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/9/2015, 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/Rassnoyarsk/365/2015, A/Blagoveshensk/19/2015, A/Kemerovo/20/2015, 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, Phylogenetic 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), 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.

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

| Region of sampling                        | Number of seropositives in an age group | Across all subtypes |
|-------------------------------------------|-----------------------------------------|---------------------|
|                                           | Type/subtype of virus   | 35 years and under | 36–59 years | 60 years and older | Total (%) | Negative (%) | Positive (%) |
| Russian Far East                          | A/H1N1pdm09\(^1\) | 225 (32%)         | 313 (45%)   | 163 (23%)    | 701 | 191 (27) | 28 (4) |
|                                           | A/H3N2\(^2\)       | 44 (7%)           | 69 (10%)    | 30 (4%)      | 139 | 33 (23) | 3 (2) |
|                                           | B/Victoria\(^3\)   | 145 (22%)         | 187 (27%)   | 65 (9%)      | 397 (57%) | 94 (13) | 5 (1) |
|                                           | B/Yamagata\(^4\)  | 102 (15%)         | 93 (13%)    | 32 (4%)      | 227 (32%) | 44 (6) | 2 (1) |
| Eastern Siberia                           | A/H1N1pdm09         | 364 (49%)         | 342 (46%)   | 44 (6%)      | 750 | 157 (21) | 77 (10) |
|                                           | A/H3N2              | 108 (15%)         | 133 (19%)   | 31 (4%)      | 272 (36%) | 50 (6) | 3 (1) |
|                                           | B/Victoria          | 139 (21%)         | 124 (17%)   | 13 (2%)      | 276 (37%) | 67 (9) | 7 (1) |
|                                           | B/Yamagata          | 222 (34%)         | 189 (26%)   | 29 (4%)      | 440 (59%) | 88 (12) | 8 (1) |
| Western Siberia                           | A/H1N1pdm09         | 531 (43%)         | 581 (47%)   | 129 (10%)    | 1241 | 226 (18) | 185 (15) |
|                                           | A/H3N2              | 330 (25%)         | 283 (21%)   | 44 (6%)      | 657 (53%) | 130 (10) | 10 (1) |
|                                           | B/Victoria          | 275 (20%)         | 251 (19%)   | 55 (8%)      | 581 (47%) | 112 (8) | 10 (1) |
|                                           | B/Yamagata          | 231 (16%)         | 221 (16%)   | 17 (2%)      | 469 (36%) | 91 (6) | 7 (1) |
| Ural and near regions                     | A/H1N1pdm09         | 263 (40%)         | 363 (56%)   | 27 (4%)      | 653 | 147 (23) | 88 (13) |
|                                           | A/H3N2              | 163 (25%)         | 138 (21%)   | 4 (0%)       | 305 (47%) | 62 (9) | 4 (1) |
|                                           | B/Victoria          | 153 (23%)         | 143 (22%)   | 5 (0%)       | 301 (46%) | 56 (8) | 4 (1) |
|                                           | B/Yamagata          | 119 (18%)         | 110 (17%)   | 7 (1%)       | 236 (36%) | 41 (6) | 3 (1) |
| European part of Russia                   | A/H1N1pdm09         | 183 (34%)         | 271 (50%)   | 89 (16%)     | 543 | 143 (26) | 23 (4) |
|                                           | A/H3N2              | 49 (8%)           | 66 (11%)    | 18 (3%)      | 133 (24) | 20 (3) | 3 (1) |
|                                           | B/Victoria          | 57 (10%)          | 73 (12%)    | 21 (4%)      | 151 (26) | 23 (4) | 3 (1) |
|                                           | B/Yamagata          | 48 (8%)           | 98 (16%)    | 17 (3%)      | 163 (30) | 23 (4) | 3 (1) |
|                                           | 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)

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 2. Analysis of sera in HI test with vaccine influenza viruses

| Region of sampling | Number of seropositives in an age group | Across all subtypes |
|--------------------|-----------------------------------------|---------------------|
|                    | Type/subtype of virus   | 35 years and under | 36–59 years | 60 years and older | Total (%) | Negative (%) | Positive (%) |
| Russian Far East   | A/H1N1pdm09\(^1\) | 225 (32%)         | 313 (45%)   | 163 (23%)    | 701 | 191 (27) | 28 (4) |
| Eastern Siberia    | A/H1N1pdm09         | 364 (49%)         | 342 (46%)   | 44 (6%)      | 750 | 157 (21) | 77 (10) |
| Western Siberia    | A/H1N1pdm09         | 531 (43%)         | 581 (47%)   | 129 (10%)    | 1241 | 226 (18) | 185 (15) |
| Ural and near regions | A/H1N1pdm09       | 263 (40%)         | 363 (56%)   | 27 (4%)      | 653 | 147 (23) | 88 (13) |
| European part of Russia | A/H1N1pdm09     | 183 (34%)         | 271 (50%)   | 89 (16%)     | 543 | 143 (26) | 23 (4) |
|                    | Total               | 1566 (40%)        | 1870 (48%)  | 452 (12%)    | 3888 | 864 (22%) | 401 (10%) |

\(^1\) A/California/07/09(H1N1) pdm09

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

| Material       | Sex | Age | Strain designation    | Subtype              | Region in Fig. | Date collected | Neuraminidase inhibition results | HA genetic group | GISAID EpiFlu isolate ID | GISAID EpiFlu/IA accession | GISAID EpiFlu NA accession |
|----------------|-----|-----|-----------------------|----------------------|----------------|----------------|--------------------------------|-----------------|--------------------------|-----------------------------|-----------------------------|
| autopsy        | f   | 11  | B/Yekaterinburg/155/2015 | B/Yamagata           | 11             | 22.04.2015     | Normal (Ose, Zan)                | Y3              | EPI_ISL_195723           | EPI643028                   | EPI643027                   |
| autopsy        | m   | 65  | A/KMAO/1/2015          | A(H1N1pdm09)         | 8              | 23.05.2015     | Normal (Ose, Zan)                | 6B              | EPI_ISL_195804           | EPI643259                   | EPI643259                   |
| nasopharyngeal | f   | 35  | A/Kurgan/149/2015      | A(H1N1pdm09)         | 9              | 01.04.2015     | Normal (Ose, Zan)                | 6B              | EPI_ISL_195800           | EPI643268                   | EPI643268                   |
| nasopharyngeal | m   | 62  | A/Buryatia/19/2015     | A(H1N1pdm09)         | 2              | 26.01.2015     | Normal (Ose, Zan)                | 5C              | EPI_ISL_197519           | EPI649668                   | EPI649668                   |
| nasopharyngeal | m   | 15  | A/Irkutsk/88/2015      | A(H1N1pdm09)         | 3              | 21.01.2015     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195710           | EPI649718                   | EPI649718                   |
| nasopharyngeal | m   | 37  | A/Krasnoyarsk/324/2015 | A(H1N1pdm09)         | 4              | 04.02.2015     | Normal (Ose, Zan)                | 4B              | EPI_ISL_195713           | EPI648884                   | EPI648884                   |
| nasopharyngeal | m   | 46  | A/Krasnoyarsk/365/2015 | A(H1N1pdm09)         | 4              | 09.02.2015     | Normal (Ose, Zan)                | 6B              | EPI_ISL_195804           | EPI643259                   | EPI643259                   |
| nasopharyngeal | f   | 46  | A/Novosibirsk/122/2015 | A(H1N1pdm09)         | 6              | 09.02.2015     | Normal (Ose, Zan)                | 6B              | EPI_ISL_195800           | EPI643268                   | EPI643268                   |
| nasopharyngeal | m   | 78  | A/Novosibirsk/64/2015  | A(H1N1pdm09)         | 6              | 27.02.2015     | Normal (Ose, Zan)                | 6B              | EPI_ISL_195800           | EPI643268                   | EPI643268                   |
| autopsy        | f   | 7   | A/Omsk/160/2015        | A(H1N1pdm09)         | 7              | 29.12.2014     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195710           | EPI649668                   | EPI649668                   |
| nasopharyngeal | m   | 16  | A/Omsk/141/2015        | A(H1N1pdm09)         | 7              | 26.01.2015     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195804           | EPI643268                   | EPI643268                   |
| nasopharyngeal | m   | 36  | A/Chelyabinsk/192/2015 | A(H1N1pdm09)         | 10             | 20.02.2015     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195804           | EPI643268                   | EPI643268                   |
| nasopharyngeal | f   | 80  | A/Yekaterinburg/239/2015 | A(H1N1pdm09)         | 11             | 05.03.2015     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195804           | EPI643268                   | EPI643268                   |
| nasopharyngeal | m   | 82  | A/Komi/99/2015         | A(H1N1pdm09)         | 12             | 27.01.2015     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195804           | EPI643268                   | EPI643268                   |
| autopsy        | f   | 82  | A/Chelyabinsk/160/2015 | A(H1N1pdm09)         | 12             | 27.01.2015     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195804           | EPI643268                   | EPI643268                   |
| nasopharyngeal | m   | 82  | A/Chelyabinsk/192/2015 | A(H1N1pdm09)         | 12             | 27.01.2015     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195804           | EPI643268                   | EPI643268                   |
| nasopharyngeal | f   | 7   | A/Nizhny Novgorod/788/2015 | A(H1N1pdm09)         | 13             | 26.01.2015     | Normal (Ose, Zan)                | 3C              | EPI_ISL_195804           | EPI643268                   | EPI643268                   |

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. Hemagglutination inhibition test of influenza A(H1N1pdm09) viruses

| Reference ferret antisera | Reference antigens CA/07 | BA/2021 | FL/62 | SA/3626 |
|--------------------------|--------------------------|---------|-------|---------|
| CA/07                    | 1280                     | 640     | 1280  | 2560    |
| A/Bangladesh/2011/2012   | 320                      | 1280    | 320   | 640     |
| A/Florida/62/2014        | 1280                     | 640     | 1280  | 2560    |
| A/South Africa/3626/2013 | 1280                     | 640     | 1280  | 2560    |

Table 4. Hemagglutination inhibition test of influenza H3 viruses

| Reference ferret antisera | Reference antigens TX/50 | HK/4801 | SZ/9715293 |
|--------------------------|--------------------------|---------|------------|
| CA/07                    | 1280                     | 320     | 320        |
| A/Hong Kong/4801/2014    | 80                       | 160     | 40         |
| A/Switzerland/9715293/2013 | 160                    | 160     | 160        |

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

| Reference ferret antisera | Reference antigens MA/02/2012 | PHU/3073 |
|--------------------------|------------------------------|----------|
| CA/07                    | 1280                         | 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.
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(H1N1)pdm were <4% (Rospotrebnadzor, 2015). These data can be easily explained with regard to previous studies on seasonal H3N2 subgroups (WHO, 2015). 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 the 2015–2016 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 A198S and N312S. Subgroup 3B viruses also have amino acid changes of N144S 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(H1N1)pdm09 viruses have continued to circulate worldwide since their emergence in 2009. In 2014–2015, A(H1N1)pdm09 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 V234I 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 cocirculate, 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 0001360 (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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