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Severe and Fatal Influenza Cases in Russia in 2014-2015

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Abstract: This paper aims to characterize herd immunity of the population inhabiting Asian part of Russia before influenza epidemic and to describe influenza viruses isolated from severe cases including cases with fatal outcomes in the 2014-2015 epidemic season. HI test enabled us to study 3888 serum samples from healthy individuals including 1939 samples collected from poultry farm workers. We showed that none of the 3888 samples produced positive results with the antigens A/H5N1, A(H5N8) and A/H7N9. 41% of the samples are positive to A/California/07/09(H1N1pdm09), 36% of the samples are positive to A/Texas/50/2012 (H3N2), 40% of the samples are positive to B/Brisbane/60/2008 (Victoria lineage) and 47% of the samples are positive to B/Massachusetts/2/2012 (Yamagata lineage). In the 2014-2015 epidemic season 25 clinical and 19 autopsy samples were collected from individuals with severe flu-like infection. Fifteen influenza A(H3N2), two influenza A(H1N1pdm09) and one influenza B (Yamagata) virus strains were isolated in Madin-Darby Canine Kidney cell culture. All viruses exhibited normal inhibition by oseltamivir and zanamivir. A/KMAO/1/2015 and A/Kurgan/149/2015 were antigenically characterized as A/California/07/2009-like. Their hemagglutinin (HA) gene sequences fell into the predominant genetic group 6B and were similar to other recent H1N1pdm09 viruses circulating in Asian region. Eight H3N2 isolated viruses (A/Omsk/160/2015, A/Krasnoyarsk/324/2015, A/NizhnyNovgorod/788/2015, A/Omsk/141/2015, A/Buryatia/19/2015, A/Komi/99/2015, A/Novosibirsk/122/2015 and A/Chelyabinsk/160/2015) were characterized as A/Hong Kong/4801/2014-like and seven viruses (A/Irkutsk/88/2015, A/Krasnoyarsk/365/2015, A/Blagoveshchensk/19/2015, A/Kemerovo/2/2015, A/Chelyabinsk/160/2015, A/Chelyabinsk/192/2015 and A/Novosibirsk/64/2015) as A/Switzerland/9715293/2013-like. Their HA gene sequences belong to 3C.2a and 3C.3a genetic groups, respectively. B/Yekaterinburg/155/2015 virus was antigenically similar to B/Phuket/3073/2013 with HA sequence belonging to Y3 genetic group. Obtained findings are important for adjustment of public health measures and vaccine strategy in Russia.

Keywords: Seasonal Influenza Viruses, HI Test, Sequencing, Phylogenic Analysis, Sensitivity to NA Inhibitors

Introduction

Since 2009 seasonal influenza has been caused by viruses A(H1N1pdm09), A(H3N2), influenza B (Victoria lineage) and influenza B (Yamagata lineage) (WHO, 2016). In the 2014–2015 epidemic season A(H3N2) was prevalent among influenza A viruses and strains of Yamagata lineage predominated among influenza B viruses. Thus, in Canada 81% of confirmed cases were caused by influenza A and 19% by influenza B viruses. The majority of circulating influenza A viruses (41.5%) belonged to H3N2 subtype, while a share of A(H1N1pdm09) viruses was only 0.3% (there were no subtyping for 58.2% of the cases) (FluWatch, 2015). In the USA similar data were registered (Appiah *et al*., 2015). In Europe 67% of approximately 16000 positive results belonged to influenza A viruses (of them 21.7% influenza A(H1N1pdm09), 72.1% influenza A(H3N2) and the rest untyped) and 33% belonged to influenza B (of them 0.6% Victoria, 25.2% Yamagata lineage and the rest untyped) (ECDC, 2015a).

In Russia the epidemic rise of influenza incidence began during the 5th calendar week in 2015 (25 January-01 February); the peak of epidemic was observed during the 8th week (16 February-22 February) and decreased activity was registered up to the 13th week. More than 59% of all isolated strains in Russia accounted for influenza A(H3N2) viruses; approximately 37% -for influenza B viruses and share of A(H1N1pdm) viruses were <4% (Rospotrebnadzor, 2015).

This research pursues an objective to isolate influenza virus strains from autopsy and clinical materials obtained from people who suffered from a severe flu-like disease, study their antigenic and biomolecular features and analyze anti-neuraminidase drug sensitivity. Besides, herd immunity just before epidemic was investigated. This paper continues our previous studies devoted to monitoring of influenza in Russia (Ilyicheva *et al*., 2011; 2013; 2016).

Materials and Methods

Investigation of the Herd Immunity to Influenza

Testing of blood sera was approved by Ethics Committee IRB 00001360. The presence of antibodies to different types/serotypes of influenza virus in the sera was tested following a standard technique, in Hemagglutination Inhibition (HI) test (WHO, 2011), to A/California/07/09(H1N1)pdm09, A/Texas/50/2012 (H3N2), B/Massachusetts/2/2012 (Yamagata lineage) and B/Brisbane/60/2008 (Victoria lineage) influenza viruses recommended by World Health Organization (WHO) as vaccine strains in 2014–2015 and to highly pathogenic influenza viruses A/Anhui/01/2013(H7N9), A/Black-Headed gull/Tyva/115/09 (H5N1) and A/wigeon/Sakha/1/2014 (H5N8).

The A/California/07/09(H1N1)pdm09, A/Texas/50/2012 (H3N2), A/Switzerland/9715293/13 (H3N2), B/Brisbane/60/2008 and B/Massachusetts/2/2012 influenza viruses were kindly provided by the WHO Collaborating Center in Atlanta, USA. The A/Anhui/01/2013 (H7N9) virus was kindly provided by the WHO Collaborating Center in Beijing, China. The A/Black-Headed gull/Tyva/115/09 (HPAI H5N1) virus (clade 2.2.3) was isolated by the authors in Western Siberia (Sharshov *et al*., 2010); the A/wigeon/Sakha/1/2014 (HPAI H5N8) virus (clade 2.2.4.4) was isolated by the authors in Eastern Siberia (Marchenko *et al*., 2015).

In total 3888 serum samples from healthy individuals were tested including 1939 samples collected from poultry farm workers. Sera were collected in various regions of Russia in October-November, 2014 (Fig. 1).

Influenza Virus Isolates from Autopsy and Clinical Materials

Vector State Research Center of Virology and Biotechnology received the autopsy materials (pieces of the bronchi, trachea and lungs) from individuals who died presumably from influenza and clinical materials from people with severe disease. Figure 1 shows regions where the materials were collected. All samples were transferred in tubes placed into thermal containers with cold pack. In total 25 clinical and 19 autopsy samples were collected from individuals with severe flu-like infection.

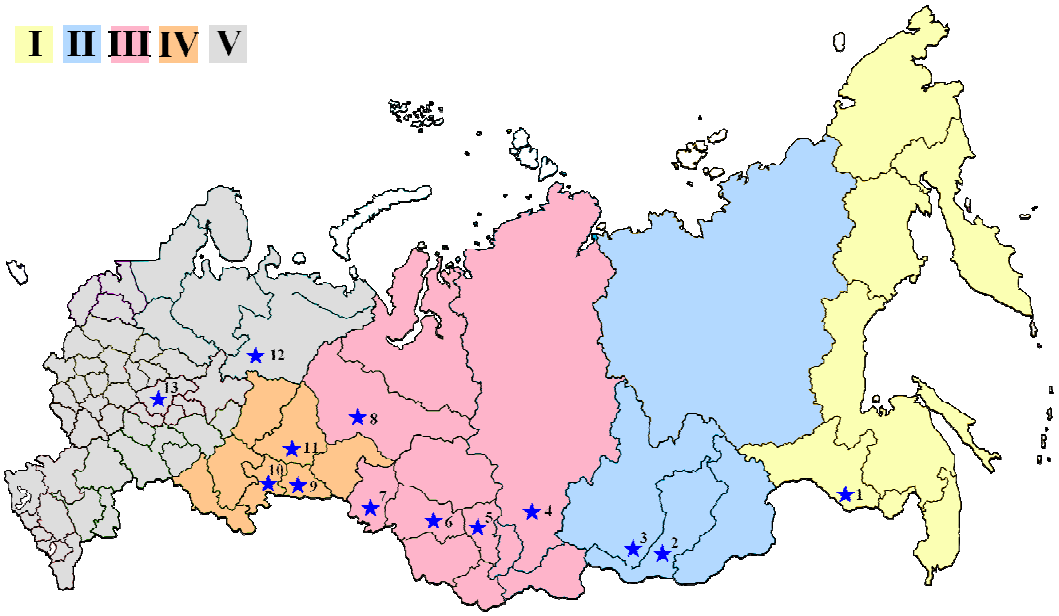

Fig. 1. Regions of sample collection. I – Russian Far East, II – Eastern Siberia, III – Western Siberia, IV – Ural and near regions, V – European part of Russia. 1 – Blagoveshchensk, 2 – Ulan Ude, 3 – Irkutsk, 4 – Krasnoyarsk, 5 – Kemerovo, 6 – Novosibirsk, 7 – Omsk, 8 – Khanty-Mansiysk, 9 – Kurgan, 10 – Chelyabinsk, 11 – Yekaterinburg, 12 – Syktyvkar, 13 – Nizhny Novgorod

The isolates were recovered in Madin-Darby Canine Kidney (MDCK) cell culture (London line) as described previously (Ilyicheva *et al*., 2016).

Studying sensitivity to anti-neuraminidase drugs was carried out using fluorescent method according to WHO recommendations (WHO, 2009).

Results

Investigation of the Herd Immunity to Influenza

None of the 3888 samples produced positive results with the antigens A/H5N1, A(H5N8) and A/H7N9 even at dilution 1:10.

The HI results with the antigens A/H1pdm09, A/H3N2, B/Victoria and B/Yamagata are shown in Table 1.

Analysis of the data from Table 1 shows that 41% of the samples were positive to A/California/07/09(H1N1pdm09), 36% of the samples were positive to A/Texas/50/2012 (H3N2), 40% of the samples were positive to B/Brisbane/60/2008 (Victoria lineage) and 47% of the samples were positive to B/Massachusetts/2/2012 (Yamagata lineage). In addition, 22% of the samples reacted in HI with all antigens with the reciprocal titer lower than 40, i.e., they were negative to all studied antigens while 10% of the samples were positive to all the antigens.

Influenza Virus Isolates from Autopsy and Clinical Materials

Fifteen influenza A(H3N2), two A(H1N1pdm09) and one influenza B (Yamagata) virus strains were isolated from autopsy and clinical materials. Table 2 demonstrates the data concerning isolated strains.

Table 1. Analysis of sera in HI test with vaccine influenza viruses

		Number of seropositives in an age group				Across all subtypes	
Region of sampling	Type/subtype of virus	35 years and under	36–59 years	60 years and older	Total (%)	Negative (%)	Positive (%)
Russian Far East							
Age range: 0.3–85 years		225 (32%)	313 (45%)	163 (23%)	701	191 (27)	28 (4)
	A/H1N1pdm09[1]	94	69	30	193 (28)		
	A/H3N2[2]	44	36	14	94 (13)		
	B/Victoria[3]	145	187	65	397 (57)		
	B/Yamagata[4]	102	93	32	227 (32)		
Eastern Siberia							
Age range: 7–76 years		364 (49%)	342 (46%)	44 (6%)	750	157 (21)	77 (10)
	A/H1N1pdm09	178	120	15	313 (42)		
	A/H3N2	108	133	21	262 (35)		
	B/Victoria	139	124	13	276 (37)		
	B/Yamagata	222	189	29	440 (59)		
Western Siberia							
Age range: 1–90 years		531 (43%)	581 (47%)	129 (10%)	1241	226 (18)	185 (15)
	A/H1N1pdm09	330	283	44	657 (53)		
	A/H3N2	275	251	55	581 (47)		
	B/Victoria	231	221	17	469 (38)		
	B/Yamagata	234	232	35	501 (40)		
Ural and near regions							
Age range: 17–79 years		263 (40%)	363 (56%)	27 (4%)	653	147 (23)	88 (13)
	A/H1N1pdm09	163	138	4	305 (47)		
	A/H3N2	153	143	5	301 (46)		
	B/Victoria	119	110	7	236 (36)		
	B/Yamagata	180	146	8	334 (51)		
European part of Russia							
Age range: 1–92 years		183 (34%)	271 (50%)	89 (16%)	543	143 (26)	23 (4)
	A/H1N1pdm09	49	66	18	133 (24)		
	A/H3N2	57	73	21	151 (28)		
	B/Victoria	48	98	17	163 (30)		
	B/Yamagata	123	155	40	318 (59)		
Total		1566 (40%)	1870 (48%)	452 (12%)	3888	864 (22%)	401 (10%)

[1] - A/California/07/09(H1N1) pdm09
[2] - A/Texas/50/2012 (H3N2)
[3] - B/Brisbane/60/2008 (Victoria lineage)
[4] - B/Massachusetts/2/2012 (Yamagata lineage)

Table 2. Viruses isolated from autopsy and clinical materials in the 2014–2015 epidemic season

Material	Sex	Age	Strain designation	Subtype	Region in Fig. 1	Date collected	Neuraminidase inhibition results	HA genetic group	GISAID EpiFlu isolate ID	GISAID EpiFlu HA accession	GISAID EpiFlu NA accession
autopsy	f	11	B/Yekaterinburg/155/2015	B/Yamagata	11	22.04.2015	Normal (Ose, Zan)	Y3	EPI_ISL_195725	EPI643028	EPI643027
autopsy	m	65	A/KMAO/1/2015	A(H1N1pdm09)	8	23.05.2015	Normal (Ose, Zan)	6B	EPI_ISL_195804	EPI643300	EPI643299
nasopharyngeal swab	f	35	A/Kurgan/149/2015	A(H1N1pdm09)	9	01.04.2015	Normal (Ose, Zan)	6B	EPI_ISL_195800	EPI643268	EPI643267
nasopharyngeal swab	m	11	A/Blagoveshchensk/19/2015	A(H3N2)	1	29.01.2015	Normal (Ose, Zan)	3C.3a	EPI_ISL_205174	EPI683443	EPI683442
autopsy	m	62	A/Buryatia/19/2015	A(H3N2)	2	26.01.2015	Normal (Ose, Zan)	3C.2a	EPI_ISL_197519	EPI649651	EPI649650
nasopharyngeal swab	m	15	A/Irkutsk/88/2015	A(H3N2)	3	21.01.2015	Normal (Ose, Zan)	3C.3a	EPI_ISL_205176	EPI683459	EPI683458
nasopharyngeal swab	m	37	A/Krasnoyarsk/324/2015	A(H3N2)	4	04.02.2015	Normal (Ose, Zan)	3C.2a	EPI_ISL_194641	EPI637162	EPI637173
nasopharyngeal swab	m	46	A/Krasnoyarsk/365/2015	A(H3N2)	4	09.02.2015	Normal (Ose, Zan)	3C.3a	EPI_ISL_194642	EPI637205	EPI637206
nasopharyngeal swab	f	5	A/Kemerovo/2/2015	A(H3N2)	5	26.02.2015	Normal (Ose, Zan)	3C.3a	EPI_ISL_194637	EPI636801	EPI636806
autopsy	f	46	A/Novosibirsk/122/2015	A(H3N2)	6	09.02.2015	Normal (Ose, Zan)	3C.2a	EPI_ISL_194631	EPI636661	EPI636662
nasopharyngeal swab	f	78	A/Novosibirsk/64/2015	A(H3N2)	6	27.02.2015	Normal (Ose, Zan)	3C.3a	EPI_ISL_197513	EPI649604	EPI649603
autopsy	f	7	A/Omsk/160/2015	A(H3N2)	7	29.12.2014	Normal (Ose, Zan)	3C.2a	EPI_ISL_194636	EPI636799	EPI636800
nasopharyngeal swab	m	16	A/Omsk/141/2015	A(H3N2)	7	26.01.2015	Normal (Ose, Zan)	3C.2a	EPI_ISL_200721	EPI667964	EPI667963
autopsy	f	82	A/Chelyabinsk/160/2015	A(H3N2)	10	10.02.2015	Normal (Ose, Zan)	3C.3a	EPI_ISL_194644	EPI637209	EPI637210
autopsy	m	36	A/Chelyabinsk/192/2015	A(H3N2)	10	20.02.2015	Normal (Ose, Zan)	3C.3a	EPI_ISL_200723	EPI667980	EPI667979
autopsy	f	80	A/Yekaterinburg/239/2015	A(H3N2)	11	05.03.2015	Normal (Ose, Zan)	3C.2a	EPI_ISL_194646	EPI637213	EPI637214
nasopharyngeal swab	m	82	A/Komi/99/2015	A(H3N2)	12	27.01.2015	Normal (Ose, Zan)	3C.2a	EPI_ISL_200722	EPI667972	EPI667971
nasopharyngeal swab	f	7	A/NizhnyNovgorod/788/2015	A(H3N2)	13	26.01.2015	Normal (Ose, Zan)	3C.2a	EPI_ISL_194643	EPI637207	EPI637208

Assessed in the fluorescent neuraminidase (NA) inhibition assay with four NA inhibitors (Ose: oseltamivir; Zan: zanamivir). NA inhibition characterized according to criteria introduced by the WHO Influenza Antiviral Working Group (WHO–AVWG) (WHO, 2012)

Table 3. Hem agglutination inhibition test of influenza A(H1N1pdm09) viruses

	Reference ferret antisera			
Reference antigens	CA/07	BA/2021	FL/62	SA/3626
A/California/07/2009	1280	640	1280	2560
A/Bangladesh/2021/2012	320	1280	320	640
A/Florida/62/2014	1280	640	1280	2560
A/South Africa/3626/2013	1280	640	1280	2560
Test antigen				
A/KMAO/1/2015	1280	320	1280	2560
A/Kurgan/149/2015	1280	160	640	2560

Table 4. Hem agglutination inhibition test of influenza H3 viruses

	Reference ferret antisera		
Reference antigens	TX/50	HK/4801	SZ/9715293
A/Texas/50/2012	1280	320	320
A/Hong Kong/4801/2014	80	160	40
A/Switzerland/9715293/2013	160	160	160
Test antigens			
A/Buryatia/19/2015	160	160	160
A/Blagoveshchensk/19/2015	160	160	160
A/Irkutsk/88/2015	1280	320	640
A/Krasnoyarsk/365/2015	160	160	320
A/Krasnoyarsk/324/2015	160	320	80
A/Kemerovo/2/2015	320	80	320
A/Omsk/160/2014	160	320	80
A/Omsk/141/2015	160	320	160
A/Novosibirsk/122/2015	160	320	80
A/ Novosibirsk/64/2015	160	20	160
A/Chelyabinsk/160/2015	320	80	320
A/Chelyabinsk/192/2015	160	160	320
A/Yekaterinburg/239/2015	160	80	160
A/Komi/99/2015	160	320	80
A/Nizhny_Novgorod/788/2015	160	160	40

Table 5. Hemagglutination inhibition test of influenza B (Yamagata lineage) viruses

	Reference ferret antisera	
Reference antigens	MA/02	PHU/3073
B/Massachusetts/02/2012	1280	1280
B/Phuket/3073/2013	160	640
Test antigen		
B/Yekaterinburg/155/2015	80	640

Antigenic features of isolated strains were studied in HI test with reference sera. Results are shown in Table 3-5. A virus is considered “reference virus-like” if its HI titer is equal to or within a 4-fold difference to the homologous HI titer of the reference strain. A virus is considered as low to the reference virus if there is an 8-fold or greater reduction in the HI titer when compared to the homologous HI titer of the reference strain.

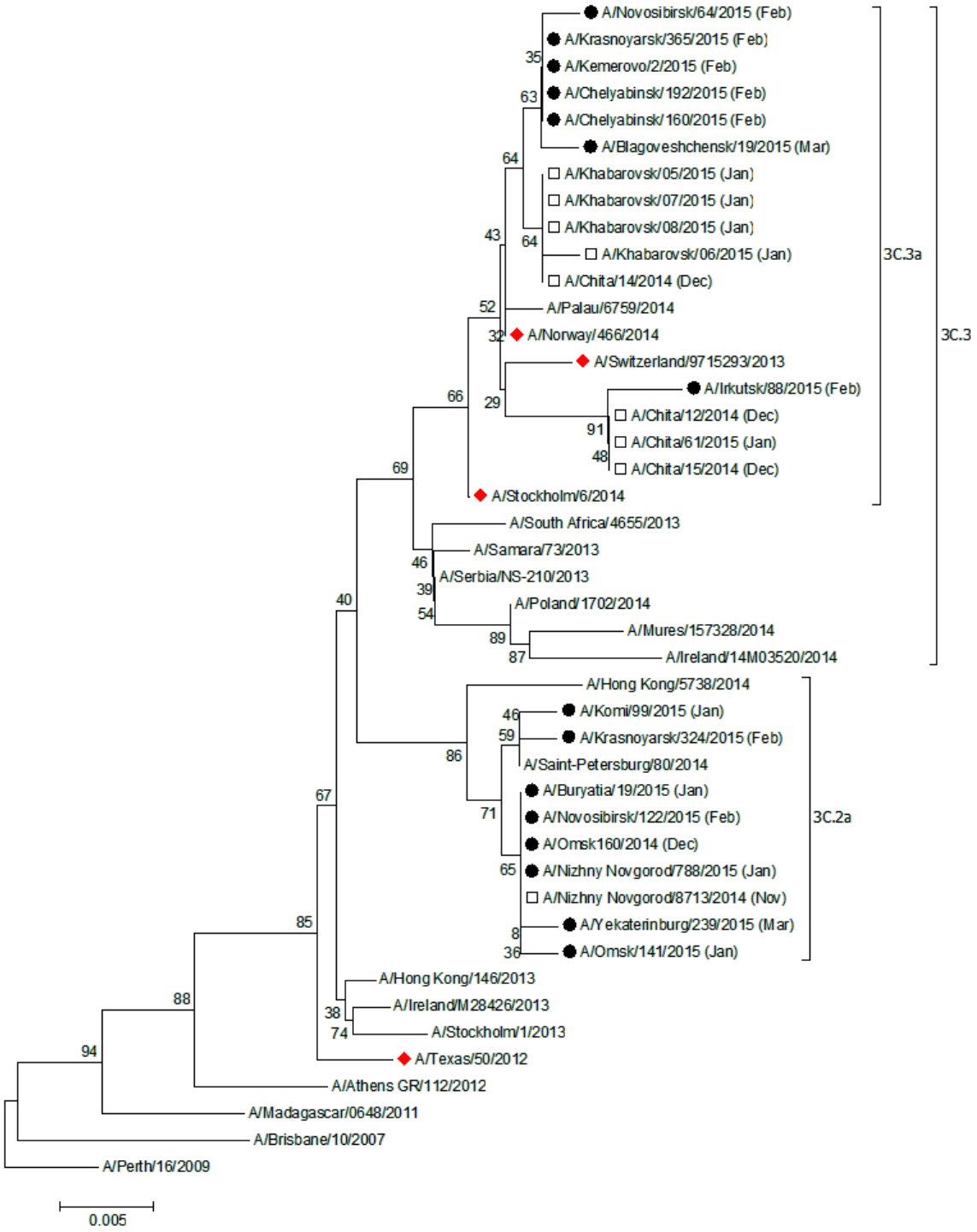

Fig. 2. Phylogenetic tree of HA gene of influenza A(H3N2) virus strains studied in 2015. Strains of 2015 are marked with black solid circles. For comparison we also included strains analyzed in 2014 (white squares), vaccine strains (red diamonds); sequences of other strains are taken from GISAID database

Table 3 shows that antigenic features of A/KMAO/1/2015 (H1N1pdm09) and A/Kurgan/149/2015 (H1N1pdm09) strains were similar to A/California/07/09 (H1N1pdm09) vaccine strain.

As Table 4 shows antigenic properties of all isolated A(H3N2) strains differed from A/Texas/50/2012 vaccine strain and were similar to A/Switzerland/9715293/2013 and A/Hong Kong/4801/2014 strains.

Table 5 demonstrates that antigenic features of isolated strain B/Yekaterinburg/155/2015 were similar to B/Phuket/3073/2013 (Yamagata lineage) strain.

All isolated strains were sensitive to anti-neuraminidase drugs oseltamivir and zanamivir (Table 2).

We conducted sequence analysis of HA and NA genes of all isolated strains (Table 2) and built phylogenetic tree for H3 (Fig. 2).

Discussion

In 2014-2015 more than 59% of all isolated strains in Russia accounted for influenza A(H3N2) viruses; approximately 37% -for influenza B viruses and share of A(H1N1pdm) viruses were <4% (Rospotrebnadzor, 2015). These data can be easily explained with regard to previously studied conditions of herd immunity before epidemics. Thus, adaptive immunity insufficiently protected Russian population against influenza A and B. New variant of influenza A(H3N2) virus was prevalent and due to antigenic drift was able to evade specific immunity targeting previous epidemic and vaccine virus strains. United States Centers for Disease Control and Prevention suggested that the 2014–2015 influenza vaccine strain A/Texas/50/2012 was essentially ineffective against the circulating A(H3N2) strains (CDC, 2015).

Influenza A(H3N2) predominated during the 2014–2015 influenza season in North America, Asia and Europe; the majority of H3N2 viruses were antigenically related to A/Switzerland/9715293/2013 virus and A/Hong Kong/4801/2014 virus (Hua *et al*., 2015). While the majority of H3N2 viruses tested were antigenically related to A/Switzerland/9715293/2013, most viruses were better inhibited by ferret antisera raised against A/Hong Kong/4801/2014, which belonged to genetic group of A(H3N2) viruses predominating globally by late 2015. A/Switzerland/9715293/2013 virus was recommended as H3N2 component for the 2015–2016 Northern Hemisphere vaccine formulations (WHO, 2015a), A/Hong Kong/4801/2014 virus was recommended as the A(H3N2) component for 2016 Southern Hemisphere influenza vaccine composition (WHO, 2015b).

The phylogenetic tree for the HA gene of H3N2 viruses can be divided into 7 genetic groups based on shared amino acid changes (compared to previous vaccine strain A/Perth/16/2009); with only group 3 viruses currently circulating. Group 3 viruses share HA amino acid change V223I and are further divided into subgroups 3A, 3B and 3C. Subgroup 3A viruses share amino acid changes of N144D (loss of a glycosylation site), N145S and D487N. Subgroup 3B and 3C viruses share amino acid changes of A198S and N312S. Subgroup 3B viruses also have amino acid changes of N145S and D487N (Stucker *et al*., 2015).

Since 2013, genetic group 3C has been the dominant subgroup circulating worldwide. Viruses from group 3C share amino acid changes of S45N (gain of a glycosylation site) and T48I. Group 3C has further diverged into three genetic subgroups (3C.1, 3C.2 and 3C.3). Genetic subgroups 3C.2 and 3C.3 share amino acid changes of Q33R, N145S and N278K. Genetic subgroup 3C.2 viruses share amino acid change D489N. Genetic subgroup 3C.3 viruses share additional amino acid changes T128A (loss of a glycosylation site) and R142G in the HA gene. Despite the genetic divergences, these three subgroups of H3N2 viruses were antigenically similar (ECDC, 2015b).

In 2014, antigenic drift variant viruses emerged from genetic subgroups 3C.2 and 3C.3. Ferret antisera raised against vaccine strain A/Texas/50/2012 showed a reduction in HI titer to these viruses. Group 3C.2 has split into two subgroups: A very small number of viruses belong to 3C.2b which shares amino acid changes L3I, N144S (loss of a glycosylation site), K160T (gain of a glycosylation site), N225D and Q311H (designated as 3C.2b) while the vast majority of viruses belong to subgroup 3C.2a which shares the same changes as 3C.2b but with an additional change of F159Y. In group 3C.3 these viruses form a new subgroup, 3C.3a and share amino acid changes A138S, F159S, N225D and K326R. An additional subgroup within 3C.3 has also emerged, 3C.3b, but the majority of viruses within this subgroup showed normal HI titers to ferret antisera raised against vaccine strain A/Texas/50/2012. Genetic subgroup 3C.3b viruses share amino acid changes E62K, K83R, N122D, L157S, R261Q and V347K. Viruses in genetic groups 3C.3, 3C.3b, 3C.2 and 3C.2b were largely antigenically similar to previous vaccine virus A/Texas/50/2012, while viruses from genetic subgroups 3C.2a, represented by A/Hong Kong/4801/2014 and 3C.3a, represented by A/Switzerland/9715293/2013, were antigenically distinct from the A/Texas/50/2012 virus (Haveri *et al*., 2015).

In this study, seven H3N2 isolated viruses were characterized as A/Switzerland/9715293/2013-like and eight viruses as A/Hong Kong/4801/2014-like. We presented a phylogenetic tree of the HA genes of these viruses with HA sequences belonging to 3C.2a and 3C.3a genetic groups. All H3N2 viruses exhibited normal inhibition by oseltamivir and zanamivir.

Influenza A(H1N1pdm09) viruses have continued to circulate worldwide since their emergence in 2009. In 2014–2015, A(H1N1pdm09) activity was variable with notable widespread outbreaks in the Indian subcontinent and in parts of Africa (Parida *et al*., 2016; Takashita *et al*., 2016). The phylogenetic tree for the HA gene of H1N1pdm09 viruses can be divided into nine major genetic groups, although recently isolated viruses belong to group 6. Genetic group 6 is represented by viruses circulating worldwide and shares amino acid changes D97N, S185T, S203T, E374K and S451N in the HA. Group 6 can be divided into three subgroups -6A, 6B

and 6C. Subgroup 6A viruses share amino acid changes H138R and V249L in the HA and are represented by A/Bangladesh/2021/2012. Subgroups 6B and 6C share amino acid changes K283E and E499K in the HA. The majority of viruses, which circulated in the 2014 season in the Southern Hemisphere and in the 2014–2015 season in the Northern Hemisphere, belonged to subgroup 6B. Subgroup 6B viruses share additional amino acid changes of K163Q and A256T in the HA and are represented by A/North Carolina/04/2014. Subgroup 6C viruses share an additional change of V2341 in the HA and are represented by A/Pennsylvania/07/2013 (McCauley *et al*., 2014). Despite the genetic diversity, the vast majority of H1N1pdm09 isolates are antigenically indistinguishable and similar to A/California/07/2009, which has been included in influenza vaccine since 2009 and remains the H1N1pdm09 vaccine component of the 2015 Southern Hemisphere and 2015–2016 Northern Hemisphere seasons (WHO, 2015a).

Two influenza A(H1N1pdm09) viruses were isolated in Russia in 2014–2015. Those viruses were antigenically characterized as A/California/07/2009-like. A/KMAO/1/2015 and A/Kurgan/149/2015 viruses exhibited normal inhibition by oseltamivir and zanamivir. Their HA gene sequences fell into the predominant genetic group 6B and were similar to other recent H1N1pdm09 viruses circulating in Asian region (Parida *et al*., 2016).

Influenza B viruses of B/Victoria/2/87 and B/Yamagata/16/88 lineages have continued to co-circulate, with B/Yamagata-lineage viruses predominating in most regions of the world. The majority of B/Yamagata lineage viruses collected recently belonged to genetic group 3 and were antigenically more closely related to the B/Phuket/3073/2013 reference virus. In the summer of 2015, the proportion of B/Victoria lineage viruses increased rapidly in Oceania countries. Therefore, at the WHO vaccine consultation meeting in September 2015, a B/Victoria lineage virus B/Brisbane/60/2008 was recommended as the influenza B component of the trivalent and quadrivalent vaccines for the 2016 Southern Hemisphere influenza season (WHO, 2015b).

The phylogenetic tree for the HA gene of B/Yamagata lineage viruses can be divided into three genetic groups. Genetic group 1 includes the former vaccine virus B/Florida/04/2006. All recent B/Yamagata strains belonged to genetic groups 2 or 3 and could be distinguished antigenically in HI test by some post-infection ferret antisera. Strains from genetic group 2 are represented by B/Massachusetts/02/2012, the B vaccine component of the 2014–2015 Northern Hemisphere and the 2014 Southern Hemisphere seasons. Group 2 strains share amino acid changes of R48K, P108A, T182A and S230G in the HA. A small number of recent viruses from Asia, Africa and South America belong to genetic group 2. The majority of recent B/Yamagata-lineage viruses belong to genetic group 3, sharing amino acid changes at positions N116K, S150I, N166Y, N203S, S230D, K299E and E313K in the HA compared to B/Florida/04/2006 (Pan *et al*., 2015). Group 3 isolates are antigenically similar to B/Phuket/3073/2013-like viruses, the recommended B vaccine component for the 2015 Southern Hemisphere and 2015–2016 Northern Hemisphere seasons. Recently within group 3 there were two separate reassortment events leading to viruses with the HA from B/Yamagata genetic group 3 and the NA from B/Victoria genetic groups 1A or 4. These reassortant viruses remain antigenically similar to the recommended Group 3 B/Yamagata component in the vaccine (Oong *et al*., 2015).

This paper presents one B/Yamagata lineage virus isolated from a fatal influenza case. It was tested by HI and antigenically similar to B/Phuket/3073/2013. We also sequenced HA and NA genes of this virus, its HA sequence belongs to Y3 genetic group. This B virus exhibited normal inhibition by oseltamivir and zanamivir.

Conclusion

Thus, in 2014–2015 in Russia A(H3N2) viruses belonging to 3C.2a and 3C.3a genetic groups predominated among influenza A viruses. Fifteen influenza A(H3N2) virus strains were isolated from people who died presumably from influenza and individuals with severe flu-like infection. All viruses differed from vaccine strain of the 2014–2015 season (A/Texas/50/2012) and were similar to A/Switzerland/9715293/2013 and A/Hong Kong/4801/2014. Although there were less than 4% of A(H1N1pdm09) viruses among all circulated influenza viruses in Russia, we isolated two influenza A/California/07/2009-like virus strains from autopsy and clinical materials. Besides, one influenza B/Phuket/3073/2013-like virus strain was isolated from a fatal case. All isolated strains were sensitive to anti-neuraminidase drugs.

Obtained findings are important for adjustment of public health measures and vaccine strategy in Russia.

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Author's Contributions

All authors have read and approved the final manuscript.

Svetlana V. Svyatchenko: Performed the experiments, analyzed the data, wrote the paper.

Alexander G. Durymanov, Natalya P. Kolosova, Natalya I. Goncharova: Performed the experiments.

Ivan M. Susloparov, Valery N. Mikheev, Alexander B. Ryzhikov: Analyzed the data.

Tatyana N. Ilyicheva: Designed the experiments, analyzed the data, wrote the paper.

Ethics

All works regarding clinical samples (nasopharyngeal swabs, blood sera) and autopsy materials were approved by Ethics Committee IRB 00001360 (protocol #7 dated 20 May 2014). We obtained patients informed consent for all samples; anonymity was guaranteed for all patients concerning studying samples and analyzing results.

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