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RESEARCH NOTE

(Re)emergence of A(H1N1)pdm09 influenza viruses with pandemic markers in the 2018/2019 flu season in the USA

[version 1; peer review: 2 approved with reservations]

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Abstract

During the 2009 pandemic, the Centers for Disease and Control and Prevention (CDC) estimated that 43 to 89 million cases of swine flu were reported during a 1-year span, with 1799 deaths in 178 countries worldwide. Now, nine years later, A(H1N1)pdm09 influenza viruses, which caused this pandemic, have reemerged and become the dominant subtype for the current 2018/19 flu season in the USA. The *in silico* analysis of A(H1N1)pdm09 viruses isolated in USA in October and November 2018, performed using the electronic biology platform “wEB”, showed that these viruses carry previously identified pandemic markers suggesting their increased pandemic potential. Possible consequences of these findings are discussed.

Keywords

influenza virus, pandemic, swine flu, mutations, electronic biology

Open Peer Review**Approval Status** ? ?

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version 1	?	?
01 Feb 2019	view	view
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Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Paessler S: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; Veljkovic V: Conceptualization, Formal Analysis, Software, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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Introduction

Each flu season represents a serious public health threat and the challenge for health workers, vaccine producers and researchers due to unpredictable behavior of influenza A viruses. Vaccination is the most effective way to protect against seasonal influenza viruses. As a consequence of the high variability of influenza viruses, it is difficult to select vaccine candidates or predict the vaccine effectiveness (VE) for the upcoming season. Accordingly, the VE against the dominant A strain H3N2 in Australia in 2017 was very low (about 10%), and this country experienced record-breaking numbers of influenza-related hospitalizations and deaths (<http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-ozflu-2017.htm>). The World Health Organization (WHO) selected the same vaccine strains for the United States for the flu season 2017–2018 and based on data coming from Australia 6 months before the beginning of the flu season, a similarly low VE against the H3N2 viruses was anticipated. This prediction of the potentially low VE in the USA, which was made by leading vaccine experts¹, and aggressively promoted by press media, resulted in a very low vaccination rate in the USA. As a consequence, according to the CDC, 2017–2018 was the worst flu season on record, with an estimated 79,000 deaths, including total of 185 pediatric deaths. This number exceeds the previously highest number of flu-associated deaths in children reported during a regular flu season (171 during the 2012–2013 season). Approximately 80% of these deaths occurred in unvaccinated children.

Previous comparison of H3N2 viruses from 2017 from Australia with viruses collected during the pre-flu season 2017–2018 in the USA was performed using the electronic biology platform “wEB”, developed using methods described previously². Our analysis demonstrated significant differences between these two groups of viruses³ allowing us to correctly predict higher VE against H3N2 virus for the flu season 2017–2018 in US than in Australia³. This prediction was in contrast with the prediction of VE of 10% based on Australian data¹, and it was confirmed at the end of the flu season. CDC officials reported an overall VE of 36%, with VE of 25% against the H3N2 strain. The Armed Forces Health Surveillance Branch Air Force (AFHSB-AF) found that the vaccine provided substantially better protection to military dependents treated at the U.S. Air Force School of Aerospace Medicine, with an overall adjusted VE of 51% and a VE of 37% against H3N2. The Naval Health Research Center (NHRC) determined that the vaccine had even greater effectiveness among the civilians who received care for febrile respiratory illnesses, with an overall adjusted VE of 55% and VE of 52% against H3N2.

In contrast to the flu season in 2017–2018, which was dominated by H3N2 strains, the 2018–2019 flu season in the USA is heavily dominated by circulating influenza A(H1N1)pdm09 viruses between October 2018 and January 2019 (to access this data, visit <https://gis.cdc.gov/grasp/fluview/fluportaldashboard.html> and select Season 2018–19). This change in the dominant flu subtype was followed by an early beginning of the flu season

and increased hospitalization and death rates for children and healthy young people in the beginning of the flu season for 2018–2019.

Herein, we analyzed sequences of HA of viruses A(H1N1)pdm09 collected in the USA during months of October and November of 2018 using our previously described electronic biology platform (wEB). The most recent analysis revealed that some of the (re)emerging viruses contain mutations previously identified as pandemic markers⁴. Moreover, the percentage of the viruses belonging to this population is significantly higher in October–November 2018 than in all past nine years. We also demonstrate that most of A(H1N1)pdm09 viruses have changed informational properties previously proposed to correlate with their biological and immunological properties. Possible consequences of these findings are discussed.

Methods

Viruses

We analyzed the hemagglutinin subunit 1 (HA1) of (i) 33197 A(H1N1)pdm09 viruses collected between May 2009 and December 2017, (ii) 224 human pH1N1 viruses collected in US from October to November 2018 (GISAID database) and 28 human pH1N1 viruses collected in Canada during the same period and (iii) vaccine virus A/Michigan/45/2015 for 2018/2019 flu season. All sequences were stored in the publicly open database GISAID.

Informational spectrum method (ISM)

The ISM is a virtual spectroscopy method for the study of the long-range protein-protein interaction. According to this method, described in detail elsewhere², sequences (protein or DNA) are transformed into signals by assignment of numerical values of each element (amino acid or nucleotide). These values correspond to electron-ion interaction potentials, determining the electronic properties of amino acid/nucleotides for their inter-molecular interactions. The signal obtained is then decomposed in periodical function by Fourier transformation. The result is a series of frequencies and their amplitudes. The obtained frequencies correspond to the distribution of structural motifs with defined physico-chemical characteristics responsible for biological function of the sequence. When compared, proteins that share the same biological or biochemical function(s) possess the code/frequency pairs specific for their common biological properties. The method is insensitive to the location of the motifs and does not require previous alignment of the sequence.

The ISM served as a base for development of the phylogenetic algorithm for the Informational Spectrum-based Phylogenetic Analysis (ISTREE)⁵.

Phylogenetic analysis

The phylogenetic tree of the HA1 influenza proteins is generated with the ISM-based phylogenetic algorithm ISTREE, previously described in detail elsewhere⁵. In the presented analysis,

we calculated the distance matrix with the distance measure between sequences X_1 and X_2 defined as:

$$d_1(X_1, X_2) = \left| \frac{A_1(F_1)}{A_1(F_2)} - \frac{A_2(F_1)}{A_2(F_2)} \right|$$

where $A_1(F_1)$ and $A_2(F_1)$ are amplitudes on frequency $F_1=0.295$; $A_1(F_2)$ and $A_2(F_2)$ are amplitudes on frequency $F_2=0.055$ in informational spectra on sequences X_1 and X_2 respectively.

Results

Previously, we showed that the IS frequency $F(0.055)$ of HA1 is responsible for interaction between H1N1/N2 and swine protein(s)/receptor(s) while the IS frequency $F(0.295)$ for the same HA subunit is responsible for the interaction with human

protein(s)/receptor(s)⁴. This suggests that the acquisition of mutations in HA1, leading to increased ratio of these amplitudes at these two frequencies $A(0.29536)/A(0.055)$, are essential for adaptation of swine A/H1N1 viruses to humans, and this is currently used for real-time monitoring of A/H1N1 viruses. It has been also suggested that positions 94, 196 and 274 in HA1 of A(H1N1)pdm09 are hotspots for advantageous mutations for human adaptation of A(H1N1)pdm09⁴. In **Figure 1** and **Table 1** we present the fraction of A(H1N1)pdm09 viruses with mutations in these hotspots that were collected between May 2009 and December 2017, and viruses isolated in between October and November 2018 (**this period corresponds to the beginning of the flu season in the USA**). Of note is that all of these mutations in 2018 appeared in A(H1N1)pdm09 viruses collected in October and November of 2018 in the USA.

Figure 1. Distribution by years of A(H1N1)pdm09 viruses with pandemic markers collected between 2009 and 2018.

Table 1. Distribution of A(H1N1)pdm09 viruses with pandemic markers by years.

Year	Total number of viruses	Viruses with markers	[%]
2009/2010	11524	177	1.53
2011	1958	7	0.35
2012	990	4	0.40
2013	2305	14	0.61
2014	1964	15	0.76
2015	3267	33	1.04
2016	7159	77	1.08
2017*	3464	42	1.21
2018 (Oct/Nov)	566	9	1.59
2018 (Oct/Nov) USA	244	9	3.69

*28 redundant HA1 with mutation D274N from viruses collected in Hungary are given as one representative sequence

In **Figure 2a** we present the consensus informational spectrum (CIS) of A(H1N1)pdm09 viruses isolated in the period of September–November 2018 in the USA. The dominant peak in this CIS corresponds to the frequency $F(0.281)$. Viruses collected in the same period in Canada are characterized with the IS frequency $F(0.295)$ (**Figure 2b**), which was previously identified as the hallmark of pandemic A(H1N1)pdm09⁴.

Previously, we showed that the amplitude on characteristic frequencies in IS of HA1 from influenza A viruses is indicative of the vaccine efficacy against these viruses^{3,6}. Accordingly, in **Figure 3** we present the ISM-based phylogenetic tree of 224 A(H1N1)pdm09 viruses isolated in October and November of 2018 in the USA and the vaccine virus A/Michigan/45/2015. As presented, 101 (45 %) viruses are co-clustered with the vaccine virus suggesting that the current vaccine can at least efficiently protect against this fraction of analyzed viruses.

Discussion

We previously showed that the ratio of amplitudes on the characteristic frequencies in IS of HA1 of influenza viruses determines adaptation of animal viruses to human^{7,8}. *In silico* analysis of pandemic A(H1N1)pdm09 viruses revealed positions 94, 196 and 274 in HA1 as hotspots for mutations, which could increase infectivity of these viruses⁴. At the very beginning of the last pandemic, when this analysis was performed (May 2009), only three viruses from three countries (Spain, Italy and USA) in the GISAID database contained the mutations in these hot-spots. At the end of the pandemic (late 2010) sequences of 350 viruses with these mutations were deposited in GISAID. This suggests better adaptation of some viruses to humans during pandemic. The fraction of the viruses with these pandemic markers sharply decreased at the end of the pandemic in 2011. However, this viral population continued to slowly grow until 2017 while reaching the same end-pandemic level of 2010 in October and November

of 2018 (**Figure 1** and **Table 1**). It is also important to note that all viruses with pandemic markers isolated in October and November of 2018 are from the USA only, and that the fraction of these viruses is significantly higher than at the end of the 2009 pandemic (**Table 1**). This is a warning sign that the pandemic potential of A(H1N1)pdm09 viruses in the USA has increased. Of note also is that 7 of 9 of these viruses are isolated in patients 5 to 13 years old, suggesting that children could be more susceptible to infection with these viruses than adults.

Each subtype of influenza A viruses is characterized with specific frequency⁹. Results presented in **Figure 2** show that HA1 from US viruses collected at the beginning of the 2018/2019 flu season are characterized with the IS frequency $F(0.281)$. On the contrary, all other A(H1N1)pdm09 viruses isolated in the last 10 years are characterized with IS frequency $F(0.295)$. This strongly suggests possible changes in the interaction profile of these viruses with host proteins. For now, it is not possible to predict how this change will influence pathological and immunological properties of A(H1N1)pdm09 virus.

Results presented in **Figure 3** demonstrated that 101 of 224 (45%) A(H1N1)pdm09 viruses isolated in October and November 2018 in the USA are clustered in the ISM-based phylogenetic tree together with the current vaccine virus A/Michigan/45/2015. This suggests that the efficacy of the current seasonal flu vaccine could be about 50%, which corresponds to common protection against A(H1N1)pdm09 viruses in the last years. The problem could arise if viruses which are not compatible with the vaccine take over during the flu season. Of great concern are viruses with very high amplitude ratio $A(0.29536)/A(0.055)$ that also carry pandemic markers (A/New Mexico/25/2018, A/California/70/2018, A/Texas/134/2018). In our opinion these viruses have increased pandemic potential and could represent precursors of the new pandemic virus.

Figure 2. The consensus informational spectrum of A(H1N1)pdm09 viruses with pandemic markers which are collected in October and November 2018 in (a) the USA and (b) Canada.

Figure 3. The schematic presentation of the informational spectrum method-based phylogenetic tree of the HA1 from human A(H1N1)pdm09 influenza viruses collected in the USA from October to November 2018.

In conclusion, presented results show: (i) that some pre-seasonal and seasonal A(H1N1)pdm09 viruses collected in October and November 2018 in the USA carry pandemic markers, indicating possibility of their evolution toward new pandemic viruses; (ii) that these US viruses have changed informational properties which determine their interacting profiles with the host, and; (iii) that about 50% of circulating viruses could escape the current flu vaccine and could evolve toward new pandemic viruses. Taking into account presented results, as well as the fact that A(H1N1)pdm09 viruses are dominant in the flu season 2018/2019, causing already high hospitalization and mortality rates in children and young healthy people, further monitoring of evolution of these viruses is urgently needed.

Data availability

Sequence data of the viruses were obtained from the [GISAID EpiFlu™ Database](#). To access the database each individual user should complete the “[Registration Form For Individual Users](#)”, which is available alongside detailed instructions. After submission of the Registration form, the user will receive a password. There are not any other restrictions for the access to GISAID. Conditions of access to, and use of, the GISAID EpiFlu™ Database and Data are defined by the [Terms of Use](#).

Grant information

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References

1. Paules CI, Sullivan SG, Subbarao K, *et al.*: **Chasing Seasonal Influenza - The Need for a Universal Influenza Vaccine**. *N Eng J Med*. 2018; 378(1): 7–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
2. Veljkovic V, Paessler S: **Possible repurposing of seasonal influenza vaccine for prevention of Zika virus infection [version 2; referees: 2 approved]**. *F1000Res*. 2016; 5: 190.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Paessler S, Veljkovic V: **Prediction of influenza vaccine effectiveness for the influenza season 2017/18 in the US [version 1; referees: 2 approved]**. *F1000Res*. 2017; 6: 2067.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
4. Veljkovic V, Niman HL, Glisic S, *et al.*: **Identification of hemagglutinin structural domain and polymorphisms which may modulate swine H1N1 interactions with human receptor**. *BMC Struct Biol*. 2009; 9: 62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. Perovic V: **Novel algorithm for phylogenetic analysis of proteins: application to analysis of the evolution of H5N1 influenza viruses**. *J Mathem Chem*. 2013; 51(8): 2238–2255.
[Publisher Full Text](#)
6. Paessler S, Veljkovic V: **Using electronic biology based platform to predict flu vaccine efficacy for 2018/2019 [version 2; referees: 2 approved, 1 approved with reservations]**. *F1000Res*. 2018; 7: 298.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Perovic VR, Muller CP, Niman HL, *et al.*: **Novel phylogenetic algorithm to monitor human tropism in Egyptian H5N1-HPAIV reveals evolution toward efficient human-to-human transmission**. *PLoS One*. 2013; 8(4): e61572.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Schmier S, Mostafa A, Haarmann T, *et al.*: **In Silico Prediction and Experimental Confirmation of HA Residues Conferring Enhanced Human Receptor Specificity of H5N1 Influenza A Viruses**. *Sci Rep*. 2015; 5: 11434.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Veljkovic V, Veljkovic N, Muller CP, *et al.*: **Characterization of conserved properties of hemagglutinin of H5N1 and human influenza viruses: possible consequences for therapy and infection control**. *BMC Struct Biol*. 2009; 9: 21.
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Informational spectrum method (ISM) was used for analyzing the HA sequences of the influenza A/H1N1 viruses. The ISM is a virtual spectroscopy method for the study of the long-range protein-protein interaction. 252 human pH1N1 viruses collected in US and Canada from October to November 2018 were compared with 33197 H1N1 pdm09 viruses of 2009-2017 years and with vaccine virus A/Michigan/45/2015 for 2018/2019 flu season. Based on this analysis, it was concluded that: 45% of analyzed viruses of 2018 are co-circulated with the vaccine virus A/Michigan/45/2015 for 2018/2019 flu season suggesting that this vaccine can at least efficiently protect against this fraction of circulated viruses. It has been also suggested that these viruses carry previously identified pandemic markers (namely substitution in positions 94, 196 and 274 in HA1 suggesting their increased pandemic potential. The authors declare that the acquisition of these mutations in HA1 are essential for adaptation of swine A/H1N1 viruses to humans, by virtue of the interaction with human protein(s)/receptor(s).

The main question that can be presented to the authors is the advantage of the Informational spectrum method in comparison with the generally accepted analysis of genomic sequences. The subdivision of circulating viruses into subpopulations is detected by building evolutionary trees. To predict the effectiveness of the vaccine, you can compare the sequence of circulating viruses with the sequence of the vaccine strain in the field of antigenic determinants.

Identification of mutations that are markers of pandemic viruses is more reliably carried out by direct analysis of sequences, than by changing the averaged characteristics. Moreover, the statement that positions 94, 196 and 274 are pandemic markers is based only on the previous work of the authors of this article, and was not confirmed by other researchers.

Even more doubtful is the claim that the positions 94, 196 and 274 are responsible for the interaction with the human receptor. The problem of the interaction of influenza viruses with the

host receptors has been well studied. Are known the amino acids that are located in the receptor-binding site of HA and form hydrogen bonds with specific atoms of the receptor molecule. The authors do not quote these works, and do not seem to know them.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: influenza viruses, receptors, host restriction.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 04 Apr 2019

Veljko Veljkovic, Biomed Protection, Galveston, USA

Interaction between virus and the host involves two steps: (i) recognition and targeting between virus proteins and receptor (long-range interactions - distances >5Å) and (ii) chemical binding between virus and receptor (short-range interactions - distances <5Å). The ISM allows analysis of the first step. Mutations, which increase amplitudes on the frequencies responsible for long-range interaction between virus and receptor, increase efficacy of the virus-host interaction, and from this point of view increase pandemic potential of influenza viruses. This concept has been experimentally proven (see ref. 8). Based on this concept mutations in positions 94, 196 and 274 are characterized as potential pandemic markers (see refs. 4 and 7) because they increase the likelihood of "finding the receptor".

All remarks from this Referee concern the second step of interaction (chemical binding) between virus and receptor and for this reason are not relevant for results presented in this

article (see previous comment).

Competing Interests: No competing interests were disclosed.

Reviewer Report 03 April 2019

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Guadalupe Ayora-Talavera

Laboratorio de Virología, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autonoma de Yucatan, Mérida, Mexico

The authors described the analysis of HA sequences from H1N1pdm09 viruses reported since 2009 to the last influenza season 2018-2019 in the USA. The analysis was performed using the electronic biology platform "wEB" and the ISM method. The aim of the study was to determine pandemic markers that could favor the (re)emergence of the H1N1pdm09 virus. They emphasize the role of three hotspots as predictors of pandemic potential according to the analysis performed.

The authors refer to residues 94, 196 and 274. They mention in figure 1 and table 1 the proportion of viruses that have mutated in these positions. It would be good to complement table 1 with the amino acid changes that have occurred in these hotspots.

When the authors say "that the pandemic potential of A(H1N1)pdm09 viruses in the USA has increased" what do they mean? They mention three viruses with pandemic markers, the same as 94, 196 and 274? Or different? On what evidence they base the assumption that these viruses could represent precursors of new pandemic viruses?

Minor comments:

The authors should mention what HA numbering are using, the H1 or H3

I recommend being cautious about the terms used as "(re)emergence" or "precursor of a new pandemic". It is desirable always to include any biological data available to reinforce any conclusion that could have an impact.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Influenza

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 04 Apr 2019

Veljko Veljkovic, Biomed Protection, Galveston, USA

Interaction between virus and the host involves two steps: (i) recognition and targeting between virus proteins and receptor (long-range interactions - distances $>5\text{\AA}$) and (ii) chemical binding between virus and receptor (short-range interactions - distances $<5\text{\AA}$). The ISM allows analysis of the first step. Mutations, which increase amplitudes on the frequencies

responsible for long-range interaction between virus and receptor, increase efficacy of the virus-host interaction, and from this point of view increase pandemic potential of influenza viruses. This concept has been experimentally proven (see ref. 8). Based on this concept mutations in positions 94, 196 and 274 are characterized as potential pandemic markers (see refs. 4 and 7) because they increase the likelihood of "finding the receptor".

Competing Interests: No competing interests were disclosed.

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