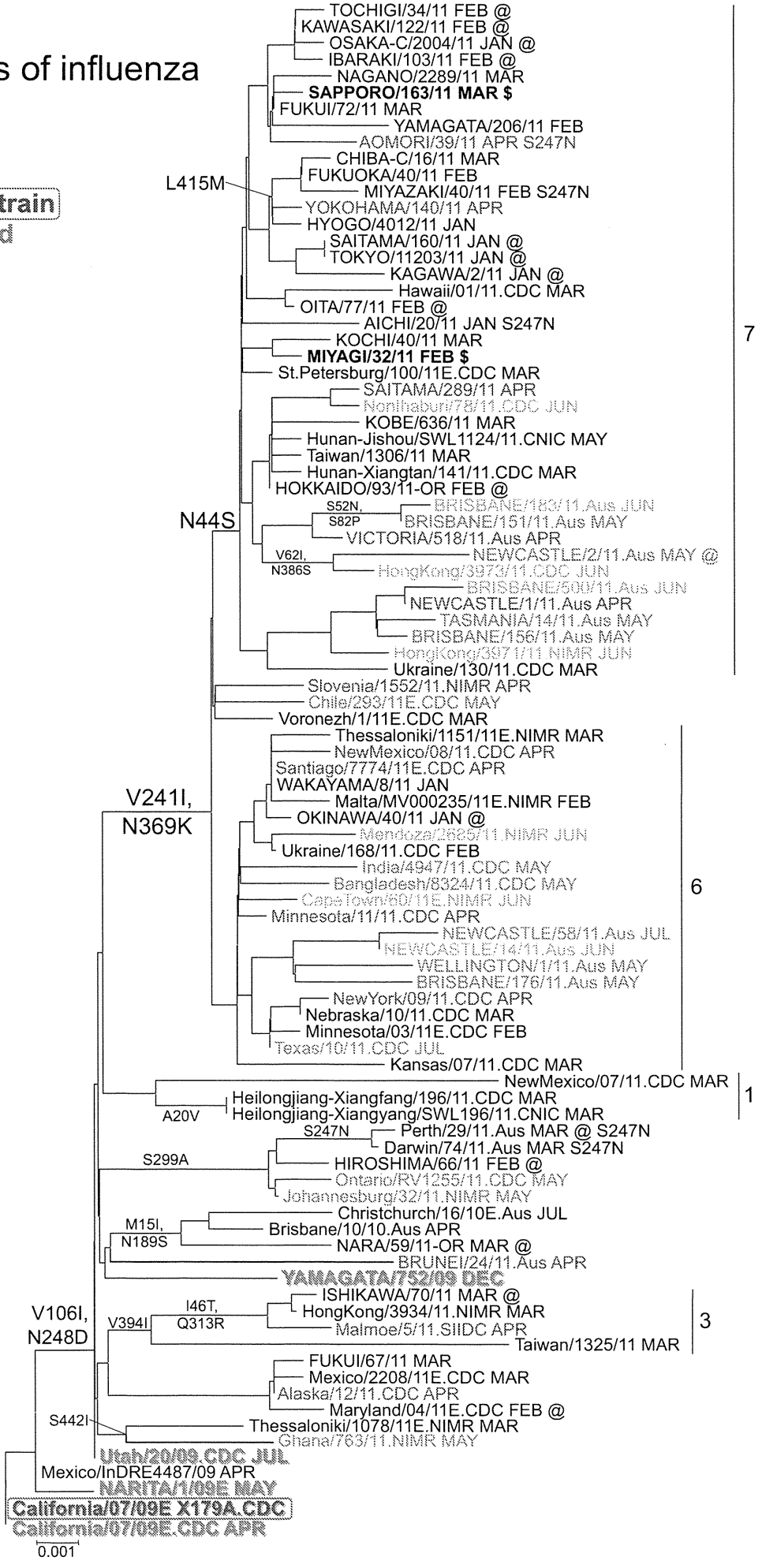
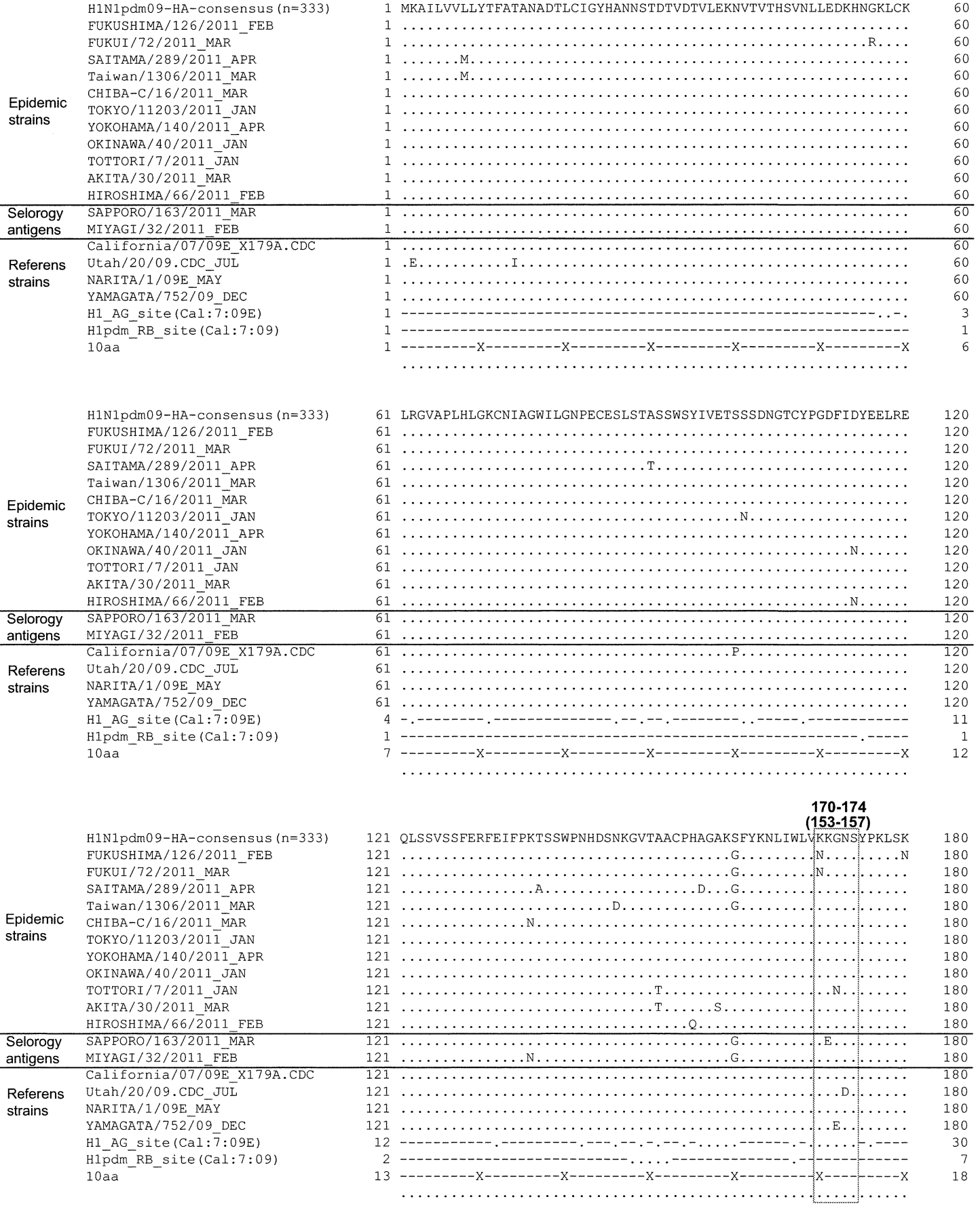
Phylogenetic analysis of influenza H1N1pdm NA genes

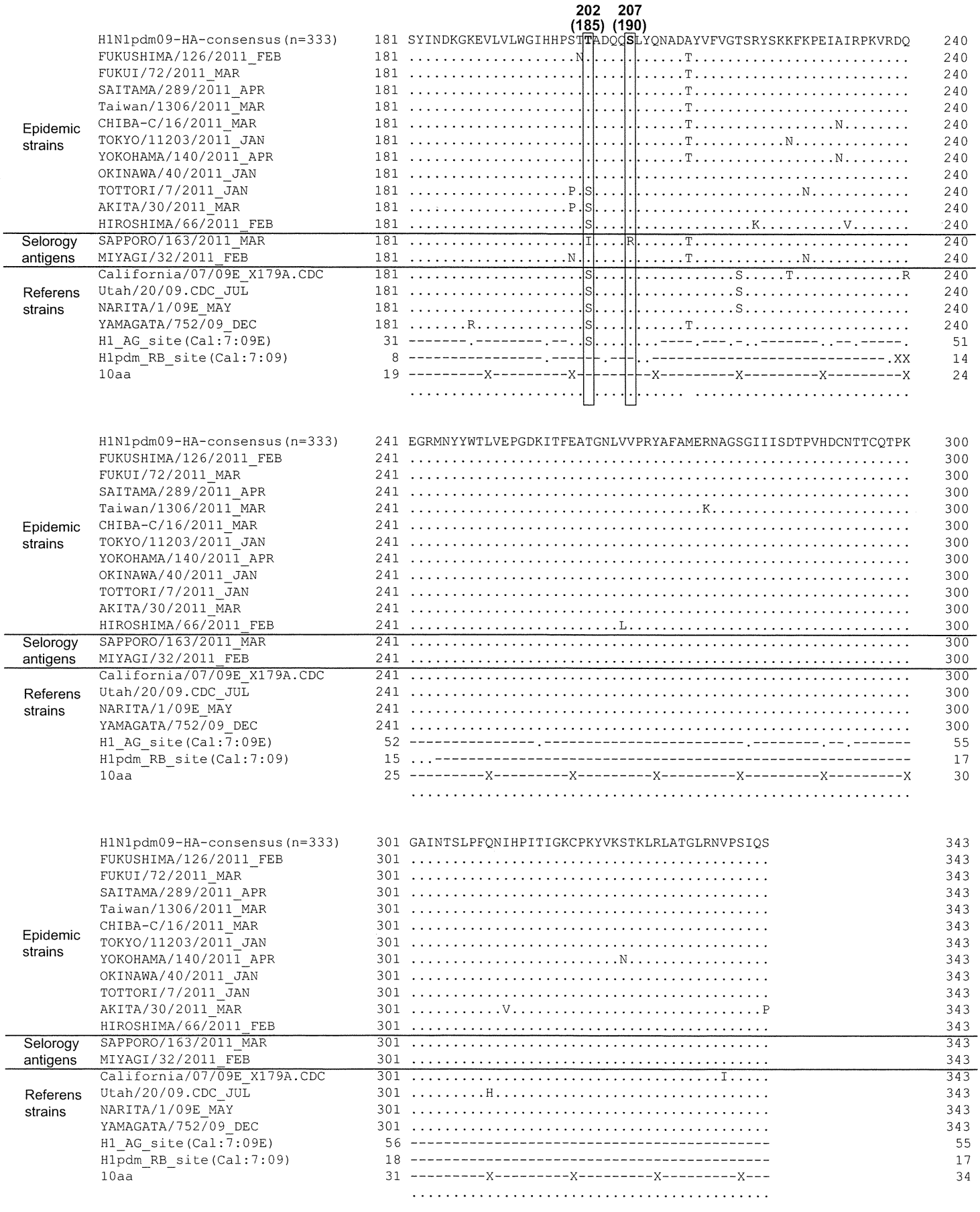
10/11 Japanese vaccine strain
HI reference strains in Red
April 2011 in Blue
May 2011 in Green
June 2011 in Orange
July 2011 in Pink
@: Oseltamivir resistant
$: Serology antigens



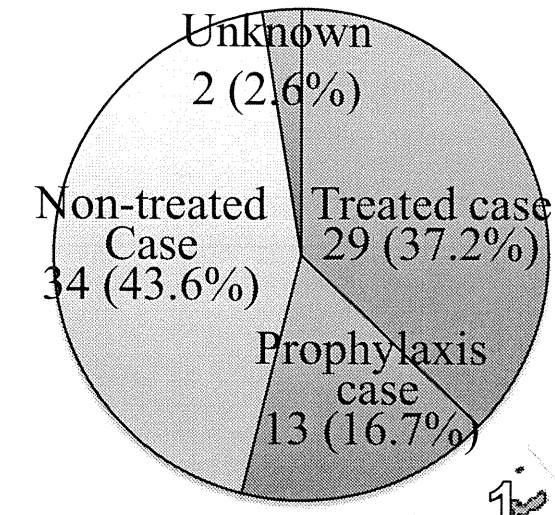
Influenza A (H1pdm) HA1 amino acid comparison



Influenza A (H1pdm) HA1 amino acid comparison



Detection of A/H1N1pdm09 viruses with H275Y mutation in Japan (Sep 2010 – Aug 2011)



Total (Sep 2010 – Aug 2011)
*) 78 / 3,748
**) 70 / 3,748 (1.9%)

Total (Mar 2011 – Aug 2011)
*) 6 / 92
**) 3 / 89 (3.4%)

*The number of H275Y mutant viruses / The number of A/H1N1pdm09 viruses tested.
**Viruses isolated from an outbreak in the same care house were counted as one virus and the percentage reflects the actual detection rate of resistant viruses.

The clinical background of A/H1N1pdm09 viruses with H275Y mutation

Clinical background	2009-2010 season			2010-2011 season		
	No. of viruses tested	No. of viruses with H275Y	Detection rate (%)	No. of viruses tested	No. of viruses with H275Y	Detection rate (%)
Treated with oseltamivir	511	52	10.2	50	19	38.0
Prophylaxed with oseltamivir	14	12	85.7	16	14	87.5
Treated with peramivir	0	0	0	13	6	46.2
Treated with zanamivir	117	0	0	22	1	4.5
Prophylaxed with zanamivir	1	0	0	0	0	0
Treated with laninamivir	0	0	0	4	0	0
Treated with oseltamivir and peramivir	0	0	0	6	2	33.3
Treated with oseltamivir and zanamivir	9	2	22.2	0	0	0
Treated with peramivir and zanamivir	0	0	0	1	0	0
Total	652	66	10.1	112	42	37.5
No known exposure to drug	2,236	13	0.6	225	33	14.7
Insufficient information	5,257	0	0	3,349	2	0.1
Total	7,493	13	0.2	3,574	35	1.0

Clinical background of Oseltamivir/Peramivir-resistant A/H1N1pdm09 viruses detected in two seasons

Summary

1. Oseltamivir/Peramivir-resistant A/H1N1pdm09 viruses detected in 2010/11 season were slightly increased than those detected in 2009/10 season.
2. The ratio of resistant viruses detected from non- drug exposure cases was increased from that of last season.
3. All resistant viruses were sporadically detected in the entire region of Japan and no geographical cluster was seen.
4. No spread of resistant viruses in community was confirmed so far.

A/H3N2 viruses

Summary

Antigenic analysis:

- Majority of recent viruses examined were well inhibited by A/Perth/16/2009 and A/Victoria/210/2009 reference ferret antisera. Some of Japanese viruses (2.4%) showed 4-fold reduced HI titer to A/Victoria/210/2009 antiserum, but no virus showing >8-fold reduced HI titer was observed.
- Ferret antiserum raised against A/Victoria/210 X-187 vaccine production virus did not cover well with MDCK grown viruses, so that most viruses grown in MDCK cells showed over 16 -32-fold lower HI titer to homologous titer of X-187.

Phylogenetic analysis

HA gene:

- H3N2 viruses were divided into A/Victoria/208 and A/Perth/16 clades. The A/Victoria/208 clade was further divided into 4 genetic groups.
- Recent Myanmar isolates belonged to group 3 (V223I), while the majority of Japanese isolates belonged to group 4 (N312S) and groups 5 and 6 (D53N, Y94H, I230V, E280A) in A/Victoria clade.
- Some viruses isolated mainly in March-May 2011 fell in group 1 (E62K, N144K) in A/Perth/16 clade.
- Three serology antigen viruses were selected from groups 4 (A/Yamagata/285/2011) and 5 (A/Sakai/20/2011, A/Montana/5/2011) in A/Victoria/208 clade, while one virus (A/Kumamoto-C/36/2100) was selected from group 1 in A/Perth/16 clade.

NA gene:

- Phylogenetic tree of the NA gene was correlated well with that of the HA gene and most viruses belonged to either group 4, 5 and 6 in A/Victoria/208 clade (S367N, K369T, I464L) or group 1-1 in A/Perth/16 clade (D127N, L338F, N342D).

Antiviral resistant (NAI) viruses:

- No NAI-resistant A/H3N2 virus was detected since March 2011.

Influenza A/H3 isolates characterized by NIID

2010.09.01-2011.08.25

	Japan	China	Taiwan	South Korea	Mongolia	Laos	Myanmar	Singapore	Total	
September 2010 - February 2011									n	%
A/Perth/16/2009 -like	76	41	8	5	8	4	0	0	142	83.0
A/Perth/16/2009 -like*	11	16	2	0	0	0	0	0	29	17.0
A/Perth/16/2009 (Low)**	0	0	0	0	0	0	0	0	0	0.0
Total	87	57	10	5	8	4	0	0	171	
A/Victoria/210/2009 -like	78	49	9	5	8	3	0	0	152	88.9
A/Victoria/210/2009 -like*	9	8	1	0	0	1	0	0	19	11.1
A/Victoria/210/2009 (Low)**	0	0	0	0	0	0	0	0	0	0.0
Total	87	57	10	5	8	4	0	0	171	

	Japan	China	Taiwan	South Korea	Mongolia	Laos	Myanmar	Singapore	Total	
March 2011 - August 2011									n	%
A/Perth/16/2009 -like	41	0	0	0	0	0	0	0	41	100.0
A/Perth/16/2009 -like*	0	0	0	0	0	0	0	0	0	0.0
A/Perth/16/2009 (Low)**	0	0	0	0	0	0	0	0	0	0.0
Total	41	0	0	0	0	0	0	0	41	
A/Victoria/210/2009 -like	40	0	0	0	0	0	0	0	40	97.6
A/Victoria/210/2009 -like*	1	0	0	0	0	0	0	0	1	2.4
A/Victoria/210/2009 (Low)**	0	0	0	0	0	0	0	0	0	0.0
Total	41	0	0	0	0	0	0	0	41	

* 4-fold low to homologous titer

** 8-fold or greater low to homologous titer

Hemagglutination inhibition tests of influenza A/H3 viruses-1%Guinea Pig RBCs

			Brisbane-lineage	Perth16 group				Victoria208 group		HK2000 group	HI test date:2011/8/25
Strains	Passage History	Sample date	Uruguay/716/07 Egg No.2	Victoria/ 210/09 Egg No.1	Victoria/ 210/09 (X-187) Egg No.1	Perth/ 16/09 Egg No.2	Niigata/ 403/09 Cell No.1	Brisbane/ 11/10 Egg No.1	Shizuoka/ 736/09 Cell No.2	Hunan-beihu/ 1313/09 Cell No.1	Remarks
REF.Ag											
A/Uruguay/716/2007	SpfCk1E3 +3	2007/06/21	1280	80	20	40	40	160	160	320	
A/Victoria/210/2009	E2 +2	2009/06/02	10	640	640	160	640	160	160	40	P*
A/Victoria/210/2009 (X-187)	E7/E2+1		20	1280	2560	640	640	320	320	40	P*
A/Perth/16/2009	E3 +2		10	320	80	160	160	80	80	20	P*
A/NIIGATA/403/2009	MDCK 2 +2	2009/03/12	20	1280	320	640	640	320	640	40	P*
A/Brisbane/11/2010	E4 +2		20	320	80	80	160	640	320	80	V*, N312S
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	80	1280	160	320	640	320	640	320	V*
A/Hunan-beihu/1313/2009	C 3 +1	2009/05/07	80	20	10	20	40	20	10	320	HK/2000 CL
TEST.Ag											
A/MIYAZAKI/92/2011	MDCK 1 +1	2011/04/28	20	1280	640	640	1280	640	640	80	P*, E50K, P162S, N81D
A/KITAKYUSYU/13/2011	MDCK 2 +2	2011/03/18	10	640	320	320	640	320	320	20	P*, E50K, P162S
A/AICHI/221/2011	MDCK 1 +2	2011/05/03	40	640	160	320	640	640	640	160	V*, (1)
A/WAKAYAMA/34/2011	MDCK 1 +2	2011/02/07	10	640	160	320	640	320	320	20	ND
A/IBARAKI/190/2011	MDCK 2 +1	2011/04/15	10	640	160	320	640	320	160	20	P*, E50K, P162S
A/Taiwan/1097/2011	MDCK 3 +1	2011/02/07	20	640	160	320	320	320	160	40	P*, E50K, P162S
A/MIYAZAKI/39/2011	MDCK 1 +2	2011/02/15	20	640	80	160	320	320	320	160	ND
A/Taiwan/1177/2011	MDCK 3 +1	2011/02/15	40	640	80	160	320	160	160	160	V*, (1)
A/NIIGATA/745/2011	MDCK 2 +1	2011/05/30	10	640	80	160	320	160	80	80	P*, E50K, P162S, N81D
A/AICHI/227/2011	MDCK 1 +1	2011/03/30	20	640	80	160	320	160	320	80	V*, (1)
A/SHIGA/69/2011	MDCK 1 +2	2011/04/18	20	640	80	160	160	320	160	80	V*, (1)
A/SHIMANE/170/2011	MDCK 1 +2	2011/03/14	20	320	80	160	160	160	160	40	V*, (1)
A/NIIGATA/630/2011	MDCK 2 +2	2011/05/02	20	320	80	80	160	160	160	80	V*, (1)
A/SHIZUOKA-C/21/2011	MDCK 1 +1	2011/05/24	20	320	40	80	160	160	160	80	V*, (1)
A/YOKOHAMA/67/2011	MDCK 3 +1	2011/01/25	20	320	40	80	80	160	40	80	V*, N312S
A/FUKUSHIMA/149/2011	MDCK 2 +1	2011/05/06	20	320	40	80	80	80	40	80	V*, N312S
A/OKAYAMA/27/2011	MDCK 3 +1	2011/03/26	10	320	40	80	80	80	40	40	P*, E50K, P162S, N81D
A/Taiwan/1096/2011	MDCK 3 +1	2011/02/08	20	160	40	80	160	80	80	80	V*, (1)

*: P: Perth/16 CL, V: Vic/208 CL, (1): D53N, Y94H, I230V, E280A

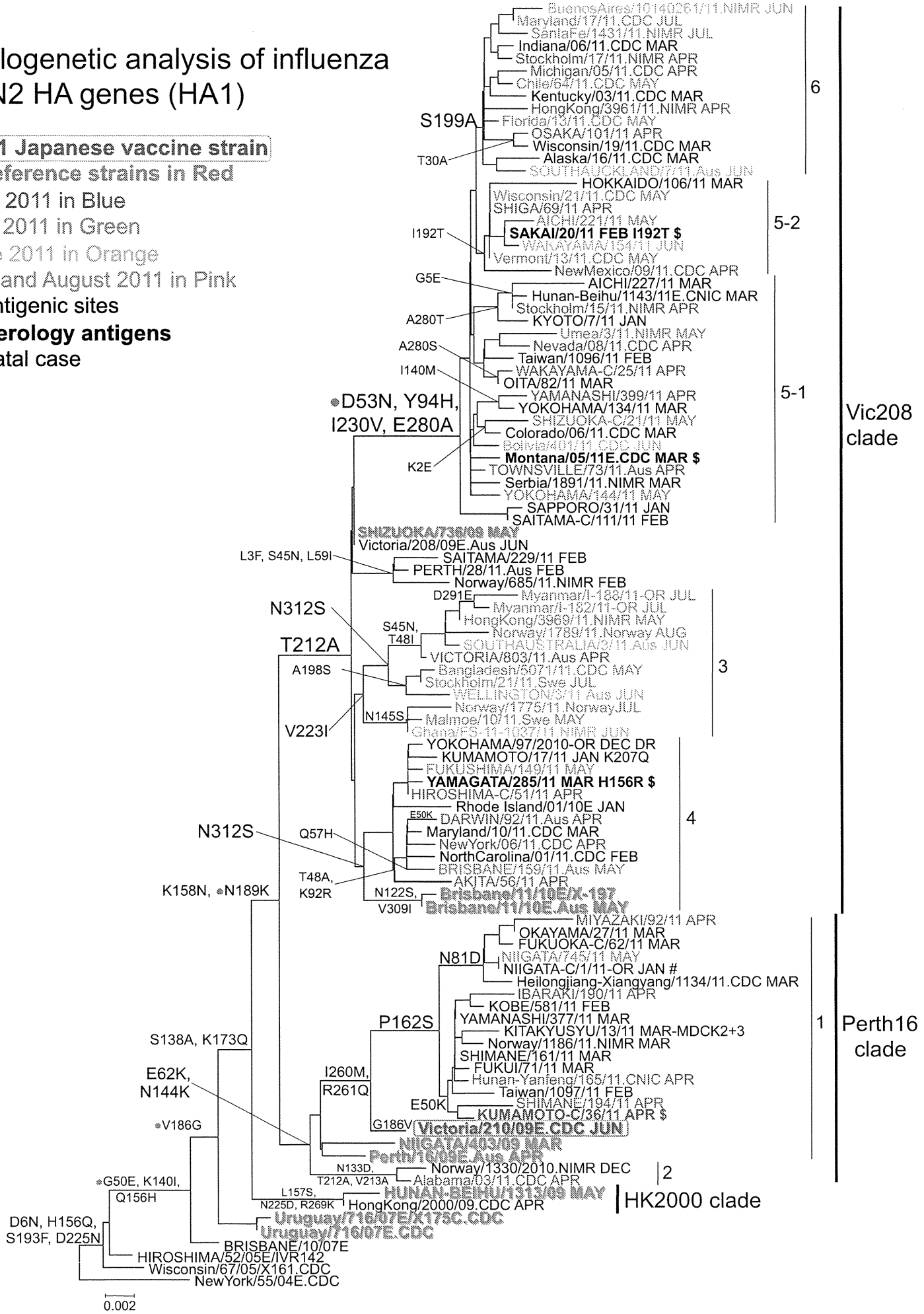
Hemagglutination inhibition tests of influenza A/H3 viruses-1%Guinea Pig RBCs

			Brisbane-lineage	Perth16 group				Victoria208 group		HK2000 group	HI test date:2011/7/7
Strains	Passage History	Sample date	Uruguay/716/07 Egg No.2	Victoria/ 210/09 Egg No.1	Victoria/ 210/09 (X-187) Egg No.1	Perth/ 16/09 Egg No.2	Niigata/ 403/09 Cell No.1	Brisbane/ 11/10 Egg No.1	Shizuoka/ 736/09 Cell No.2	Hunan-beihu/1313/09 Cell No.1	Remarks
REF.Ag											
A/Uruguay/716/2007	SpfCk1E3 +3	2007/06/21	640	40	10	20	20	80	80	320	
A/Victoria/210/2009	E2 +2	2009/06/02	20	640	640	160	640	160	320	80	P*
A/Victoria/210/2009 (X-187)	E7/E2+1		20	1280	2560	320	640	320	320	40	P*
A/Perth/16/2009	E3 +2		10	320	80	160	160	80	80	20	P*
A/NIIGATA/403/2009	MDCK 2 +2	2009/03/12	20	1280	320	320	640	320	320	40	P*
A/Brisbane/11/2010	E4 +2		20	320	80	160	320	640	320	80	V*, N312S
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	80	1280	160	320	640	320	640	320	V*
A/Hunan-beihu/1313/2009	C 3 +1	2009/05/07	160	40	20	20	40	40	20	640	HK/2000 CL
TEST.Ag											
A/SHIMANE/194/2011	MDCK 2 +1	2011/04/15	20	1280	640	640	1280	640	640	40	P*, E50K, P162S
A/CHIBA/1061/2011	MDCK 1 +1	2011/03/19	10	1280	320	640	1280	320	640	20	P*, E50K, P162S
A/NIIGATA-C/21/2011	MDCK 2 +1	2011/01/29	40	1280	160	640	640	320	320	160	ND
A/HOKKAIDO/106/2011	MDCK 2 +1	2011/03/07	40	1280	160	320	640	640	640	160	V*, (1), I192T**
A/NIIGATA-C/45/2011	MDCK 2 +1	2011/03/01	40	1280	160	320	640	320	160	80	ND
A/KAWASAKI/84/2011	MDCK 1 +1	2011/01/27	10	640	320	640	1280	320	320	20	ND
A/KUMAMOTO-C/36/2011	**CaCo-2 2 +1**	**2011/04/15**	**10**	**640**	**320**	**320**	**640**	**320**	**320**	**20**	**P*, E50K, P162S, $**
A/NIIGATA-C/48/2011	MDCK 2 +1	2011/03/12	20	640	160	320	320	320	320	80	ND
A/YAMANASHI/399/2011	CaCo-2 1 +1	2011/04/11	40	640	160	160	320	320	320	160	V*, (1)
A/YOKOHAMA/144/2011	MDCK 2 +1	2011/05/23	40	640	160	160	320	320	320	160	V*, (1)
A/TOCHIGI/49/2011	MDCK 4 +2	2011/02/21	40	640	80	160	320	80	160	80	ND
A/FUKUSHIMA/131/2011	MDCK 2 +1	2011/03/15	20	320	80	160	160	80	80	80	ND
A/AKITA/56/2011	MDCK 1 +1	2011/04/25	40	320	80	80	160	160	80	160	V*, N312S
A/SAPPORO/153/2011	MDCK 1 +1	2011/03/05	20	320	40	160	160	160	160	80	ND
A/SAITAMA/229/2011	MDCK 2 +1	2011/02/25	40	320	40	80	80	80	40	160	V*
A/SAITAMA-C/111/2011	MDCK 3 +1	2011/02/19	20	320	40	80	80	80	80	80	V*, (1)
A/OSAKA/99/2011	MDCK 2 +1	2011/04/06	20	320	40	80	80	80	40	160	V*, (1)
A/HIROSHIMA-C/51/2011	MDCK 2 +1	2011/04/01	40	320	40	80	80	80	40	160	V*, N312S
A/KYOTO/7/2011	MDCK 3 +1	2011/01/26	20	320	40	80	80	80	80	80	V*, (1)
A/OSAKA/101/2011	MDCK 2 +1	2011/04/19	40	320	40	80	160	160	80	80	V*, (1)
A/YAMAGATA/285/2011	**MDCK 2 +1**	**2011/03/17**	**20**	**160**	**40**	**80**	**80**	**160**	**80**	**80**	**V*, N312S, H156R**, $**

*: P: Perth/16 CL, V: Vic/208 CL, (1): D53N, Y94H, I230V, E280A
**: Antigenic site
$: Serology Antigen

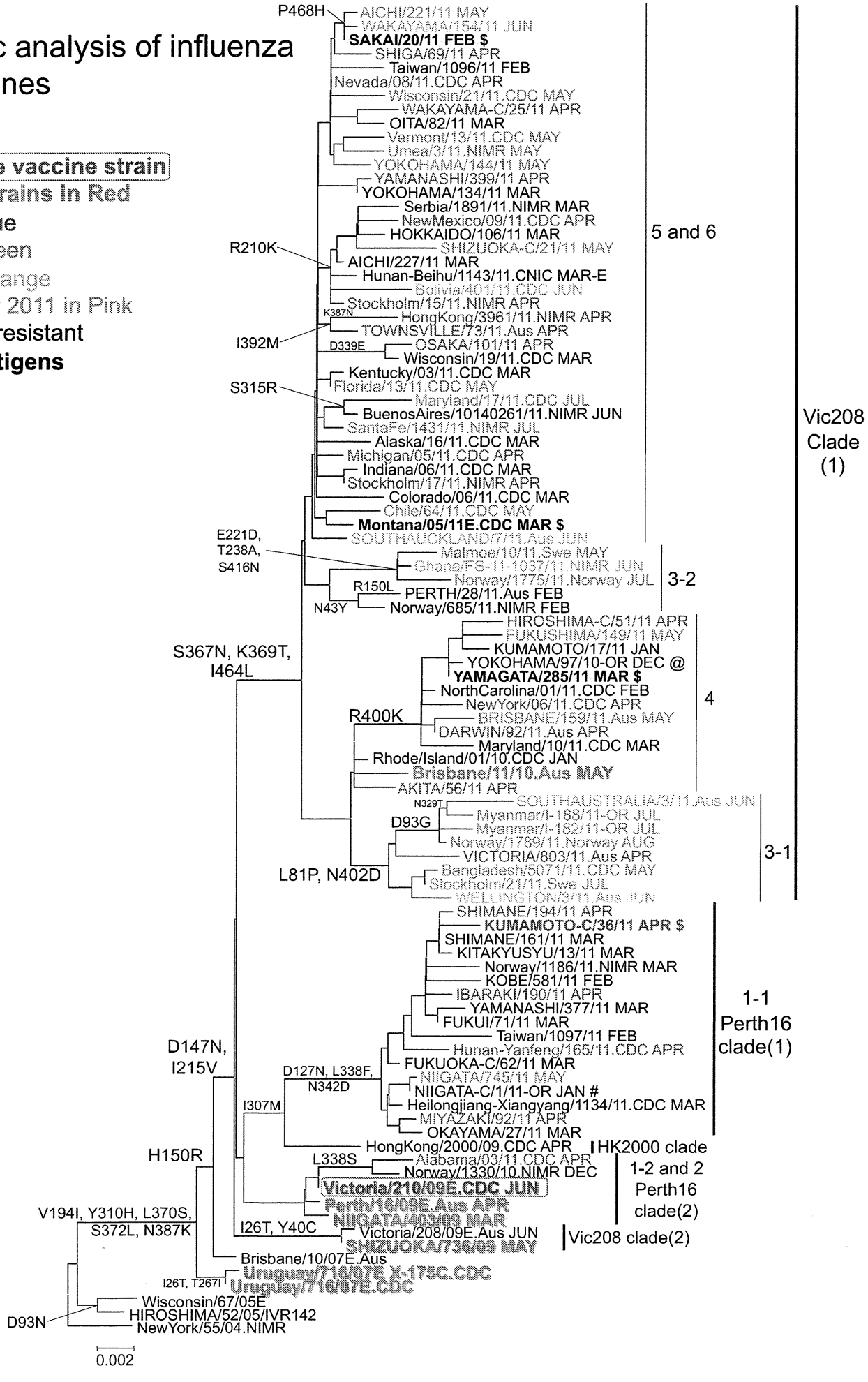
Phylogenetic analysis of influenza H3N2 HA genes (HA1)

10/11 Japanese vaccine strain
HI reference strains in Red
April 2011 in Blue
May 2011 in Green
June 2011 in Orange
July and August 2011 in Pink
⊛: Antigenic sites
$: Serology antigens
#: Fatal case

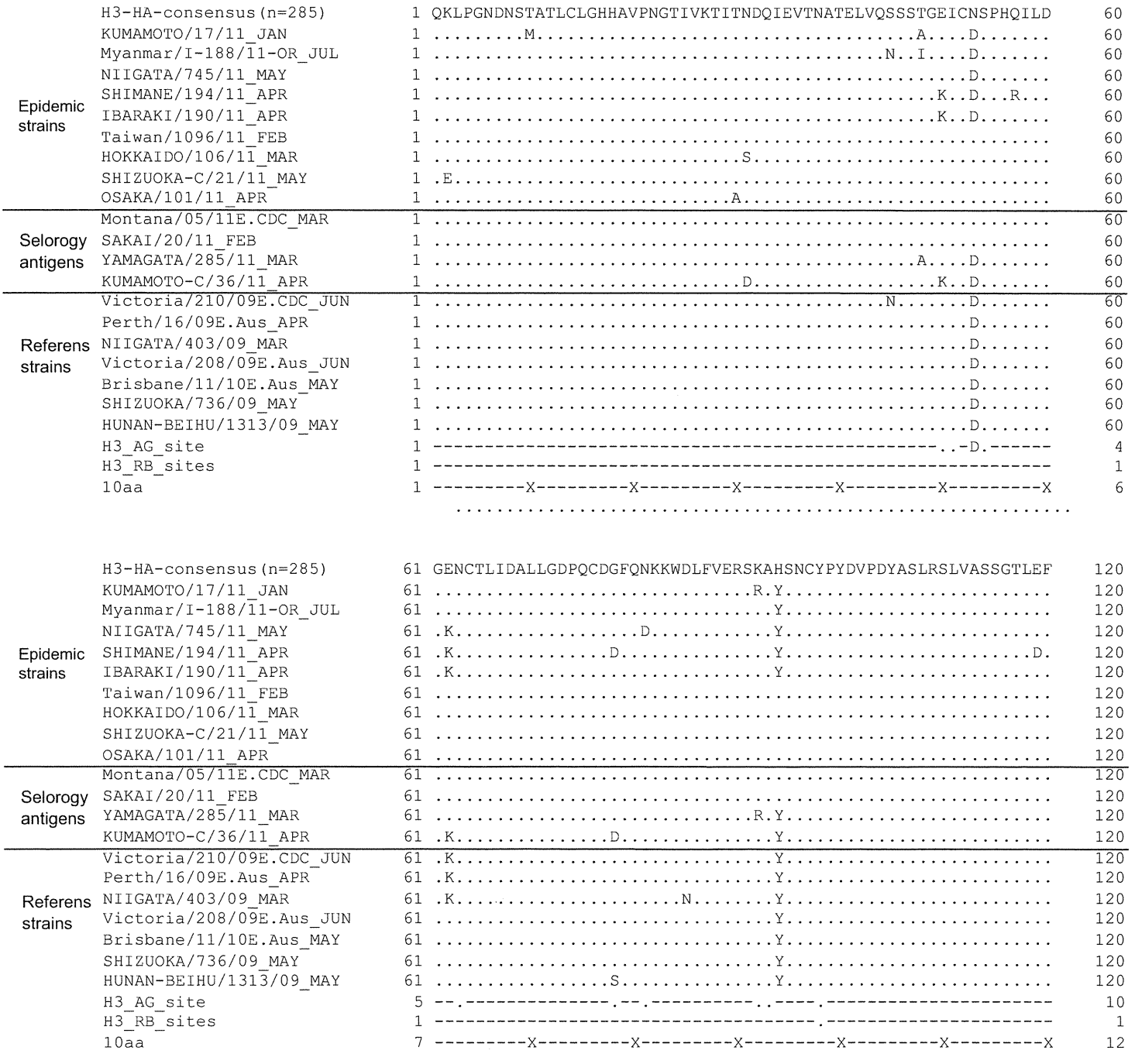


Phylogenetic analysis of influenza H3N2 NA genes

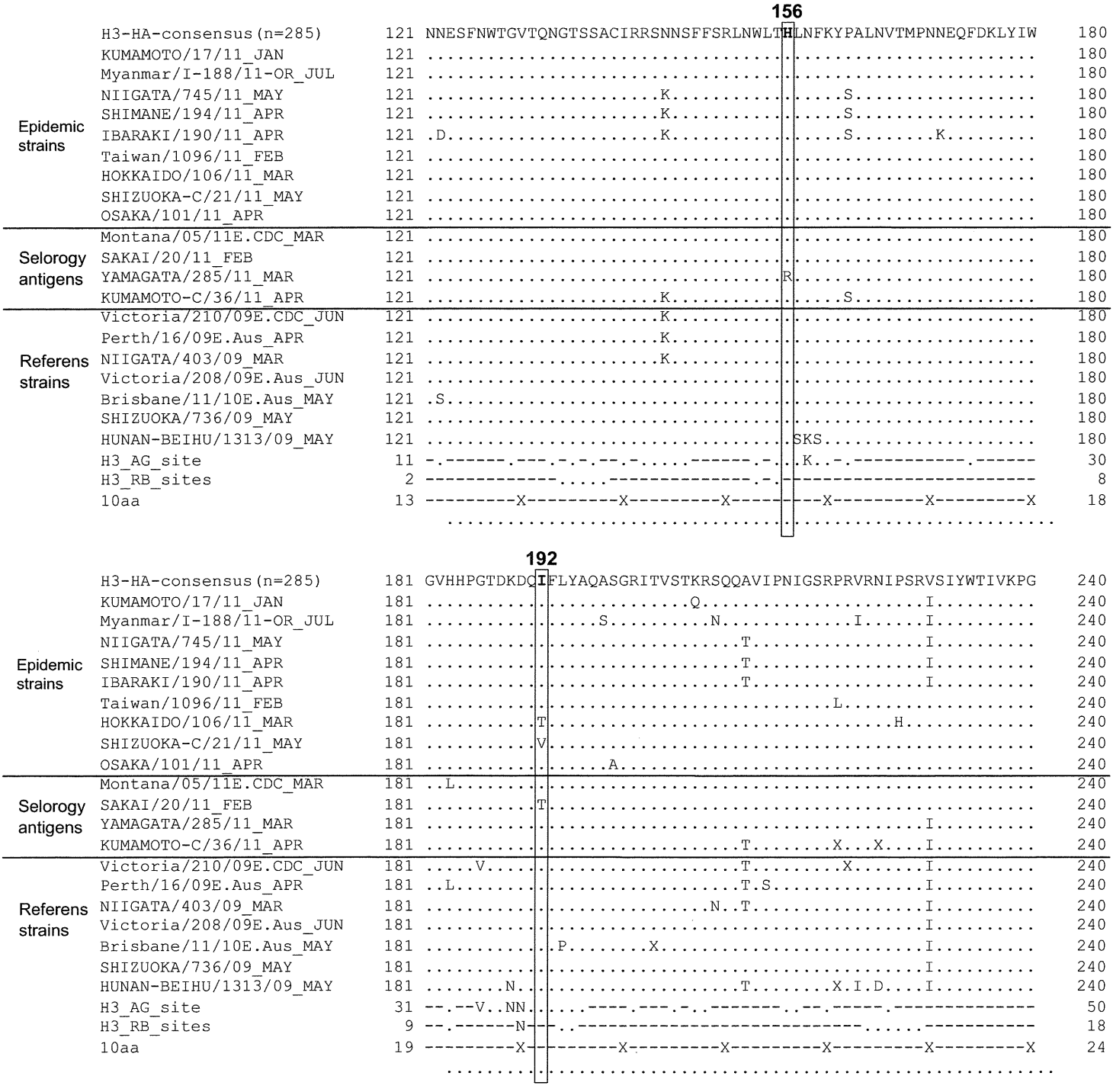
10/11 Japanese vaccine strain
HI reference strains in Red
April 2011 in Blue
May 2011 in Green
June 2011 in Orange
July and August 2011 in Pink
@: Oseltamivir resistant
$: Serology antigens
#: Fatal case



Influenza A (H3) HA1 amino acid comparison



Influenza A (H3) HA1 amino acid comparison



Influenza A (H3) HA1 amino acid comparison

	H3-HA-consensus(n=285)	241	DILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSACITPNGSIPNDKPFQNVNRI	300
	KUMAMOTO/17/11_JAN	241	.....E.....	300
	Myanmar/I-188/11-OR_JUL	241	.....E.....E.....	300
	NIIGATA/745/11_MAY	241	.....MQ.....E.....	300
Epidemic strains	SHIMANE/194/11_APR	241	.....MQ.....E.....	300
	IBARAKI/190/11_APR	241	.....MQ.....E.....	300
	Taiwan/1096/11_FEB	241	.....	300
	HOKKAIDO/106/11_MAR	241	.....Q.....	300
	SHIZUOKA-C/21/11_MAY	241	.....	300
	OSAKA/101/11_APR	241	.....	300
	Montana/05/11E.CDC_MAR	241	.....	300
Selorogy antigens	SAKAI/20/11_FEB	241	.....	300
	YAMAGATA/285/11_MAR	241	.....E.....	300
	KUMAMOTO-C/36/11_APR	241	.....MQ.....E.....	300
	Victoria/210/09E.CDC_JUN	241	.....MQ.....E.....	300
	Perth/16/09E.Aus_APR	241	.....E.....	300
Referens strains	NIIGATA/403/09_MAR	241	.....E.....	300
	Victoria/208/09E.Aus_JUN	241	.....E.....	300
	Brisbane/11/10E.Aus_MAY	241	.....E.....	300
	SHIZUOKA/736/09_MAY	241	.....E.....	300
	HUNAN-BEIHU/1313/09_MAY	241	.....X.....K.....E.....	300
	H3_AG_site	51	-.-------.-------.--.-------	54
	H3_RB_sites	19	----------	18
	10aa	25	---------X---------X---------X---------X---------X---------X	30
			.....	

	H3-HA-consensus(n=285)	301	TYGACPRYVKQNTLKLATGMRNVPEKQTR	329
	KUMAMOTO/17/11_JAN	301	.....S.....	329
	Myanmar/I-188/11-OR_JUL	301	.....S.....	329
	NIIGATA/745/11_MAY	301	.....	329
Epidemic strains	SHIMANE/194/11_APR	301	.....	329
	IBARAKI/190/11_APR	301	.....	329
	Taiwan/1096/11_FEB	301	.....	329
	HOKKAIDO/106/11_MAR	301	.....K.....	329
	SHIZUOKA-C/21/11_MAY	301	.....	329
	OSAKA/101/11_APR	301	.....	329
	Montana/05/11E.CDC_MAR	301	.....	329
Selorogy antigens	SAKAI/20/11_FEB	301	.....	329
	YAMAGATA/285/11_MAR	301	.....S.....	329
	KUMAMOTO-C/36/11_APR	301	.....	329
	Victoria/210/09E.CDC_JUN	301	.....	329
	Perth/16/09E.Aus_APR	301	.....	329
Referens strains	NIIGATA/403/09_MAR	301	.....	329
	Victoria/208/09E.Aus_JUN	301	.....	329
	Brisbane/11/10E.Aus_MAY	301	.....I..S.....	329
	SHIZUOKA/736/09_MAY	301	.....	329
	HUNAN-BEIHU/1313/09_MAY	301	.....	329
	H3_AG_site	55	----------	54
	H3_RB_sites	19	----------	18
	10aa	31	---------X---------X---------	32
			.....	

B viruses

Summary

Antigenic analysis:

Victoria-lineage:

- Most B/Victoria-lineage viruses tested (90%) showed similar HI titers to the homologous titers of antisera raised against MDCK grown B/Brisbane/60 and B/Sakai/43/2008 reference viruses.
- Ferret antiserum raised against egg-grown vaccine virus B/Brisbane/60 poorly reacted with MDCK grown viruses.
- A few viruses isolated in March were 4-fold low to all B/Brisbane/60-like reference antisera. Those viruses were included in antigenic variant group B/Taiwan/55/2009 (clade 5) and were still sporadically isolated after March, too.

Yamagata-lineage:

- A quite few number of B/Yamagata-lineage viruses were isolated. The majority of isolates (79%) poorly reacted with B/Bangladesh/3333/2007 ferret antiserum, while all test viruses were well inhibited with B/Wisconsin/1/2010 antisera.

Phylogenetic analysis

Victoria-lineage HA gene:

- All test viruses, except for a few clade 5 viruses, fell into B/Brisbane/60 clade. This clade was further divided into two groups as group 1 (L58P) and group 2.
- Majority of Asian viruses belonged to group 1, while viruses isolated in USA, Africa and EU regions mainly belonged to group 2.
- A few viruses with 4-fold reduced HI titer to B/Brisbane/60 antiserum and belonging to clade 5 (T37I clade, so called B/Taiwan/55 clade) were sporadically detected after March.
- Two serology antigen viruses (B/Shanghai-Jingan/1392/2011, B/Shizuoka/57/2011) were selected from group1 and one (B/Aichi/64/2011) was selected from group 2.

Victoria-lineage NA gene:

- B/Brisbane/60 clade was divived into three groups as group 1 (I204V, N220K, D329N, A358E), group 2 (A389T, S397R) and group 3 (D384N, A465T).
- The majority of Japanese viruses fell into group 1, while the majority of Chinese viruses fell into group 3.

Yamagata-lineage HA gene:

- Most viruses isolated April-May belonged to B/Bangladesh/3333 clade (clade 3) and formed N202S, N116K subclade.

Yamagata-lineage NA gene:

- Phylogenetic tree of the NA gene was correlated well with that of the HA gene.

Antiviral resistant (NAI) viruses:

- One oseltamivir/peramivir resistant virus each from B/Yamagata-lineage and B/Victoria-lineage was detected on Feb, 2011 in China and May, 2011 in Japan, respectively.
- The Chinese resistant virus possessed I221T and showed elevated IC_{50} values against all

four NAIs.

- The Japanese resistant virus exhibited remarkably high IC_{50} to peramivir and zanamivir. Detection of the amino acid substitution in the NA gene is in progress.

Influenza B isolates characterized by NIID

2010.10.21-2011.9.15

	Japan	China	Taiwan	South Korea	Mongolia	Laos	Myanmar	Singapore	Total	
September 2010 - February 2011									n	%
B/Brisbane/60/2008 -like Cell	35	4	2	0	0	20	0	0	61	84.7
B/Brisbane/60/2008 -like* Cell	4	0	2	0	0	1	0	0	7	9.7
B/Brisbane/60/2008 (Low)** Cell	3	0	1	0	0	0	0	0	4	5.6
B/Wisconsin/1/2010 -like	14	25	1	0	0	0	0	0	40	100.0
B/Wisconsin/1/2010 -like*	0	0	0	0	0	0	0	0	0	0.0
B/Wisconsin/1/2010 - (Low)**	0	0	0	0	0	0	0	0	0	0.0
B/Bangladesh/3333/2007 -like	4	15	1	0	0	0	0	0	20	50.0
B/Bangladesh/3333/2007 -like*	10	6	0	0	0	0	0	0	16	40.0
B/Bangladesh/3333/2007 - (Low)**	0	4	0	0	0	0	0	0	4	10.0
Vic Total	42	4	5	0	0	21	0	0	72	64.3
Yam Total	14	25	1	0	0	0	0	0	40	35.7
Total	56	29	6	0	0	21	0	0	112	

	Japan	China	Taiwan	South Korea	Mongolia	Laos	Myanmar	Singapore	Total	
March 2011 - August 2011									n	%
B/Brisbane/60/2008 -like Cell	61	19	3	0	0	3	0	0	86	89.6
B/Brisbane/60/2008 -like* Cell	3	5	1	0	0	0	0	0	9	9.4
B/Brisbane/60/2008 (Low)** Cell	0	0	1	0	0	0	0	0	1	1.0
B/Wisconsin/1/2010 -like	7	7	5	0	0	0	0	0	19	100.0
B/Wisconsin/1/2010 -like*	0	0	0	0	0	0	0	0	0	0.0
B/Wisconsin/1/2010 - (Low)**	0	0	0	0	0	0	0	0	0	0.0
B/Bangladesh/3333/2007 -like	0	1	1	0	0	0	0	0	2	10.5
B/Bangladesh/3333/2007 -like*	3	2	1	0	0	0	0	0	6	31.6
B/Bangladesh/3333/2007 - (Low)**	4	8	3	0	0	0	0	0	15	78.9
Vic Total	64	24	5	0	0	3	0	0	96	83.5
Yam Total	7	7	5	0	0	0	0	0	19	16.5
Total	71	31	10	0	0	3	0	0	115	

* 4-fold low to homologous titer

* * 8-fold low to homologous titer

Hemagglutination inhibition tests of influenza B viruses (Victoria lineage)-0.5%TRBCs

Rabbit serum

HI test date:2011/9/15

Strains	Passage History	Sample date	Malaysia/ 2506/04 Egg No.05-1	Brisbane/ 60/08 Cell NIID No.4	Brisbane/ 60/08 Egg No.2	Sakai/43/08 Cell No.2 Boosted	FujianGulou/ 1272/08 Egg No.1	Taiwan/ 55/09 Cell No.10281-2	Bangladesh/ 3333/07 Egg No.2	Remarks
REF.Ag										
B/Malaysia/2506/2004	E3/E1+2		640	< 10	160	20	320	640	< 10	
B/Brisbane/60/2008	MDCKx/1 +2	2008/08/04	40	160	20	160	< 10	80	< 10	Bri/60 CL, V146I
B/Brisbane/60/2008	E4 +1	2008/08/04	640	40	320	80	160	640	< 10	Bri/60 CL, V146I
B/SAKAI/43/2008	MDCK 1 +2	2008/11/24	20	80	10	160	< 10	80	< 10	Bri/60 CL, V146I
B/Fujian Gulou/1272/2008	E1/E2 +1	2008/03/13	640	10	160	20	320	640	< 10	FujianGulou/1262 CL
B/Taiwan/55/2009	MDCK 2 +2	2009/11/15	640	40	320	20	320	1280	< 10	Taiwan/55 CL
B/Bangladesh/3333/2007	E4 +2	2007/08/19	20	< 10	< 10	< 10	< 10	< 10	640	Yamagata-lineage
TEST.Ag										
B/Hunan-Hecheng/1207/2011	C 3 +1	2011/06/07	40	160	20	160	10	80	< 10	Bri/60 CL, L58P
B/Jiangsu-Chongchuan/11113/2011	C 3 +1	2011/06/21	20	160	20	160	10	80	< 10	Bri/60 CL, L58P
B/Zhejiang-Linhai/1308/2011	C 2 +1	2011/07/18	20	160	20	160	10	80	< 10	Bri/60 CL
B/IWATE/2/2011	MDCK 1 +2	2011/04/13	40	160	20	80	< 10	80	< 10	ND
B/Guangdong-Chancheng/1381/2011	C 2 +1	2011/07/11	40	160	10	160	10	80	< 10	Bri/60 CL, L58P, R80G
B/Hunan-Lusong/1401/2011	C 1 +1	2011/06/27	40	160	10	160	10	80	< 10	Bri/60 CL, L58P
B/Guangdong-Zhongshan/1272/2011	C 2 +1	2011/07/05	20	160	10	160	10	80	< 10	Bri/60 CL, L58P, R80G
B/NAGANO/2363/2011	MDCK 2 +1	2011/04/23	40	160	10	80	10	80	< 10	Bri/60 CL, L58P
B/Zhejiang-Haishu/1475/2011	C 1 +1	2011/06/22	20	80	10	80	10	40	< 10	Bri/60 CL, L58P

Hemagglutination inhibition tests of influenza B viruses (Victoria lineage)-0.5%TRBCs

Rabbit serum — HI test date:2011/6/9

Strains	Passage History	Sample date	Malaysia/ 2506/04 Egg No.05-1	Brisbane/ 60/08 Cell NIID No.4	Brisbane/ 60/08 Egg No.2	Sakai/ 43/08 Cell No.2 Boosted	Brisbane/ 33/08 Cell No.2	Brisbane/ 33/08 Egg No.1	FujianGulou/ 1272/08 Egg No.1	Taiwan/ 55/09 Cell No.10281-2	Bangladesh/ 3333/07 Egg No.2	Remarks
REF.Ag												
B/Malaysia/2506/2004	E3/E1+2		1280	10	320	20	40	160	160	640	< 10	
B/Brisbane/60/2008	MDCKx/1 +2	2008/08/04	40	160	20	160	40	40	< 10	80	< 10	Bri/60 CL, V146I
B/Brisbane/60/2008	E4 +1	2008/08/04	640	80	320	80	160	320	160	640	< 10	Bri/60 CL, V146I
B/SAKAI/43/2008	MDCK 1 +2	2008/11/24	20	80	20	160	< 10	20	< 10	80	< 10	Bri/60 CL, V146I
B/Brisbane/33/2008	MDCKx/2 +3	2008/07/13	320	80	320	40	80	160	160	640	< 10	Bri/60 CL, V146I
B/Brisbane/33/2008	E3 +1	2008/07/13	320	80	320	80	160	320	160	640	< 10	Bri/60 CL, V146I
B/Fujian Gulou/1272/2008	E1/E2 +1	2008/03/13	640	10	320	20	40	160	320	640	< 10	FujianGulou/1262 CL
B/Taiwan/55/2009	MDCK 2 +2	2009/11/15	640	40	320	20	40	160	160	640	< 10	Taiwan/55 CL
B/Bangladesh/3333/2007	E4 +2	2007/08/19	20	10	< 10	< 10	< 10	< 10	10	< 10	320	Yamagata-lineage
TEST.Ag												
B/Shanghai-Luwan/173/2011	E4 +1		160	40	320	40	80	160	40	80	< 10	Bri/60 CL, L58P
B/NAGANO/2282/2011	MDCK 2 +1	2011/03/15	40	160	40	160	40	80	10	160	< 10	ND
B/HOKKAIDO/12/2011	MDCK 1 +1	2011/03/22	40	160	40	160	40	40	10	80	< 10	Bri/60 CL
B/SAPPORO/13/2011	MDCK 1 +1	2011/02/10	40	160	40	160	40	20	10	160	< 10	ND
B/ISHIKAWA/69/2011	MDCK 1 +1	2011/02/26	40	160	20	160	40	40	10	80	< 10	ND
B/KITAKYUSYU/2/2011	MDCK 2 +1	2011/03/03	40	160	20	160	40	40	10	80	< 10	ND
B/KOBE/647/2011	MDCK 1 +1	2011/03/28	40	160	20	160	40	40	10	80	< 10	ND
B/KOCHI/43/2011	MDCK 1 +1	2011/03/02	40	160	20	160	40	40	10	80	< 10	ND
B/HIROSHIMA-C/20/2011	MDCK 1 +1	2011/03/23	40	160	20	160	40	20	< 10	80	< 10	ND
B/OITA/16/2011	MDCK 1 +1	2011/03/03	40	160	20	160	40	40	10	80	< 10	ND
B/HAMAMATU-C/131/2011	MDCK 2 +1	2011/02/10	40	160	20	160	40	40	10	80	< 10	ND
B/KOBE/43/2011	MDCK 1 +1	2011/04/20	40	160	20	160	40	40	< 10	160	< 10	Bri/60 CL, L58P, R80G
B/HOKKAIDO/5/2011	CaCo-2 1 +1	2011/03/15	20	160	20	160	40	40	< 10	80	< 10	ND
B/KAWASAKI/17/2011	MDCK 1 +1	2011/02/22	40	160	20	160	20	40	< 10	160	< 10	ND
B/HYOGO/3184/2011	MDCK 2 +1	2011/03/09	40	160	20	80	40	20	10	80	< 10	ND
B/SHIZUOKA/53/2011	MDCK 1 +1	2011/03/06	40	80	20	160	20	40	10	160	< 10	Bri/60 CL, L58P
B/CHIBA-C/15/2011	MDCK 2 +1	2011/03/18	40	80	20	160	40	40	10	160	< 10	Bri/60 CL, L58P
B/YAMANASHI/251/2011	CaCo-2 1 +1	2011/02/07	40	80	20	160	20	20	10	160	< 10	Bri/60 CL, L58P
B/SAITAMA/26/2011	MDCK 1 +1	2011/03/09	20	80	20	80	40	20	< 10	40	< 10	Bri/60 CL
B/NIIGATA/326/2011	MDCK 2 +1	2011/02/27	40	40	10	10	< 10	< 10	10	80	< 10	Taiwan/55 CL
B/KUMAMOTO-C/3/2011	CaCo-2 3 +1	2011/01/05	40	40	10	10	< 10	< 10	10	80	< 10	Taiwan/55 CL
B/KUMAMOTO-C/30/2011	CaCo-2 2 +1	2011/03/11	40	40	10	10	< 10	< 10	10	80	< 10	Taiwan/55 CL
B/CHIBA/17/2011	MDCK 1 +1	2011/03/18	40	40	10	10	< 10	< 10	10	80	< 10	Taiwan/55 CL
B/SHIZUOKA/57/2011	**MDCK 1 +1**	**2011/03/14**	**40**	**40**	**< 10**	**40**	**< 10**	**< 10**	**< 10**	**20**	**< 10**	**Bri/60 CL, L58P, K203N*, $**

*: Antigenic site
$: Serology Antigen