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An epidemic surge of influenza A(H3N2) virus at the end of the 2016–2017 season in Taiwan with an increased viral genetic heterogeneity

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ABSTRACT

Background: The epidemic of the 2016–2017 influenza season in Taiwan started early with moderate activity and was predominated by the influenza A(H3N2) virus. However, the influenza activity increased dramatically during the late stage of the 2016–2017 season.

Objectives: The genetic and antigenic characteristics of the influenza A(H3N2) virus circulating in Taiwan during the 2016–2017 season were investigated. The relationship between virus clades and the patients' 2016–2017 vaccination histories was determined.

Study design: Respiratory samples from patients with influenza-like illness in the community, clustered outbreaks, and inpatients with severe complications were tested for influenza virus. Influenza gene sequencing, phylogenetic analysis and hemagglutination inhibition assay were performed.

Results: A total of 1185, 690 and 353 cases of outpatients, inpatients and cluster events were tested positive for the A(H3N2) virus in this report. Multiple clades of the H3N2 virus co-circulated. New genetic variants were detected, including clade 3C.2a.1 with additional N121 K, K92R or T135 K mutations, 3C.2a.3a with T135 K and R150 K mutations and 3C.2a.4. The proportions of N121 K and T135 K mutations were continuously increasing. Most of the viruses (85.4%, 111/130) were antigenically related to the current vaccine strain. Infection by different clade H3N2 viruses did not correlate with immunization with the 2016–2017 vaccine.

Conclusions: The data in this study indicate that antigenic drift is not the primary determinant of the epidemic wave at the end of the 2016–2017 season. The fitness changes in new variants, waning immunity and climatic changes are considered as possible contributors to the resurgence of the influenza A(H3N2) virus.

1. Background

Influenza is an annually occurring infectious disease. During seasonal epidemics, typically, 5–15% of the worldwide population is infected, resulting in 3–5 million cases of severe illness and between 250,000–500,000 deaths every year around the world [1]. Currently, the influenza A(H1N1), A(H3N2) and influenza B viruses are responsible for seasonal epidemics in humans [2,3]. Among the three, the A(H3N2) viruses cause more severe illness and have a higher genetic mutation rate [4]. The A(H3N2) virus also affects the elderly, with increased hospitalization and case fatality rates reported in patients aged 65 years or older [5].

In the influenza season of 2014–2015, a newly emerged A(H3N2) virus belonging to the genetic 3C.2a clade, whose antigenic properties were distinct from the contemporary vaccine component, rapidly predominated in humans worldwide and resulted in low (1%) vaccine effectiveness against illness caused by influenza A(H3N2) viruses [6]. Another concern associated with the current conventional inactivated vaccines is the resultant short-lived immunity. Intraseason waning of host immunity conferred by influenza vaccines has been reported [7], [8]. The vaccine protection against viral types or subtypes has been observed to decrease with increasing time since vaccination, leading to a decline in vaccine effectiveness of approximately 7% per month for influenza A(H3N2) and influenza B and 6%-11% per month for influenza A(H1N1)pdm09 viruses [7]. Furthermore, this decreased vaccine effectiveness may relate to the late influenza epidemic that has occurred in some northern hemisphere seasons [8].

In Taiwan, the influenza virus is identified throughout the year by the country-wide laboratory surveillance network coordinated by the Taiwan Centers for Disease Control (CDC) [9]. In Taiwan, the peak activity of influenza is usually observed in the winter, with many more virus-infected cases identified from December to the next March

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[9–11]. The epidemic of the 2016–2017 influenza season in Taiwan started early with moderate activity and was marked by the viral subtype A(H3N2). However, the number of inpatients and outpatients infected by the influenza A(H3N2) virus peaked dramatically in the late stage of the 2016–2017 season, from May to July 2017. The suddenly increasing virus activity raised a public health concern.

2. Objectives

In this study, we analyzed the genetic and antigenic characteristics of the influenza A(H3N2) viruses circulating in Taiwan during the 2016–2017 season, as well as the evolutionary trends of the viruses. The virus strains detected in patients who had received the 2016–2017 trivalent influenza vaccine (TIV) were also analyzed to determine potential factors contributing to the surge in activity of the A(H3N2) viruses.

3. Study design

3.1. Definition of the annual period of the influenza season

In this study, the duration of an influenza season was defined as the period from July 1 to June 30 of the next year. Therefore, the study period (the 2016–2017 influenza season) started on July 3 (week 27), 2016, and extended to July 1 (week 26), 2017.

3.2. Laboratory surveillance of influenza viruses/collection of clinical specimens and virus isolates

In Taiwan, data regarding influenza isolates and laboratory-confirmed cases were obtained from three major influenza laboratory surveillance systems that target outpatients with influenza-like illness in the community, inpatients with severe complications and clustered outbreaks in densely populated places. For community-based surveillance, a national influenza surveillance network was established in 1999 and coordinated by the Taiwanese CDC [9,11]. Taiwan CDC was notified of the clustered cases through a syndromic surveillance system. Influenza with severe complications is a notifiable disease. Patients who exhibit influenza-like illness and need treatment in an intensive care unit due to symptoms of complications, including pulmonary complications, neurological complications, myocarditis or pericarditis and invasive bacterial infection, should be reported to the National Notifiable Disease Surveillance System (NNDSS). Clinical specimens collected through the three surveillance systems were inoculated onto Madin-Darby canine kidney (MDCK) cells for virus isolation and/or tested by real-time RT-PCR [12].

3.3. Influenza gene sequencing and phylogenetic analysis

Viral RNA extraction and a nucleotide sequence analysis of viral genes were processed using methods described previously [13,14]. Multiple sequence alignments, protein translation and phylogenetic analysis were performed on the basis of nucleotide sequences using MEGA6 software [15] and BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Phylogenetic trees were constructed using the neighbor-joining method, and 1000 bootstrap replications were performed to evaluate the robustness.

3.4. Hemagglutination inhibition (HI) assay

Antigenic characterization of the influenza A(H3N2) virus was conducted using HI assays described previously [14] with ferret antisera raised against reference virus strains and 0.75% guinea pig RBCs.

3.5. Proteotyping analysis of the A(H3N2) viruses

The proteotyping map was constructed from the amino acid sequences of viral HA genes to monitor the genetic mutations chronologically and to detect the emergence of a new viral variant. The detailed method was described previously [16]

3.6. Statistical analysis

Statistical analyses were performed using chi-square and Fisher's exact tests. Data were considered to be statistically significant at a p value of less than 0.05.

3.7. Sequence information

Information about the influenza viruses used for phylogenetic analysis in this study, including nucleotide sequence accession numbers, collection dates and evolutionary clades are listed in Supplementary Table 1.

4. Results

Using data from the national influenza surveillance system in Taiwan, the circulation of influenza viruses was analyzed. During the 2016–2017 influenza season, a total of 59 (4.2%), 1185 (84.3%) and 161 (11.5%) cases tested positive in outpatients in the community (mild cases) for the influenza A(H1N1)pdm09, A(H3N2) and influenza B viruses, respectively, using real-time RT-PCR and/or virus isolation. From the inpatients with severe complications (severe cases), a total of 43 (5.4%), 690 (86.8%) and 62 (7.8%) cases were laboratory-confirmed as infected by the influenza A(H1N1)pdm09, A(H3N2) or influenza B viruses, respectively. Regarding the cluster outbreaks, a total of 8 (2.0%), 353 (86.5%) and 47 (11.5%) events were caused by influenza A(H1N1)pdm09, A(H3N2) or influenza B viruses, respectively. The monthly distribution of virus type/subtype among outpatients, inpatients and clustered cases are shown in Fig. 1A–C. Based on the results, the predominant type/subtype in the three populations was consistent, and the influenza A(H3N2) virus was more prevalent than either influenza A(H1N1)pdm09 or influenza B viruses (Fig. 1A–C). The winter influenza epidemic of the 2016–2017 season began to occur in week 43 of 2016 (Fig. 1A) and peaked in week 47 (Fig. 1A). Of note, an epidemic of influenza A(H3N2) virus resurged outside of winter at the end of the 2016–2017 season (Fig. 1A) and reached a peak during weeks 24–26 of 2007 in the surveillances of outpatients, inpatients and cluster events (Fig. 1). Such an epidemic surge of influenza in the summer time was unusual in Taiwan, compared with the previous community surveillance data of the 2009–2010 to 2015–2016 seasons (Fig. 1D).

To investigate the possible viral factors contributing to the prominent epidemic wave of the influenza A(H3N2) virus in this summer, first, the evolutionary patterns of the circulating viruses were explored. Based on the representative phylogenetic topology obtained through analysis of the full-length viral hemagglutinin (HA) gene sequences, the H3N2 viruses belonged exclusively to clade 3C.2, which could be further classified into five clades: 3C.2a.1, 3C.2a.2, 3C.2a.3, 3C.2a.3a and 3C.2a.4 (Fig. 2). The signatures of these viruses were those of the 3C.2a clade (145S-159Y-160T-225D) with additional signatures of 171 K, 131K–142 K, 121K–144 K, 121K-144K-135K–150 K and 142G-144R-I192T-197H, respectively (Fig. 2). The monthly distribution of these clades showed that during the early stage of the 2016–2017 season in Taiwan, the mild winter influenza epidemic was mainly caused by viruses of the clades 3C.2a.1 and 3C.2a.2 (Fig. 3). However, the clade 3C.2a.1 viruses harboring additional N121 K, K92R or T135 K mutations increased in April 2017, accompanied by a new emergence of the clade 3C.2a.3a virus (3C.2a.3 with additional T135 K and R150 K mutations), both of which co-dominated during the epidemic resurgence,