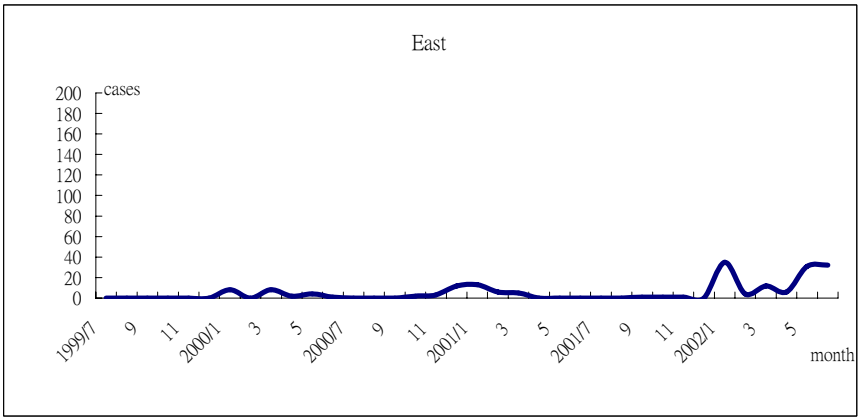


Figure 2. No. of Influenza Virus Strains Isolated by Region, Taiwan, 1999-2002






South
cases
200
150
100
50
0
1999/7
9
11
2000/1
3
5
2000/7
9
11
2001/1
3
5
2001/7
9
11
2002/1
3
5
month


East
cases
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160
140
120
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80
60
40
20
0
1999/7
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2000/7
9
11
2001/1
3
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11
2002/1
3
5
month

Figure 3. No. of Types A and B Influenza Viruses Isolated by Month, Taiwan, 1999-2002

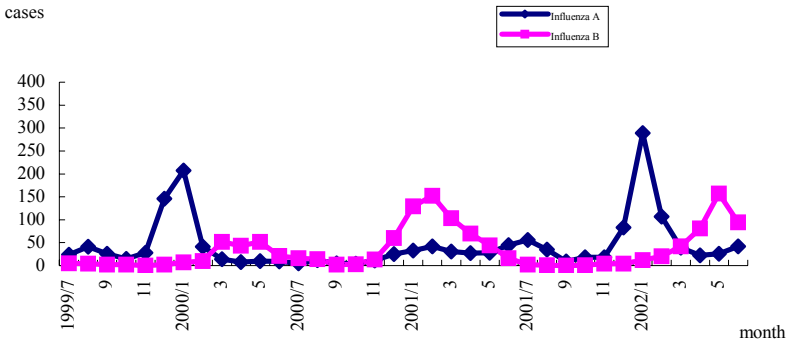

Table 1. Major Types of Influenza Viruses in the North Hemisphere, 1999

season	Taiwan	Japan	Hong Kong	US	Europe
1999/2000	A（H1N1）	A（H1N1）	A（H1N1）and A（H3N2）	A（H3N2）	A（H3N2）
2000/2001	B	B	B	A（H1N1）	A（H1N1）
2001/2002	A（H1N1）	A（H1N1）	A（H3N2）	A（H3N2）	A（H3N2）

Subtyped Influenza Virus Isolates in Taiwan, 1999-2002