Local Spread of Influenza A (H1N1) Viruses Without A Mutation For The Maximum Duration of An Epidemic Season In Japan

Seiji Kageyama (✉ skageyama@tottori-u.ac.jp)

Tottori University <https://orcid.org/0000-0002-5220-6137>

Akeno Tsuneki-Tokunaga

Tottori University: Tottori Daigaku

Kyosuke Kanai

Tottori University: Tottori Daigaku

Research Article

Keywords: hemagglutinin, epidemic, influenza, transmission

Posted Date: August 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-817416/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Close observation of the local transmission of influenza A(H1N1) viruses enabled an estimation of the transmission period of the virus without a mutation. Of 4,448 isolates from 11 consecutive years, 237 isolates could be categorized into 57 strain groups with identical hemagglutinin genes. Transmission of these 57 strains was chased for the maximum duration of an epidemic season. In addition, 35 identical strains were recognized at the study site and other countries within 147 days. Consequently, it can be postulated that once an influenza virus enters a temperate country, the strain rarely mutates until the end of the season.

Main Text

The Global Burden of Disease Study estimated that influenza-related lower respiratory tract infections (LRTIs) were responsible for an estimated 145,000 deaths among all ages in 2017. The influenza LRTI mortality rate was highest among adults older than 70 years. This study estimated that influenza LRTIs accounted for 9,459,000 hospitalizations due to LRTIs and 81,536,000 hospital days, and that 11.5% of LRTI episodes were attributable to influenza. This study also showed the substantial annual effect of influenza on global health and advised that vaccine use should be considered [5].

Influenza viruses are capable of evading antibody-mediated immunity induced during previous infections or vaccinations by gradually accumulating mutations in hemagglutinin and neuraminidase segments. This process, termed antigenic drift, necessitates frequent updates of influenza vaccines to ensure sufficient antigenic relatedness between the vaccine and emerging virus variants [1]. Frequent updates should also be done to reduce the risk drug resistance. For example, the emergence of oseltamivir resistance has become of particular concern for influenza A H1N1 viruses because it has happened in the past; in 2007, resistant influenza A H1N1 viruses began to circulate and quickly became dominant by the 2008–2009 season [6].

An analysis characterized 100 countries with one influenza peak season (85%), 13 with two influenza peak seasons (11%), and five with year-round influenza activity (4%) [10]. Many tropical populations seem to have significant influenza activity year-round in contrast to temperate regions. Therefore, the question of whether influenza persists in temperate countries all year long or it is reintroduced from the tropics each year at the beginning of winter is important in relation to vaccine composition and influenza control in the tropics as well as temperate countries. The influenza vaccination strategy may have to be tailored to each individual tropical region, based on local prevalence, timing, cycling of influenza strains, and how they match the strains circulating in temperate areas [17]. It has been hypothesized that new lineages are seeded from a persistent influenza reservoir in the tropics to sink populations in temperate regions [15]. Studies on the global dynamics of A/H3N2 viruses provided further evidence of local extinction of viruses between epidemics and identified East and Southeast (E-SE) Asia as the global source of A/H3N2 [2].

We carried out a 3-year study previously, which isolated 288 strains, successfully traced 38 groups of strains through frequent isolations, and found a strain transmitted without a mutation for a maximum duration of 94 days. Two strains were found to be identical to those isolated in Mongolia and Thailand in the same epidemic season [20]. The present study enlarged the previous study scale and continued over an additional 8 years and tried to estimate the length of viral spread without mutations.

After written informed consent was obtained, nasal swabs and/or aspirated nasal discharges were collected from influenza virus antigen-positive and patients with suspected influenza living in Tottori Prefecture, Japan, during 11 consecutive winters from 2009 to 2020. Each swab or aspirate was kept in 3 ml of virus transportation medium (2% Difco™ Veal Infusion Broth, 0.4% gelatin, 0.06% bovine serum albumin, 200 unit/ml penicillin G, and 100 µg/ml streptomycin in Dulbecco's modified Eagle's medium) at 4°C prior to virus isolation. The Institutional Review Board of Tottori University Faculty of Medicine approved this study protocol (No. 1981).

The procedure for influenza virus isolation was based on the manual issued by the World Health Organization, with slight modifications [18]. Briefly, an aliquot (500 µl) of diluted sample in transportation medium was inoculated into a Madin-Darby canine kidney (MDCK) cell line for 1 hour. The culture was maintained in Dulbecco's modified Eagle's medium supplemented with 5 µg/ml trypsin (Difco™ Trypsin 250, Becton Dickinson, Tokyo, Japan), 0.2% heat-inactivated bovine serum albumin, 4 mM L-glutamine, 200 units/ml penicillin G, and 100 µg/ml streptomycin at 34°C in a 5% CO₂/95% air atmosphere until the recognition of cytopathic effects. Culture medium was kept frozen at -80°C as a virus stock prior to further studies.

Total RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Tokyo, Japan). Eight RNA segments of influenza A virus were simultaneously reverse-transcribed and amplified using a SuperScript™III One Step RT-Polymerase Chain Reaction (PCR) System (Life Technologies, Tokyo, Japan) with the MBTuni-12/MBTuni-13 primer pair [21]. The first round PCR products were subjected to second PCR in order to amplify A(H1N1) (266 bases) for sub-typing [9].

The nucleotide sequences of the amplified hemagglutinin fragments were determined using a BigDye® Terminators v3.1 Cycle Sequence kit in accordance with the manufacturer's instructions (Life Technologies). M13 and PCR primers were used for the sequencing reaction. Nucleotide sequences except primer regions (1,701 bases of A(H1N1)) were aligned with those retrieved from the International Nucleotide Sequence Database. Inspection and manual modification and evolutionary analyses were conducted in Molecular Evolutionary Genetics Analysis Version X (MEGA X). A phylogenetic tree was constructed using the neighbor-joining method (1,000 bootstrap replications) in MEGA X. An estimation of the mean evolutionary diversity was also conducted using MEGA X [7].

The sequences described in this study have been deposited in the International Nucleotide Sequence Database under accession numbers: LC637996 to LC638404.

Isolation of influenza A(H1N1) viruses was carried out for 11 consecutive seasons in the study area, Tottori prefecture, Japan, and plotted out in a phylogenetic tree. A/South Carolina/1/1918(H1N1) was used as an out-group. An A(H1N1) tree was composed of 409 strains. Each cluster of influenza A (H1N1) viruses spread annually is shown in shaded squares. Each cluster did not overlap in phylogenetic tree although the epidemic source was unknown (Fig. 1).

Sequential isolations of epidemic influenza viruses were carried out during epidemic seasons in Tottori Prefecture from 2009 to 2020 in addition to the previous study (2009-2011, 288 isolates). The number of isolates was 4,448. Close observation of influenza A viruses from patients admitted at two clinics and a hospital in the study region enabled the identification of 57 transmission chains of influenza A viruses with 100% homology in the coding region of the hemagglutinin segment (1.7 kb). The transmission chains could be observed for up to 94 days (Fig. 2).

Nucleotide sequences of influenza A (H1N1) virus hemagglutinin (1.7 kb) were compared with those in the International Nucleotide Sequence Databases. Strains showing 100% homology were found in the USA (n=28), Canada (n=2), Thailand (n=2), Mongolia (n=1), Taiwan (n=1), and Italy (n=1). The longest time gap was 147 days between the time of a virus isolation in the present study and that in another country (Table 1).

As described above, each cluster of influenza A (H1N1) viruses spread annually did not overlap in the phylogenetic tree although the epidemic source is unknown. Of the 4,448 isolates in 11 consecutive years (2009-2020), 237 were categorized into 57 strain groups harboring identical hemagglutinin genes in the successful observation period from 1 to 94 days. Among the 57 strain groups, an identical strain was isolated 11 times and the total observation time was 94 days. The nucleotide sequences of some isolates in the study area were identical to those in USA and 5 other countries/areas after a long lag time (147 days for example).

It has been estimated that influenza A virus mutates at 2×10^{-6} to 2×10^{-4} nucleotides per site per genome replication [3, 12-14, 19]. The value is equivalent to 2.6×10^{-3} mutations per site per year according to Nobusawa et al [11] and it takes about 83 days for the virus to mutate at one site. This value fits well with the observation of influenza A virus transmission without a mutation in the hemagglutinin gene for 94 days identified in local transmissions in the current study and 147 days between a strain in the current study and another in international database.

Influenza viruses disappeared in most areas in Japan after the epidemic season in winter and re-appeared as variants in the following season except for the southern part of the country [4, 16]. At the end of winter, the spread of influenza viruses may be terminated in Japan or transmit to the susceptible population in other countries around the globe [2, 8]. The stable transmission of influenza viruses in Japan and other some temperate countries suggests that antigenic drift of influenza viruses is less likely to occur within an influenza epidemic season in temperate countries. However, the source of these variants is unknown. A seasonal influenza epidemic in a temperate country may occur with the introduction of

variants originating in variant-prevalent areas or as the result of global circulation. Therefore, it is indispensable to compare the difference in the nucleotide sequences of epidemic strains in a temperate country and a tropical country annually.

Of the 57 influenza A viruses repeatedly isolated that harbored identical hemagglutinin genes, one of these strains was recognized at the local study area in Japan 11 times in 94 days. Thereby, it can be postulated that once an influenza virus enters a temperate country, it rarely mutates until the end of the epidemic season. The long-term stable transmission of influenza A viruses may have an advantage in identifying the possible epidemic strains at a timepoint further in advance of the following epidemic season in a temperate country.

Declarations

Acknowledgments

The authors are grateful to the patients who agreed to participate in this study.

Funding

This work was supported in part by JSPS KAKENHI (Grant Numbers: 15K19228 and 18K17353), a Grant-in-Aid for Environmental Health Research from Tottori prefecture, and a Grant-in-Aid for Scientific Research on Infection Control and Prevention by the International Platform for Dryland Research and Education, Tottori University.

Compliance with ethical standards

Conflict of interest

The authors report no declarations of interest.

Ethical approval

The present study was conducted with the approval and under the control of the Institutional Review Board of the Faculty of Medicine, Tottori University, Japan (No. 1981). All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Sample collection was conducted after obtaining informed consent from patients in collaborating medical facilities consisting of a hospital and three clinics. Samples were then shipped to the laboratory without patient identification.

References

1. Ampofo WK, Baylor N, Cobey S, Cox NJ, Daves S, Edwards S, Ferguson N, Grohmann G, Hay A, Katz J, Kullabutr K, Lambert L, Levandowski R, Mishra AC, Monto A, Siqueira M, Tashiro M, Waddell AL, Wairagkar N, Wood J, Zambon M, Zhang W (2012) Improving influenza vaccine virus selection: report of a WHO informal consultation held at WHO headquarters, Geneva, Switzerland, 14-16 June 2010. *Influenza Other Respir Viruses* 6:142-152, e141-145.
2. Bedford T, Riley S, Barr IG, Broor S, Chadha M, Cox NJ, Daniels RS, Gunasekaran CP, Hurt AC, Kelso A, Klimov A, Lewis NS, Li X, McCauley JW, Odagiri T, Potdar V, Rambaut A, Shu Y, Skepner E, Smith DJ, Suchard MA, Tashiro M, Wang D, Xu X, Lemey P, Russell CA (2015) Global circulation patterns of seasonal influenza viruses vary with antigenic drift. *Nature* 523:217-220.
3. Bloom JD (2014) An experimentally determined evolutionary model dramatically improves phylogenetic fit. *Mol Biol Evol* 31:1956-1978.
4. Cai J, Zhang B, Xu B, Chan KKY, Chowell G, Tian H, Xu B (2019) A maximum curvature method for estimating epidemic onset of seasonal influenza in Japan. *BMC Infect Dis* 19:181.
5. Collaborators GI (2019) Mortality, morbidity, and hospitalisations due to influenza lower respiratory tract infections, 2017: an analysis for the Global Burden of Disease Study 2017. *Lancet Respir Med* 7:69-89.
6. Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, Palese P, Shaw ML, Treanor J, Webster RG, García-Sastre A (2018) Influenza. *Nat Rev Dis Primers* 4:3.
7. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol* 35:1547-1549.
8. Lindstrom SE, Hiromoto Y, Nerome R, Omoe K, Sugita S, Yamazaki Y, Takahashi T, Nerome K (1998) Phylogenetic analysis of the entire genome of influenza A (H3N2) viruses from Japan: evidence for genetic reassortment of the six internal genes. *J Virol* 72:8021-8031.
9. Mahony JB, Chong S, Luinstra K, Petrich A, Smieja M (2010) Development of a novel bead-based multiplex PCR assay for combined subtyping and oseltamivir resistance genotyping (H275Y) of seasonal and pandemic H1N1 influenza A viruses. *J Clin Virol* 49:277-282.
10. Newman LP, Bhat N, Fleming JA, Neuzil KM (2018) Global influenza seasonality to inform country-level vaccine programs: An analysis of WHO FluNet influenza surveillance data between 2011 and 2016. *PLoS One* 13:e0193263.
11. Nobusawa E, Sato K (2006) Comparison of the mutation rates of human influenza A and B viruses. *J Virol* 80:3675-3678.
12. Parvin JD, Moscona A, Pan WT, Leider JM, Palese P (1986) Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. *J Virol* 59:377-383.

13. Pauly MD, Procario MC, Luring AS (2017) A novel twelve class fluctuation test reveals higher than expected mutation rates for influenza A viruses. *Elife* 6
14. Petrova VN, Russell CA (2018) The evolution of seasonal influenza viruses. *Nat Rev Microbiol* 16:47-60.
15. Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC (2008) The genomic and epidemiological dynamics of human influenza A virus. *Nature* 453:615-619.
16. Sunagawa S, Iha Y, Taira K, Okano S, Kinjo T, Higa F, Kuba K, Tateyama M, Nakamura K, Nakamura S, Motooka D, Horii T, Parrott GL, Fujita J (2016) An Epidemiological Analysis of Summer Influenza Epidemics in Okinawa. *Intern Med* 55:3579-3584.
17. Tamerius J, Nelson MI, Zhou SZ, Viboud C, Miller MA, Alonso WJ (2011) Global influenza seasonality: reconciling patterns across temperate and tropical regions. *Environ Health Perspect* 119:439-445.
18. Webster R, Cox N, Stohr K (2002) WHO Manual on Animal Influenza Diagnosis and Surveillance. In: World Health Organization DoCDSaR (ed) WHO Global Influenza Programme. World Health Organization, pp 1-97
19. Xue KS, Bloom JD (2020) Linking influenza virus evolution within and between human hosts. *Virus Evol* 6:veaa010.
20. Yoshida Y, Tsuneki A, Itagaki A, Tsuchie H, Okada T, Narai S, Kasagi M, Tanaka K, Ito A, Ryoke K, Kageyama S (2015) Frequent Isolations of Influenza A Viruses (H1N1)pdm09 with Identical Hemagglutinin Sequences for More Than Three Months in Japan. *Yonago Acta Med* 58:165-171.
21. Zhou B, Donnelly ME, Scholes DT, St George K, Hatta M, Kawaoka Y, Wentworth DE (2009) Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and Swine origin human influenza a viruses. *J Virol* 83:10309-10313.

Tables

Table 1. Influenza A (H1N1) viruses harboring identical hemagglutinins were also isolated in the following countries/regions

Country/ region	Number of strains	Time lag (days)*	Accession numbers of recorded strains identical to those isolated in the study area in Tottori Prefecture, Japan
USA	28 (including 3 in Guam)	1–147	CY187197, CY187651, CY187652, CY187653, CY210003, CY218881, CY218889, CY223405, CY242486, CY243790, CY244244, CY244782, KT274568, KT274640, KX004065, KX406995, KX407043, KX407163, KX407443, KX410051, KX410091, KX411403, KX411555, KX411891, KX918420, KX918668, KX918732, KY04473
Canada	2	56 and 117	KJ396003, MN538791
Thailand	2	1 and 20	CY054591, KP941704
Mongolia	1	Same day	CY080420
Taiwan	1	7 and 49	KY950223
Italy	1	Unknown**	KU322291

Accession numbers of the 409 strains studied in the present study: LC637996–LC638404

*: Between two isolation dates in the study area and in another area in the world

** : Collection date was not documented.

Figures

Figure 1

Phylogenetic analysis of hemagglutinin of influenza A(H1N1) viruses This phylogenetic tree is composed of 409 influenza A(H1N1) viruses (Accession Nos.: LC637996- LC638404) (2009–2010, n=167; 2010–2011, n=144; 2013–2014, n=18; 2015–2016, n=18; 2016–2017, n=4; 2017–2018, n=16; 2018–2019, n=11; 2019–2020, n=31). A/South Carolina/1/1918(H1N1) was used as the out-group.

Figure 2

Identical strains displaying 100% homology in hemagglutinin nucleotide sequences of A(H1N1) isolated in Tottori Sequential isolation of epidemic influenza viruses was carried out during epidemic seasons in Japan. Of the 409 influenza A(H1N1) viruses isolated in eleven consecutive years (2009-2020, Fig. 1), 237 could be grouped into 57 (X-axis) harboring identical hemagglutinin genes in the successful observation period from 1 (on the same day, left end of X-axis) to 94 days (right end). Closed circles (●) denote each time point of viral isolation from the initial isolation (bottom).