Infection, Genetics and Evolution 20 (2013) 177–187

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Monitoring of influenza viruses in Western Siberia in 2008–2012

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ARTICLE INFO

Article history:
Received 16 July 2013
Received in revised form 23 August 2013
Accepted 27 August 2013
Available online 5 September 2013

Keywords:
Human anti-H5 antibodies
Influenza virus
Molecular epidemiologic monitoring
Western Siberia

ABSTRACT

Western Siberia is of great importance in ecology and epidemiology of influenza. This territory is nesting area for great amount of bird species. Territorial relations of Western Siberian birds that are established during seasonal migration are extremely wide since this region is an intersection point of bird migration flows wintering in different regions of the world: Europe, Africa, Middle East, Central Asia, Hindustan, and South East Asia. Reassortant influenza viruses that can cause outbreak among population may emerge in Western Siberia with high probability. Thus, it is extremely important to carry out widespread study of circulated viruses, their molecular biological properties, phylogenetic links in this region, as well as herd immunity to influenza virus serotypes with epidemic potential.

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1. Introduction

Western Siberia is of great importance in ecology and epidemiology of influenza. The South of this region (the Ob and Irtysh river valleys, Ob–Irtysh interfluves) is replete with rivers and lakes that lie in migration paths of many bird species. Thus, this territory is nesting area for great amount of bird species ecologically connected with water. Forest steppe of Western Siberia represents both huge "incubator" where millions of nestlings are brought out in nesting periods, and vast "station" where even more amount of birds stop during their migration and nest in boreal coniferous forests and Arctic prairie. Territorial relations of Western Siberian birds that are established during seasonal migration are extremely wide since this region is an intersection point of bird migration flows wintering in different regions of the world: Europe, Africa, Middle East, Central Asia, Hindustan, and South East Asia. Furthermore, the South of Western Siberia is a region with extended infrastructure and relatively high (for Siberia) population density. Therefore, there is high probability of emergence of reassortant strains between human and animal influenza viruses, as well as emergence of local outbreaks of human morbidity caused by uncommon variants of influenza viruses.

In view of the above facts, it seems obvious that it is necessary to carry out widespread study of circulating viruses, their molecular biological properties, and phylogenetic links in Western Siberia.

We have studied epizootology and ecology of avian influenza viruses since 2002. We showed large variety of influenza A viruses among different wild bird species (Marchenko et al., 2012; Sharshov et al., 2010; Sivay et al., 2013, 2012). It was revealed that during 10 years influenza A/H3N8 and A/H4N6 virus subtypes prevail among birds (Marchenko et al., 2012; Sivay et al., 2012). Genome reassortation was detected in a number of influenza viruses (Marchenko et al., 2012; Sivay et al., 2013). Interestingly, we isolated in Western Siberia reassortant influenza H15N4 virus subtype, previously having isolated only in the Southern hemisphere (Marchenko et al., 2012). Additionally, we showed that several Western Siberian lakes are of key importance in highly pathogenic influenza A/H5N1 epizootology. Therefore research of circulating influenza virus strains and herd immunity in Western Siberia is of great importance, since it enables to detect nontypical virus variants that are likely to emerge in this region.

The objective of this work is to analyze results of molecular epidemiologic monitoring of influenza in human population of Western Siberia in 2008–2012.

2. Materials and methods

2.1. Biosafety

Work with influenza A/H1N1, A/H1N1pdm, and A/H3N2 was carried out in BSL-2 virological laboratories. All studies of influenza A/H5N1 viruses were conducted in BSL-3 lab. The use of clinical samples (nasopharyngeal swabs, autopsy material, blood sera)

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1567-1348/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.meegid.2013.08.025

was approved by Ethics Committee IRB 00001360 (protocol #2 d.d. 20.05.2008).

Nasopharyngeal swabs and autopsy material were collected in hospitals of Novosibirsk, Tomsk, Omsk, Krasnoyarsk, Barnaul, and in hospitals from several towns of Novosibirsk region: Dovolnoe, Zdvinsk, Karasuk (230, 280, and 400 km to the South–West of Novosibirsk, respectively), and Koltsovo (the nearest suburb). In total 1132 samples were collected. Ten percent of patients were vaccinated. Google map (Appendix A) demonstrates collection sites.

Virus was isolated from nasopharyngeal swabs collected from patients with provisional diagnosis of "influenza" or from autopsy material (10% homogenate in Hanks' solution). Homogenate or transport medium containing clinical material was centrifuged at 400*g* for 10 min and inoculated into culture plates with a monolayer MDCK cells. Virus reproduction was checked visually on the base of cytopathic effect in the hemagglutination of goose and human erythrocytes. All nasopharyngeal swabs and isolated influenza virus strains were tested in PCR.

Blood sera were collected in towns Dovolnoe, Zdvinsk, Karasuk, Kogalym, Novoagansk, Khanty–Mansiysk, and Rostov-on-Don. In total 2282 blood sera samples were collected.

Sera from participants were tested for antibodies to H5 virus by microneutralization assay (MN) and hemagglutinin inhibition test (HI). In test work we used A/Commongull/Chany/06 (HPAI H5N1) (clade 2.2) virus isolated in Western Siberia from dead Common gull – European and Asian subspecies of *Larus canus* (Sharshov et al., 2010).

Sera were also tested for antibodies to human influenza A viruses of the H1 and H3 subtypes for control purposes (data not shown). Ferret antisera raised against homologous viruses were used as positive control sera for the assays. Human antisera were tested at a starting dilution of 1:10.

2.2. Hemagglutination-inhibition test

Presence of antibodies to influenza A/H5N1 in blood sera was detected according to WHO recommendations in HI test with horse erythrocytes as described in (Rowe et al., 1999). Before study all tested sera were treated with RDE (Denka Seiken, Tokyo, Japan) that destroys non-specific inhibitors. After that twofold sera dilutions were mixed with four HAU of inactivated by β-propiolactone virus (Rowe et al., 1999) and incubated for 60 min at room temperature. Then equal amount of one percent erythrocytes suspension was added in each well of the plate. Sera were considered positive if the antibody titer was equal to or greater than 40.

2.3. Microneutralization test

Sera were analyzed in microneutralization assay as described in (Rowe et al., 1999). Twofold sera dilutions previously heated for 30 min at 56 °C were mixed with 100 TCID50/100 μl of virus, kept for 60 min at 37 °C in 5% CO_2 atmosphere. After that we added 1.5×10^4 MDCK cells and incubated for 18–20 h. Then cells were fixed; presence of virus antigen in cells was detected using ELISA. Mice anti-NP monoclonal antibodies (CDC) were used as the first antibodies, goat antimouse IgG conjugated by horseradish peroxidase (Sigma) – as the second ones. Sera were considered positive if the antibody titer was equal to or greater than 80.

Influenza virus RNA isolation was carried out with the kit PROMEGA SV Total RNA Isolation System (Promega Corporation, Madison, WI, USA) in compliance with the manufacturer's recommendations.

To type and subtype influenza virus strains we used a set of reagents for the detection of influenza A and influenza B virus RNA in clinical materials using PCR with hybridization-fluorescence detection AmpliSens® Influenza virus A/B-FL and the kit for typing (subtype identification of H1N1 and H3N2) of influenza A viruses AmpliSens® Influenza virus A-type-FL made by Central research institute of epidemiology of the Ministry of health of Russia (Moscow, Russia).

To produce cDNA from influenza virus RNA matrix reverse transcription was carried out with the kit Fermentas RevertAid (Fermentas International Inc., Vilnius, Lithuania).

To amplify certain gene segments encoding influenza virus surface glycoproteins PCR with gene-specific primers was used (Ghedin et al., 2005). We used the following primer sequences:

Primers for amplification of HA gene (segment 4) of influenza A/H1N1pdm09	
SW-HA-F1	TGTAAAACGACGGCCAGTATACGACTAGCAAAAGCAGGGG
SW-HA-F351	TGTAAAACGACGGCCAGTACRTGTTACCCWGGRGATTTCA
SW-HA-F736	TGTAAAACGACGGCCAGTAGRATGRACTATTACTGGAC
SW-HA-R943	CAGGAAACAGCTATGACCGAAAKGGGAGRCTGGTGTTTA
SW-HA-R1204	CAGGAAACAGCTATGACCTCTTTACCYACTRCTGTGAA
SW-HA-R1340	CAGGAAACAGCTATGACCTTCTKCATTRTAWGTCCAAA
SW-HA-R1541	CAGGAAACAGCTATGACCTCATAAGTYCCATTTYTGA
SW-HA-R1778	CAGGAAACAGCTATGACCGTGTCAGTAGAAACAAGGGTGTTT
Primers for amplification of NA gene (segment 6) of influenza A/H1N1pdm09	
SW-NA-F0	TGTAAAACGACGGCCAGTAGCAAAAGCAGGAGT
SW-NA-F318	TGTAAAACGACGGCCAGTTACACAAAAGACAAYAGC
SW-NA-F536	TGTAAAACGACGGCCAGTGGTCAGCAAGCGCATGYCATG A
SW-NA-F941	TGTAAAACGACGGCCAGTTAGGATACATCTGCAGTGG
SW-NA-R740	CAGGAAACAGCTATGACCGGRCCATCGGTCATTATG
SW-NA-R1063	CAGGAAACAGCTATGACCCATATYTGTATGAAAACC
SW-NA-R1346	CAGGAAACAGCTATGACCGCTGCTYCCRCTAGTCCAGAT
SW-NA-R1452	CAGGAAACAGCTATGACCAGTAGAAACAAGGAG
Primers for amplification of HA gene (segment 4) of influenza A/H3N2	
HA_F_1BM13	TGTAAAACGACGGCCAGTAGCARAAGCAGGGGA
HA_R_589M13	CAGGAAACAGCTATGACCTTGTTTGGCATRGTCACGTTC
HA_F_453BM13	TGTAAAACGACGGCCAGTTTCRAYTGGRCTGGRGTCRC
HA_R_975M13	CAGGAAACAGCTATGACCTTTTGAAADGGYTTGTCATTGG

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