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Surveillance of influenza viruses isolated from travellers at Nagoya International Airport

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SUMMARY

In order to conduct a survey of influenza viruses entering Japan via travellers arriving by airplanes, gargle solutions were collected from passengers who reported to the quarantine station of Nagoya International Airport complaining of respiratory symptoms. From 504 samples collected between August 1996 and March 1999, 30 influenza virus strains were isolated. Twenty-eight of the isolates were influenza A (H3N2) viruses and two were influenza B viruses. No H1N1 virus was isolated. Among 28 isolates of H3N2 virus, 3 strains were obtained outside the influenza season. Nucleotide sequences of the haemagglutinin (HA) genes of these isolates along with those from domestic patients were analysed in order to determine the influence of imported influenza viruses by travellers on epidemics in Japan. From the phylogenetic and chronological aspects, the possibility was suggested in one case in 1997/8 and two in the 1998/9 season that imported virus by travellers may have influenced the domestic influenza epidemics.

INTRODUCTION

Influenza viruses migrate constantly among the human populations of the world. It is not easy to determine the source or to trace the movement of these viruses in detail, and the advanced means of transportation of our modern era further complicates the subject. On the other hand, because of the developments in methodology combining PCR and sequencing, relationships among viruses can now be examined in a quantitative manner and evolutionary analysis has been made possible [1]. In Japan, influenza epidemics (influenza-season) occur from November to March, with a few viruses sporadically appearing in the off-season. Glezen reported that isolates of influenza virus in the late epidemic or in the off-season sometimes become the epidemic strain of the next season and can be considered a herald wave [2, 3]. The phenomenon of herald waves has also been observed in Japan [4, 5]. However, since Nakajima and colleagues [6, 7] showed that the isolates in the off-season did not necessarily become the epidemic viruses of the next influenza season in Japan, it was thought that influenza viruses coming from outside the country each year might play an important role in the subsequent epidemics. Although the surveillance of influenza virus isolation in Japan has been systematized [8], the influenza viruses carried into Japan by travellers using airplanes have not been surveyed.

In 1997, an H5N1 influenza A virus caused an epidemic in chickens, infected 18 people in Hong Kong [9–12] but did not cause a pandemic. However,

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since the outbreak of H3N2 influenza A virus, 30 years have passed and it is thought that a new influenza pandemic may occur at any time [13]. Therefore, an influenza prevention strategy is being planned worldwide [14]. When H3N2 viruses appeared in Hong Kong in 1968, the first infected person carrying the H3N2 virus entered Japan by sea 15 days after the occurrence of outbreak in Hong Kong [15]. In these days it is possible that a new pandemic virus could enter Japan before the first outbreak could be detected as a pandemic threat. In this study, we isolated viruses from returning travellers from abroad who reported to the quarantine station at Nagoya International Airport complaining of respiratory symptoms. The purpose of this study was to survey influenza viruses entering Japan via travellers by airplane and to analyse the relationships between these viruses and the domestic isolates and to construct a system for tracking new influenza viruses entering Japan.

METHODS

Samples

Gargle samples were collected from 504 travellers on their return from foreign countries who reported respiratory symptoms at the quarantine station of Nagoya International Airport, Aichi prefecture, Japan. Nagoya International Airport has been ranked the third in importance among the international airports of Japan, and about two million people passed through in 1998. Samples were collected between August 1996 and March 1999. The ages of the passengers ranged from 2 to 77 years (average $29{\cdot}6 \pm 11{\cdot}3$ years). Of these travellers, 271 were male and 229 were female and the sex of 4 was unknown. The area visited included Asia (81·3%), Hawaii and Guam (5·4%), Europe (4·4%), other countries (6·0%), and unknown (2·2%). Among the Asian countries, Thailand, Indonesia, Hong Kong, Singapore and Malaysia were visited by 22·9, 18·0, 14·2 and 11·9% of these travellers, respectively. Influenza viruses isolated in Aichi prefecture were collected from throat swabs of domestic patients as part of the epidemiological surveillance of infectious disease in Aichi prefecture.

Virus isolation and antigenic analysis

Specimens were collected and stored at -30 °C at the quarantine station in Nagoya International Airport. They were kept frozen and transported to Aichi Prefectural Institute of Public Health once per week, and virus isolation, antigenic analysis, and nucleotide sequencing of the HA gene were performed. They were seeded in MDCK cells with trypsin (2·5 μg/ml) at 34 °C to isolate influenza viruses. The isolated viruses were identified by the haemagglutination inhibition (HI) test with turkey erythrocytes using anti-H1, H3 and B influenza antisera. Since 1998, anti-H5 antiserum was also used. Anti-A/Beijing/262/95 serum was used as H1 antiserum. Anti-A/Aichi/46/96 (A/Wuhan/359/96-like) and anti-A/Sydney/05/97 sera were used. Anti-A/tern/S.Africa/61 serum was used as H5 antiserum. Anti-B/Harbin/7/94 and B/Guangdong/5/94 sera were used as influenza B antisera. Anti-A/Sydney/05/97, A/Beijing/262/95, A/tern/S. Africa/61, B/Harbin/7/94 and B/Guangdong/5/94 sera were supplied by The National Institute of Infectious Diseases, Japan.

Nucleotide sequencing and phylogenetic analysis of the HA genes

Complementary DNAs of the HA genes from the viruses were amplified by the reverse transcription (RT)–PCR method as described previously [16]. For the HA1 region of the H3 gene, seven primers employed in the previous study were used [17]. Nucleotide sequences of the HA gene were determined from virion RNA by the di-deoxy chain termination method as described previously [17].

Amino-acid sequences of the HA1 region were deduced from the nucleotide sequences of the H3N2 isolates from all travellers and 38 domestic viruses which represented 10% of isolates from each influenza season, balanced with sampling date and area. A phylogenetic tree was constructed in such a way as to give priority to mainstream changes inherited by most of the subsequent strains rather than to strain-specific changes, thus minimizing the possibility that a strain-specific change shared by two viruses had occurred independently [6]. This tree constructed by mainstream and sidestream changes was originally proposed by Both and colleagues [18]. This method based on the hypothesis that frequency of back mutation is low than additional mutation and mainstream changes remained to the isolates in following season. The constructed tree is similar to a tree by Neighbour-Joining methods. Our method has one advantage compared to NJ method, readers know which amino-acid change occurred in the evolution.

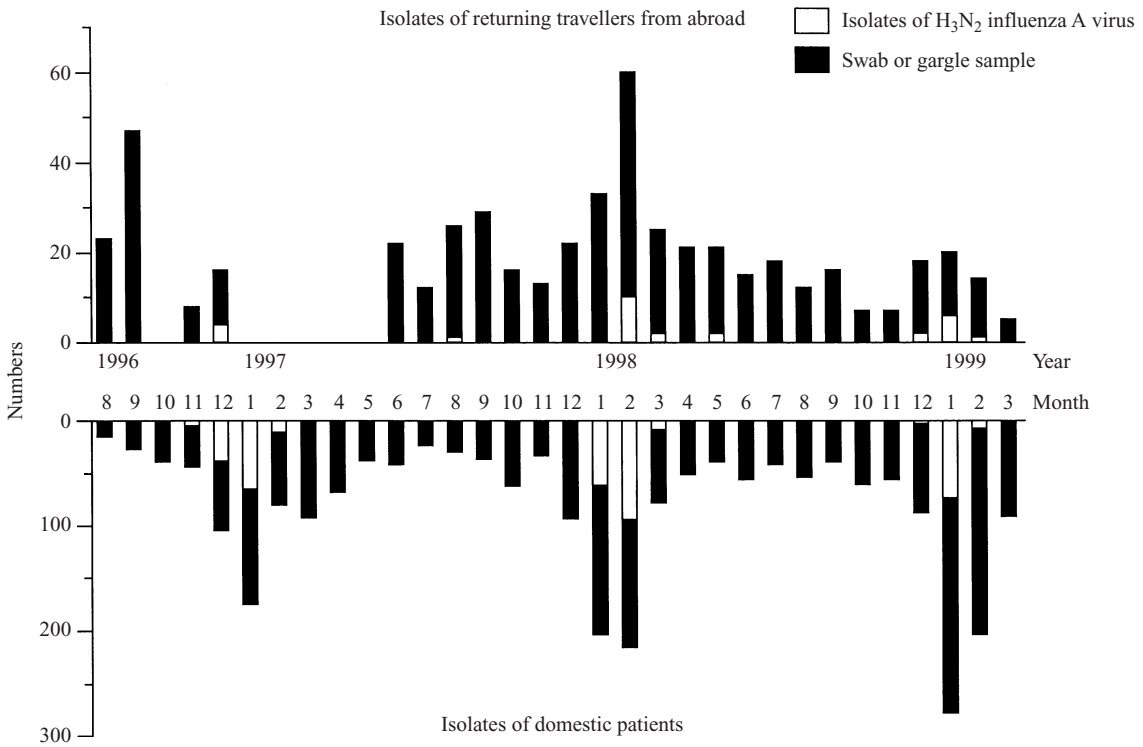

Fig. 1. Monthly report of H3N2 influenza A viruses isolated from returning travellers from abroad and from non-traveller patients. The upper portion of the graph indicates the isolates from returning travellers from abroad, and lower portion indicates the isolates from local epidemiological surveillance. The white box shows the number of H3N2 viruses. The black box shows the number of swab and gargle samples.

RESULTS

Survey of influenza viruses isolated from returning travellers from abroad

Among 504 samples, 30 were found to contain influenza viruses. HI tests showed that 28 were A (H3N2) and two were influenza B viruses. A (H1N1) virus was not isolated. Figure 1 shows the chronological surveillance of influenza A viruses isolated in Aichi prefecture and from returning travellers from abroad. From traveller samples collected during the 1996/7 season, four (T1–T4) H3N2 viruses were isolated. In the following off-season, one (T5) H3N2 virus was isolated. During the 1997/8 season, 12 (T6–T17) H3N2 viruses were isolated and in the following off-season, two H3N2 viruses (T18, T19) were isolated. During the 1998/9 season, nine H3N2 viruses (T20–T28) were isolated as well as two influenza B viruses (B/AI/72/99 and B/AI/73/99) in February 1999. Profiles of persons from whom viruses were isolated are shown in Table 1. At the quarantine station, physicians asked all the returning passengers with respiratory symptoms to participate in our survey but gargle specimens were obtained only from Japanese. During the survey period, Japanese citizens comprised 85% of the international travellers coming to Nagoya International Airport at which gargle samples were collected. The date of onset of symptoms was reported by the patients themselves. Since influenza B viruses were isolated from only two travellers in this study, we omitted further analysis of them in this paper.

Isolation of influenza viruses from domestic patients

In 1996/7 season, 119 H3N2 viruses were isolated from 285 swabs and 114 gargle samples as a routine epidemiological surveillance in Aichi prefecture. The H3N2 viruses were isolated from November 1996 to February 1997 and the peak of isolation was January 1997 (65 isolates). In the 1997/8 season, 167 H3N2 viruses were isolated from 355 swabs and 139 gargle samples from January to March of 1998 and the peak of isolation was January (61 isolates) and February (94 isolated) in 1998. In the 1998/9 season, 84 H3N2 viruses were isolated from 413 swabs and 153 gargle samples from January to March 1999, and the peak was in January (74 isolates) (Fig. 1).

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Table 1. *Profiles of Japanese travellers returning from abroad from whom influenza A H3N2 viruses were isolated and identification numbers of selected H3N2 virus isolates from domestic patients*

(A) Onset date and the periods from departure to onset were declared by the travellers.

Identification number	Virus strain*	Sampling date	Country visited	Onset date	The periods from departure to onset (days)
T1	AI/129/96	Dec 96	Hong Kong	Unknown	Unknown
T2	AI/130/96	Dec 96	Saipan	Unknown	Unknown
T3	AI/131/96	Dec 96	Hong Kong	Unknown	Unknown
T4	AI/132/96	Dec 96	Philippines	Unknown	Unknown
T5	AI/89/97	1 Aug 97	Thailand	Unknown	Unknown
T6	AI/74/98	7 Feb 98	Hong Kong	6 Feb 98	3
T7	AI/77/98	8 Feb 98	Singapore	Unknown	Unknown
T8	AI/78/98	10 Feb 98	Indonesia	Unknown	Unknown
T9	AI/79/98	11 Feb 98	Thailand	Unknown	Unknown
T10	AI/80/98	11 Feb 98	Thailand	Unknown	Unknown
T11	AI/81/98	11 Feb 98	Thailand	Unknown	Unknown
T12	AI/82/98	12 Feb 98	Singapore	11 Feb 98	3
T13	AI/83/98	16 Feb 98	Maldives	9 Feb 98	1
T14	AI/84/98	18 Feb 98	Hong Kong	17 Feb 98	3
T15	AI/86/98	19 Feb 98	Thailand	12 Feb 98	2
T16	AI/85/98	10 Mar 98	Hawaii	7 Mar 98	3
T17	AI/179/98	25 Mar 98	Canada	24 Mar 98	Unknown
T18	AI/178/98	6 May 98	India	2 May 98	7
T19	AI/180/98	17 May 98	Hong Kong	16 May 98	6
T20	AI/21/99	30 Dec 98	Hong Kong	29 Dec 98	3
T21	AI/19/99	31 Dec 98	Singapore	27 Dec 98	2
T22	AI/25/99	1 Jan 99	Hong Kong	31 Dec 98	4
T23	AI/22/99	2 Jan 99	Thailand	2 Jan 99	5
T24	AI/28/99	3 Jan 99	Italy	2 Jan 99	5
T25	AI/27/99	7 Jan 99	Indonesia	Unknown	Unknown
T26	AI/24/99	9 Jan 99	Malaysia	8 Jan 99	3
T27	AI/73/99	16 Jan 99	Malaysia	13 Jan 99	6
T28	AI/58/99	10 Feb 99	Philippines	7 Feb 99	10
(B)					
D1	AI/94/96	10 Dec 96	D20	AI/50/98	4 Feb 98
D2	AI/113/96	18 Dec 96	D21	AI/65/98	3 Jan 98
D3	AI/14/97	9 Jan 97	D22	AI/76/98	10 Feb 98
D4	AI/15/97	9 Jan 97	D23	AI/87/98	10 Mar 98
D5	AI/20/97	13 Jan 97	D24	AI/100/98	5 Feb 98
D6	AI/22/97	13 Jan 97	D25	AI/120/98	15 Feb 98
D7	AI/25/97	21 Jan 97	D26	AI/151/98	25 Feb 98
D8	AI/36/97	22 Jan 97	D27	AI/176/98	10 Mar 98
D9	AI/50/97	11 Jan 97	D28	AI/177/98	24 Mar 98
D10	AI/66/97	18 Jan 97	D29	AI/3/99	6 Jan 99
D11	AI/80/97	8 Feb 97	D30	AI/4/99	8 Jan 99
D12	AI/3/98	12 Jan 98	D31	AI/15/99	18 Jan 99
D13	AI/9/98	20 Jan 98	D32	AI/17/99	19 Jan 99
D14	AI/11/98	7 Jan 98	D33	AI/20/99	25 Jan 99
D15	AI/14/98	20 Jan 98	D34	AI/36/99	30 Jan 99
D16	AI/19/98	26 Jan 98	D35	AI/48/99	17 Feb 99
D17	AI/29/98	1 Jan 98	D36	AI/49/99	30 Jan 99
D18	AI/31/98	10 Feb 98	D37	AI/54/99	22 Jan 99
D19	AI/35/98	4 Feb 98	D38	AI/61/99	18 Jan 99

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Table 2. *Antigenic analysis of the AH3N2 influenza A virus isolates*

	Antisera	
Virus strain	Aichi/46/96	Sydney/5/97
Aichi/46/96 …	80	20
Sydney/5/97 …	10	160
T2	40	20
T3	40	20
T4	40	20
T5	40	20
T7	20	80
T9	20	80
T14	20	80
T17	20	80
T18	20	80
T19	< 10	80
T20	10	40
T21	10	40
T22	10	80
T23	< 10	40
T25	< 10	80
T27	10	80
D4	40	10
D6	40	20
D14	20	40
D18	20	80
D29	10	80
D32	10	80

The identification numbers of isolated viruses are shown in Table 1.
All viruses of travellers were tested, except for viruses with the same HA1 sequence. A representative strain was selected from same sequenced travellers' viruses with the same sequence. Representative strains of viruses from domestic patients were randomly selected.

Antigenic analysis of the viruses isolated from travellers

The results of antigenic analysis of the viruses isolated from travellers are shown in Table 2. Domestic isolates of H3N2 virus in Aichi prefecture in the 1996/7 season were antigenically similar to A/Wuhan/359/95 (A/Aichi/46/96-like). Viruses isolated from travellers in the 1996/7 season were also antigenically similar to A/Wuhan/359/95. In the following off-season, of 1997, 1 H3N2 (T5) virus was isolated from a traveller in August and it was also antigenically similar to the Wuhan/359/95. In the 1997/8 season, viruses isolated from travellers and domestic-patients were antigenically similar to A/Sydney/05/97. In the following off-season of 1998, 2 viruses isolated from travellers were antigenically similar to A/Sydney/05/97. In the 1998/9 season, all H3N2 isolates were A/Sydney/05/97 type.

Comparison of the amino-acid sequences of H3 HA1 polypeptides of viruses from travellers and domestic patients

A phylogenetic tree of amino-acid changes relative to A/Wuhan/359/95 is displayed in Figure 2 and the changes are shown on the tree. The isolates from travellers (T1–T28) are enclosed with grey squares, and the isolates from domestic patients (D1–D38) are enclosed within white squares. The isolates of the 1996/7 season and the following off-season are marked with open triangles, the isolates of the 1997/8 season and the following off-season with closed circles, and the isolates of the 1998/9 season with open circles. Three off-season isolates from travellers are marked by double asterisks (T5**, T18**, T19**). The isolates of 1996/7 had 2–8 amino acids which differed in the mainstream sequence of A/Wuhan/359/95. Most isolates in this season had eight amino-acid changes in the mainstream. The isolates of 1997/8 had 0–6 amino acids differing from the mainstream sequence of the major isolates of 1996/7 and the largest number of isolates had 4 amino acid (15 isolates) and 5 amino-acid changes (13 isolates) in the mainstream. The isolates of 1998/9 had 0–3 amino acids differing in the mainstream of the major isolates of 1997/8 and the majority of the isolates had 3 amino-acid changes in the mainstream. Distribution of the isolates on each branch showed no significant differences between the viruses isolated from travellers and domestic patients. The off-season virus in 1997 (T5**) and the virus during 1996/7 season (T1) from travellers were revealed to have the same amino-acid sequences of the HA1 region. These two viruses were demonstrated to belong to the largest group of the isolates of 1996/7 season. One of the two off-season viruses in 1997/8 (T18**) belonged to the largest group of the isolates of 1997/8 season and was closely related to an isolate (D31) from a domestic patient obtained in the following season. The other virus (T19**) belonged to a minor group of isolates of 1997/8 season. As shown in Figure 2, after the 1996/7 season, different types of amino-acid changes occurred at residue 137 and resulted in three branches which were termed A, B and C, having amino-acid residues, serine (S), asparagine (N) and phenylalanine (F), respectively. Position 137 (138 [19]) is positively

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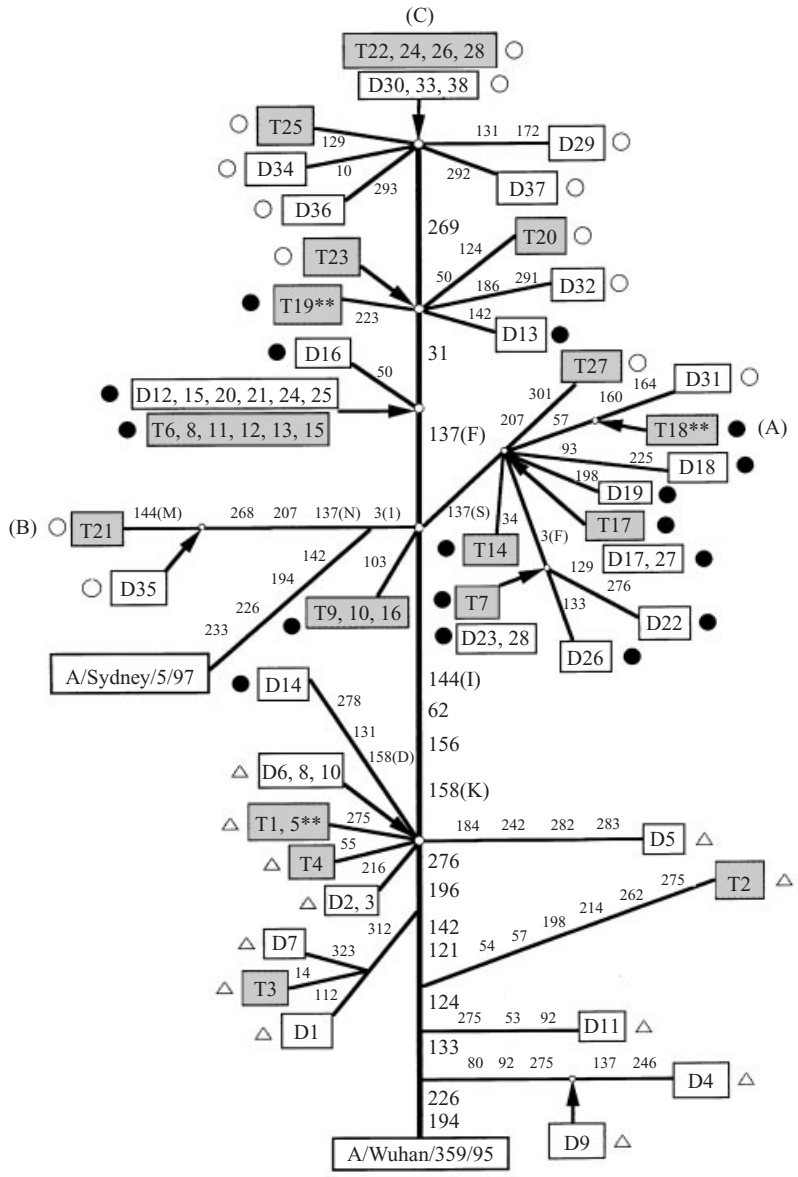

Fig. 2. The evolutionary tree for HA1 polypeptides of influenza A (H3N2) virus. Numbers refer to the mainstream amino-acid changes which became fixed in most of the subsequent strains (vertical lines), or to strain-specific amino-acid changes (side branch) which appeared in a few strains.

selected codons [19] on the site A. The branch A includes 12 strains of the 1997/8 and 2 strains of 1998/9, the branch B includes none of 1997/8 season but 2 strains of 1998/9, and the branch C includes 15 strains of 1997/8 and 15 strains of the 1998/9 season.

DISCUSSION

In Japan, it is thought that the influenza viruses coming from outside the country each year may cause subsequent epidemics. In order to conduct a survey of influenza viruses entering Japan via travellers arriving by airplane and the relationships of epidemic strains, we collected specimens from 504 passengers who reported to the quarantine station of Nagoya International Airport complaining of respiratory symptoms and analysed the isolated influenza viruses. The isolation rate was about 6% from passengers; this value was low compared to isolation rate 30% from domestic patients. One of the reasons is that samples were stored at −30 °C at the airport for 1 week.

The chronological pattern of surveillance of the viruses of travellers and domestic patients showed that the peak isolation period for travellers was similar to that of domestic patients except in off-seasons. The phylogenetic tree constructed with

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isolates of both groups showed that the viruses were very similar. The length of stay in the foreign countries for travellers was relatively short, therefore some travellers might have been infected before their trip abroad. Table 1 showed the length of time from departure from Japan to the date of onset of symptoms. In the case of three travellers, symptoms appeared only 1 and 2 days after departure, but for other travellers more than 3 days had passed after leaving Japan. The relationships with travellers and domestic strains were considered from the view of phylogenetic and chronological aspects. In the 1996/7 season, the parental relationships were not observed among travellers and domestic strains. In the 1997/8 season, T7 may have parental relationships with D22, -23, -26 and -28 strains chronologically and phylogenetically. On the other hand D17, -18, -19 and -27 looked to be derived from T17 isolate from Figure 2. However, T17 was isolated on 25 March but the D-strains were isolated earlier than T17. Therefore chronologically, T17 was not a parental strain of these D-strains. T6, -8, -11, -12, -13, and -15 and D12, -15, -20, -21, -24 and -25 had the same amino-acid sequence in the HA1 region. However D12, -15, -21 and -24 were isolated earlier than any of the T-strains. Therefore, these T-strains could not affect the domestic epidemic. In the 1998/9 season, T23 was chronologically and phylogenetically a parental strain of D32 strain. T22, -24, -26 and -28 may have a parental relationship with D30, -33 and -38. From the phylogenetic and chronological aspects, the possibility was suggested in 1 case in the 1997/8 and 2 in the 1998/9 season that influenza viruses imported by travellers may influence the domestic influenza epidemics. However, the following cases were shown in Figure 2. One is that the HA1 sequences of several strains were the same but isolation times were different (for example, T6, -8, -11, -12, -13, -15 and D12, -15, -20, -21, -24 and -25) and two was that phylogenetic and chronological data did not fit (for example T17 and D18, -19, -17). Therefore, even if T7 strain seems to have parental relationships with D23, D28 by means of phylogenetic and chronological aspects, we could not exclude the possibility that similar virus of T7 was already present in Aichi prefecture before the isolation of T7. However, we could say that viruses of foreign origin entered Aichi prefecture in the epidemic season which could affect the domestic epidemic.

In the off-season of 1996/7 and in the 1997/8 season, three H3N2 viruses were isolated. The travellers from whom these viruses were isolated had visited Thailand, India, and Hong Kong. Of the three countries, two were reported to have epidemics of H3N2 viruses during the travel periods [20, 21]. Off season T18** was phylogenetically and chronologically the possible parental strain of D31 in the next season. However, no off-season isolates from domestic patients in Aichi prefecture were recorded. We do not have any evidence that influenza viruses can survive during summer season in Japan. However, the off-season isolates were characterized as belonging to the largest group of isolates of former influenza seasons (T5** in 1996/7, T18** in 1997/8) and having the largest number of mainstream changes of the former influenza seasons (T5** in 1996/7, T19** in 1997/8). Therefore, surveys of off-season viruses brought into Japan by travellers may be useful for predicting the next epidemic viruses in Japan. In the present survey, the greatest number of air passengers arrived from Southeast Asia, and a few arrived from the southern hemisphere. Further surveillance of this latter group is necessary in order to clarify the relationship between influenza viruses isolated from travellers arriving in the off-season and the epidemic strains of the next influenza season. Over the period of surveillance presented here, we isolated only a few isolates of influenza B. Because most of the travellers were adults (over 16 years), and only 21 (4·2%) were children (under 15 years), the lower rate of isolation of influenza B virus can be explained. Present surveillance at Nagoya International Airport could not give direct evidence that the epidemics in Japan were caused by the viruses imported from foreign countries. Ninety percent of Japanese travellers were cooperative and we could collect many samples. Although, foreigners coming to Japan represented 15% of the total persons entering the country, we are going to operate the surveillance to include these people in the future. It is expected that the next pandemic will originate in Southern China [22]. Our surveillance system may be useful in detecting causative viruses for the next pandemic because of the large number of travellers come back from Hong Kong to Nagoya International Airport (about 200000 passengers in 1998).

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