J Vet Sci. 2023 Nov;24(6):e78
https://doi.org/10.4142/jvs.23105
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Rapid Communication
Virology

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Evolution and international transmission of H3N2 canine influenza A viruses from Korea during 2014–2017

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OPEN ACCESS

Received: Apr 12, 2023
Revised: Sep 1, 2023
Accepted: Sep 10, 2023
Published online: Oct 5, 2023

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ABSTRACT

Avian-origin H3N2 canine influenza A viruses (CIVs) have become enzootic in China and Korea and have sporadically transmitted to North America, causing multiple epidemics. We isolated six CIVs in Korea from CIV-infected patients during 2014–2017 and conducted whole genome sequencing and phylogenetic analyses. Results revealed that CIVs have circulated and evolved in Korea since the early 2000s and then diversified into a new clade, probably contributing to multiple epidemics in China, the USA, and Canada. Our findings bridge an evolutionary gap for understanding the global transmission of CIVs, emphasizing the significance of continuous monitoring of CIVs.

Keywords: H3N2 virus; phylogenetic analysis; infectious disease transmission; molecular evolution

INTRODUCTION

Avian-origin H3N2 canine influenza A virus (CIV) was first reported in 2007 in Korea [1], and a subsequent study revealed that these CIVs circulated in China around 2006 [2]. CIVs became enzootic in China and Korea and were first introduced into the USA in 2015 [3]. Since then, several epidemics of H3N2 CIVs have been reported in North America, which are believed to be driven by multiple introductions of the viruses from Asia [3-5].

Despite the worldwide spread of H3N2 CIVs, their pandemic potential is currently believed to be low, and there have been no reported human infections to date. Nevertheless, the ongoing outbreaks may drive additional adaptive evolution in mammalian hosts. Moreover, coinfection of dogs with novel influenza A virus subtypes may facilitate genetic reassortment, potentially causing the emergence of novel viral strains. The close relationship between humans and their companion animals is a cause for the risk of transmission of CIVs to the human population through close contact. Hence, continual surveillance and genetic characterization of H3N2 CIVs are essential. Nonetheless, the number of publicly available whole genome sequences of H3N2 CIVs deposited in public databases such as NCBI (National Center for Biotechnology Information) and GISAID (Global Initiative on Sharing All Influenza Data) remains relatively low compared with that of other animal-origin viruses,

Journal of Veterinary Science

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Conflict of Interest
The author declares no conflicts of interest.

Funding
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (RS-2023-00210169).

which could potentially complicate the precise inference of transmission and evolution patterns. We isolated six H3N2 CIVs during 2014–2017 in Korea and performed time-scaled phylogenies to infer their global transmission and evolution. Our study provides valuable insights into the international spread of H3N2 CIVs and emphasizes the significance of continued surveillance of these viruses.

MATERIALS AND METHODS

Virus isolation, sequencing, and phylogenetic analyses

Six CIVs were isolated from the nasal swabs of CIV-infected dogs at the Veterinary Teaching Hospital, Seoul National University, from 2014 to 2017. All the infected dogs were confirmed to be CIV-positive using reverse transcription quantitative polymerase chain reaction targeting the matrix gene (forward primer: 5′-AGA TGA TTC TAA CCG AGG TCG-3′, reverse primer: 5′-TGC AAA AAC ATC TTC AAG TCT CTG-3′) [6]. The viruses were passaged two or three times into 10-day-old chicken embryonated eggs and subjected to next-generation sequencing.

For whole genome phylogenetic analyses, the sequences of all eight gene segments of H3N2 CIVs were collected from GISAID on December 23, 2022. Sequence alignments were generated using MUSCLE in MEGA11 and manually confirmed for accuracy [7]. In total, 207 complete coding sequences (201 from GISAID and 6 from this study) were included in the phylogenetic analysis. Maximum likelihood phylogenies of each gene segment were constructed using IQ-TREE v2.2.0, with the best-fit models for each gene segment determined using ModelFinder [8]. Node support values were generated using 1,000 ultrafast bootstrap replicates [9] and examined using the SH-aLRT branch test [10] in IQ-TREE v2.2.0. Time-scaled phylogenies of the concatenated gene segments of CIVs were inferred by Bayesian Markov Chain Monte Carlo sampling using BEAST v.1.10.4 with an uncorrelated lognormal relaxed molecular clock [11]. The GTR + G nucleotide substitution model was used, and four independent runs with 50 million steps were combined to obtain an adequate effective sample size (> 200) for all parameters. A time-scaled maximum clade credibility (MCC) tree was generated using TreeAnnotater v.1.10.4 in BEAST and visualized using Figtree 1.4.4.

Data availability statement

The six CIV genome sequences isolated in this study were deposited in GISAID with isolate IDs, as listed in **Table 1**.

RESULTS

The phylogenetic trees for each gene segment exhibited a similar tree topology, except for two virus strains (**Supplementary Fig. 1**). The matrix gene of A/canine/Korea/MV1/2012

Table 1. Canine influenza A viruses isolated in this study

Name	Isolate ID	Subtype	Collection date
A/canine/Korea/SH1/2014	EPI_ISL_17244468	H3N2	2014-09-01
A/canine/Korea/SH5/2015	EPI_ISL_17244667	H3N2	2015-11-20
A/canine/Korea/SH2/2016	EPI_ISL_17244523	H3N2	2016-07-22
A/canine/Korea/SH3/2016	EPI_ISL_17244524	H3N2	2016-07-22
A/canine/Korea/SH4/2016	EPI_ISL_17244648	H3N2	2016-07-22
A/canine/Korea/SH6/2017	EPI_ISL_17244742	H3N2	2017-11-07

(MV1) originated from the pandemic H1N1/09 virus (pdm09 H1N1), which was reported as a reassortant virus between H3N2 CIV and pdm09 H1N1 [12]. The PB1 gene of A/canine/Guangdong/GY01/2018 (GY01) was remarkably distant from other PB1 sequences. Interestingly, the second half (1270-2273) of the GY01 PB1 gene was more similar to A/duck/Hubei/ZYSYF2/2015 (H3N6) than its closest strain A/canine/Guangdong/1/2018 (97.8% and 89.3%, respectively) in contrast to the first half (1-1269) (91.1% and 99.6%, respectively). This could be evidence of segment exchange in the GY01 PB1 gene; however, it could also be an artifact produced by random mutations [13].

To investigate the global dissemination of H3N2 CIV, we constructed an MCC tree using the concatenated eight gene segments of 205 CIVs. The MV1 and GY01 strains were excluded to improve the accuracy of our analysis. The analysis revealed the existence of eight major global transmission events involving H3N2 CIVs (**Fig. 1**). The phylogenetic tree revealed that clades B and C diverged around 2007 and circulated separately in China and Korea, respectively. Subsequently, clade C diverged into clades D and E, and clade D was introduced to the USA, causing the 2015 CIV epidemic in the country. Clade E further diverged into clades E1 and E2, causing multiple international spread of CIVs.

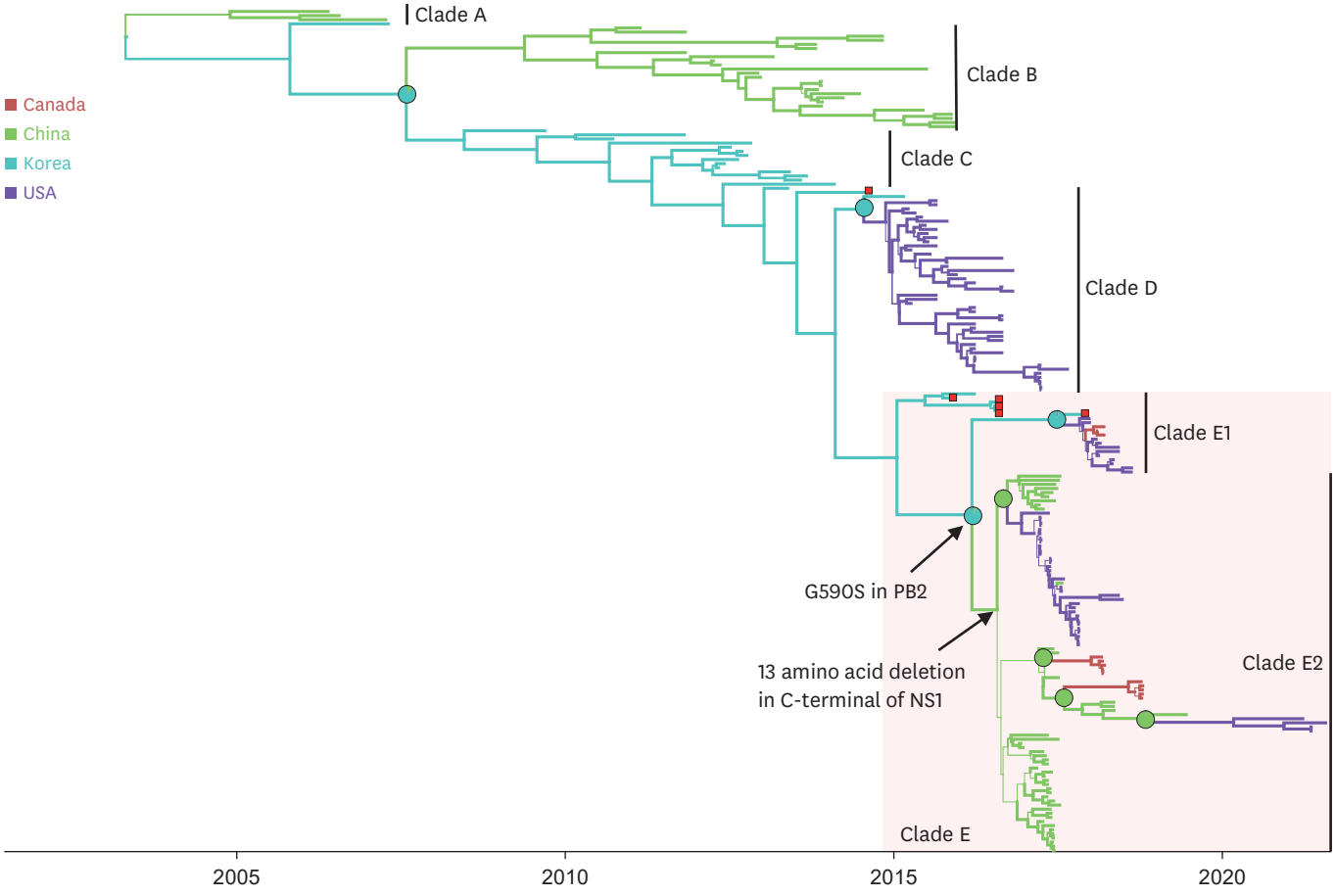

Fig. 1. Bayesian phylogenetic tree of the concatenated eight gene segments of 205 H3N2 CIVs. The branches are color-coded to represent the country of origin, and their widths correspond to the posterior support. The pie charts at the node indicate the probability of the root country for the virus distribution. The tips with red rectangle are the Korean CIVs isolated in this study.
CIV, canine influenza A virus.

https://doi.org/10.4142/jvs.23105

The six viruses isolated in Korea during 2014–2017 belonged to clades D and E1 and were closely related to the multiple introductions of H3N2 CIVs to North America during 2015–2018. No well-known mammalian adaptive substitutions were detected in the six Korean CIV isolates, such as Q591R/K, E627K, and D701N in PB2 or Q226L and G228S substitutions in hemagglutinin (HA) Nonetheless, a serine substitution at position 590 (590S) in PB2 was detected in CIVs circulating in Korea, with the time to the most recent common ancestor inferred as February 25, 2016 (95% HPD: 2015.09.10–2016.07.24). Furthermore, clade E2 with a 13-amino-acid deletion in the C-terminal region of NS1 emerged on July 21, 2016 (95% HPD: 2016.05.01–2016.10.01). Remarkably, this clade spread worldwide, causing multiple outbreaks in China, the USA, and Canada.

DISCUSSION

Since their first detection in 2007, H3N2 CIVs have become enzootic pathogens in Asia, and their introduction has caused periodic epidemics in North America. Their intercontinental transmissions might be associated with dog importation, which increases the risk of their global spread. Despite the continuous CIV circulation in Korea, active surveillance and ongoing sequencing analysis to track their evolution are relatively insufficient [14]. Our study revealed that the CIVs have circulated in Korea during 2014–2017 and belong to clades D and E, and clade E further diverged into two subclades, E1 and E2. Clades D and E1 have been disseminated to North America, causing multiple epidemics in Canada and the USA. Meanwhile, clade E2 has also been introduced to China, where the viruses have evolved and spread to North America multiple times.

Chen et al. reported that an H3N2 CIV strain isolated in 2019, closely related to the SH6 virus in our study, exhibited a high zoonotic potential [15]. The virus acquired a preference for human-like sialic acid receptor, enhanced replication capacities in human lung epithelial cells, and achieved a 100% transmission rate through aerosol transmission in a ferret model. During the viral circulation in Korea around 2016, the H3N2 CIVs harbored two distinctive mutations, G590S in PB2 and a 13-amino-acid deletion in the C-terminal of NS1. The G590S substitution increases polymerase activity and viral replication in mammalian cells and has been associated with the acquisition of the Q591R substitution observed in the 2009 pandemic H1N1 virus [16]. Moreover, the 13-amino-acid deletion in NS1 C-terminal increased the virulence of H5N8 avian influenza A viruses in mice [17]. Therefore, the emergence of these mutations in a recent cluster of CIVs may be related to an increased risk for zoonotic transmission of H3N2 CIVs. Further studies are required to clarify the specific role of these mutations in the adaptation of CIVs to mammals, which would be considerably interesting.

Altogether, the continuous circulation of H3N2 CIVs in Korea during the mid-2010s caused multiple international spread events and the acquisition of several substitutions in the viral genome. These data suggest an ongoing evolution of H3N2 CIVs and emphasize the importance of active surveillance of CIVs to effectively control canine flu and monitor the risk of cross-species transmission of CIVs to humans.

ACKNOWLEDGMENTS

The author is grateful for the technical advice from Junghoon Kwon at Kyungpook National University, College of Veterinary Medicine. The author also appreciates Jin-Ha Song and Seung-Eun Son for their technical assistance.

SUPPLEMENTARY MATERIAL

Supplementary Fig. 1

Maximum likelihood trees of eight segments of canine influenza A virus (H3N2). Branches are colored according to the country of virus isolation.

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