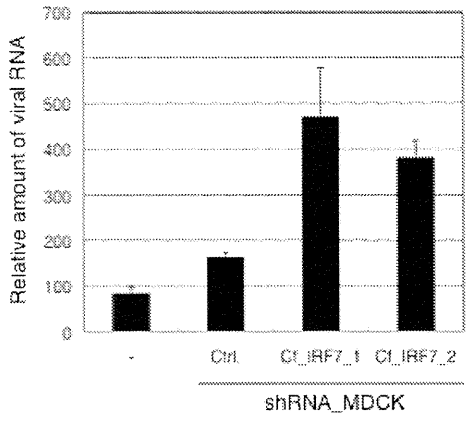

Fig. 4 The amount of A/Narita/1/2009 pdm in MDCK-based cell line expressing shRNA IRF7.

D. 考察

本研究では、新型インフルエンザの大流行に備え、より高い効率でのシードウイルスの分離・増殖とワクチン製造を行うことを可能とする細胞の樹立を目的として、MDCK 細胞の改変によるウイルス高増殖性細胞の取得を試みた。ヒトゲノムについてのデーターベースが充実しているため、まず A549 細胞におけるヒト I 型 IFN 誘導性遺伝子群を標的とした 78 種類の標的遺伝子に対する siRNA ライブラリーを作製した。A549 においてウイルス産生量を増加させる遺伝子を絞り込み、その結果に基づいて、MDCK における siRNA ライブラリーを用いたスクリーニングを行った。その結果、IRF7 をノックダウンすると従来の MDCK 細胞に比べてウイルス産生量が 4 倍まで増加することが明らかとなった。一方、IRF3 をノックダウンしてもウイルス産生量への変化は認められなかったことから、今後は IRF7 と IRF3 によるウイルス産生の制御機構及び IRF7 低発現 MDCK 細胞におけるウイルス産生量の増加機構の解析を推進したいと考えている。

E. 結論

本研究では、インフルエンザウイルスの産生量を増加させる遺伝子として IRF7 を同定した。MDCK 細胞においても A549 細胞と同様に IRF7 をノックダウンするとウイルス産生量が約 4 倍増加したことから IRF7 低発現細胞ではウイルス産生量が増加することが示唆された。本研究を進めていくことにより、ウイルス増殖効率が向上した新規 MDCK 細胞を開発できる可能性がある。

F. 研究発表

1. 論文発表

   なし

2. 学会発表

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G. 知的財産権の出願・登録状況

1. 特許取得

   なし

2. 実用新案登録

   なし

3. その他

   なし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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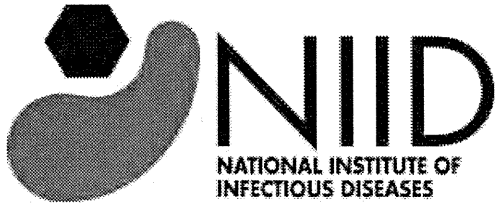

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Summary

Epidemiology

Influenza activity from September 2010 to February 2011 in Japan

- Influenza activity in 2010/11 season in Japan has been increasing from week 42, 2010 and the reported cases per sentinel became 31.88 at week 4, 2011. Cumulative number of ILI patients who visited clinics or hospitals was estimated to be 4.9 million after week 36, 2010.
- At the beginning of 2010/11 season, influenza patients with H3N2 virus were predominant until week 48-49, 2010 and thereafter the patients with pandemic H1N1 (A/H1N1pdm) became predominant; H1N1pdm was attributable to more than 80% of ILI cases at week 5, 2011. Relative proportion of isolates to date was 62.4% for H1N1pdm, 33.9% for H3N2 and 3.7% for B. In B virus, B/Victoria-lineage was predominant (87.8%) and only 12 viruses of B/Yamagata-lineage were isolated in Japan in this moment. Old seasonal H1N1 virus was not isolated at all in 2010/11 season as well as 2009/10 season.
- NIID received viruses isolated in 2010/11 season from China (42 H3N2, 13 B), Laos (5 H1N1pdm, 4 H3N2, 11 B), Mongolia (10 H3N2), Myanmar (9 H1N1pdm, 10 B), South Korea (5 H1N1pdm, 5 H3N2), and Taiwan (5 H1N1pdm, 12 H3N2, 6 B).

Characterizations of viruses

● *Pandemic H1N1 viruses*

Antigenic analysis:

- Most H1N1pdm viruses tested (94.4%) showed similar HI titers to the homologous titer of A/Cal/7/2009 and A/Narita/1/2009 ferret antisera and no virus showed >8-fold reduced HI titer to those reference antisera. The viruses with reduced HI titer possessed an amino acid change from G to E or G/E mixture at position 155 in the HA protein.

Phylogenetic analysis

HA gene:

- Since September 2010, all viruses tested exclusively fell into S203T clade and recent viruses were discernible three subgroups: S185T group, N125D group, and A134T+
- S183P group. Most recent Japanese viruses isolated after November fell into the S185T group.
- Viruses possessing G155E or G/E mixture and oseltamivir resistant viruses were detected in both S185T and N125D groups.
- No virus possessing D222G or E substitution was detected since March 2010.

NA gene:

- All recent test viruses belonged to N248D clade and majority of the viruses fell into V241I+N369K subclade. A few viruses fell into N397K+S442I subclade.

◆ Conclusions:

Most recent viruses were antigenically similar to A/Cal/7/2009 and there has been no tendency to increase antigenic variant viruses. Phylogenetically, recent viruses were discerned into three subclades of HA gene and one major clade of NA gene. Sporadically detected NAI resistant viruses neither occurred in a particular subclade nor formed a

genetic cluster.

- *H3N2 viruses*

Antigenic analysis:

- Majority of recent viruses examined were well covered by A/Perth/16/2009 (81.4%), A/Victoria/210/2009 (85.7%) and A/Brisbane/11/2010 reference ferret antisera. Some of Chinese and Japanese viruses showed 4-fold reduced HI titers to these reference antisera. The low reactor viruses possessed amino acid changes L194L/P or K207R in the antigenic site of HA protein.
- Ferret antiserum raised to X-187 vaccine production virus did not cover well MDCK grown viruses, so that most viruses grown in MDCK cells showed over 16 -32-fold lower HI titer to homologous titer to X-187. This was associated with two amino acid changes in the vicinity of receptor binding site in the HA protein of X-187 virus; S223T and N156H change. These changes would result in structural change of the receptor binding site.
- In contrast, ferret antiserum raised to A/Brisbane/11 X-197 inhibited broadly both egg-grown and MDCK-grown viruses and could detect low reactors similarly to antiserum to A/Brisbane/11/2010 wild type virus.

Phylogenetic analysis

HA gene:

- H3N2 viruses were divided into A/Victoria/208 and A/Perth/16 clades. Majority of recent viruses fell into A/Victoria/208 clade, but they were discerned further two subclades; D53N, Y94H, I230V, E280A subclade and N312S subclade.
- In Perth/16 clade, recent Japanese, Chinese and Korean viruses formed P162S subclade.
- No virus belonging to the Kong Kong/2000 clade represented by A/Hunan-Beihu/1313 variant virus was observed.

NA gene:

- A/Perth/16 and A/Victoria/208 clades were subdivided into two NA subclades, respectively. Majority of recent viruses in A/Victoria/208 clade formed S367N, K369T, I464L subclade (Vic/208 group 1), while majority of A/Perth/16 clade formed D127N, I307N subclade (Perth/16-group 1).

◆ **Conclusions:**

Majority of recent viruses was antigenically similar to A/Perth/16/2009 and a recent reference virus A/Brisbane/11/2010. Some viruses with 4-fold reduced HI titer were seen, but the proportion of these viruses have not increased since the period of Mar-Aug, 2010. Recent viruses formed three phylogenic subclades of HA gene, but antigenic property was not different among these subclades; all retaining the antigenicity of A/Perth/16

- *B viruses:*

Antigenic analysis:

Victoria-lineage:

- Most B/Victoria-lineage viruses tested (85.7%) showed similar HI titer to the homologous titer of antiserum raised to MDCK grown B/Brisbane/60 reference viruses, although virus with 8-fold reduced HI titer has slightly increased since September

2010.

- Ferret antiserum raised to egg-grown vaccine virus B/Brisbane/60 poorly reacted with MDCK grown viruses.
- A few viruses possessing K165N change in the antigenic site B were low reactors to all B/Brisbane/60-like reference antisera. Other low reactors reacted with antiserum raised to B/Taiwan/55/2009 virus and were confirmed to belong to Clade 5 (Taiwan/55 clade).

Yamagata-lineage:

- Number of B/Yamagata-lineage viruses isolated in Japan was quite low since September in Japan. Most viruses tested were provided from China and Taiwan.
- Seventy-five percent of recent viruses showed similar or less than 4-fold reduced HI titers to the homologous titer of B/Bangladesh/3333/2007 and recent prototype virus B/Wisconsin/1/2010 antisera.

Phylogenetic analysis

Victoria-lineage HA gene:

- In Victoria-lineage viruses, majority of viruses belonged to B/Brisbane/60 clade and they were further discerned group 1 (L58P group) and group 2 represented by B/Brisbane/60 and our reference virus B/Sakai/43. Recent viruses isolated in Japan fell into group 2.
- A few antigenic variant viruses belonging to clade 5 (T37I clade, so called B/Taiwan/55 clade) were sporadically detected after September.

Victoria-lineage NA gene:

- Majority of recent B/Victoria-lineage viruses formed two subclades in B/Brisbane/60 clade ; subclade 1 with I204V, N220K, A358E substitutions and subclade 2 with T8M substitution. November and December isolates in Japan belonged to N340D group in subclade 1.

Yamagata-lineage HA gene: All viruses belonged to B/Bangladesh/3333 clade (clade 3) and formed N202S subclade represented by B/Hubei-Wujiagang/158/2009 and B/Wisconsin/1/2010.

Yamagata-lineage NA gene:

- Phylogenetic tree of NA gene was quite similar to that of HA gene and recent viruses belonged to A68T, D463N, A465T in clade 3.

◆ **Conclusions:**

- The majority of B viruses was of B/Victoria-lineage and they were antigenically similar to B/Brisbane/60, while virus with 8-fold reduced HI titer has slightly increased since September 2010.
- Yamagata-lineage viruses were antigenically and genetically similar to B/Hubei-Wujiagang/158/2009 and B/Wisconsin/1/2010.

Antiviral resistant viruses:

H1N1pdm:

- Since September 2010, 494 H1N1pdm viruses have been analyzed to detect oseltamivir and peramivir resistant viruses with 275Y substitution and followed by NAI susceptibility assay using NA-star. Eleven resistant viruses with high IC_{50} were detected (2.2% of test viruses). Those resistant viruses were sensitive to zanamivir and

laninamivir.

- Of 11 resistant viruses, 3 cases were oseltamivir treatment, 1 case was prophylaxis with oseltamivir, 1 case was peramivir treatment and 6 cases were non-treated with any drugs.
- No NAI resistant was detected from viruses provided from China, Taiwan, Korea, Laos, Myanmar and Mongolia.
- By sequencing of M2 gene, all H1N1pdm viruses tested were amantadine resistant.

H3N2 and B:

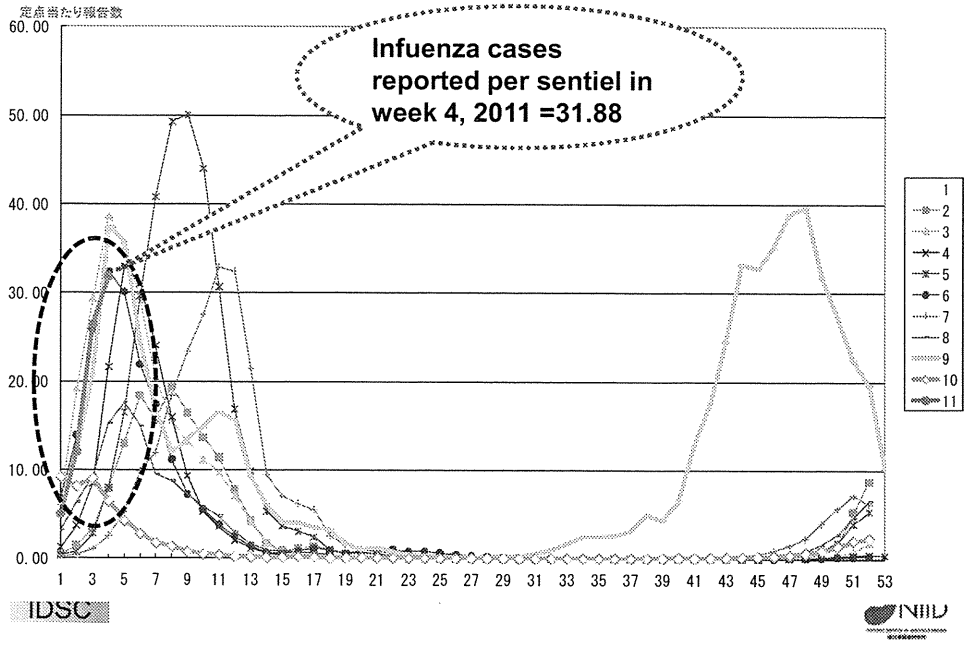
- No NAI resistant was detected with H3N2 and B viruses.

Highly pathogenic H5N1 virus:

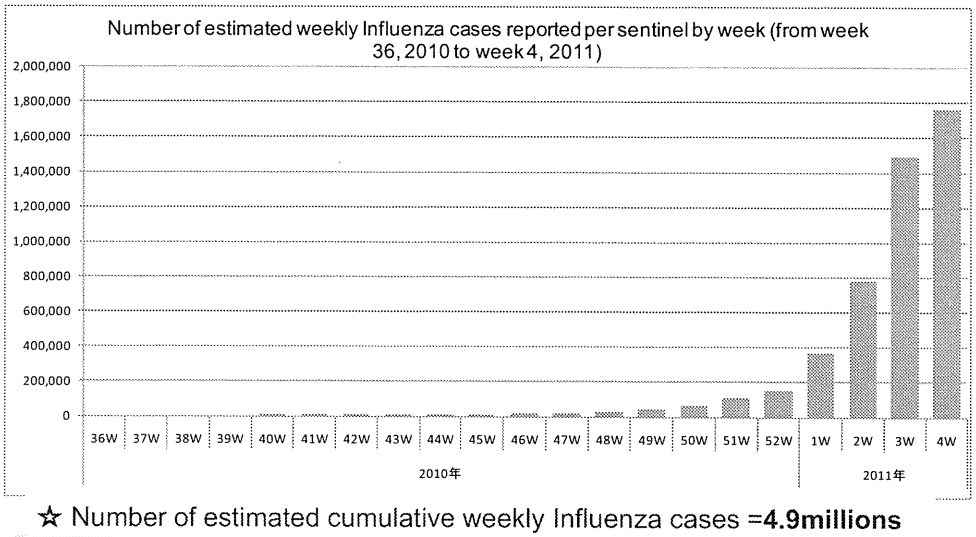
- Since October 2010, highly pathogenic H5N1 virus has been detected from dead wild birds and poultry farms in Japan. Sporadic outbreaks in poultry farms have increased in 2011, particularly in Kyushu Island (Southern part of Japan).
- H5N1 viruses detected were genetically quite similar to each other and classified as clade 2.3.2.
- Of those, HA sequence data of A/whooper swan/Hokkaido/4/2011 were available and the virus genetically closely related to viruses isolated from Mongolian wild birds in 2009, but phylogenetically formed distinct subcluster from Korean chicken viruses isolated in 2008.

Epidemiology of 2010/11 season

Weekly Influenza cases reported per sentinel



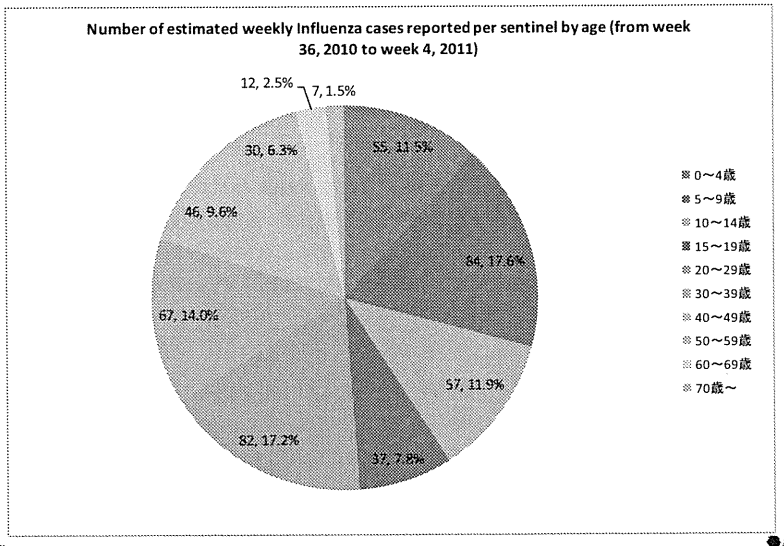
Number of estimated weekly Influenza cases reported per sentinel by week (from week 36, 2010 to week 4, 2011)



☆ Number of estimated cumulative weekly Influenza cases =**4.9millions**

IDSC NIID

Number of estimated weekly Influenza cases reported per sentinel by age (from week 36, 2010 to week 4, 2011)



IDSC

NIID

Summary 1

- The reported cases per sentinel of influenza in week 4, 2011 was 31.88（actual reported cases 15,7381） which continue to increase from 42 week, 2010. Miyazaki (60.88↓), Nagasaki (56.61↑) , Fukuoka (47.71↓), Saga (46.64↓), Gunma (45.30), Oita (44.36↓), and Saitama (43.66) were the top seven prefecture.

- 37 prefecture reported more cases compared to previous week.

- Weekly estimated number of patients were about 176 million. The cumulative number of estimated patients who visited to clinics or hospital after week 36, 2010 is 4.9 million [95% CI; 4.72 – 5.08 million] .

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NIID

Summary 2

- Male patients were reported more than female (51.8% vs 48.2%) and 5-9 years of age group was most reported (840 ,000) followed by 20 – 29 years of age group (820,000) , 30 – 39 years (670,000), 10 – 14 years (570,000), and 0-4 years (550,000).

- After week 36, 2010, influenza virus was detected from 3,475 cases and 2,169 (62.4%) were AH1pdm, and after week 52, 2011, 1,466 (82.2%) were AH1pdm. This indicates that most of the current influenza cases occur in Japan is caused by pandemic influenza (A/H1N1) 2009.

- Number of reported cases from sentinel are increasing from 36 week,2010. 37 prefecture is continuously reporting more cases of influenza, but 10 prefecture is less than previous week. This suggest that Japan may be the peak of influenza for this wave.

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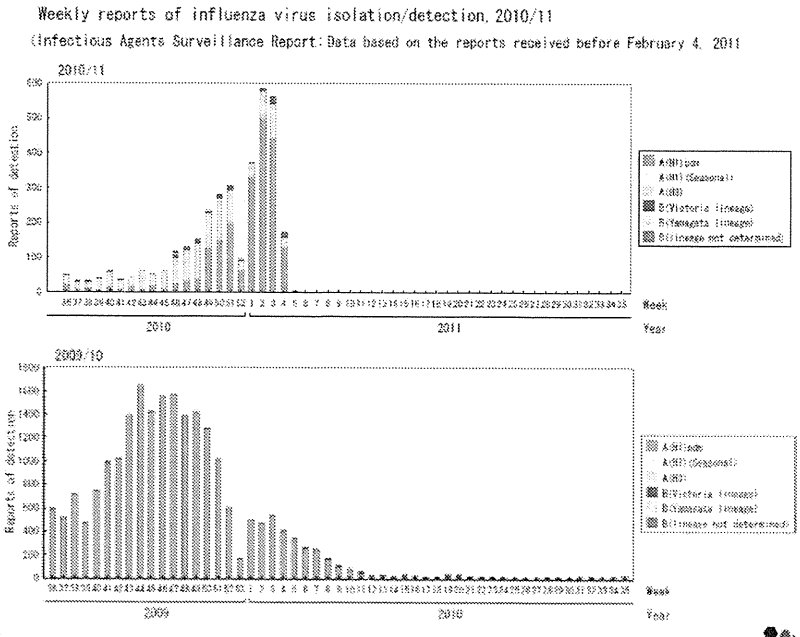
NIID



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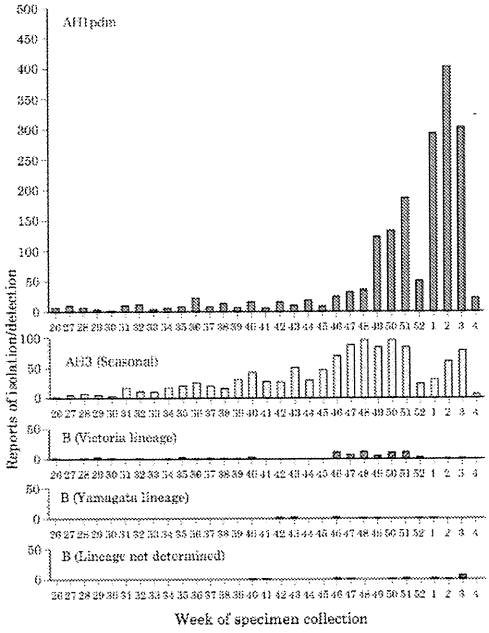
NIID

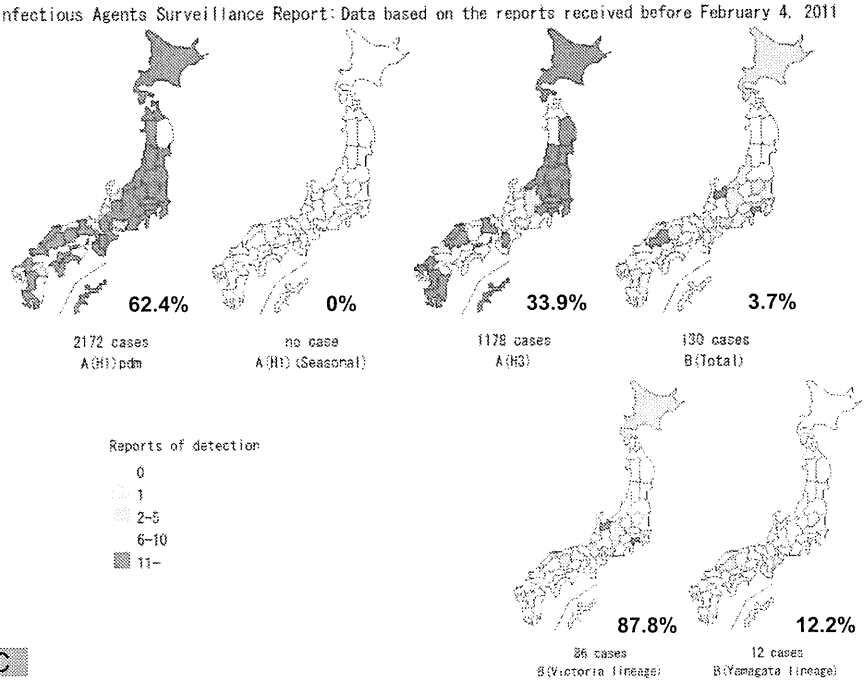
Weekly reports of influenza virus isolation/detection,
from week 26 of 2010 to week 4 of 2011, Japan
(Infectious Agents Surveillance Report: Data based on the reports received before January 27, 2011 from public health institutes)



IDSC

NIID

Isolation/detection of influenza virus by prefecture, 2010/11
(Infectious Agents Surveillance Report: Data based on the reports received before February 4, 2011



IDSC

Countries of Origin of Isolates Characterized by NIID

Month of Collection	A/H1N1	A/H1N1pdm	A/H3N2	B/Victoria	B/Yamagata
2009/2010 season viruses (HI-test before Sept.2010)		Japan 33 Myanmar 23 Taiwan 2	Japan 11 China 3 Laos 2 Taiwan 2	Japan 8 China 8 Taiwan 2	China 5 Taiwan 1
September, 2010		Japan 10 Taiwan 3	Japan 6 China 18 Taiwan 4	Japan 6 Laos 5 Taiwan 2	China 9 Taiwan 1
October, 2010		Japan 4 Laos 1 South Korea 1	Japan 11 China 11	Japan 1 China 2	Japan 1 China 1
November, 2010		Japan 3 Laos 1 South Korea 1	Japan 8 China 1	Japan 3	
Decemder, 2010		Japan 8 South Korea 2	Japan 4 Mongolia 7	Japan 2	
January, 2011		Japan 1			
February, 2011					
March, 2011					
April, 2011					
May, 2011					
June, 2011					
July, 2011					
August, 2011					
September, 2011					
Total	**0**	**93**	**88**	**39**	**18**

Influenza A/H1N1 pdm Viruses

Influenza A/H1pdm isolates characterized by NIID

	Japan	China	Taiwan	South Korea	Mongolia	Laos	Myanmar	Singapore	Total	
March 2010 - August 2010									n	%
A/California/7/2009 -like	71	0	4	1	0	0	23	0	99	99.0
A/California/7/2009 -like*	0	0	0	1	0	0	0	0	1	1.0
A/California/7/2009 (Low)**	0	0	0	0	0	0	0	0	0	0.0
Total	71	0	4	2	0	0	23	0	100	

	Japan	China	Taiwan	South Korea	Mongolia	Laos	Myanmar	Singapore	Total	
September 2010 - February 2011									n	%
A/California/7/2009 -like	26	0	3	3	0	2	0	0	34	94.4
A/California/7/2009 -like*	1	0	0	1	0	0	0	0	2	5.6
A/California/7/2009 (Low)**	0	0	0	0	0	0	0	0	0	0.0
Total	27	0	3	4	0	2	0	0	36	

* 4-fold low to homologous titer

** 8-fold or greater low to homologous titer

Hemagglutination inhibition tests of influenza A/H1pdm viruses-0.5%TRBCs

Rabbit serum

HI test date:2011/02/03

Strains	Passage History	Sample date	Wisconsin/ 10/98 Cell&Egg No.9930-2	California/ 07/09 pdm Egg No.1	Narita/ 1/09 pdm Egg No.3	Narita/ 1/09 pdm Cell No.6	Utah/ 20/09 pdm Cell No.1	Yamagata/ 752/09 pdm Cell No.1	Remarks
REF.Ag									
A/Wisconsin/10/1998	C3/C3E2 +2		2560	1280	2560	1280	160	160	
A/California/07/2009pdm	E2 +3	2009/04/09	160	640	640	640	640	320	
A/Narita/1/2009pdm	E2 +1	2009/05/08	320	1280	1280	1280	640	320	
A/Narita/1/2009pdm	MDCK 1 +2	2009/05/08	320	1280	2560	1280	640	640	
A/Utah/20/2009pdm	C 2 +1	2009/07/25	320	320	320	320	2560	1280	N156D**
A/YAMAGATA/752/2009pdm	MDCK 2 +1	2009/12/03	40	160	160	320	640	2560	(1)*, A197T
TEST.Ag									
A/WAKAYAMA/49/2010	MDCK 1 +1	2010/12/07	1280	1280	2560	1280	1280	1280	(1)*, N125D
A/HIROSHIMA/50/2010	MDCK 1 +1	2010/11/28	640	1280	2560	1280	640	640	(1)*, S185T, A197T
A/TOCHIGI/105/2010	MDCK 2 +1	2010/11/20	320	640	2560	1280	320	320	(1)*, S185T, A197T, S143G
A/NIIGATA/1581/2010	MDCK 2 +1	2010/12/09	320	640	2560	1280	640	320	(1)*, S185T
A/KOBE/435/2010	MDCK 1 +1	2010/12/27	640	640	2560	1280	640	640	(1)*, S185T, A197T, S143G
A/SAKAI/47/2010	MDCK 1 +1	2010/12/20	1280	640	2560	1280	640	640	(1)*, N125D
A/Laos/O072/2010	MDCK 1 +1	2010/11/22	320	640	2560	640	320	320	(1)*, S185T
A/HIROSHIMA/1/2011	MDCK 1 +1	2011/01/06	160	640	1280	1280	640	320	(1)*, S185T
A/FUKUOKA-C/37/2010	MDCK 2 +1	2010/11/09	160	640	1280	640	320	160	(1)*, S185T, A197T, S143G
A/Chungbuk/2826/2010	MDCK 2 +1	2010/12/14	160	640	1280	640	640	320	(1)*, S185T
A/KANAGAWA/90/2010	MDCK 2 +2	2010/12/08	160	640	1280	640	320	640	(1)*, S185T, A197T, S143G
A/Laos/I969/2010	MDCK 1 +1	2010/10/04	320	640	1280	640	320	320	(1)*, A134T, S183P
A/NIIGATA/1563/2010	MDCK 2 +1	2010/12/06	40	320	640	640	320	320	(1)*, S185T
A/YAMAGUCHI/34/2010	MDCK 2 +1	2010/12/03	160	320	320	640	1280	2560	(1)*, S185T, A197T, G155E**
A/Gyeongnam/3110/2010	MDCK 2 +1	2010/12/12	160	320	160	320	320	320	(1)*, S185T, A197T, S143G, G155G/E**
A/Gyeonggi/2623/2010	MDCK 2 +1	2010/11/24	160	160	160	320	1280	1280	(1)*, S185T, G155G/E**
A/FUKUSHIMA/138/2010	MDCK 2 +1	2010/12/07	160	160	160	320	640	1280	(1)*, S185T, A197T, G155G/E**

*: S203T

**Antigenic site

Hemagglutination inhibition tests of influenza A/H1pdm viruses-0.5%TRBCs

TC (1)

Rabbit serum

HI test date:2010/12/16

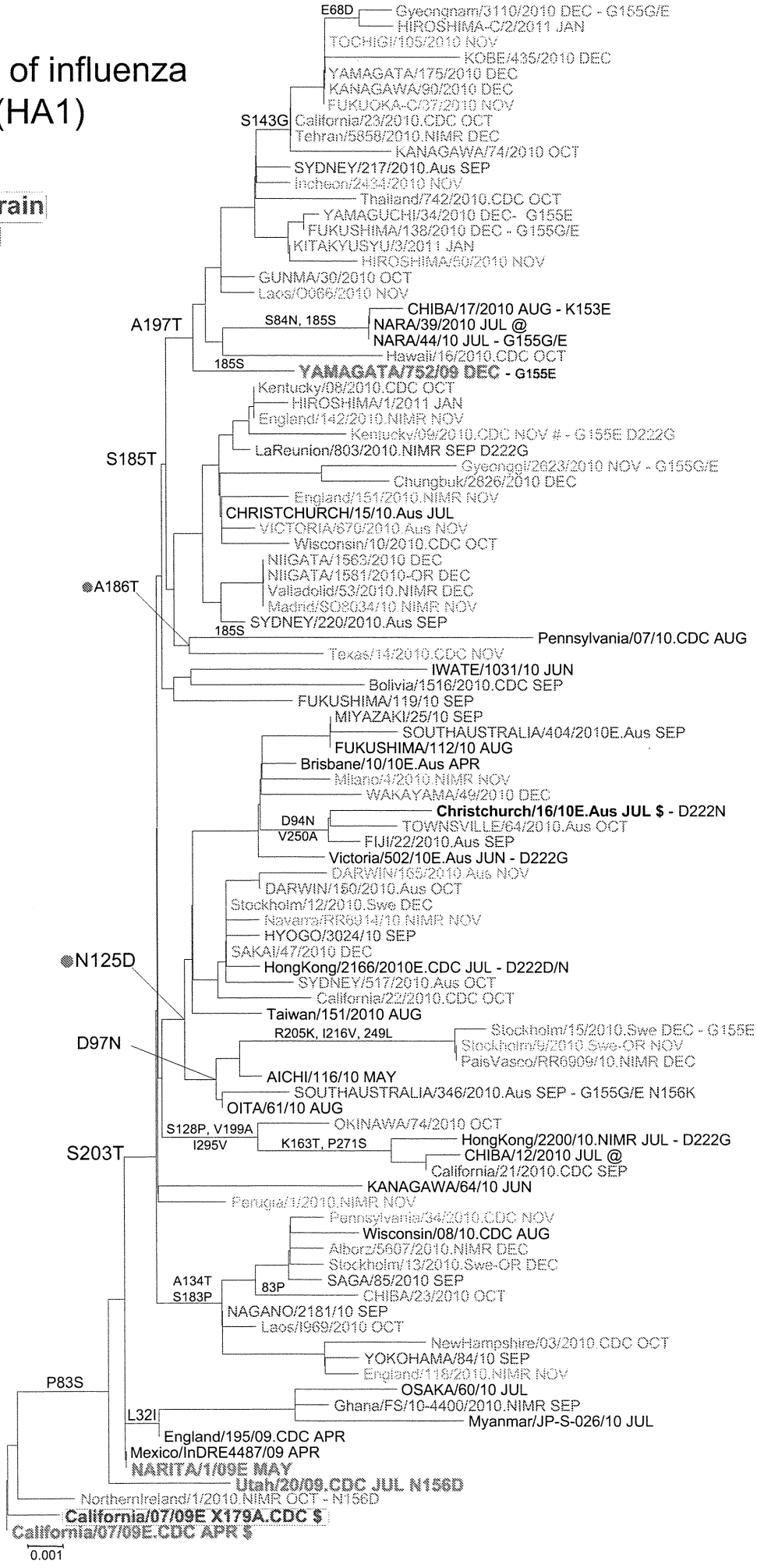
Strains	Passage History	Sample date	Wisconsin/ 10/98 Cell&Egg No.9930-2	California/ 07/09 pdm Egg No.1	Narita/ 1/09 pdm Egg No.3	Narita/ 1/09 pdm Cell No.6	Utah/ 20/09 pdm Cell No.1	Yamagata/ 752/09 pdm Cell No.1	Remarks
REF.Ag									
A/Wisconsin/10/1998	C3/C3E2 +2		5120	640	2560	1280	160	160	
A/California/07/2009pdm	E2 +3	2009/04/09	160	320	320	320	640	320	
A/Narita/1/2009pdm	E2 +1	2009/05/08	320	1280	2560	1280	320	320	
A/Narita/1/2009pdm	MDCK 1 +2	2009/05/08	320	640	2560	1280	640	640	
A/Utah/20/2009pdm	C 2 +1	2009/07/25	320	160	160	320	1280	1280	N156D**
A/YAMAGATA/752/2009pdm	MDCK 2 +1	2009/12/03	160	160	160	640	640	2560	(1)*, A197T
TEST.Ag									
A/Taiwan/305/2010	MDCK 3 +1	2010/08/21	1280	1280	2560	2560	1280	2560	(1)*, N125D, D97N
A/CHIBA/23/2010	MDCK 2 +1	2010/10/06	320	1280	2560	1280	320	320	(1)*, A134T, S183P
A/Taiwan/733/2010	E2 +1	2010/09/25	640	1280	2560	1280	640	1280	(1)*, N125D
A/OKINAWA/74/2010	MDCK 1 +1	2010/10/08	320	640	2560	1280	320	160	(1)*, S128P, I295V
A/SAGA/89/2010	MDCK 2 +1	2010/09/27	320	640	2560	1280	320	160	ND
A/SAGA/90/2010	MDCK 2 +1	2010/09/28	320	640	2560	1280	640	320	(1)*, A134T, S183P
A/Taiwan/454/2010	MDCK 3 +1	2010/09/08	1280	640	2560	1280	640	1280	(1)*, N125D
A/Taiwan/729/2010	MDCK 2 +1	2010/09/10	640	640	2560	1280	640	640	(1)*, N125D
A/KANAGAWA/74/2010	MDCK 2 +1	2010/10/16	160	640	1280	640	160	160	(1)*, A197T
A/Taiwan/151/2010	MDCK 3 +1	2010/08/04	1280	640	1280	640	640	640	(1)*, N125D
A/SAGA/85/2010	MDCK 2 +1	2010/09/24	320	320	1280	640	320	320	(1)*, A134T, S183P
A/CHIBA/17/2010	MDCK 1 +1	2010/08/10	320	160	320	640	1280	1280	(1)*, A197T, S84N, K153E**

*: S203T

**Antigenic site

Phylogenetic analysis of influenza H1N1pdm HA genes (HA1)

10/11 Japanese vaccine strain
HI reference strains in Red
September 2010 in Blue
October 2010 in Green
November 2010 in Orange
December 2010 and
January 2011 in Pink
●: Antigenic sites
$: Serology antigens
@: Oseltamivir resistant
#: Fatal case



Phylogenetic analysis of influenza H1N1pdm NA genes

10/11 Japanese vaccine strain
HI reference strains in Red
September 2010 in Blue
October 2010 in Green
November 2010 in Orange
December 2010 and
January 2011 in Pink
●: Antigenic sites
$: Serology antigens
@: Oseltamivir resistant